

Bone Mineral Status and Renal Tubular Dysfunction in HIV- Positive Men

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Abstract

Background: As HIV-positive patients live longer, they are experiencing age-related comorbidities, including bone and renal disease. The aetiology is multifactorial, comprising traditional and HIV-related factors, including antiretroviral therapy (ART). However, whether reduced bone mineral density (BMD) translates into a higher fracture risk remains unclear.

Aims: My main aim in my thesis was to conduct a cross-sectional study with changes over time to investigate BMD and renal tubular dysfunction (RTD) in a relatively homogenous group of white, ART-experienced HIV-positive men in the UK, who were mostly men who have sex with men (MSM) and mainly on tenofovir disoproxil fumarate (TDF). The aims I investigated were:

1. The prevalence and risk factors associated with reduced BMD at baseline and the change in BMD over 12 months and the factors associated with loss of BMD.
2. The utility of the FRAX[®] score and peripheral dual-energy x-ray absorptiometry (pDXA) as screening tools.
3. The utility of albumin/protein ratio (APR) in differentiating RTD from other proteinuria and the relationship between RTD and bone.

Methods: I designed a prospective cohort study of HIV-positive men attending an HIV outpatient clinic. Participants underwent central DXA (cDXA) and pDXA, fasting blood and urine tests (including bone turnover markers [BTMs] and retinol binding protein creatinine ratio [RBPCR]) and completed a questionnaire at baseline and at 12 months.

Results: I found a low prevalence of reduced BMD. Mainly 'traditional' risk factors were associated with reduced BMD. The change seen in absolute BMD at 12 months was small, reflecting the short follow-up period. The only factor associated with a greater than smallest detectable difference (SDD) reduction in BMD was a detectable HIV viral load. FRAX[®] was not sensitive enough as a screening tool, and pDXA was slightly more sensitive, although combining FRAX[®] and pDXA was not much better than FRAX[®] alone. RTD prevalence was too low to conduct meaningful analyses. Overall, 20.7% had RBPCR-defined RTD and there was a borderline association between severe RTD and BMD at the lumbar spine, but not with BTMs.

Conclusions: In my cohort of mainly white, ART-experienced (mainly exposed to TDF) HIV-positive MSM in the UK, the prevalences of reduced BMD and RTD were low. The factors associated with reduced BMD were mainly 'traditional' factors and probably reflects a 'return to health' with ART in these men. There was not much change in BMD over 12 months, which is probably reflective of the short follow-up period. Using FRAX[®] and pDXA may be useful as screening tools, but further work is needed before any firm conclusions can be made in this cohort. Although one-fifth had RBPCR-defined RTD, the clinical significance of these findings and the impact on bone health is yet to be fully elucidated.

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Glossary of Abbreviations

1,25(OH) ₂ D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
95% CI	95% confidence interval
ACR	Albumin/creatinine ratio
ACTG	AIDS Clinical Trials Group
AIDS	Acquired immunodeficiency syndrome
AKI	Acute kidney injury
ALLRT	ACTG Longitudinal-Linked Randomized Trial
ALP	Alkaline phosphatase
ANRS	Agence Nationale de Recherche sur le Sida
APR	Albumin/protein ratio
ART	Antiretroviral therapy
ASSERT	Assessment of Safety and Efficacy of Abacavir/Lamivudine and Tenofovir/Emtricitabine
ATN	Acute tubular necrosis
AUROC	Area under the receiver-operating characteristic curve
BHIVA	British HIV Association
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMP-7	Bone morphogenic protein 7
BMU	Basic multicellular unit
BSUH	Brighton and Sussex University Hospitals
BTM	Bone turnover marker
cDXA	Central dual-energy x-ray absorptiometry
CHAMPS	Cohort of HIV at-risk Aging Men's Prospective Study
CKD	Chronic kidney disease
CKD-Epi	Chronic Kidney Disease Epidemiology Collaboration
CKD-MBD	Chronic kidney disease-mineral and bone disorder
CRF	Case report form
CT	Computed tomography
CTX	C-terminal cross-linking telopeptides of type I collagen
CTX-MMP	C-terminal cross-linking telopeptides of type I collagen generated by metalloproteinase

dATP	2'-deoxyadenosine triphosphate
dAMP	2'-deoxyadenosine monophosphate
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded deoxyribonucleic acid
DXA	Dual-energy x-ray absorptiometry
EDTA	Ethylenediaminetetraacetic acid
EFV	Efavirenz
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
FePO ₄	Fractional excretion of phosphate
FGF-23	Fibroblast growth factor-23
FRAX [®]	Fracture Risk Assessment Tool
FSGS	Focal segmental glomerulosclerosis
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GP	Glomerular proteinuria
HBV	Hepatitis B
HCV	Hepatitis C
HIV	Human immunodeficiency virus
HIVAN	HIV-associated nephropathy
HIVICK	HIV-associated immune complex kidney disease
HOPS	HIV Outpatient Study
HOPS-DIDC	HIV Outpatient Study-Denver Infectious Diseases Consultants
HR	Hazard ratio
ICH	International Conference on Harmonisation
IFCCLM	International Federation of Clinical Chemistry and Laboratory Medicine
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-11	Interleukin-11
IOF	International Osteoporosis Foundation
IOM	Institute of Medicine
iPrEx	Iniciativa Profilaxis Pre-Exposición
IQR	Interquartile range
IR(ME)R	Ionising Radiation (Medical Exposure) Regulations
IRR	Incidence rate ratio

ISCD	International Society for Clinical Densitometry
IVDU	Intravenous drug use
KDIGO	Kidney Disease: Improving Global Outcomes
K/DOQI	Kidney Disease Outcomes Quality Initiative
LMWP	Low molecular weight protein
M-CSF	Macrophage colony stimulating factor
MDRD	Modification of Diet in Renal Disease
MEDICLAS	Metabolic Effects of Different Classes of Antiretrovirals
MONET	MONotherapy in Europe with Tmc114
MRI	Magnetic resonance imaging
MRP2	Multidrug resistance protein-2
MRP4	Multidrug resistance protein-4
MSM	Men who have sex with men
NGAL	Neutrophil gelatinase-associated lipocalin
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NKF	National Kidney Foundation
NNRTI	Non-nucleoside reverse inhibitor
NPV	Negative predictive value
NRTI	Nucleoside reverse transcriptase inhibitor
NOGG	National Osteoporosis Guideline Group
NOS	National Osteoporosis Society
NTX	N-terminal cross-linking telopeptides of type I collagen
OAT1	Organic anion transporter-1
OAT3	Organic anion transporter-3
OCT1	Organic cation transporter-1
OCT2	Organic cation transporter-2
OR	Odds ratio
P1CP	C-terminal propeptide of type I procollagen
P1NP	N-terminal propeptide of type I procollagen
PCP	<i>Pneumocystis jirovecii</i> (<i>carinii</i>) pneumonia
PCR	Protein/creatinine ratio
pDXA	Peripheral dual-energy x-ray absorptiometry
PGE2	Prostaglandin E2
Pgp	P glycoprotein
PHI	Primary HIV infection
PIS	Participant information sheet

PI	Protease inhibitor
PPAR γ	Peroxisome proliferator-activated receptor gamma
PPV	Positive predictive value
PrEP	Pre-exposure prophylaxis
PTH	Parathyroid hormone
QA	Quality assurance
RADAR	Efficacy of RAltegravir Combined with Boosted DARunavir Compared to Tenofovir/Emtricitabine Combined with Boosted Darunavir in Antiretroviral-Naïve Patients
RANK	The receptor for RANKL
RANKL	Receptor activator for nuclear factor κ B ligand
RBP	Retinol binding protein
RBPCR	Retinol binding protein creatinine ratio
RCT	Randomised controlled trial
REC	Research Ethics Committee
RLFP	Remaining lifetime fracture probability
RNA	Ribonucleic acid
ROC	Receiver-operating characteristic
ROI	Region of interest
RTD	Renal tubular dysfunction
RUNX-2	Runt-related transcription factor-2
SD	Standard deviation
SDD	Smallest detectable difference
SMART	Strategies for Management of Antiretroviral Therapy
ssRNA	Single-stranded ribonucleic acid
START	Strategic Timing of AntiRetroviral Treatment
STEAL	Simplification of Antiretroviral Therapy with Tenofovir-Emtricitabine or Abacavir-Lamivudine
SUN	Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy
T ₃	Free triiodothyronine
T ₄	Free thyroxine
TAF	Tenofovir alafenamide
TDF	Tenofovir disoproxil fumarate
TGF- β	Transforming growth factor- β
TNF	Tumour necrosis factor
TP	Tubular proteinuria

TRACP	Tartrate-resistant acid phosphatase
TSH	Thyroid stimulating hormone
UK CHIC	UK Collaborative HIV Cohort
ULN	Upper limit of normal
VACS-VC	Veterans Aging Study Virtual Cohort
VDD	Vitamin D deficiency
VHA CCR	Veterans Health Administration's Clinical Case Registry
WHO	World Health Organization
WIHS	Women's Interagency HIV Study
WMA	World Medical Association

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For your unconditional love, support and encouragement always

To my husband

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For your continued love and support, especially through some tough times

To my daughter

Amaya

For putting everything into perspective

To my late supervisor

Prof Martin Fisher

May your star forever shine bright

Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed: 

Dated: 27th July 2017

Chapter 1: Introduction

1.1 Background

In the course of this study, which has extended over eight years, knowledge of human immunodeficiency virus (HIV) infection, long-term complications of the disease and HIV treatment have changed considerably as new data have emerged. This Introductory chapter describes the understanding of HIV infection, bone mineral density (BMD) and kidney disease as it was at the commencement of my study. Subsequent developments will be described and discussed in the relevant Results chapters and in my final Discussion chapter (Chapter 8).

1.2 HIV infection

1.2.1 Introduction

HIV was first recognised as causing a disease of unknown aetiology in the USA in 1981 [1]. This infection was associated with fatal disease in young, previously well men who have sex with men (MSM). It was characterised by emaciation, overwhelming opportunistic infection, in particular, *Pneumocystis jirovecii* (*carinii*) pneumonia (PCP), and rare aggressive tumours, such as Kaposi's sarcoma. The USA Center for Disease Control named this clinical phenomenon acquired immunodeficiency syndrome (AIDS). In 1983, the ribonucleic acid (RNA) virus responsible for AIDS was identified as a lentivirus belonging to the retrovirus family, and was demonstrated to cause AIDS by selective destruction of immune cells expressing the CD4 cell trans-membrane receptor [2,3]. The term HIV infection began to be used in 1986 [3]. There are two types of HIV infection: HIV-1 and HIV-2. HIV-1 is present worldwide, whilst HIV-2 is primarily found in West Africa [4]. HIV-1 is more likely to cause disease and a more rapid course than HIV-2 [4]. HIV-1 is responsible for the global epidemic and will be the focus of this thesis, and will henceforth be referred to as 'HIV'.

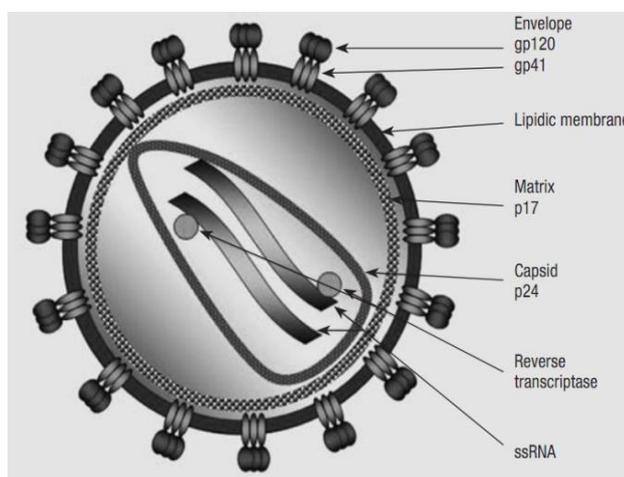
1.2.2 HIV pathogenesis

HIV is a retrovirus which uses the enzyme reverse transcriptase to produce proviral deoxyribonucleic acid (DNA) from single-stranded RNA. HIV primarily infects mononuclear cells in the immune system, particularly T-helper lymphocytes containing the CD4 cell receptor. It also infects other cells expressing CD4 cell receptors, including macrophages, Langerhans cells, monocytes and microglial cells [5]. HIV infection leads to low levels of CD4 T-lymphocytes via three main mechanisms which involve the direct viral killing of infected cells, increased rates of apoptosis in infected cells and killing of infected CD4 T-lymphocytes by CD8 cytotoxic lymphocytes that recognise infected cells [6,7]. As a result of this selective destruction of the immune system, individuals infected with HIV infection are more susceptible to illnesses and opportunistic infections [8].

The HIV virion contains three components comprising the core, the matrix and the outer lipid envelope (Figure 1.2.2.1) [7]. The glycoprotein envelope consists of a lipid bilayer membrane and is made up of two glycoproteins, gp120 and gp41 [7]. These two glycoproteins are essential for the binding of HIV to CD4 T-lymphocytes and fusing with target host cells [5]. The matrix is made up of viral protein p17 and is surrounded by the envelope. The viral core contains two single strands of viral RNA and is encapsulated by the capsid comprised of viral protein p24 and viral enzymes (reverse transcriptase, integrase and protease), which are essential for viral replication [7]. The structure of HIV is determined by three major genes called gag, pol and env, which replicate essential HIV components. The RNA genome also contains other genes (e.g. tat, rev, nef, vif, vpr, vpu) and polyproteins (e.g. p55-gag).

Figure 1.2.2.1 Structure of the HIV virion [7]

Structure of the HIV virion showing all the major components.
ssRNA: single-stranded RNA

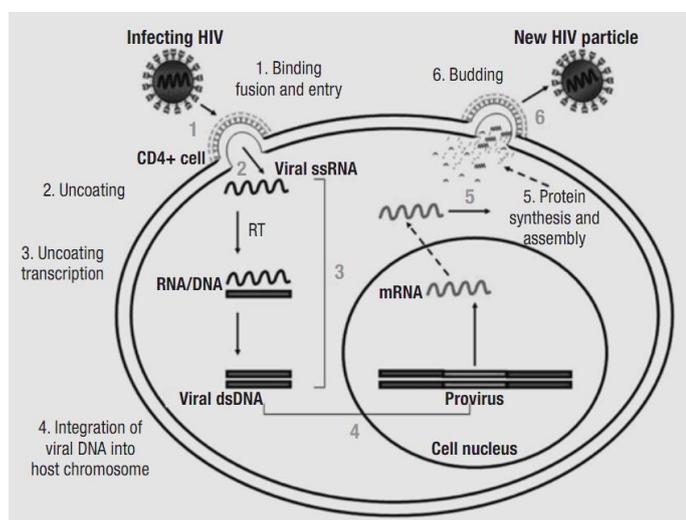


HIV can only replicate inside human cells. Its lifecycle is divided into two phases, comprising HIV entry and establishment of infection, followed by active viral assembly and budding (Figure 1.2.2.2) [7]. The HIV particle binds to a host cell by attaching its gp120 to the host's CD4 cell receptor site. After attachment, gp41 facilitates fusion of the HIV envelope with the CD4 cell membrane. Fusion of these membranes is followed by release of the viral RNA contents into the cell, leaving the envelope behind. The HIV viral core then uncoats, releasing two single strands of viral RNA into the host's cytoplasm. Since HIV requires DNA for replication, the enzyme reverse transcriptase converts the viral RNA into DNA. This DNA is transported to the nucleus, where it is incorporated into the human genome by the enzyme integrase. This HIV provirus can lie dormant within the cell for a long period but once activated, it uses the host enzyme RNA polymerase to create copies of the HIV genomic material and messenger RNA. These are transported outside the nucleus and are translated into new HIV proteins and enzymes [5]. The enzyme protease cleaves long protein strands into smaller ones. At the host cell surface, the new HIV proteins and enzymes combine with viral RNA to form new HIV particles, which when newly matured are released from the host cell by a process called 'budding'. These new HIV particles then infect other cells, thereby restarting the replication cycle. The entire process is very active and large numbers of HIV particles are released daily [9].

Figure 1.2.2.2 The HIV life cycle [7]

This figure shows how the HIV virion replicates. It binds to the host cell and then enters the host cell via fusion (1). The viral core uncoats (2), releasing viral RNA into the host's cytoplasm. The HIV RNA is converted to DNA using reverse transcription (3). This proviral DNA is integrated into the host cell chromosome (4). Using RNA polymerase, the provirus creates copies of the HIV genomic material and messenger RNA, which leads to protein synthesis and the assembly of new HIV virions (5). The new HIV virions are released from the host cell via budding (6).

DNA: deoxyribonucleic acid; dsDNA: double-stranded deoxyribonucleic acid; RNA: ribonucleic acid; ssRNA: single-stranded ribonucleic acid



HIV infection is transmitted by exposure to body fluids, including serum, seminal fluid, vaginal secretions, amniotic fluid and breast milk, and occurs via unprotected sexual intercourse, sharing of intravenous needles or transfusion of contaminated blood products [6]. Additionally, pregnant women infected with HIV can transmit the infection to their foetus or infant during the perinatal period.

1.2.3 Natural history of HIV infection

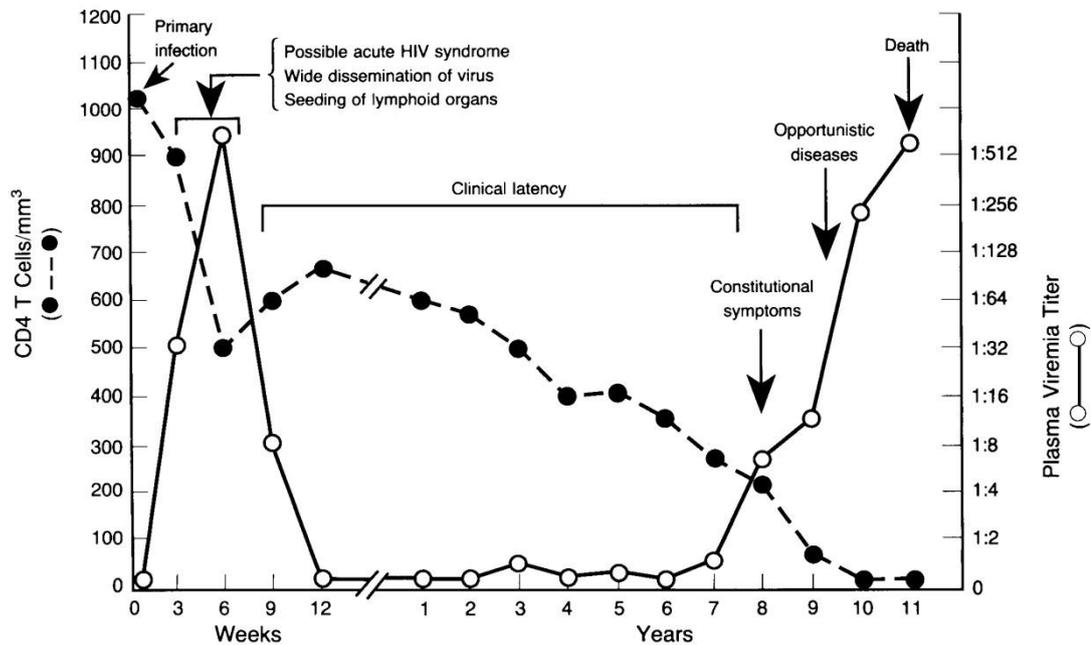
Figure 1.2.3.1 shows the course of HIV infection, which can be separated into five stages as below:

1. Primary HIV infection (PHI)
2. Early stage
3. Middle stage
4. Advanced stage
5. Late stage.

During the seroconversion phase of PHI, there is widespread dissemination of HIV infection [10]. This leads to a very high HIV viral load and a rapid decrease in CD4 cell count, which eventually reverses, although pre-infection levels are rarely reached. The early stage of infection (CD4 cell count >500 cells/ μ L) which follows is usually asymptomatic, except for the appearance of, or deterioration of, certain skin disorders (e.g. seborrhoeic dermatitis, aphthous ulcers, psoriasis). In the middle stage of infection, the CD4 cell count decreases to between 200 and 500 cells/ μ L. This phase becomes mildly symptomatic, with the worsening of skin disorders from the previous stage, as well as the emergence of certain conditions, such as recurrent herpes simplex and varicella zoster infections, diarrhoea, weight loss, intermittent fever, and respiratory infections caused by community-acquired pathogens, including tuberculosis. During the advanced phase, the HIV viral load increases and the CD4 cell count further drops to between 50 and 200 cells/ μ L. At this point, patients become susceptible to opportunistic infections (e.g. PCP, Kaposi's sarcoma) and AIDS. In the late stage (CD4 cell count <50 cells/ μ L), very high levels of viraemia are seen in conjunction with conditions associated with severe immunodeficiency (e.g. cytomegalovirus retinitis, disseminated *Mycobacterium avium* complex infection). Although these clinical stages have been arbitrarily chosen, evidence indicates that inflammation associated with chronic HIV infection is what is important and that this is highly predictive of outcome [11].

Figure 1.2.3.1 Natural history of HIV infection [10]

Following the primary infection, in the early stage there is widespread dissemination of HIV and a sharp reduction in the CD4 cell count. There is then a reduction in the HIV viral load as the body mounts an immune response to the infection. This is followed by a long latent period. As the infection progresses, the HIV viral load continues to increase and the CD4 cell count continues to decrease until a critical level is reached, when there is a risk of developing opportunistic infections and/or AIDS-related conditions.



1.2.4 HIV epidemiology

Since the start of the HIV epidemic, 60 million people have been infected, with the majority living in sub-Saharan Africa, and 25 million people have died worldwide [3]. In 2009, there was a global pandemic, with 33.4 million individuals estimated to be infected with HIV [12].

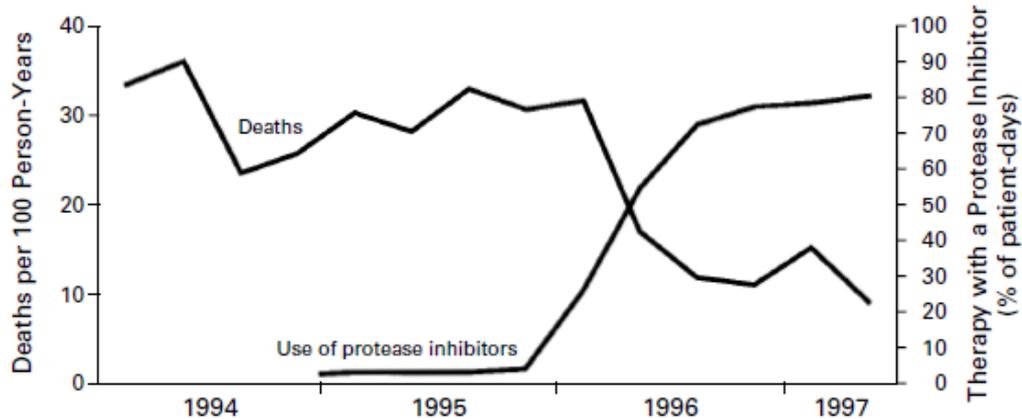
By 2009, an estimated 86,500 individuals had been infected in the UK, which is equivalent to a crude rate of 1.4 people per 1,000 population (1.9 per 1,000 men and 0.91 per 1,000 women) [13]. Approximately a quarter of HIV-positive people were estimated to be unaware of their diagnosis [13]. While the rates of new diagnoses in people infected heterosexually had declined, the incidence in MSM remained high. However, the proportion diagnosed late was lower among MSM (39%) compared with heterosexual women (59%) and heterosexual men (66%) [13].

Despite the yearly increase in rates of HIV-positive individuals in the UK, hospitalisation and mortality rates have fallen over the last decade, and the number of deaths from AIDS has fallen from 1715 in 1995 to 525 in 2008 [13,14]. These

reductions in morbidity and mortality were attributable to the introduction of antiretroviral therapy (ART), as shown in Figure 1.2.4.1 [14].

Figure 1.2.4.1 Change in the rate of death from AIDS with the introduction of ART [14]

This figure shows that the death rate dramatically reduced with the introduction of PIs in the 1990s. PI: protease inhibitor



In the ART era, mortality in HIV-positive patients has dramatically declined [15]. A young person diagnosed with HIV infection in a resource-rich country can expect a longer life expectancy than in the pre-ART era, and median survival is predicted to be more than 35 years after diagnosis [16]. An analysis of 14 cohort studies in Europe and North America has estimated the life expectancy of young HIV-positive patients to be at least two-thirds that of the general population [15]. Interestingly, a study from the Netherlands has suggested that asymptomatic HIV-positive patients who are ART-naïve 24 weeks after diagnosis have an expected life expectancy that is similar to that in the general population [17].

Improved survival has seen a shift in disease progression. Mortality associated with opportunistic infections and AIDS has declined, and HIV-positive patients are more likely to die from non-AIDS-defining conditions, of which some may be related to HIV infection or ART [13,18,19]. The life expectancy of asymptomatic HIV-positive ART-naïve patients is similar to that of HIV-negative individuals after 24 weeks of diagnosis [17]. As HIV-positive patients continue to age, they are at increased risk of developing chronic conditions, such as cardiovascular disease, renal disease and osteoporosis [20]. Additionally, ART has been associated with numerous metabolic complications, including hyperlipidaemia, lipodystrophy, insulin resistance and altered bone metabolism [21,22]. Reduced BMD and osteoporosis are complications that are of concern in an ageing population [23]. The consequence of reduced BMD

is that it can cause an increased incidence of fragility fractures, which could worsen morbidity and mortality in an ageing HIV population. There continues to be improved survival and the risk from long-term complications may increase as HIV-positive patients continue to age [24].

1.2.5 ART

The mainstay of management of HIV infection is with ART. There are a number of different classes, with nucleoside reverse inhibitors (NRTIs), non-nucleoside reverse inhibitors (NNRTIs) and boosted protease inhibitors (PIs) being the most commonly used (Table 1.2.5.1). There are also a number of newer classes of drugs which act via different mechanisms to prevent viral replication. Over the years, treatment has evolved from the use of single agents to using combination treatment. In 2009, the British HIV Association (BHIVA) guidelines recommended prescribing a combination of two NRTIs with either an NNRTI (preferred regimen) or a PI boosted with ritonavir (alternative regimen) [25]. There are also specific reasons for prescribing different regimens or classes, such as viral resistance, side effects, pill burden and patient preference.

Table 1.2.5.1 Classes of ART and individual drugs available in 2009

These were the individual ART drugs which were available in 2009, although there are newer drugs available now in some classes.

NRTIs	NNRTIs	PIs	Fusion/entry inhibitors	Integrase inhibitors
Abacavir	Delavirdine	Amprenavir	Enfuvirtide	Raltegravir
Didanosine	Efavirenz (EFV)	Atazanavir	Maraviroc	
Emtricitabine	Etravirine	Darunavir		
Lamivudine	Nevirapine	Fosamprenavir		
Stavudine	Rilpivirine	Indinavir		
Tenofovir (TDF)*		Lopinavir/ritonavir		
Zalcitabine		Nelfinavir		
Zidovudine		Ritonavir		
		Saquinavir		
		Tipranavir		

EFV: efavirenz; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors; PI: protease inhibitor

*NB: TDF refers to tenofovir disoproxil fumarate

Although Table 1.2.5.1 shows antiretroviral drugs which were available in 2009, the treatment options have since evolved. Some drugs (e.g. stavudine, zalcitabine, delavirdine, fosamprenavir, enfuvirtide) are rarely or no longer used. Newer drugs (e.g. dolutegravir, tenofovir alafenamide [TAF]) have become available, and some of these will be discussed in Chapter 8. The current BHIVA guidelines recommend starting ART with tenofovir disoproxil fumarate (TDF)/emtricitabine or TAF/

emtricitabine as the backbone with the third drug being either an NNRTI (rilpivirine), a boosted PI (atazanavir/ritonavir or darunavir/ritonavir) or an integrase inhibitor (raltegravir, dolutegravir or elvitegravir/cobicistat) [26].

1.2.5.1 Tenofovir disoproxil fumarate (TDF)

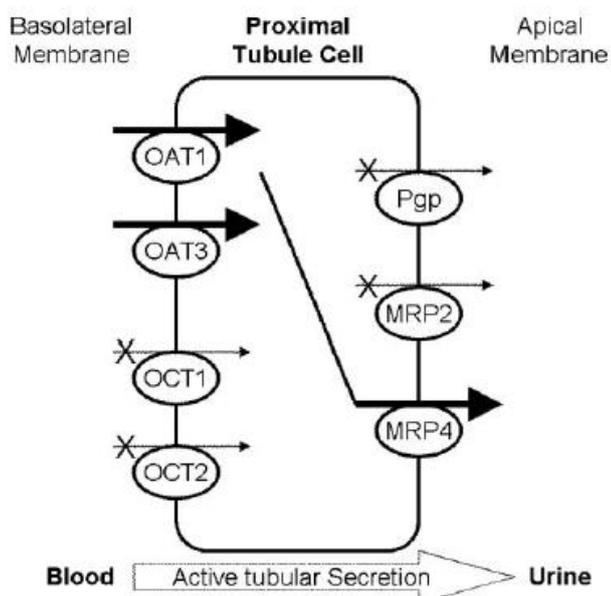
Although TDF is in the NRTI class, it is technically a nucleotide reverse transcriptase inhibitor as it is an acyclic nucleotide analogue of 2'-deoxyadenosine monophosphate (dAMP) [27]. The active moiety is tenofovir-diphosphate. However, as this has poor permeability of the lipid membrane and hence low bioavailability, it is orally administered as the fumarate salt of its disoproxil prodrug, TDF. After phosphorylation, tenofovir-diphosphate competes with endogenous 2'-deoxyadenosine triphosphate (dATP) to be incorporated by virally encoded reverse transcriptase enzymes where it acts as a chain terminator of viral DNA [27].

TDF is transported from the blood into the proximal tubule by the organic anion transporters organic anion transporter-1 (OAT1) and organic anion transporter-3 (OAT3) on the basolateral membrane, and secreted out in the urine via the multidrug resistance protein-4 (MRP4), which is an apical membrane transporter (Figure 1.2.5.1) [28]. TDF can also be transported via multidrug resistance protein-2 (MRP2), but this pathway is blocked by ritonavir, which is used to boost PIs [28].

Figure 1.2.5.1 Mechanism of active renal tubular secretion of TDF [28]

TDF is transported from the blood into the proximal tubule by OAT1 and OAT3, where it is effluxed into the urine by the efflux pump MRP4 on the apical membrane.

MRP2: multidrug resistance protein-2; MRP4: multidrug resistance protein-4; OAT1: organic anion transporter-1; OAT3: organic anion transporter-3; OCT1: organic cation transporter-1; OCT2: organic cation transporter-2; Pgp: P glycoprotein



Data from randomised controlled trials (RCTs) have not shown any serious adverse events [29-32]. However, TDF has been reported to cause renal tubular dysfunction (RTD) [33]. Several case reports and studies emerged which described the association of TDF with Fanconi syndrome, a severe form of RTD characterised by glycosuria, renal phosphate wasting and increased urinary excretion of low molecular weight protein (LMWPs) [33-38]. These studies have shown that the risk of Fanconi syndrome is increased by the concomitant prescription of boosted PIs.

TDF can also affect BMD. This can occur directly via its actions on osteoclasts and osteoblasts due to altered gene metabolism, which have been demonstrated *in vitro* [39,40]. TDF also has an indirect effect on BMD via RTD and Fanconi syndrome, and renal phosphate wasting leading to osteomalacia [33]. Finally, TDF can have an effect on the vitamin D/PTH pathway. TDF has been shown to cause secondary hyperparathyroidism and increased bone turnover, which is worse in patients with vitamin D deficiency (VDD) [41].

There is now a newer drug called TAF that is a prodrug of TDF [42]. It has better bone and renal profiles and will be discussed in Chapter 8.

1.3 Bone biology

1.3.1 Structure and function of normal bone

Bone consists of bone cells and a mineralised bone matrix comprised of organic protein and inorganic material [43]. There are two main types of bone: cortical (compact) bone and trabecular (cancellous) bone [43]. Cortical bone, found in the shaft of long bones and in the outer shell of flat bones, is formed of concentric rings of bone and is able to sustain the strain of bending and is responsible for the support function of bone. Trabecular bone, located at the ends of long bones and inside flat bones (e.g. vertebral bodies), is the main site of bone remodelling [44]. It is made up of an interconnecting network of bone, which is able to withstand compressive loads. Different bones are made up of different compositions of cortical and trabecular bone. For example, the femoral neck is comprised of 70% cortical bone, with its proportion increasing nearer the greater trochanter [45].

There are three main types of bone cells: osteoclasts, osteoblasts and osteocytes [46]. Osteoclasts are derived from haematopoietic stem cells [47]. They fix to bone and actively secrete hydrogen ions. This lowers the pH and in turn increases the

solubility of the inorganic matrix. Osteoclasts then produce a variety of proteolytic enzymes (e.g. cathepsin K, tartrate-resistant acid phosphatase [TRACP]) which degrade the organic matrix [48,49]. Osteoblasts originate from undifferentiated mesenchymal stem cells and are responsible for the synthesis and mineralisation of the osteoid matrix, which makes it rigid and hard [50]. Osteocytes are derived from osteoblasts and are found embedded in the bone matrix [43]. They play a key role in bone turnover. In response to mechanical injury, they either undergo apoptosis or alter cell signalling, which then activates bone formation.

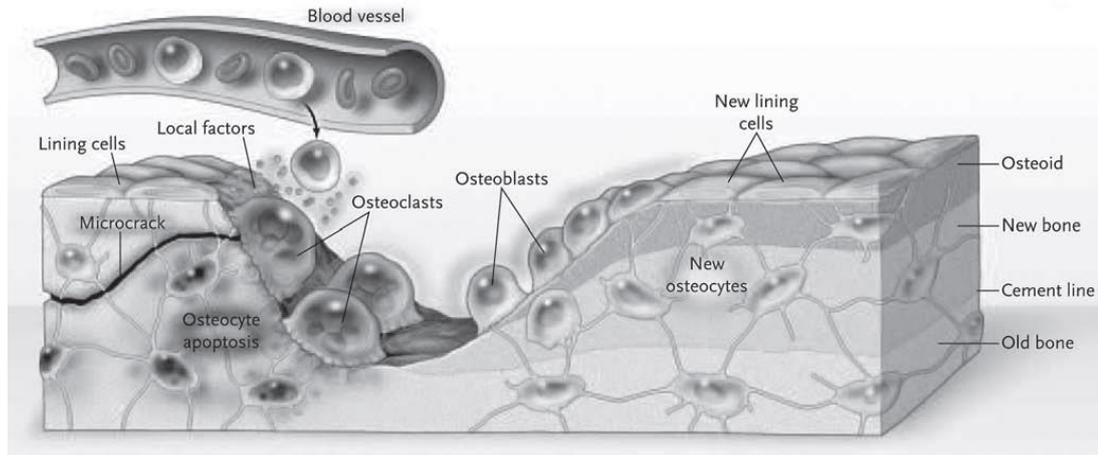
The bone matrix is mainly made up of type I collagen [43]. This protein forms parallel lamellae, which impairs cracks from spreading. The matrix also contains a number of non-collagen proteins (e.g. osteopontin, osteocalcin, fibronectin). Bone mineral is mainly comprised of calcium hydroxyapatite.

1.3.2 Bone remodelling and homeostasis

Normally, the strength and integrity of adult bone is maintained by continuous bone remodelling by the basic multicellular unit (BMU) [51]. Bone remodelling is said to be coupled, which ensures that the synthesis of new bone (bone formation) matches the removal of old bone (bone resorption) to keep bone mass constant (Figure 1.3.2.1) [46]. Osteoblasts are responsible for bone formation and osteoclasts for bone resorption [51]. This cross-talk between osteoclasts and osteoblasts is key to bone remodelling [52]. Most bone remodelling occurs during growth in puberty and early adulthood [51]. After longitudinal growth ceases in early adulthood, a small percentage of the adult skeleton undergoes remodelling annually, which is necessary to maintain structural integrity, calcium homeostasis, acid-base balance and enable repair of micro-architectural damage [46,51].

Figure 1.3.2.1 The bone remodelling cycle on a trabecula [46]

A microcrack in the bone causes apoptosis of osteocytes. Osteocytes, together with lining cells, release local factors into the BMU to enable osteoclastogenesis to occur. Osteoclasts resorb the bone matrix at the microcrack. This is followed by osteoblasts laying down new bone. Osteoblasts trapped in the matrix become osteocytes, whilst others die or form new, flattened osteoblast lining cells. BMU: basic multicellular unit

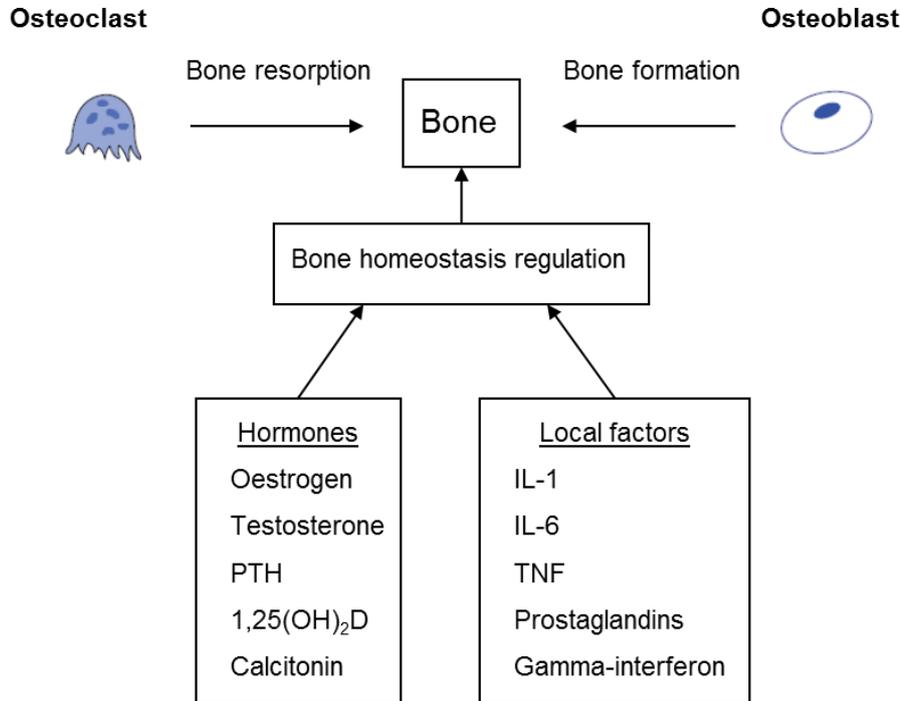


Regulation of bone remodelling is complex, and involves the interaction of numerous systemic hormones, including oestrogen, testosterone, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D (1,25[OH]₂D) and calcitonin, as well as local factors, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor (TNF), prostaglandins and gamma-interferon (Figure 1.3.2.2) [46,51,53]. Osteoblasts contain alkaline phosphatase (ALP) and express receptors for PTH, oestrogen, glucocorticoids, vitamin D, inflammatory cytokines and transforming growth factor- β (TGF- β) [54]. Osteoblasts also synthesise a number of molecules that regulate osteoclast activity, namely receptor activator for nuclear factor κ B ligand (RANKL) and its antagonist, osteoprotegerin [55]. By binding to its receptor, RANK, on the osteoclast surface, RANKL stimulates osteoclast differentiation and activation and bone resorption. In opposition, osteoprotegerin competes with and inhibits RANKL, thus preventing osteoclastogenesis and bone resorption, thereby acting as a regulator of osteoclast activation. As well as needing RANKL, osteoclast differentiation also requires macrophage colony stimulating factor (M-CSF), which is a cytokine that acts through its transmembrane tyrosine kinase receptor on the osteoclast [55]. RANKL is a member of the TNF family and is produced in response to PTH, vitamin D, oestrogen, glucocorticoid, prostaglandin E₂ (PGE₂), IL-1 and interleukin-2 (IL-2) [55].

Figure 1.3.2.2 Regulation of bone homeostasis (adapted from [53])

Bone homeostasis is regulated by a number of hormones and local factors that enable bone remodelling to occur.

1,25(OH)₂D: 1,25-dihydroxyvitamin D; IL-1: interleukin-1; IL-6: interleukin-6; PTH: parathyroid hormone; TNF: tumour necrosis factor



Newer osteoblast differentiation pathways have also been discovered. The canonical *wnt*/β-catenin pathway is a signal transduction pathway which is made of proteins [51,55]. When it is activated by *wnt* ligands (lipid modified glycoproteins), it is involved in osteoblast differentiation. Additionally, runt-related transcription factor-2 (RUNX-2) is a transcription factor that has an important role in the differentiation of mesenchymal cells into osteoblasts [55]. Although this cross-talk between the cellular components in bone is essential for determining bone mass, the precise mechanism of regulation of bone remodelling is yet to be fully determined.

1.4 Reduced bone mineral density (BMD)

1.4.1 Definition of reduced bone mineral density (BMD)

Osteoporosis is a systemic skeletal disorder associated with reduced BMD and microarchitectural deterioration of bone tissue, resulting in an increased risk of fracture [56-58]. In 1994, the World Health Organization (WHO) defined low BMD according to the T-score, which is based on the number of standard deviations (SDs) from the mean peak bone mass to which BMD is reduced on dual-energy x-

ray absorptiometry (DXA) in comparison to a young, healthy sex-matched population (Table 1.4.1.1) [57]. The T-score can be used to diagnose reduced BMD at the lumbar spine, femoral neck and total hip. A T-score between <-1.0 and >-2.5 is defined as osteopenia and ≤ -2.5 as osteoporosis.

Table 1.4.1.1 WHO defined diagnostic categories of bone density assessed by DXA [57]

Diagnostic T-score categories for assessing BMD using DXA.

Bone disorder	T-score	Clinical relevance
Normal	≥ -1	Baseline fracture risk
Osteopenia	<-1.0 and >-2.5	Up to 2-fold risk of fracture compared with normal BMD
Osteoporosis	≤ -2.5	More than 4-fold risk of fracture compared with normal BMD
Severe osteoporosis	≤ -2.5 and ≥ 1 fragility fracture	Very high risk of fracture

BMD: bone mineral density; DXA: dual-energy x-ray absorptiometry

The T-score is accurate when used in post-menopausal women and older men [59]. For pre-menopausal women and men <50 years, the T-score is not as useful, and the Z-score is preferred. This is an age-, ethnicity- and sex-matched score comparing BMD to adults of the same age and defines reduced BMD as ≤ -2 [60].

1.4.2 Pathogenesis of reduced BMD

The underlying mechanism of reduced BMD results from an imbalance in the activity of osteoclasts and osteoblasts, leading to increased bone resorption and/or decreased bone deposition [53]. This imbalance can result from either increased activity or differentiation of osteoclasts, or increased apoptosis and/or decreased activity and differentiation of osteoblasts [51]. The process of bone remodelling is influenced by both systemic and local regulation, involving hormones, growth factors and cytokines. For example, PTH enhances osteoblast differentiation and reduces osteoblast apoptosis [53]. It is through influencing these tightly regulated local and/or systemic regulators that an imbalance in bone remodelling occurs.

1.4.3 Risk factors for reduced BMD

The aetiology of reduced BMD is multifactorial, with a number of modifiable, non-modifiable, disease and medication-related risk factors contributing to an individual's risk of developing low BMD (Table 1.4.3.1) [61]. A prime determinant of skeletal

health is peak bone mass which is achieved in early adulthood, and therefore, nutrition and exercise in childhood and adolescence is also important [61].

Table 1.4.3.1 Risk factors for reduced BMD (adapted from [61])

Risk factors for low BMD are multifactorial, and include non-modifiable and modifiable causes, as well as secondary causes (e.g. diseases, drugs).

Risk factor category	Type of risk factor
Non-modifiable	Ageing (especially post-menopausal women) Gender (female > male) Ethnicity Family history (especially maternal history of hip fracture) Heritable factors Previous fractures (especially spine or wrist)
Modifiable	Alcoholism (>3 units/day) Smoking VDD Diet/malnutrition Lack of exercise/immobilisation Low BMI (<20 kg/m ²)
Diseases	CKD (including RTD) Malignancies (especially those that result in bone loss e.g. leukaemia, lymphoma, multiple myeloma) Endocrine disorders (e.g. diabetes mellitus, hypogonadism, hyperthyroidism, hyperparathyroidism, prolonged secondary amenorrhea, hyperprolactinaemia, Cushing syndrome) Gastrointestinal disorders (e.g. malabsorption, coeliac disease) Chronic liver disease Rheumatologic disorders (e.g. rheumatoid arthritis) Haematologic disorders (e.g. sickle cell anaemia) Genetic disorders (e.g. haemochromatosis, haemophilia, idiopathic scoliosis, osteogenesis imperfecta, thalassaemia) Connective tissue diseases Eating disorders (e.g. anorexia nervosa) HIV infection
Drugs	Corticosteroid therapy (e.g. prolonged high-dose prednisolone) Chemotherapy Antiepileptics (e.g. phenytoin, carbamazepine) Aromatase inhibitors Anticoagulants Proton pump inhibitors

BMI: body mass index; CKD: chronic kidney disease; RTD: renal tubular dysfunction; VDD: vitamin D deficiency

1.4.4 Reduced BMD and fracture epidemiology

Reduced BMD typically affects elderly, white, post-menopausal women, with lower rates seen in black populations [58]. Most studies have used BMD as a surrogate marker of fracture risk because reduced BMD has been shown to be associated with fragility fractures. In older patients, there is a strong relationship between low BMD and fractures, especially as these patients are at increased risk of falls [58]. The sites most commonly affected by fragility fractures are the lumbar spine, the distal forearm and the hip [58]. Figures show that 75% of all hip fractures occur in white, post-menopausal women and 90% of all hip fractures occur in those aged >50 years

[56,58]. Of all fragility fractures, hip fractures are the most serious, resulting in hospitalisation and fatality in 20% of cases and leaving 50% permanently disabled. Worldwide, fragility fractures are a significant cause of morbidity and mortality in the elderly, and with an ageing population, forecasts suggest that there will be an estimated 8.2 million hip fractures worldwide by 2050 [58]. The economic burden of fragility fractures is huge, not to mention the physical, financial and psychosocial implications for the individual and their family.

Assessment of fracture risk is important. Although several assessment tools have been created, the WHO has approved the international use of the Fracture Risk Assessment Tool[®] (FRAX[®]) score (www.shef.ac.uk/frax) [62]. The FRAX[®] tool calculates the 10-year absolute risk of both a major osteoporotic fracture and a hip fracture [63,64]. It is discussed in more detail in Chapter 5.

1.5 Reduced BMD in men in the general population

1.5.1 Introduction

Reduced BMD in men is an important public health problem. Using the National Health and Nutrition Examination Survey (NHANES) 2005-2006 database, the prevalence rates of femoral osteopenia and osteoporosis in American men aged ≥ 50 years were 30% and 2%, respectively [65]. Although it has been under-recognised compared to osteoporosis in women, osteoporosis research in men has started receiving more attention in recent years [66]. This has led to the development of guidelines aimed specifically at diagnosis, treatment and prevention of reduced BMD in men, and has also led to the better understanding of differences in men and women.

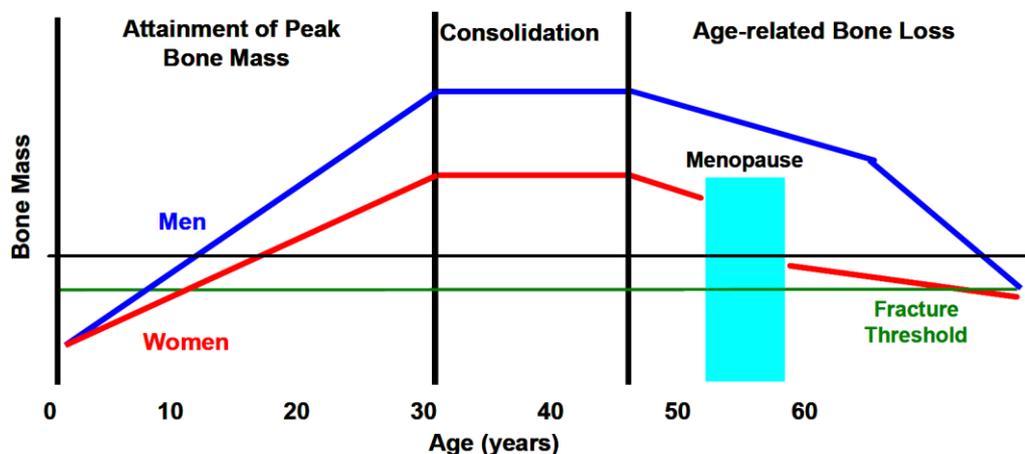
1.5.2 Skeletal development in men

Bone mass accumulation varies between males and females (Figure 1.5.2.1) [67]. Although in both sexes there is a gradual accumulation of bone mass from childhood, pubertal changes at adolescence lead to a rapid increase in bone mass, which occurs slightly later in boys at an average age of 18 years, as opposed to an average age of 16 years in girls [68]. Peak bone mass in men is higher than in women, and even after adjustment for body size, boys have thicker cortices and

larger bones than girls. The reasons for these differences are not fully understood, but may be due to differences in sex steroids, growth hormones and mechanical forces on bone. These differences may explain why men have a lower fracture risk in later life than women.

Figure 1.5.2.1 Changes in bone mass in men and women with age (adapted from [67])

This graph shows the change in bone mass (y-axis) with age (x-axis) in men (dark blue) and women (red). Both men and women attain peak bone mass from childhood to early adulthood, although men have a higher peak bone mass than women. There is a period of stable bone mass (consolidation) before bone mass reduces from mid-adulthood. There is a faster rate of bone loss in women associated with the menopause (pale blue), but both men and women reach similar rates of bone mass in later life, which are associated with increased risk of fracture (green).



1.5.3 Effects of ageing in men

In both men and women, ageing is associated with a loss of bone mass and a change in bone architecture [69]. However, men have a gradual reduction in bone volume of 0.5% to 1.0% per year, and do not have an equivalent phase to the accelerated bone loss experienced by women after the menopause [70,71]. The mechanism for loss of trabecular bone is different between men and women: women lose trabecular bone with age, whilst men experience a reduction in bone formation, which leads to thinning of trabecular bone [72]. During bone loss, there is also better preservation of trabecular architecture in men compared to women [73]. Additionally, there is a greater increase in periosteal bone expansion in men compared to women, which preserves bone strength, and may help to explain why men experience lower fracture rates than women [71]. In the elderly (aged >70 years), there is a similar rate of accelerated bone loss in both men and women [74].

1.5.4 Causes of reduced BMD in men

Although studies have reported rates of reduced BMD in men of unknown aetiology of up to 40% (termed primary or idiopathic osteoporosis), an underlying cause is frequently found [70,75]. Although the causes are similar to those described in Table 1.4.3.1, the commonest secondary causes are hypogonadism, excessive alcohol use and corticosteroid therapy [75]. Primary osteoporosis can affect men of all ages, including younger men who would be otherwise unaffected [76]. The aetiology is unknown, but genetic factors are thought to be the most likely.

1.5.5 Assessment of low BMD in men

As in women, measuring BMD remains the cornerstone of evaluating osteoporosis in men [70,75]. At the time my study commenced, guidelines from the International Society for Clinical Densitometry (ISCD) recommended measuring BMD in men aged >70 years or in younger men with fragility fractures and risk factors [59]. Although screening for reduced BMD in older men was not recommended, a cost-effectiveness study has suggested that screening in elderly men may be beneficial [77]. In this study, DXA scanning was recommended for men aged ≥ 65 years with a previous history of a fracture and for men aged 80 to 85 years with no prior fracture.

Assessment of potential risk factors and diagnosis of secondary causes of reduced BMD is also important. Recommended laboratory tests include a bone profile (calcium, phosphate and ALP), vitamin D, PTH, testosterone and sex hormone binding globulin, renal function, full blood count and urinary calcium and creatinine.

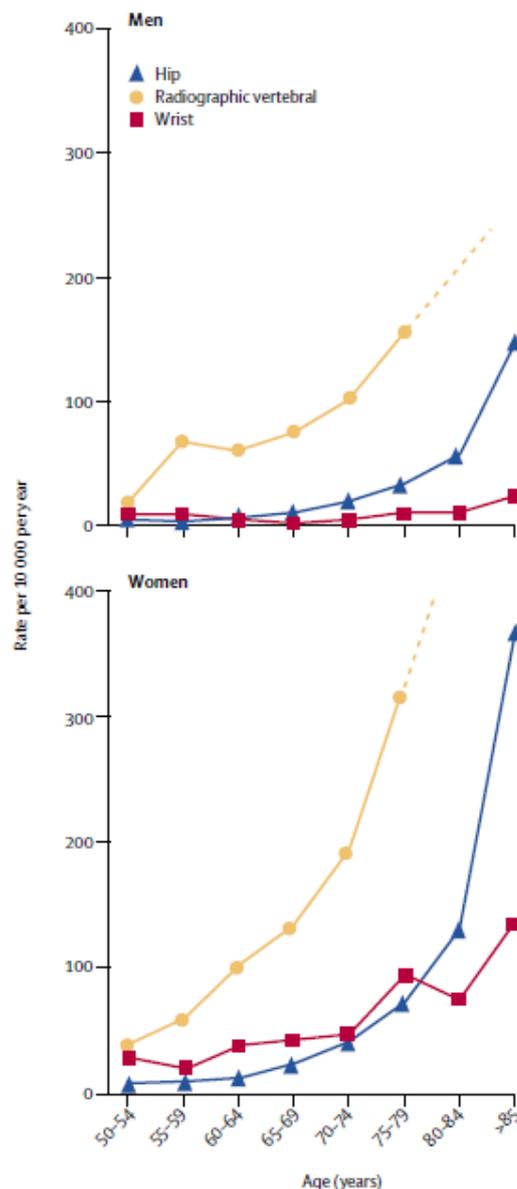
1.5.6 Fracture epidemiology in men

In men, the incidence of fracture is bimodal, with a peak in fractures in adolescence and mid-adulthood, followed by a second peak after the age of 70 years [78]. There is a difference in the types of fractures sustained, with young men experiencing mainly long bone fractures, whilst elderly men suffer from hip and vertebral fractures. This difference suggests that the aetiology may be different, with fractures at a younger age being related to trauma, whilst those in the elderly may be due to increased skeletal fragility and falls.

The lifetime risk of an osteoporotic fracture in the British general population is similar to that in the USA [79]. Estimates suggest 1 in 5 men aged >50 years old will experience an osteoporotic fracture in their lifetime [58] and one-third of all hip fractures occur among men [80]. The exponential increase in the incidence of fractures in older men is similar to that seen in women, but occurs 5 to 10 years later in age (Figure 1.5.6.1) [58,78]. Although the age-adjusted incidence of hip fracture is lower in men than in women, morbidity and mortality in the first year following a hip fracture are much greater among men [81].

Figure 1.5.6.1 Age- and sex-specific incidence of radiographic vertebral, hip and distal forearm fractures [58]

The incidence of osteoporotic fractures increases with age at the hip, the spine and the distal forearm in both men and women, with hip and distal forearm fractures increasing exponentially. In all three types of fracture, the rates are higher in women.



The aetiology of fractures in men is multifactorial. Race and geography have been implicated, with black men having a lower risk of fractures compared to white men, and Asian men having a lower risk of hip fractures than white men [67,82]. The majority of fractures occur in men whose BMD is not osteoporotic [83,84], implying that factors other than skeletal fragility and reduced BMD are also involved. Other factors associated with the probability of future fractures are ageing, a previous history of fracture, lower body mass index (BMI) and falls [85,86].

1.5.7 Treatment and prevention of low BMD in men

The principles of treating and preventing reduced BMD are the same in men and women. These include lifestyle measures and preventative strategies, such as fall prevention [75,87]. Studies have shown that exercise (weight-bearing exercise, resistance training, or both) increases BMD in older men compared to controls [88]. Good nutrition (adequate calcium and vitamin D intake) is a factor. A systematic review of 64,000 participants showed that the daily ingestion of calcium (1200 mg or more) or calcium with vitamin D (800 IU or more) led to a 12% reduction in osteoporotic fractures in both men and women aged ≥ 50 years old [89]. Finally, the avoidance of factors associated with bone loss (Table 1.4.3.1) is also important in preventing the development of low BMD and fractures in later life [70,75].

1.6 Diagnosis of reduced BMD

1.6.1 Measurement of BMD with DXA

Although there are different bone densitometry techniques, the most widely used is DXA [90]. This uses dual-energy x-ray beams to correct for overlying soft tissue when calculating BMD [91]. Different manufacturers use different methods to optimise separation of mineralised bone and soft tissue during the scanning process. Areal BMD, expressed in g/cm^2 , is calculated by measuring bone mineral content (BMC, g), which is divided by the bone area (cm^2) under examination [91,92]. Additionally, whole body DXA scanning can be used to measure body composition (lean mass and fat mass), as well as whole body and regional BMC (g).

DXA can be used to measure different sites of the skeleton which are affected by osteoporotic fractures [90]. These include the lumbar spine (L1-L4) and the proximal

femur (total hip, femoral neck, trochanter and Ward's area) in the central skeleton and the forearm and calcaneus in the peripheral skeleton. Although BMD at the lumbar spine, femoral neck and total hip are assessed for diagnosing osteoporosis, measurement of BMD at the femoral neck is considered the gold standard site as it has the highest predictive value for hip fracture [61].

DXA measures areal BMD of cortical and trabecular bone. As the ratios of cortical and trabecular bone differ between skeletal sites, coupled with variable rates of change, measurements at different sites in one individual will not produce the same BMD measurements [93]. With correlations between BMD measurements in an individual patient varying between $r=0.4$ and $r=0.9$, it is not possible to use DXA to predict BMD at a different site even in a single patient [93].

It is important to assess the precision, accuracy and sensitivity of bone densitometry. The reproducibility of DXA is measured by precision and varies depending on the site scanned (Table 1.6.1.1) [94,95]. Precision can be optimised by reducing inter-operator variability by using a small number of trained radiographers.

Table 1.6.1.1 Precision of DXA at different skeletal sites [94,95]

The precision of DXA measurements varies depending on the site scanned.

Skeletal site	Precision of DXA
Lumbar spine	1 – 2%
Total hip	1 – 2%
Femoral neck	2.5%
Trochanter	2.5%
Ward's area	2.5 – 5%
Distal forearm	1%
Ultra-distal forearm	2.5%
Calcaneus	1.4%

DXA: dual-energy x-ray absorptiometry

Accuracy determines how close the BMD measured by DXA is to the actual calcium content of the bone [96]. The accuracy of DXA is between 3% and 7% [96]. It is reduced by the presence of marrow fat and DXA taking into account soft tissue as a reference [96,97]. As DXA makes some assumptions about soft tissues and body composition, accuracy is affected by patients who are under- or overweight, and in those who have experienced large changes in weight between scans [96-98]. In particular, HIV-positive patients are at risk of developing peripheral lipodystrophy which leads to redistribution of fat [99]. However, as DXA is less able to assess central fat changes, which may include an increase in visceral fat, as well as a decrease in subcutaneous fat, the ISCD do not currently recommend using DXA for

monitoring trunk fat changes in HIV-positive patients [100]. Instead, they recognise that computed tomography (CT) or magnetic resonance imaging (MRI) is the gold standard in this scenario. MRI has been shown to be precise in quantifying lipodystrophy in HIV-positive patients [101]. Additionally, patients could self-report body changes which may help identify those at risk of fat redistribution.

Sensitivity is the ability of the DXA measurement to distinguish between patients with and without fractures and the ability to measure small changes over time and/or with treatment [102]. As changes in BMD over time are relatively small, sensitivity is improved by leaving an adequate time interval (e.g. 18 to 24 months) between measurements [102].

Once BMD is measured, the bone densitometer interprets the result using normal reference databases. These databases are mainly derived from a white, female, post-menopausal American population [56]. In an effort to standardise results, the NHANES reference database was selected as the reference database for interpreting hip data [103].

1.6.2 Clinical application of DXA

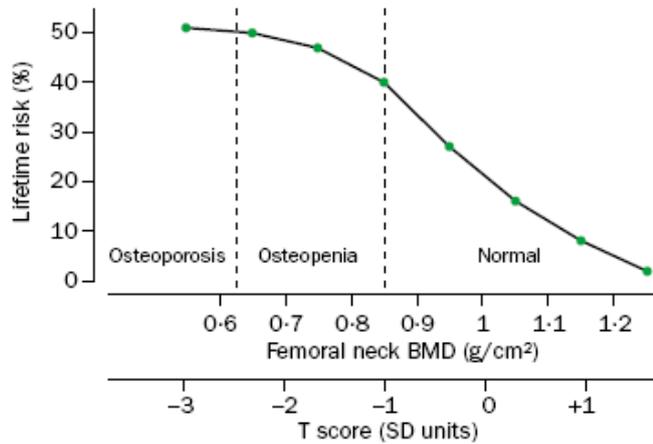
As DXA has high specificity but low sensitivity, there has been much debate concerning its use for population screening, for example, in post-menopausal women [90,104]. Currently, patients are selected for DXA screening using a case-finding strategy based on the presence of osteoporotic risk factors and the presence of fragility fractures [61]. Areal DXA is considered the gold standard measurement for diagnosing reduced BMD and osteoporosis and assessing the risk of fracture [90,95,104].

Absolute BMD is converted into T-score values, which enable stratification of absolute BMD into clinically relevant estimates of fracture risk (Table 1.4.1.1 and Figure 1.6.2.1) [57,61]. As there are factors additional to BMD that are involved in developing a fracture (e.g. age, falls, nature of the fall, etc.), BMD alone cannot completely determine fracture risk [61]. However, patients with lower BMD on DXA are more at risk of sustaining a fracture [105]. BMD measurements at any skeletal site are predictive of fracture, with the relative risk per 1 SD decrease in BMD below the age-adjusted mean being between 1.4 and 2.6 [105].

Figure 1.6.2.1 Lifetime risk of fracture in women aged 50 years according to BMD and T-scores of the femoral neck [61]

The lifetime risk of fracture in women >50 years old with the femoral neck BMD and T-score cut-offs for normal, osteopenia and osteoporosis.

BMD: bone mineral density; SD: standard deviation



Another application of DXA is using it to measure changes in BMD over time and treatment [102]. Again, there is currently no consensus on this and policies vary between countries [106]. In assessing change in BMD, it is best to use absolute BMD. In longitudinal studies, it is recommended that patients are scanned at adequate time intervals (e.g. 18 to 24 months) to enable changes in BMD to be accurately diagnosed [102].

1.6.3 Limitations of DXA

Bone densitometry has its limitations. DXA uses radiation, although the radiation doses patients are exposed to are very small (Table 1.6.3.1), and comparable to natural background radiation (2400 $\mu\text{Sv}/\text{year}$; $\sim 7 \mu\text{Sv}/\text{day}$) [107,108]. Although BMD accounts for 70% of bone strength, DXA is a two-dimensional measurement. It ignores qualitative three-dimensional qualities of bone, such as macroscopic structure, microscopic structure (including micro-fractures and abnormalities in trabecular architecture), the degree of bone remodelling, composition of bone proteins and cortical bone width [105,107]. Therefore, one of the main limitations of DXA is that it is size-dependent. BMD serves as a proxy measure of bone strength but it cannot by definition measure or assess the quality of bone. Additionally, BMD cannot distinguish between low calcium content in bone secondary to osteoporosis with that due to osteomalacia [61].

Table 1.6.3.1 Radiation doses of DXA [107,108]

Although the radiation doses patients are exposed to at different anatomical sites vary, the levels are very small and equivalent to background radiation from the sun.

Skeletal site	Radiation dose (μSv)
Lumbar spine	2 – 4
Femur	2 – 5
Forearm	0.5
Calcaneus	0.03
Whole body	1 - 3

The normal reference databases used by bone densitometers to interpret BMD results are mainly based on data from white, post-menopausal American women [56]. This means that BMD results in men, ethnic minorities (e.g. black, Asian) and pre-menopausal women need to be interpreted with caution, with some patients with reduced BMD not being correctly identified [95]. In addition, as different manufacturers use varying algorithms and measure different regions of interest (ROIs), the results from different DXA scanners are not directly comparable. This means that it is not always possible to detect changes in BMD over time even in the same patient. This problem can be overcome by ensuring the same bone densitometer is used in longitudinal studies and cross-calibrating between machines [109,110].

1.6.4 Peripheral dual-energy x-ray absorptiometry (pDXA)

Although the gold standard investigation for measuring BMD is areal DXA measured using central dual-energy x-ray absorptiometry (cDXA) [90,95,104], studies have indicated that pDXA of the non-dominant distal forearm may be useful as a screening investigation in high-risk populations [111] and that BMD of the distal forearm is a good predictor of the risk of a future hip fracture [112,113]. As pDXA uses very low levels of radiation, it can be performed by non-radiographers and can be used in an outpatient setting. This may be a useful tool, especially when access to cDXA is limited. The National Osteoporosis Society (NOS) guidelines recommend using pDXA as an adjunct to cDXA [114]. A combination of pDXA and the assessment of risk factors have been shown to reduce the need for cDXA [115]. As it is cheaper than cDXA [115,116], pDXA may be useful as a screening tool, with cDXA being reserved for patients who have reduced BMD on pDXA. However, as most studies evaluating pDXA have been performed in post-menopausal women and older institutionalised adults [117,118], its utility in HIV-positive men is not known.

The distal forearm is a trabecular-rich site whilst the proximal forearm is a cortical-rich site [119]. As HIV infection has been shown to be associated with a loss of trabecular BMD [120], and DXA measurements represent the sum of both cortical and trabecular compartments, pDXA of the distal forearm may be a good screening tool in HIV-positive patients.

1.7 Bone turnover markers (BTMs)

1.7.1 Introduction

As mentioned in Section 1.3, bone metabolism occurs at the BMU and involves two opposing mechanisms. During bone resorption, osteoclasts dissolve the bone matrix, which produces a resorptive cavity and releases a number of bone matrix components. This process is followed by bone formation, in which osteoblasts synthesise new bone. This bone matrix undergoes mineralisation and fills in the resorptive cavity. BTMs either measure bone resorption or bone formation (Table 1.7.1.1) [121]. Depending on the BTM, they can be measured in serum and/or in urine.

Table 1.7.1.1 BTMs (adapted from [121])

There are numerous bone markers, with some measuring resorption and others measuring formation. Serum CTX and serum P1NP have been recommended to be used as reference markers (highlighted).

Bone resorption markers	Bone formation markers
Degradation products of bone collagen Hydroxyproline Pyridinoline Deoxypyridinoline N-terminal cross-linking telopeptides of type I collagen (NTX) C-terminal cross-linking telopeptides of type I collagen (CTX) C-terminal cross-linking telopeptides of type I collagen generated by metalloproteinase (CTX-MMP)	Products of active osteoblasts Osteocalcin Bone-specific ALP Total ALP N-terminal propeptide of type I procollagen (P1NP) C-terminal propeptide of type I procollagen (P1CP)
Non-collagenous proteins of bone matrix Bone sialoprotein Osteopontin Osteocalcin fragments (urine)	
Osteoclast enzymes TRACP 5b Cathepsin K	

ALP: alkaline phosphatase; CTX: C-terminal cross-linking telopeptides of type I collagen; CTX-MMP: C-terminal cross-linking telopeptides of type I collagen generated by metalloproteinase; NTX: N-terminal cross-linking telopeptides of type I collagen; P1CP: C-terminal propeptide of type I procollagen; P1NP: N-terminal propeptide of type I procollagen; TRACP 5b: tartrate-resistant acid phosphatase

In order to standardise the use of BTMs, The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCCCLM) have recommended using serum CTX and serum P1NP as reference markers [122].

1.7.2 Utility of BTMs

Although their main benefit has been in assessing response to treatment, some studies have shown that BTMs may be useful as surrogate markers to assess fracture risk involving all types of fracture, including vertebral and hip fractures [123-125]. Increased BTM levels have been shown to predict fragility fractures in prospective cohort and case-control studies involving post-menopausal and elderly women [126,127]. The BTMs that are best at predicting fracture risk are the bone resorptive markers and the more specific bone formation markers (e.g. bone-specific ALP) [128].

However, when comparing the association between BTM levels and BMD, the relationship is less clear. Some studies have shown that BTM levels at baseline correlate with subsequent bone loss [129]. This suggests that it is the rate of bone turnover that determines the rate of bone loss. However, at an individual level, there is a large scatter of values [130] so it is not yet clear whether BTMs are good predictors of bone loss in individual patients.

1.7.3 Limitations of BTMs

BTMs have several limitations which make it difficult to interpret their results accurately and to routinely use them in clinical practice [123,125]. They can produce variable results, with large inter-laboratory variations reported, making comparison of results from different laboratories difficult. BTMs are affected by modifiable and non-modifiable risk factors, such as season, time of day, smoking, exercise, alcohol consumption and fasting status [123,131,132]. To overcome some of these problems, standardising collection procedures (e.g. collecting fasted samples at a set time in the morning) is advisable.

1.7.4 BTMs in men

BTM levels in men are different to those in women. Between the ages of 20 and 25 years, men have more bone turnover than women, which is reflected in higher levels of BTMs [133,134]. From then on, BTM levels decrease, with the lowest levels occurring between the ages of 50 and 60 years. After 60 years of age, bone formation remains stable or increases only slightly, whilst there is an increase in bone resorption with time. However, the data on BTMs in elderly men are discordant, which is probably due to the majority of the studies being conducted with small numbers of subjects of limited age range. Additionally, although reference ranges for BTMs exist for healthy pre-menopausal women, there are none for men, making their use unclear [124]. In addition, because of the differences in BTM levels in men and women, it is not possible to extrapolate results from women to men. A prospective cohort study has shown that BTMs are not good predictors of fragility fractures in elderly men [135], whilst a case-control study showed that C-terminal cross-linking telopeptides of type I collagen generated by metalloproteinase (CTX-MMP) was associated with an increased risk of fractures [121].

1.8 Reduced BMD and HIV infection

1.8.1 Introduction

Since 2000, HIV infection has been emerging as a possible cause of reduced BMD in both HIV-positive men and women. As HIV-positive patients continue to live longer, they are at increased risk of developing long-term complications meaning HIV infection may become an important cause of secondary osteoporosis in this group of individuals. Cross-sectional studies indicate that the prevalence of reduced BMD in HIV-positive patients is greater than in HIV-negative individuals [136]. The aetiology is multifactorial, with HIV-positive patients being at risk of the traditional risk factors associated with reduced BMD. However, they are also at risk from HIV-related factors, including the virus itself and exposure to ART. Although studies indicate that HIV-positive patients are more likely to have reduced BMD, whether this translates into a higher risk of fracture is unclear.

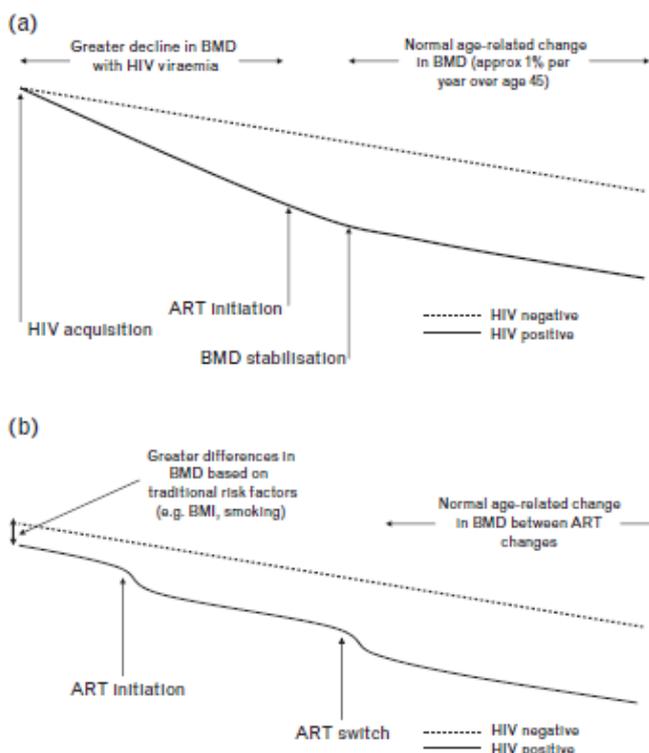
1.8.2 Pathogenesis of reduced BMD in HIV-positive patients

The exact mechanisms involved in bone loss in HIV-positive patients have not been fully elucidated, but a number of mechanisms have been postulated. These include the failure to reach peak bone mass in people who acquire HIV infection before 30 years of age, the age by which peak bone mass is usually achieved. Several cross-sectional studies have demonstrated that BMD and BMC are lower in patients with perinatally-acquired HIV infection compared to HIV-negative children [137-140].

Another mechanism that has been suggested is the effect of HIV infection, either directly affecting bone cells or indirectly causing a pro-inflammatory state, both of which can lead to increased loss of BMD (Figure 1.8.2.1a) [141]. The stabilisation of BMD loss on commencing ART may help to explain this phenomenon. Finally, an increased preponderance of traditional risk factors, as well as those relating to ART exposure, is also thought to contribute to the pathogenesis of reduced BMD in HIV-positive patients (Figure 1.8.2.1b). These mechanisms are discussed in detail in Chapter 3.

Figure 1.8.2.1 Proposed mechanisms of the pathogenesis of reduced BMD in HIV-positive patients [141]

Various mechanisms for loss of BMD in HIV-positive patients have been postulated. This included the direct and indirect effects of HIV infection (a) and an increased prevalence of traditional risk factors, as well as those caused by exposure to ART (b).
ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index



Despite more recent research, the exact mechanisms are still not known [24], but it is likely that more than one mechanism is at work in reducing BMD in an HIV-positive patient.

1.8.3 Prevalence of low BMD in HIV-positive patients

Several cross-sectional studies have indicated that HIV-positive patients are at a higher risk of developing reduced BMD (both osteopenia and osteoporosis) than HIV-negative individuals (Table 1.8.3.1). Although the prevalence of reduced BMD varied considerably from study to study, they all reported higher prevalence of reduced BMD, osteopenia and osteoporosis in HIV-positive patients compared to HIV-negative controls.

Table 1.8.3.1 Prevalence of reduced BMD in HIV-positive patients compared to HIV-negative controls

Author	Year	Location	HIV-positive patients							HIV-negative controls					
			N	% male	Mean age (years)	% on ART	% reduced BMD	% osteopenia	% osteoporosis	N	% male	Mean age (years)	% reduced BMD	% osteopenia	% osteoporosis
Tebas [142]	2000	USA	95	100	39	-	40	-	-	17	100	33	29	-	-
Huang [143]	2001	USA	28	0	37	75	54 ^a /48 ^b	50 ^a /48 ^b	4 ^a /0 ^b	21	0	36	19 ^a /10 ^b	14 ^a /10 ^b	5 ^a /0 ^b
Knobel [144]	2001	Spain	80	73	41	68	89	68	21	100	-	-	30	25	5
Loiseau-Peres [145]	2002	France	47	66	41	87	68	60	9	47	-	-	34	32	2
Bruera [146]	2003	Argentina	111	80	34	70	73 ^c	58 ^c	15 ^c	31	77	31	15 ^c	15 ^c	0 ^c
Teichmann [147]	2003	Germany	50	0	37	0	76 ^a	62 ^a	14 ^a	50	0	35	4 ^a	4 ^a	0 ^a
Amiel [148]	2004	France	148	100	40	68	82	66	16	81	100	40	36	32	4
Brown [149]	2004	USA	51	86	40	100	63	55	8	22	82	39	32	32	0
Dolan [150]	2004	USA	84	0	41	80	63	54	10	63	0	41	35	30	5
Madeddu* [151]	2004	Italy	172	65	38	88	51 ^a /47 ^d	37 ^a /37 ^d	15 ^a /10 ^d	64	61	-	8	8	0
Yin [152]	2005	USA	31	0	56	84	-	36 ^a /36 ^b	42 ^a /10 ^b	186	0	57	-	33 ^a /29 ^b	23 ^a /1 ^b
Arnsten* [153] (Menopause)	2006	USA	263	0	44	78	27	-	-	232	0	45	19	-	-
Bolland [154]	2006	New Zealand	59	100	50	100	-	29	3	118	100	50	-	21	1
Anastos* [155]	2007	USA	274	0	42-44	72	-	-	5 ^e	152	0	36	-	-	1 ^e
Arnsten* [156] (CHAMPS)	2007	USA	328	100	55	87	55	-	-	231	100	56	51	-	-
Jones [157]	2008	USA	57	60	61	82	60 ^a /60 ^b	39 ^a /54 ^b	28 ^a /5 ^b	47	30	62	47 ^a /25 ^b	26 ^a /26 ^b	13 ^a /0 ^b
Yin* [158]	2010	USA	92	0	56	79	-	78 ^a /45 ^b /64 ^c	-	95	0	60	-	64 ^a /29 ^b /46 ^c	-
Yin* [159] (WIHS)	2010	USA	100	0	40	59	23 ^a /18 ^b	14 ^a /9 ^b	9 ^a /9 ^b	68	0	36	6 ^a /9 ^b	6 ^a /7 ^b	0a/9 ^b

ART: antiretroviral therapy; BMD: bone mineral density; CHAMPS: Cohort of HIV at-risk Aging Men's Prospective Study; WIHS: Women's Interagency HIV Study

*Longitudinal studies reporting cross-sectional baseline data

^aLumbar spine; ^btotal hip; ^cfemoral neck; ^dfemoral head; ^eosteoporosis only

It is important to note that the studies varied considerably in their study populations, and the definitions of reduced BMD used. A meta-analysis, which included several of the studies comparing HIV-positive to HIV-negative patients, found marked heterogeneity amongst these studies, including grouping of osteopenia with osteoporosis, variations in classification of reduced BMD, men and women analysed together and BMD measured at different sites [136]. Despite these limitations, this review evaluated 11 studies comparing 884 HIV-positive patients to 654 HIV-negative controls. HIV-positive patients had a 3.7-fold increased risk of osteoporosis (95% confidence interval [95% CI] 2.3-5.9) and a 6.4-fold increased risk of reduced BMD (95% CI 3.7-11.3) compared to HIV-negative controls [136]. Among HIV-positive patients, the overall prevalence of osteoporosis and reduced BMD were 15% and 67%, respectively [136]. These figures are similar to those obtained from a pilot study of 168 HIV-positive men from my cohort, which compared ART-naïve patients to those on ART for <3 years to those on long-term ART (>3 years) [160]. Short *et al* reported that the prevalence of osteoporosis and osteopenia was 12% (all at the lumbar spine, none at the hip) and 58% (again mostly at the lumbar spine), respectively [160]. The latest prevalence data are discussed in Chapter 8.

1.8.4 Risk factors for reduced BMD in HIV-positive patients

As the aetiology of reduced BMD is multifactorial, several hypotheses have been postulated to explain why HIV-positive patients are at increased risk. These included a higher prevalence of traditional risk factors, as well as HIV-related factors, such as the host response to the virus, immunologic effects of HIV and the effect of ART [161]. These factors are discussed in detail in Chapter 3.

1.8.5 Fracture rates in HIV-positive patients

When I commenced this study, despite increasing evidence showing that HIV-positive patients have a higher prevalence of reduced BMD, there was a paucity of data relating to fragility fractures. Most studies used BMD as a surrogate marker of fracture risk, but it was not known whether this increased prevalence of low BMD resulted in an increased risk of fragility fractures. There is now more evidence suggesting that HIV-positive patients are at increased risk of incident fragility fractures [162], and these data are discussed further in Chapter 5.

1.9 RTD and bone in HIV infection

1.9.1 Renal disease in HIV-positive patients

Although survival rates have improved, HIV-positive patients are increasingly developing long-term chronic conditions such as renal disease [163] and are at increased risk of a number of renal diseases, including glomerular, tubulo-interstitial and vascular diseases [164-166]. The prevalence of chronic kidney disease (CKD) varies greatly depending on a number of factors, including the study population, the definitions used (including whether proteinuria is included in the definition) and whether the population is ART-naïve or not [163]. With the introduction of ART, the spectrum of renal diseases has changed [165]. In the pre-ART era, HIV-associated nephropathy (HIVAN), HIV-associated immune complex kidney disease (HIVICK) and focal segmental glomerulosclerosis (FSGS) predominated [164,167,168]. In the post-ART era, traditional causes of CKD seen in the general population (e.g. diabetes, hypertension), as well as those relating to ART exposure, have become more common [31]. The definition of CKD, its prevalence and risk factors are discussed in Chapter 6.

1.9.2 RTD

Proximal tubular dysfunction (commonly known as RTD or tubular proteinuria [TP]) is associated with an inability to reabsorb phosphate and LMWPs from the proximal tubule [169]. Several antiretroviral drugs have been implicated in causing RTD [163]. Although the exact mechanism is not known, TDF, one of the most commonly used drugs, has been associated with RTD [33]. In its severest form, RTD can lead to Fanconi syndrome, which is characterised by glycosuria, renal phosphate wasting and increased urinary concentrations of LMWPs [33]. Fanconi syndrome is usually associated with concomitant use of TDF and a boosted PI [33]. Chapters 6 and 7 discuss RTD in more detail.

1.9.3 Diagnosis of RTD

As TDF is a commonly used first-line drug, RTD is an important cause of renal disease in HIV-positive patients. It is therefore important to be able to diagnose RTD accurately and easily. In RTD, there is increased excretion of LMWPs (e.g. β_2 -microglobulin, retinol binding protein [RBP], neutrophil gelatinase-associated lipocalin [NGAL], cystatin C) in the urine [170,171]. Although higher levels of LMWPs have been reported

in HIV-positive patients with RTD [163], they can be expensive and are not always routinely available. In contrast, protein/creatinine ratio (PCR) and albumin/creatinine ratio (ACR) are relatively cheap and easily accessible. Work from our group has shown that the albumin/protein ratio (APR) can be calculated using PCR and ACR and that it is useful in distinguishing RTD from glomerular proteinuria (GP) in the general population [172]. Chapter 6 describes the utility of APR in diagnosing RTD and its utility in differentiating RTD from GP.

1.9.4 Relationship between bone and renal disease

The relationship between the bones and the kidneys is complex, and is linked to calcium and phosphate homeostasis [173]. RTD can affect bone mineralisation, which can lead to bone pain, osteomalacia, reduced BMD and an increased risk of fragility fractures [33,35,37].

Additionally, TDF can also have an adverse effect on bone. It can have a direct effect on osteoclasts and osteoblasts [39,40]. TDF can also have an indirect effect on bone by causing RTD or Fanconi syndrome, which can cause renal phosphate wasting and osteomalacia [33]. Excess loss of phosphate can stimulate increased bone resorption, which can lead to reduced BMD [141]. Finally, TDF can alter the vitamin D/PTH axis, causing secondary hyperparathyroidism and increased bone turnover, which has been shown to be worse in patients with VDD [41].

RBP is a LMWP that is increased in RTD [174]. The effects of RTD (measured using both RBP and renal phosphate wasting) on bone are discussed in detail in Chapter 7.

1.10 Context of the study

1.10.1 Key themes of the study

1.10.1.1 HIV infection

With the introduction of ART, the natural course of HIV infection has dramatically changed. Mortality relating to AIDS-defining conditions and opportunistic infections has declined, with HIV-positive patients more likely to die from non-AIDS-defining conditions. As HIV-positive patients live longer, they are at increased risk of developing long-term chronic conditions associated with ageing including cardiovascular disease, renal disease, cognitive impairment and bone disease.

At the time my study started, there was interest in a range of long-term complications of HIV infection. ART had been found to be associated with several metabolic complications, including insulin resistance, diabetes mellitus, hyperlipidaemia, lipodystrophy and altered bone metabolism. As reduced BMD can lead to fragility fractures, and HIV-positive patients were beginning to age, this was seen to be a complication that was of concern.

1.10.1.2 Reduced BMD and fracture risk

Since 2000, there have been numerous studies suggesting that HIV infection is associated with an increased prevalence of reduced BMD. The reported prevalence has varied considerably from study to study, mainly due to the marked heterogeneity amongst these studies. However, a meta-analysis of 11 studies had reported that the prevalence of reduced BMD and osteoporosis was 67% and 15%, respectively (Brown AIDS 2006). Additionally, the authors found that HIV-positive patients had an increased risk of osteoporosis and reduced BMD compared to HIV-negative controls.

The aetiology of reduced BMD is likely to be multifactorial and is discussed in detail in Chapters 3 and 4. A number of studies have shown that the prevalence of 'traditional' risk factors for low BMD is higher in HIV-positive patients, including low BMI, hypogonadism and VDD. HIV-positive patients are also at increased risk of factors associated with the inflammatory nature of HIV infection, its effects on the immunologic system and the long-term effects of ART. Although the exact mechanisms involved are still not fully known, HIV infection appears to have an effect on uncoupling bone homeostasis. This leads to an increase in bone resorption, which results in bone loss. Additionally, studies of seroconverters have shown that BMD is reduced during seroconversion, when the viral load is very high, and that this stabilises as the viral load reduces to the set point level. This too suggests that HIV itself may have an effect on BMD, possibly by inducing an inflammatory state which accelerates bone resorption.

There are many studies investigating the effect of ART on BMD, with some of these in ART-naïve patients and some in ART-experienced patients. Although the results vary from study to study, many of these suggest that ART has an effect on reducing BMD, and that certain drugs, such as TDF, seem to have more of an effect than others.

However, there are fewer studies investigating fracture rates in HIV-positive populations. In the late 2000s, it was not known whether the higher prevalence of reduced BMD seen in HIV-positive patients translated into an increased risk of fragility fractures, which is the main outcome of interest with regards to reduced BMD. The use

of the FRAX[®] tool, which calculates the 10-year absolute risk of both a major osteoporotic fracture and a hip fracture, was emerging, although it had not been validated in HIV-positive populations.

At the start of my study, although there were data relating to BMD in HIV-positive patients, many were from cross-sectional studies, and data from longitudinal studies were lacking. There were no published data on BMD from HIV-positive cohorts in the UK. My study investigates reduced BMD in a homogeneous UK cohort of mainly white HIV-positive MSM with longstanding HIV infection, but who were ART-experienced and therefore had good virologic control and immune function.

1.10.1.3 RTD

RTD is common in HIV-positive patients and has been found to be a complication of ART. It has been particularly associated with TDF and boosted PIs, with the greatest risk occurring when TDF and boosted PIs are co-prescribed. In its severest form, RTD can lead to Fanconi syndrome, which can cause hypophosphataemia and osteomalacia.

There are different LMWPs that can specifically detect RTD and differentiate it from GP. In the late 2000s, data relating to LMWPs in HIV-positive cohorts were lacking. The few studies that had examined LMWPs used various different biomarkers, making comparison between studies difficult. RBP is a LMWP that had been shown to be effective in identifying patients with RTD. At the time my study started, there were very few studies that investigated the use of RBP in HIV-positive patients, and those that had been published had small patient numbers or did not look at the association between RTD and bone.

Although LMWPs are not routinely used in clinical practice, PCR and ACR are relatively cheap and effective tests used to screen for renal disease. PCR measures total proteinuria and ACR measures albuminuria. A combination of these tests would enable APR to be calculated and would specifically identify RTD. Although there were no published data relating to APR when my study commenced, work from within our group was investigating the utility of APR in differentiating RTD from GP in the general population. As HIV-positive patients were at risk of RTD, it made sense to investigate the utility of APR in this cohort.

There is a complex relationship between the bones and the kidneys. Studies showed that TDF can cause RTD, which can lead to renal phosphate wasting. As phosphate loss can stimulate compensatory bone resorption, it can lead to a reduction in BMD

over time. With RTD effecting bone mineralisation, it could contribute to reduced BMD and an increased risk of fragility fractures. However, at the time my study commenced, it was unclear whether sub-clinical RTD was associated with altered bone homeostasis and reduced BMD.

1.10.2 Rationale for the study

1.10.2.1 Aims

My principal aim in my thesis was to conduct a cross-sectional study with changes over time to investigate BMD and renal tubular dysfunction (RTD) in a relatively homogenous group of white, ART-experienced HIV-positive men in the UK, who were mostly men who have sex with men (MSM) and mainly on tenofovir disoproxil fumarate (TDF). My aims included calculating the prevalence and risk factors associated with reduced BMD at baseline, the change in BMD over 12 months and the factors associated with loss of BMD, calculating FRAX[®] scores in these patients, assessing FRAX[®] and pDXA as screening tools, and evaluating RTD, including the utility of APR in differentiating RTD from other proteinuria and the relationship between RTD and bone.

1.10.2.2 Objectives

The main objectives were to:

1. Identify the prevalence of, and the factors associated with, reduced BMD in this population (Chapter 3), as well as changes in BMD and the factors associated with loss of BMD at 12 months (Chapter 4).
2. Calculate FRAX[®] scores in this cohort and to assess the utility of both FRAX[®] and pDXA as screening tools for identifying HIV-positive men at risk of reduced BMD (Chapter 5).
3. Investigate the utility of APR in diagnosing RTD (Chapter 6) and to assess the association between RTD and bone (Chapter 7).

Chapter 2: Methods

2.1 Study design

2.1.1 Study design rationale

Following a pilot study conducted in 2008 [160], a prospective cohort study was designed to investigate BMD in HIV-positive men. Study participants were assessed annually, and the original plan was to conduct assessments at baseline, 12 months and 24 months. The results of this thesis relate to the study participants' visits at baseline (Chapters 3, 5, 6 and 7) and at 12 months (Chapter 4). The 24-month follow-up was not done and the rationale for this is discussed in Section 2.6.

In order to ensure that the men selected for the study were truly representative of the diverse clinic population, study participants were chosen in two ways. First, men who had participated in the pilot study were invited to participate in this study. Second, the remaining men who were current attendees of the HIV outpatient clinic were chosen randomly to minimise selection bias and to ensure that a range of men were selected, including those that were relatively new diagnoses, those with longstanding HIV infection, those that were ART-naïve as well as those that were ART-experienced.

2.1.2 Study population

Brighton is a city on the south-east coast of England, situated 55 miles south of London. In 2001, the locality of Brighton and Hove had a population of 247,817 [175]. Over 10 years, the population had increased by 10.3% to 273,369 [176]. Due to a large community of lesbian, gay, bisexual and transgender people, Brighton is known as the 'gay capital' of the UK. In 2011, Brighton and Hove had the highest percentage of same-sex households in England and Wales at 0.9%, corresponding to 2346 individuals [176].

In 2013, Brighton had the highest prevalence of HIV infection outside London, with a prevalence rate of 7.96 per 1,000 people aged 15 to 59 years [177]. The Department of Sexual Health in Brighton is situated at the Royal Sussex County Hospital, part of Brighton and Sussex University Hospitals (BSUH) NHS Trust, which is an acute tertiary referral teaching hospital. The Trust treats more than 750,000 patients each year [178]. The HIV outpatient clinic within the Department of Sexual Health is one of the largest in

the UK. It is a research active unit which is recognised both nationally and internationally. Over 2,200 HIV-positive patients (both new and follow-up patients) attend the HIV outpatient clinic between 1 and 3 times per year, with the number of patients increasing by 5% annually and patients >50 years accounting for over 25% of the clinic population [179].

2.1.3 Inclusion and exclusion criteria

2.1.3.1 Inclusion criteria

All HIV-positive men aged 18 years or over who attended the HIV outpatient clinic in 2010 were eligible for recruitment into the study. To minimise selection bias, it was decided that patients involved in other research projects within the HIV outpatient clinic and the sexual health clinic were also eligible for recruitment into this study.

2.1.3.2 Exclusion criteria

All HIV-positive women and all HIV-positive men <18 years old, unable to give written, informed consent or considered unsuitable to participate in a clinical study (e.g. living abroad, having multiple medical co-morbidities making them unsuitable to take part in a clinical study as determined by their clinic doctor or having medical conditions deemed unsuitable to participate in a clinical study, such as aggressive behaviour) were excluded.

2.1.4 Selection of participants

HIV-positive men were recruited from the HIV outpatient clinic in two ways.

2.1.4.1 Pilot study HIV-positive men

All 168 HIV-positive men who participated in the pilot study conducted between 1st May 2008 and 31st October 2008 were eligible to join my study to gain further long-term data. These participants had been consecutively chosen from the HIV outpatient clinic between May and August 2008. Eligible patients were male, aged ≥ 18 years and with known chronic HIV infection. In the pilot study, patients were excluded if they were unable to give written informed consent or had undergone a DXA scan for diagnostic purposes within the preceding 12 months. Patients were purposively selected to represent a range of ART exposures, and were stratified into three groups:

1. ART-naïve
2. New and recent ART exposure (<3 years)
3. Long-term ART exposure (>3 years).

As part of the pilot study, each patient completed a questionnaire which assessed risk factors for low BMD, including details of previous fractures, family history of fracture, smoking, alcohol use, history of hypogonadism, renal or liver disease, and exposure to oral glucocorticoids. Details relating to demographics, HIV and ART regimens were extracted from the HIV clinic database and from clinic notes. Height and weight were measured to calculate their BMI. Each patient had BMD measured at the lumbar spine and femoral neck using a Hologic QDR 4500C DXA scanner (Hologic Incorporated, Bedford, USA) and at the non-dominant forearm using the Lunar PIXI pDXA scanner (GE Healthcare, Madison, USA).

By the start of my study in January 2010, four participants had transferred their care elsewhere, and were therefore no longer available to participate. The remaining 164 participants were all invited to join the study.

2.1.4.2 Randomly selected HIV-positive men

HIV-positive men were randomly selected to minimise selection bias from the list of patients who were listed as currently attending the HIV outpatient clinic in January 2010. These study participants inherently represented a spectrum of risk levels for low BMD.

In January 2010, there were 1,493 HIV-positive men (including 164 pilot study participants) listed as currently attending the HIV outpatient clinic in the clinic database. Therefore, there were 1,329 HIV-positive men eligible to join this study. Using the estimates from the power calculation (Section 2.5.1), it was decided to randomly select 600 patients to join the study. As a patient's HIV outpatient clinic number was assigned by date of first attendance at the clinic, to minimise selecting patients more recently diagnosed (who had more frequent attendance in clinic compared to patients with stable HIV infection), all patients were listed using their surname and first name. Random numbers were generated using a random number table, where consecutive three digits were recorded. As this gave values between 000 and 999, the patient list was divided into two. Using the randomly selected numbers, 300 patients were chosen from the first list and a further 300 patients from the second list. These 600 HIV-positive men were all invited to participate in the study.

2.1.5 Recruitment

2.1.5.1 Pilot study HIV-positive men

Before participation in my study, each patient was sent a letter (Appendix 10.3.2) or contacted by telephone if they did not wish to receive letters from the HIV outpatient clinic. During the telephone call, the details of the study were discussed with the patient and a pre-piloted participant information sheet (PIS) detailing the study (Appendix 10.3.3) was either sent in the post or emailed for the patient to read. Patients were given a minimum of 48 hours (if contacted by telephone) or one week (if contacted directly by post) to consider taking part in the study and were then contacted by telephone to discuss whether they wanted to participate in the study. During the telephone call, if they agreed to be recruited, patients were given an appointment to attend for a research appointment. They were also given instructions regarding the correct fasting procedure before blood and urine samples were to be taken.

2.1.5.2 Randomly selected HIV-positive men

For eligible patients, their next routine HIV outpatient clinic visit was identified. The initial approach regarding recruitment into the study was made by a member of the patient's clinical team, usually a doctor or nurse, during their routine clinic. At this time, a pre-piloted PIS detailing the study (Appendix 10.3.3) was given to each patient. All patients were asked if they were willing to be contacted by telephone by the research team in the following 48 hours to discuss whether they wanted to participate in the study. During the telephone call, if they agreed to be recruited, patients were given an appointment to attend for a research appointment and instructions regarding the correct fasting procedure to which to adhere to prior to their appointment.

The only exception to this approach was patients receiving their HIV care via the email clinic at the HIV outpatient clinic. This relatively well group of patients had stable HIV infection with a reported HIV viral load <40 copies/ml for >6 months on ART, and only attended the HIV outpatient clinic once a year for a doctor's appointment. Eligible patients who attended this clinic were sent an email (Appendix 10.3.4) and a copy of the PIS in advance of their appointments to ensure they were not missed, and therefore be subjected to negative selection bias. Patients were given one week to consider taking part in the study. They were then contacted by telephone to discuss whether they were willing to participate in the study, and during this telephone call, if they agreed to be recruited, were given an appointment to attend for a research appointment. They were also given instructions regarding the correct fasting procedure for blood and urine samples that would be taken during their study visit.

2.1.6 Consent

Voluntary informed written consent (Appendix 10.3.5) was obtained during the baseline visit (Year 1). Each participant was given a copy of the signed consent form. At the start of the first year follow-up visit (Year 2), willingness to continue participating in the study was checked with each patient.

Participation in the study was entirely voluntary. All patients were assured that if they decided not to take part, their care at the HIV outpatient clinic or the sexual health clinic would not be affected. Participants were free to withdraw from the study at any time, but their data to the point of withdrawal was used for analysis, unless the participant specifically expressed their wish for the data to not be used.

2.1.7 Schedule of visits

Participants with HIV infection recruited into the study were assessed at two time points:

1. Baseline visit (Year 1)
2. Follow-up visit at 12 months (Year 2).

The two visits were 12 months apart. Although the majority of participants' visits were one calendar year apart, not all strictly adhered to this timeline. Some participants did not keep their scheduled follow-up visit, and attended only when reminded, which occurred after they had missed their appointment. Rather than exclude participants whose visits were more than one year apart, it was decided to include patients in the analyses who returned within one month of their scheduled follow-up visit.

2.1.8 Study visits and procedures

Table 2.1.8.1 shows the study visits and the procedures performed at each visit. Further details regarding the different procedures are discussed in Section 2.2.

Table 2.1.8.1 Study visits and procedures

Apart from formal consent, participants underwent all the same tests at both visits.

Study visit	Consent	Questionnaire	Clinical measurements	BMD measurements		Fasted samples	
				cDXA	pDXA	Blood	Urine
Baseline	✓	✓	✓	✓	✓	✓	✓
12-month follow-up	Verbally checked but not formally re-taken	✓	✓	✓	✓	✓	✓

BMD: bone mineral density; cDXA: central DXA; pDXA: peripheral DXA

2.1.9 Date of closure of analysis

HIV-positive men were recruited into the study between 1st March 2010 and 28th February 2011. This constituted their baseline visit (Year 1). These participants re-attended 12 months later between 1st March 2011 and 29th February 2012 for their 1st year follow-up visit (Year 2).

2.2 Study procedures at each visit

2.2.1 Demographic details

Demographic details were obtained from the HIV clinic or sexual health clinic database as well as from the study's case report form (CRF). These included date of birth (to calculate age at each visit) and ethnicity.

2.2.2 HIV-related details

A full HIV-related history was obtained from the HIV clinic database. Route of infection was determined to assess HIV risk exposure. Date of HIV diagnosis was used to calculate duration of HIV infection at each study visit. Clinical HIV stage using the WHO classification was assessed using baseline CD4 count, nadir CD4 count and diagnosis of an AIDS-defining illness. Baseline HIV viral load was recorded. A detailed history of ART exposure was taken, including date of starting ART, the names of antiretroviral drugs taken, the duration of exposure to each antiretroviral drug and whether the participant was on ART at each study visit. Each participant's hepatitis B (HBV) and hepatitis C (HCV) statuses were determined using their latest HBV (surface antigen, core antibody, surface antibody and DNA levels) and HCV (antibody and RNA levels) serology, respectively. At recruitment, HBV positivity was determined as HBV surface

antigen positive and HCV positivity as HCV antibody positive, respectively. Although HCV RNA data were collected, these were incomplete and were not used to determine HCV status.

2.2.3 Questionnaire

Study participants completed a detailed questionnaire designed to obtain accurate information regarding risk factors for low BMD (Appendix 10.4). As there were no 'gold standard' questionnaires validated for obtaining information regarding low BMD risk factors in HIV-positive men available for use, the questionnaire used in the study was constructed from previously used questionnaires [117,180]. The questionnaire was peer-reviewed and its content was validated by my supervisors. Most sections of the questionnaire were validated in the pilot study [160]. Additionally, detailed sections pertaining to exercise and dietary intake of calcium and vitamin D were added, which were not included in the questionnaire used in the pilot study.

2.2.3.1 Traditional risk factors for reduced BMD

The following questions relating to traditional risk factors for low BMD were included:

1. Smoking history
2. Alcohol intake
3. Family history of low BMD and osteoporotic-related fractures.

There was a detailed section relating to past fractures, including dates of fractures and details of which bones were fractured. As the mode of injury to ascertain whether these fractures were fragility fractures related to low BMD (i.e. low impact) or were high impact fractures was poorly recorded, it was decided to categorise fractures using site. Therefore, fractures of the distal forearm, femoral head and/or neck and lumbar spine were categorised as fragility fractures, in keeping with the sites most commonly associated with osteoporotic fractures, and is discussed in more detail in Chapter 5.

2.2.3.2 Secondary risk factors for reduced BMD

There were questions related to secondary causes of low BMD. These included a history of certain medical conditions, such as diabetes, low testosterone, hyperthyroidism, hypothyroidism, hyperparathyroidism, hypoparathyroidism, kidney disease, liver disease, inflammatory bowel disease, coeliac disease, depression, anorexia nervosa, rheumatoid arthritis and hypercholesterolaemia. Although this was not

an exhaustive list, it included the majority of medical conditions associated with reduced BMD and seen in HIV-positive patients. There was a detailed section relating to drugs associated with reduced BMD, including steroids (inhalers, tablets and anabolic steroids), growth hormones, ketoconazole, chemotherapy, antidepressants, anticonvulsants, bendrofluazide, testosterone, calcium tablets, vitamin D supplements, bisphosphonates, antacids containing aluminium and any other medication. Questions relating to recreational drug use, in particular the use of intravenous drugs (e.g. opiates) and methadone, were also included. A further detailed section relating to recreational drug use was submitted to the National Health Service (NHS) Research Ethics Committee, but was rejected as the questions were deemed too intrusive.

2.2.3.3 Mobility and exercise

The questions on mobility and exercise were used to assess the amount of exercise each participant undertook, including the minimum amount of exercise recommended by the NOS, as well as questions relating to weight-bearing and muscle-toning exercise. There was also a question about walking-related problems and falls.

2.2.3.4 Dietary intake of calcium and vitamin D

The detailed dietary section was compiled with the assistance of a nutritionist. As there was a lack of suitable questionnaires that existed to determine the dietary intake of calcium and vitamin D, a set of dietary questions was specifically devised.

In the UK, there were reference nutrient intakes for calcium, but not for vitamin D. Portion sizes were calculated using UK food portion sizes [181] and the dietary software program Microdiet (Downlee Systems Limited, High Peak, UK). Standard food tables for the UK, including those devised by McCance and Woodison [182], and data from the Food Standards Agency [183] were used. The questions used were based on major food groups that contain calcium and vitamin D for this study population [184], as well as information from food-frequency questionnaires from other population studies in the UK [185-187].

The frequency of intake was divided into the following categories for most questions:

1. Never or rarely
2. Once in 2 weeks
3. 1 to 3 times a week
4. 4 to 7 times a week
5. Once a day
6. More than once a day.

More detailed questions were used to determine daily intake of milk, bread and fat intake.

The results from the dietary intake questions have not been analysed for this thesis as laboratory data on calcium and vitamin D were available. Although calcium and vitamin D intake may have an effect on the body's calcium and vitamin D levels, it is the actual levels in the blood that are of importance when relating to reduced BMD.

2.2.3.5 Validity and reliability

Whilst designing the questionnaire, I was aware that some sections (e.g. past fracture history, medication history and recreational drug use history) would be subjected to recall bias. Drug histories are notoriously subjected to recall bias. I expected that the participants' recall regarding medication would be higher than that seen in the general population due to the high level of commitment required to take ART, as well as the motivated nature of these participants with regards to their health care. To reduce recall bias, I rechecked their answers against their medications and repeat prescriptions, and used the clinic database to validate the information relating to ART.

2.2.4 Clinical measurements

2.2.4.1 Biometric measurements

The clothed weight (kg) and height (m) of each subject were determined using standard scales. BMI was calculated using the formula:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height}^2 \text{ (m)}}$$

2.2.4.2 Blood pressure measurements

Resting blood pressure was measured using a standard automated blood pressure machine with an appropriately sized cuff. The patient was seated and had two measurements taken with a resting period in-between as per the recommendations by the European Society of Hypertension [188]. The higher value was recorded.

2.2.5 BMD measurements

BMD measurements were made using two DXA machines.

2.2.5.1 Central DXA (cDXA)

cDXA measurements of absolute BMD (g/cm^2) at the hips, lumbar spine and non-dominant forearm were made. Ethnicity, age, height and weight were entered to calculate BMI, T- and Z-scores. For lumbar BMD, the BMD, T- and Z-scores of the composite of L1 to L4 were used. If a vertebra was incompletely scanned, or there was an artefact (e.g. metal body piercing, metalwork from operations) overlying a vertebra, this vertebra was excluded and the composite of the remaining vertebrae was used. Femoral neck BMD, total hip BMD, T- and Z-scores were used to define femoral BMD. Where the femoral shaft was inadequately scanned in the ROI, the total hip BMD could not be calculated and is missing for these patients. In patients with an artefact (e.g. metalwork from operations) on either or both sides, their femoral BMD (neck, total or both) was excluded from the analyses. Total body BMD (g/cm^2) was measured to assess body composition, including total mass (kg) fat mass (g), lean mass (g), BMC (g) and fat free mass (g). Fat distribution, including percentage of total body fat, and fat mass ratios, including trunk/total, legs/total and (arms and legs)/trunk were calculated.

A sample of the complete cDXA results printout per study subject is shown in Appendix 10.5.1.

2.2.5.2 Peripheral DXA (pDXA)

Absolute BMD (g/cm^2) measurements at the non-dominant forearm were made using pDXA. Ethnicity, age, height and weight were entered to calculate T- and Z-scores. If a patient had an artefact (e.g. metal work from operations) on the non-dominant forearm, the dominant forearm was measured and this was noted for purposes of analysis.

A sample of the DXA results printout per study subject is shown in Appendix 10.5.2.

2.2.6 Blood and urine tests

Study participants were asked to provide fasting blood and urine samples. These were taken between 8.30am and 10am on the day of the study visit to ensure consistency, especially as some tests were affected by diurnal variations. Before blood and urine samples were taken, each participant was asked if they had fasted from midnight of the day of the study visit and had ensured that they had not eaten or drunk anything except water. If this was not the case, study participants were invited to return at a later date to have their blood and urine samples collected when they had correctly fasted.

At each study visit, a number of blood and urine tests were taken, including those that were done as part of routine clinical care, as well as more specialised tests (Table 2.2.6.1). Samples were also taken for storage purposes for use in the future, subject to ethical approval.

Table 2.2.6.1 Blood and urine tests

Details of the standard and specialised tests performed, as well as details of samples stored for future use.

Type of test	Type of sample	Volume of sample	Tests done and units of measurement	Company
Standard	EDTA blood	1 x 4 mL	Full blood count: haemoglobin (g/dL), white blood count ($\times 10^9/L$), platelets ($\times 10^9/L$)	Sysmex UK Limited, Milton Keynes, UK
Standard	EDTA blood	1 x 4 mL	Total vitamin D (nmol/L), PTH (ng/L)	Roche Group Limited, Basel, Switzerland
Standard	EDTA blood	1 x 4 mL	CD4 count: absolute (cells/ μ L), percentage (%)	Becton, Dickinson and Company, New Jersey, USA
Standard	Lithium heparin blood	2 x 6 mL	Urea and electrolytes: sodium (mmol/L), potassium (mmol/L), urea (mmol/L), creatinine (μ mol/L) Bone profile: ALP (IU/L), calcium (mmol/L), phosphate (mmol/L) Lipids: total cholesterol (mmol/L), triglyceride (mmol/L), high density lipoprotein cholesterol (mmol/L), low density lipoprotein cholesterol (mmol/L) Thyroid function tests: TSH (m μ /L), T ₄ (pmol/L), T ₃ (pmol/L) Sex hormone profile: sex hormone binding globulin (nmol/L), testosterone (pmol/L) Urate (mmol/L)	Roche Limited, Basel, Switzerland
Standard	Fluoride oxalate blood	1 x 2 mL	Glucose, mmol/L	Roche Group Limited, Basel, Switzerland
Standard	Clotted blood	1 x 5 mL	HIV viral load, copies/mL.	Abbott Laboratories, Illinois, USA
Standard	Urine	1 x 5 mL	PCR, mg/mmol; ACR, mg/mmol; phosphate, mmol/L	Roche Group Limited, Basel, Switzerland
Specialised	Clotted blood	1 x 5 mL	CTX (ng/mL), P1NP (ng/mL)	USCN Life Science Incorporated, Wuhan, China
Specialised	Urine	1 x 2 mL	RBP (μ g/L)	Cambridge University, Cambridge, UK
Specialised	Urine	1 x 10 mL	Metabolomics, proteomics	University of Sussex, Falmer, UK
Storage	Clotted blood	1 x 5 mL	Serum (for storage)	
Storage	Lithium heparin blood	1 x 5 mL	Plasma (for storage)	
Storage	Urine	2 x 5 mL	Urine (for storage)	

ACR: albumin/creatinine ratio; ALP: alkaline phosphatase; CTX: C-terminal cross-linking telopeptides of type I collagen; EDTA: ethylenediaminetetraacetic acid; P1NP: N-terminal propeptide of type I procollagen; PCR: protein/creatinine ratio; PTH: parathyroid hormone; RBP: retinol binding protein; T₃: free triiodothyronine; T₄: free thyroxine; TSH: thyroid stimulating hormone

2.2.6.1 Standard tests

Blood and urine tests that were normally performed as part of routine clinical care within the HIV outpatient clinic were performed during this study, with an emphasis on markers that were useful for identifying low BMD or risk factors for low BMD. These samples were tested using automated analysers in the Pathology Department at BSUH.

CD4 and HIV viral load were only performed on study participants who did not have a result from within the preceding 3 months. Both the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) formulae were used to calculate estimated glomerular filtration rate (eGFR), expressed in ml/min/1.73m². Free triiodothyronine (T₃) was only measured if free thyroxine (T₄) was abnormal. ACR was only performed if uPCR was >30 mg/mmol as per laboratory protocol. Urine phosphate was used to calculate the fractional excretion of phosphate (FePO₄) using the following equation:

$$\text{FePO}_4 = \frac{\text{urine phosphate} \times \text{serum creatinine} \times 100}{\text{serum phosphate} \times \text{urine creatinine}}$$

2.2.6.2 Specialised tests

Specialised blood and urine tests were performed to gain further information regarding low BMD and RTD. These were conducted in two different laboratories. Serum samples stored at -80°C were analysed for CTX and P1NP. Testing was performed at the laboratory of the Research Unit. Prior to testing study samples, optimisation checks were conducted on the assays to determine the optimal sample concentrations and to ensure that the values were within the standard dilution curve (Appendix 10.6). Urine samples stored at -80°C were analysed for RBP at the end of the study using the dual monoclonal antibody DELFIA[®] assay. RBP was expressed as a ratio relative to urinary creatinine concentration (/L) to determine retinol binding protein creatinine ratio (RBPCR, µg/mmol).

2.2.6.3 Stored samples

Serum, plasma and urine samples were stored at -80°C for use in the future. These may provide useful comparator samples for long-term follow-up.

2.3 Study preparation

2.3.1 Ethical approval

The study was scientifically peer reviewed by the Protocol Review Panel at the Department of HIV and Genitourinary Medicine's Research Management Meeting on 29th July 2009 prior to submission for ethical approval. The meeting was attended by the multidisciplinary team within the Department who were involved in research, including doctors, nurses and pharmacists.

Ethical approval was sought in line with guidelines from the NHS Research Ethics Committee (REC). Ethical approval was granted on 7th October 2009 by the Brighton East Research Ethics Committee (REC Reference Number 09/H1107/101). A substantial amendment (Am01) was submitted on 28th January 2010, which was approved on 22nd February 2010. The study was conducted in accordance with the principles of Good Clinical Practice (GCP) as set out by the International Conference on Harmonisation (ICH) in 1996 [189] and the Declaration of Helsinki by the World Medical Association (WMA) [190].

2.3.2 Ionising radiation exposure

2.3.2.1 Bone densitometers

BMD measurements were made using two DXA machines.

2.3.2.1.1 Lunar iDXA bone densitometer

The GE Healthcare Lunar iDXA bone densitometer (GE Healthcare, Madison, Wisconsin, USA) was used to make cDXA measurements of absolute BMD (g/cm^2) at the hips, lumbar spine and non-dominant forearm, as well as total body composition to measure lean mass and fat mass. The reference range was set to the UK reference range.

In order to ensure methodological consistency, all DXA scans were conducted by five appropriately trained radiographers using the iDXA bone densitometer, with full adherence to Ionising Radiation (Medical Exposure) Regulations (IR[ME]R) and manufacturer's operating instructions.

2.3.2.1.2 Lunar PIXI bone densitometer

The GE Healthcare Lunar PIXI bone densitometer (GE Healthcare, Madison, Wisconsin, USA), with version 2.2 software, was used to make pDXA measurements of absolute BMD (g/cm^2) at the non-dominant forearm.

In order to ensure good precision, all pDXA scans were conducted by five healthcare professionals (four research nurses and me) who had received appropriate training in using the PIXI bone densitometer. All scanning was conducted with full adherence to IR(ME)R regulations and manufacturer's operating instructions.

2.3.2.2 Ionising radiation doses for DXA scans

Table 2.3.2.1 shows the radiation doses received by each study participant for each scan during the study. The doses estimated were evaluated by a medical physics expert for standard size patients and were based on the manufacturer's data for entrance skin doses for the GE Lunar PIXI and the GE Lunar iDXA bone densitometers, with conversions to effective doses. The total research protocol dose for a study participant in the study did not exceed $60 \mu\text{SV}$. The risks from this level of dose were classed as trivial, and were approximately equivalent to nine days natural background radiation (4.5 days natural background radiation every 12 months).

Table 2.3.2.1 Radiation doses for DXA scans

The radiation dose received by each participant for each scan during the study.

Type of scan	Area to be scanned	Estimated procedure dose (μSV)	Total dose during study (μSV)	Maximum dose approved (μSV)
cDXA	lumbar spine, femoral neck and non-dominant wrist	10	20	40
cDXA	whole body (fat composition and muscle mass)	3	6	12
pDXA	non-dominant wrist (2 views)	1 x 2 views = 2	4	6

cDXA: central dual energy x-ray absorptiometry; pDXA: peripheral dual energy x-ray absorptiometry

2.3.2.3 Procedure for operating bone densitometers

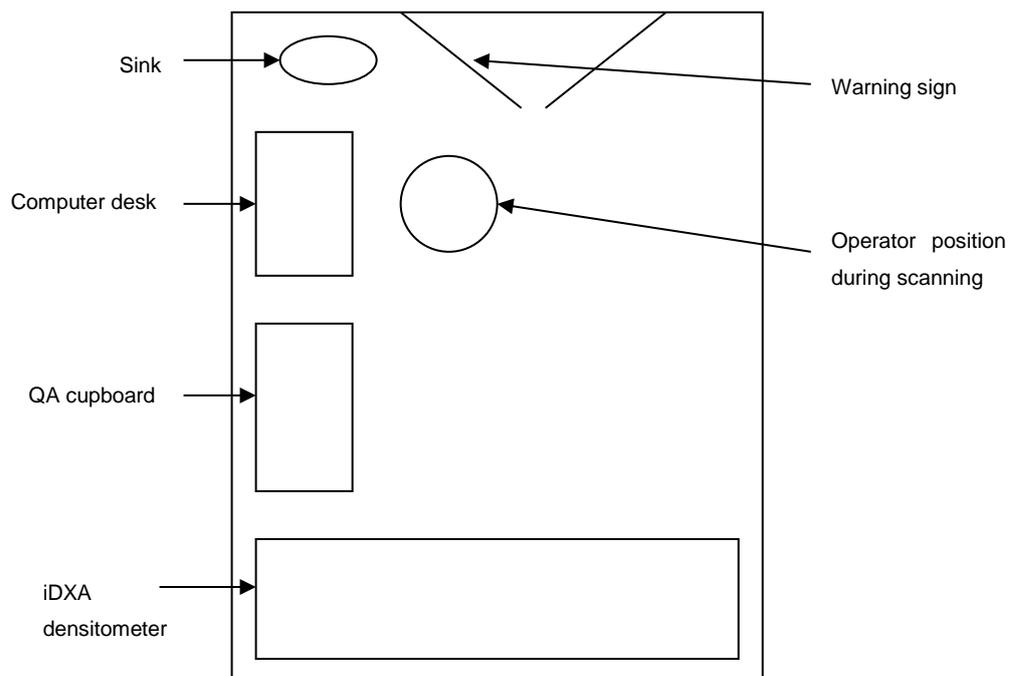
2.3.2.3.1 Lunar iDXA bone densitometer

The Lunar iDXA bone densitometer was set up in a designated room in the Research Unit, and all measurements were approved to be taken only in this room to ensure consistency of results. To reduce the risk of exposure to ionising radiation to the operators of the bone densitometer, the furniture in the room was arranged as shown in Figure 2.3.2.1. The computer was placed on the desk near to the sink so that the

operator could use it while remaining at least two metres from the bone densitometer. Prior to connecting the bone densitometer to the mains supply, a radiation warning sign was displayed outside the door. When the bone densitometer was connected to the mains supply, the whole room was designated a supervised area, and access to the room was controlled. Only appropriately trained staff designated as approved operators were able to operate the bone densitometer and perform the scans. Only those persons whose presence was judged to be essential were present in the room during an exposure. Normally this was the operator and the study participant. Whilst a radiation exposure was being made (including exposure for quality control), staff had to stay at least two metres from the bone densitometer, which condition was ensured if the operator remained by the computer during the exposure.

Figure 2.3.2.1 Arrangement of room for housing Lunar iDXA bone densitometer

The configuration of the room in which the Lunar iDXA bone densitometer was situated.
QA: quality assurance



At the start of each study session, before a participant was scanned, an eight minute quality assurance scan using a phantom block and an internal calibration system was performed to conduct a calibration check of the Lunar iDXA bone densitometer. The radiographer ascertained whether the participant had undergone a nuclear medicine isotope scan or a barium examination in the preceding one week, both of which would have interfered with the bone densitometer and produced an inaccurate scan. The scanning procedure was explained to the participant and their details were obtained relating to name, date of birth, previous fracture, presence of surgery requiring the

insertion of metalwork and hand dominance. The participant was asked to change into an examination gown and to remove any metalwork that could be removed (e.g. piercings). If an item of metalwork could not be removed, the participant was informed that they would need to have the same metalwork for their subsequent scans, to enable comparisons to be made.

Participants were correctly positioned in the middle of the examination couch using gridlines. In total, four scans were performed in the following order:

1. Whole body scan (8–10 minutes)
2. Lumbar spine scan (3–5 minutes)
3. Hip scan (3–5 minutes each side)
4. Non-dominant forearm scan (3–5 minutes).

The whole body scan was conducted with the study participant lying flat and supine on the examination couch (Figure 2.3.2.2). The lumbar spine scan was performed with a padded block placed under the lower legs to straighten the spine (Figure 2.3.2.3). If a participant was unable to lift their legs, the lumbar spine scan was conducted with their legs straight. Before the hips were scanned a metal block was securely placed by the participant's feet and the participant was asked to place their hands on their chests (Figure 2.3.2.4). Each hip was scanned separately, but formed into a dual hip scan using the program software. For the forearm scan, a plastic block was securely placed under the lower forearm and the participant was asked to make a fist with their hand to ensure the forearm was correctly aligned (Figure 2.3.2.5). Whilst the participant was being scanned, the scans were checked to ensure they were correct.

Figure 2.3.2.2 cDXA whole body scan

For the whole body scan, the participant had to lie flat and supine.



Figure 2.3.2.3 cDXA lumbar spine scan

A padded block was placed under the lower legs to straighten the spine prior to the lumbar spine scan being performed.



Figure 2.3.2.4 cDXA dual hips scan

A metal block was securely placed by the participant's feet and the participant was asked to place their hands on their chests before the hips were scanned.



Figure 2.3.2.5 cDXA forearm scan

A plastic block was securely placed under the lower forearm and the participant made a fist with their hand to ensure the forearm was correctly aligned prior to the forearm scan being performed.



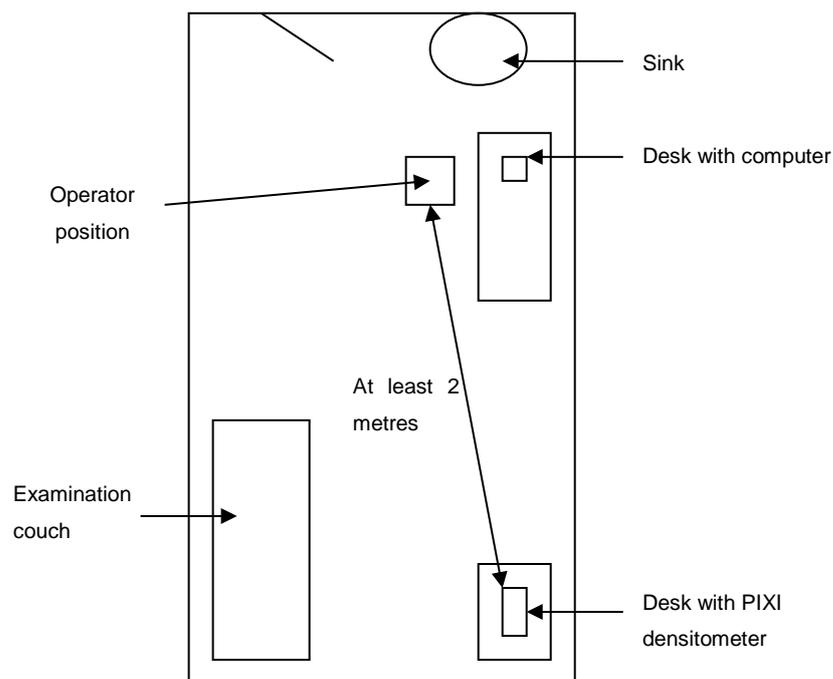
2.3.2.3.2 Lunar PIXI bone densitometer

The Lunar PIXI bone densitometer was set up in a designated room in the Research Unit, and all measurements were approved to be taken only in this room to ensure results were consistent. To reduce the risk of exposure to ionising radiation to the operators of the bone densitometer, the furniture in the room was arranged as shown in Figure 2.3.2.6. The computer was placed on the desk so that the operator could use it while remaining at least 2 metres from the bone densitometer. The bone densitometer was placed securely on a table, and not on a wheeled trolley. Prior to connecting the

bone densitometer to the mains supply, a radiation warning sign was displayed outside the door. When the bone densitometer was connected to the mains supply, the whole room was designated a supervised area, and access to the room was controlled. Only appropriately trained staff designated as approved operators were able to operate the bone densitometer and perform the scans. Only those persons whose presence was judged to be essential were present in the room during an exposure. Normally this was the operator and the study participant. While a radiation exposure was made (including an exposure for quality control), staff had to stay at least two metres from the bone densitometer, which condition was ensured if the operator remained by the computer during the exposure. A risk assessment showed that operators whose only exposure to ionising radiation was using this bone densitometer were not required to wear personal dosimeters, since doses were low and were unlikely be measurable.

Figure 2.3.2.6 Arrangement of room for housing Lunar PIXI bone densitometer

Configuration of the room in which the Lunar PIXI bone densitometer was situated.



At the start of each study session, prior to scanning a study participant, a five minute calibration check of the Lunar PIXI bone densitometer was conducted using a forearm phantom block and an internal quality assurance calibration system. The scanning procedure was explained to the participant and their details relating to name, date of birth, previous fracture, presence of surgery requiring the insertion of metalwork and hand dominance were ascertained.

Depending on whether the patient was right- or left-handed, the hand block was put into the appropriate position on the machine. Each patient was shown how to hold the hand block tightly to ensure that as much of their distal forearm was in the scanning field (Figure 2.3.2.7).

Figure 2.3.2.7 pDXA forearm scan

The positioning for the forearm scan performed using the pDXA densitometer.



2.3.2.4 IR(ME)R approval

Before the study was approved, a medical physics expert and a clinical radiation expert assessed the exposure to ionising radiation for each study participant, as well as for those conducting the scans. They assessed that all operators of the bone densitometers were appropriately trained according to IR(ME)R 2000 and Ionising Radiations Regulations 1999. Approval for the study was granted by the BSUH NHS Trust's Radiation Safety Committee on 23rd November 2009.

2.3.3 Training of staff in conducting the study

All members of the research team conducting the study were trained according to GCP.

2.3.3.1 Training of research staff

All research nurses, healthcare assistants and medical students were trained by me. Research nurses were trained in obtaining consent, venepuncture, measuring clinical parameters (height, weight and blood pressure) and performing pDXA scans using the Lunar PIXI bone densitometer. Healthcare assistants and medical students were

trained in venepuncture and measuring clinical parameters (height, weight and blood pressure). Training involved observing me carrying out the appropriate tasks a minimum of three times, followed by me observing each member conducting the various tasks three times, before each researcher was deemed competent in conducting the appropriate tasks. Once the training was complete, the researcher's details were logged in the delegation log, which included details of the tasks they were trained and competent in.

2.3.3.2 Training of operators in using the bone densitometers

2.3.3.2.1 Lunar iDXA bone densitometer

Each operator trained in performing cDXA scans using the Lunar iDXA densitometer were already fully trained according to IR(ME)R 2000 and Ionising Radiations Regulations 1999. In-house training in using the iDXA bone densitometer was conducted. Each radiographer observed the trainer perform a scan on three occasions, and the trainer then observed them scanning three participants, before each radiographer was trained to conduct scans by themselves. Additional training of all radiographers was performed by an applications specialist from GE Healthcare.

2.3.3.2.2 Lunar PIXI bone densitometer

Each operator trained in performing pDXA scans using the Lunar PIXI densitometer underwent training according to IR(ME)R 2000 and Ionising Radiations Regulations 1999. Once this was completed, all operators were trained in using the bone densitometer by an applications specialist from GE Healthcare. Each nurse observed me conduct a scan on three occasions, and I then observed them scanning three participants, before each nurse was trained to perform them independently.

2.3.4 Safety assessment

2.3.4.1 Ionising radiation exposure

Scans on both bone densitometers were deemed safe by the medical physics expert and the clinical radiation expert. They were conducted in accordance to the manufacturer's operating instructions and IR(ME)R 2000. Both bone densitometers were under a service and maintenance contract with GE Healthcare and underwent an *in situ* operation check prior to their initial use.

Patients were made aware of the levels of ionising radiation they would be exposed to during the study in the PIS. This exposure was categorised and verified as trivial by the medical physics expert and the clinical radiation expert respectively. Informed consent was obtained from study participants during recruitment and participants had the opportunity to withdraw from the study at any time.

2.3.4.2 Venepuncture

Venepuncture, including the potential risk of associated pain and bruising, was conducted according to appropriate BSUH NHS Trust safety policies, and researchers abided by BSUH NHS Trust infection control policies.

2.3.4.3 Psychological distress

There was a theoretical risk of associated psychological distress created by increased awareness of susceptibility to low BMD. If such morbidity was identified, reassurance was provided by the research team, as adequate information and the opportunity for discussion would help alleviate such anxieties. Participants were informed that their clinic doctor would be informed of any abnormal results, including abnormal BMD, blood and urine results.

2.3.5 Handling of abnormal results

It was decided that abnormal results relating to cDXA or standard blood and urine tests would be acted upon. Abnormal results from pDXA and specialised blood and urine tests were not acted upon as these were done purely for research purposes and there were no clinical guidelines indicating that abnormal results from these tests had any clinical impact.

All results from standard blood and urine samples were checked by me and sent to the participant's doctor in the HIV outpatient clinic. All cDXA results were checked by me and verified by Dr Karen Walker-Bone, and the results sent to the participant's doctor in the HIV outpatient clinic.

Any study participants identified as having low BMD were referred for further investigation, which provided a direct benefit at an individual level. As there were no guidelines for the screening or management of low BMD in HIV-positive men, these results may confer additional benefits in the future regarding the management of low BMD in HIV-positive men.

2.4 Data handling

The different study procedures produced different datasets.

2.4.1 Databases

A separate Excel database was used for each data source. The databases were:

1. Data from the clinic database
2. Data from the CRF
3. cDXA data
4. pDXA data
5. Questionnaire data
6. Laboratory data.

All datasets were cleaned and merged using Excel and converted to STATA version 12.1 (StataCorp LP, Texas, USA) or SAS version 9.3 (SAS Institute Incorporated, Cary, USA) format for analysis.

2.4.2 Confidentiality

Data were treated confidentially in accordance with Caldicott principles [191]. In order to maintain confidentiality, each participant was assigned a unique study number. During the study, three unique identifiers were used to identify each participant:

1. Study number
2. Date of birth
3. HIV clinic or sexual health clinic number.

The HIV clinic or sexual health clinic number for each patient was available as data from the clinic database was used. No other person identifiable data were recorded.

Each participant's study visit was recorded in a CRF, which was entirely separate from the participant's hospital or clinic notes. The CRF was securely kept in a locked room within the Research Unit where the study was conducted. Although each participant's name and address was recorded in the CRF and on DXA request forms and reports, these details were not used on blood or urine samples or on laboratory test request forms. Databases were password protected and stored on BSUH NHS Trust approved

computers, which were password protected and located in secure areas. All data entry was validated before analysis according to GCP. Names and addresses were not recorded in databases with results from blood tests, urine tests or questionnaire answers. Names were removed from the DXA databases after completion of merging and consistency checks. HIV clinic or sexual health clinic numbers were removed from all databases after merging of data and consistency checks were completed. This meant that the study number and the date of birth of each participant remained the only unique identifiers during analysis.

2.4.3 Data cleaning

Data were cleaned at several stages using Excel and STATA. For all databases, random manual checks were conducted after data entry and extraction to ensure consistency. Discrepancies were manually checked against CRFs, laboratory reports, cDXA reports, pDXA reports and questionnaires, and errors rectified prior to statistical analysis.

2.4.3.1 Data from the clinic database

Data were extracted from the HIV outpatient clinic database into an Excel spreadsheet. The data were clinic number, date of birth, ethnicity, sexuality, route of infection, date of HIV diagnosis, baseline CD4 count, nadir CD4 count, AIDS-defining illness, baseline HIV viral load, and HBV and HCV status. In addition, details relating to ART exposure, including date of starting ART, the names of antiretroviral drugs taken and the duration of exposure to each antiretroviral drug, were extracted.

2.4.3.2 Data from the CRF

Data recorded in the CRF comprised height, weight, blood pressure and hand dominance. These were manually entered on to an Excel database.

2.4.3.3 cDXA data

Each participant's results were checked by the radiographer conducting the scanning and by me. If there were queries, these were first assessed by another radiographer, and any remaining queries were assessed by a consultant radiologist. The data were then extracted from the Lunar iDXA scanner's software as an Excel database.

The data extracted for each participant consisted of forename, surname, date of birth and ethnicity. Date of scan, age, height, weight, sex and ethnicity were extracted for each study visit. For each ROI, the following data were extracted:

1. Absolute BMD
2. Area
3. BMC
4. T-score
5. Z-score.

At the lumbar spine, the ROIs were:

1. L1–L4
2. L2–L4
3. L1, L3–L4
4. L1–L2, L3
5. L1–L3
6. L1–L2
7. L1, L3
8. L1, L4
9. L2–L3
10. L2, L4
11. L3, L4.

If a participant had a composite involving all four vertebrae, then this was used in preference over composites with three vertebrae and two vertebrae, respectively.

For femoral BMD, the ROIs were:

1. Right femur neck
2. Left femur neck
3. Right femur total
4. Left femur total.

For forearm BMD, the forearm side was extracted, as well as data relating to 'both', which was the region that corresponded best with the region scanned using the PIXI scanner.

With regards to total body composition, the following data were extracted for each ROI:

1. Fat mass
2. Lean mass
3. Bone mass.

The ROIs were:

1. Arms
2. Legs
3. Trunk
4. Total.

2.4.3.4 pDXA data

Each participant's data, consisting of study number, date of birth, HIV or sexual health clinic number, ethnicity, date of scan, forearm side scanned, height, weight, total BMD, T-score and Z-score, were manually entered on to an Excel database. Each result was checked against the pDXA report by me.

2.4.3.5 Questionnaire data

The questionnaire was designed using Formic Fusion scanning software. This enabled each completed questionnaire to be scanned, rather than manually entered, which reduced the risk of errors. Any anomalies were detected by the program, checked and verified, and corrected if necessary. The data were then extracted from the program into an Excel database.

2.4.3.6 Laboratory data

Data for standard blood and urine tests were extracted from the BSUH pathology database into an Excel database. Any missing values were identified and manually checked against the pathology database, and data were entered by hand where appropriate.

For the specialised blood and urine tests (CTX, P1NP and RBP), the data were provided in an Excel database. Any missing values were identified and checked with the laboratories performing the tests. Data were entered by hand where appropriate.

2.5 Statistical analysis

2.5.1 Power calculation and estimated sample size

Based on published datasets [136,192], the rate of BMD loss was calculated to be approximately 0.75 - 1.0% over the 24-month study period, with a standard deviation (SD) of 6%. Based on data from the pilot study [160] and published estimates [136], a sample size of 400 participants enabled a 1.1% reduction in BMD to be detected over the 24-month study period (SD 6%) at the 5% level with >95% power, and a 0.75% reduction with 80% power. With an estimated dropout rate of 20%, recruitment of 500 participants would have enabled a final sample size of 400. This final sample size (after dropout) of 400 was consistent with other published studies [192], and would have enabled multivariable regression analyses to be performed and reasonably small effect sizes to be detected.

2.5.2 Inclusions and exclusions

The data from all patients with HIV infection were considered for inclusion in the statistical analyses. In general, if participants were missing laboratory data that was needed for analysis of the main hypothesis within a chapter, they were excluded. Laboratory data were missing due to samples not being processed, and as this was deemed a random process, it was not thought to affect selection bias.

Details of specific inclusion and exclusion criteria are given in each Results chapter (Chapters 3 to 7).

2.5.3 Hypotheses and statistical tests

The hypotheses and statistical tests used in each chapter analysis are discussed in detail in each Results chapter (Chapters 3 to 7).

2.5.4 Handling of missing data

For missing covariate data, a code for 'not known' was created, so that all participants' data may be included in the analyses. Although this approach could introduce bias, data were complete or near complete for the majority of cases.

2.6 Evolution of study methodology and my contribution

I commenced my three year PhD fellowship in April 2009 and immediately started working on the study design. This involved devising the study, writing the study protocol, meeting with the statistician to discuss different study designs and to calculate the power, amending the questionnaire used in the pilot study, and designing and writing all the relevant documents needed for the study, including the PIS and the consent form. The study protocol and the documentation were completed by 24th July 2009. I presented the details of the study for scientific peer review by the Protocol Review Panel at the Department of HIV and Genitourinary Medicine's Research Management Meeting on 29th July 2009 prior to submission for ethical approval.

I also concurrently wrote the application for ethical approval. Prior to submission, I had to ensure that the radiation exposure from DXA was considered safe, so I liaised closely with a medical physics expert and a clinical radiation expert. Their assessments involved ensuring that the bone densitometers were correctly installed, and that the doses of ionising radiation each participant and operators of the bone densitometers were exposed to were deemed safe. The iDXA machine had to be moved and a specially fortified floor built to ensure that there was no radiation exposure to the room directly beneath it. I also worked with a dietician to devise the diet-related questions included in the questionnaire, and this was completed on 11th September 2009.

I submitted the ethical application on 11th September 2009. I and Dr Karen Walker-Bone attended the Brighton East REC on 1st October 2009. Ethical approval was granted on 7th October 2009 subject to minor amendments.

Once ethical approval was granted, all healthcare professionals involved in the study had to be trained in GCP if not already trained. All operators of the bone densitometers were trained according to IR(ME)R 2000 and Ionising Radiations Regulations 1999. Approval for the study was granted by the BSUH NHS Trust's Radiation Safety Committee on 23rd November 2009.

Prior to submitting the changes requested by the REC, I further met with the dietician and amended the questions relating to diet on the questionnaire. This was because she suggested new questions that were more accurate in calculating calcium and vitamin D intake than the original questions. Additionally, I decided to devise an invitation letter for the pilot study participants and an email invitation for patients having their HIV care via the email clinic, both of which were also submitted. I submitted a substantial amendment (Am01) on 28th January 2010, which was approved on 22nd February 2010.

Recruitment into the study commenced on 1st March 2010. During the recruitment phase, I identified all eligible patients and when they were next due to attend the HIV outpatients clinic, and contacted all physicians with patients due to attend their clinic on a weekly basis. Once clinicians had made the initial approach and had given the patients the PIS, I personally contacted all those patients who had expressed an interest in participating in the study. If they wanted to participate, I booked them into an appointment for their first study visit. I then printed all their labels to ensure that they were ready for their study visit.

During the study visits, I trained all research nurses, healthcare assistants and medical students who worked alongside me. Research nurses were trained in obtaining consent, venepuncture, measuring clinical parameters (height, weight and blood pressure) and performing pDXA scans using the Lunar PIXI bone densitometer. Healthcare assistants and medical students were trained in venepuncture and measuring clinical parameters (height, weight and blood pressure). During the recruitment phase, I contacted patients who failed to attend and re-booked them for future appointments. I checked all blood and urine results and sent copies of the results to the patient's HIV physician. I checked all cDXA results which were verified by Dr Karen Walker-Bone, and the results sent to the participant's HIV doctor. All abnormal results from cDXA or standard blood and urine tests were acted upon.

Although I attempted to recruit 500 men into the study within six months, this proved to be too ambitious and recruitment occurred between 1st March 2010 and 28th February 2011. These participants re-attended a year later between 1st March 2011 and 29th February 2012 for their first year follow-up visit. By the start of the first year follow-up visits in 2011, it was obvious that the second year follow-up phase was no longer feasible as my fellowship was due to end in April 2012. Therefore, although the study was powered to calculate BMD changes at two years follow-up, as the study was stopped after the first follow-up visit, the analyses conducted were for the baseline and first year follow-up visits only.

However, during the follow-up visits, I fell ill and had to take a year of sick leave from 22nd October 2011. I returned to my fellowship part-time on 21st October 2012 and continued until 1st May 2014, when I returned to my clinical commitments. On my return, I spent my time collating the data into several Excel spreadsheets, cleaning and merging the data, and analysing the data. Once working in my clinical job, I worked on my thesis in my own time. I then went on maternity leave for a year on 20th June 2016. I completed the thesis on my return from maternity leave and submitted it on 19th July 2017.

Chapter 3: A cross-sectional analysis of risk factors associated with reduced BMD

3.1 Background

3.1.1 Introduction

Since the early 2000s, HIV infection has been suggested as a cause of reduced BMD in HIV-positive men and women. The causes of this reduction are likely to be multifactorial, and several have been postulated. These include a higher prevalence of traditional risk factors, as well as HIV-specific factors, such as viral effects, the immunologic effect of HIV and the direct effect of ART on bone [161].

3.1.2 Traditional risk factors for reduced BMD in HIV-positive patients

Studies have shown that several traditional factors associated with reduced BMD in the general population (Table 1.4.3.1) are more prevalent in HIV-positive patients. Some are modifiable risk factors associated with the demographics of this population, such as cigarette smoking [193,194], excess alcohol consumption [158] and opiate use [195]. Others are thought to be a result of HIV infection and ART exposure, such as viral hepatitis [196], reduced BMI [197,198], hypogonadism [199] in men, early menopausal state in women [192] and VDD [200]. Additionally, some factors are thought to occur due to a combination of both HIV infection and demographic effects. These include steroid use [192,193], HBV or HCV co-infection [196], increased use of medications associated with reduced BMD (e.g. antidepressants, opiates) [153,156,195,201] and reduced mobility/physical activity [157].

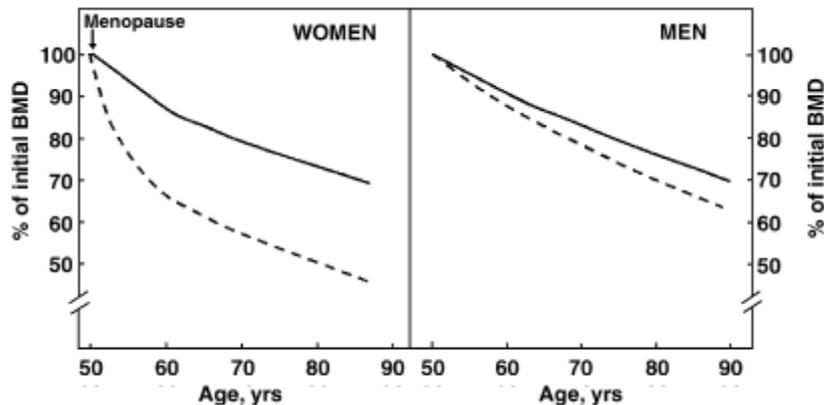
3.1.2.1 Non-modifiable risk factors

Gender and age are well-described, non-modifiable risk factors. Women are at greater risk of reduced BMD, especially after the menopause, when there is a period of accelerated bone loss lasting between 5 and 10 years, which can lead to loss of trabecular bone (Figure 3.1.2.1) [69]. Men, however, do not have an equivalent hormonal menopausal stage. They instead experience a gradual loss of both trabecular and cortical bone [202]. Although cross-sectional studies have estimated BMD loss to occur at a rate of 1% to 3% per decade, several longitudinal studies have reported a higher rate of between 5 and 10% per decade [70].

Figure 3.1.2.1 Age-related loss of BMD in men and women [69]

Women experience a greater loss of BMD after the menopause when compared with men of a similar age, especially in trabecular bone. Dashed lines represent trabecular bone and solid lines represent cortical bone.

BMD: bone mineral density



Studies have consistently shown an association between reduced BMD at the hip and increasing age, with a linear reduction at a rate of approximately 1.5% to 2.5% per decade in men aged >50 years [203,204]. On the other hand, results at the lumbar spine have not been consistent, with some showing a reduction in BMD with age [205,206], and others an increase in BMD at a rate of 1.5% to 3.5% per decade in men >60 years old [86,204]. The increase in BMD at the lumbar spine may be due to age-related degenerative changes (e.g. osteophytes), which can lead to falsely elevated BMD being reported. This was the case in one study where an initial increase in BMD disappeared when men with severe arthritis were excluded, and lumbar spine BMD was found to actually decrease significantly with advancing age [207]. Older age has also been associated with lower BMD in HIV-positive patients [153,156,208-211].

Another non-modifiable factor is ethnicity. Studies have shown an association between lower BMD and white ethnicity in children [212] and in adults [213,214]. Similar results have also been reported in HIV-positive cohorts, with non-white ethnicity associated with a higher BMD than white ethnicity [153,156,215].

Family history of osteoporotic fracture and a past history of a fragility fracture, which are factors more associated with fracture risk than BMD, are discussed in detail in Chapter 5.

3.1.2.2 Modifiable risk factors

Smoking is an important modifiable risk factor, especially as it occurs at a higher prevalence in HIV-positive patients [216-219]. A cross-sectional study in female monozygotic twins who were discordant for smoking showed that the twin who was the

heavier smoker had a greater reduction in BMD at the hip and the lumbar spine [220]. Studies in the general population in men >50 years have confirmed that current smokers have the highest risk compared to non-smokers, followed by ex-smokers [221]. Additionally, a meta-analysis has found that the fracture risk associated with smoking is greater than that explained by a reduction in BMD [222]. This may be due to a number of reasons, including the possibility of lower levels of exercise or the existence of other co-morbidities that may lead to an increased risk of falls. In HIV-positive patients, smoking has also been reported as a risk factor for reduced BMD [193,194].

Alcohol can have a direct effect on osteoblasts by suppressing their function [223]. In the general population, studies have shown an inconsistent association between alcohol and reduced BMD, with some showing a positive relationship and others showing no association [221]. Interestingly, although alcohol has been shown to be associated with an increased risk of osteoporotic fractures [61], this relationship does not appear to be linear and is U-shaped [224]. Several studies in HIV-positive patients have also confirmed an association between excessive alcohol consumption and fracture risk [158,218], some of which may be associated with an increased risk of falls secondary to alcohol consumption [225].

Increased physical activity has been associated with increased BMD in the general population [221]. Cross-sectional studies have reported that astronauts experiencing microgravity lose approximately 2% of hip BMD per month [226], whilst men who walked and exercised regularly had higher BMD than those who did not [227]. A meta-analysis in men has shown that exercise interventions can improve or maintain BMD [228] and another meta-analysis in postmenopausal women concluded that aerobic and resistance training can slow the rate of BMD loss [229]. There are relatively few studies investigating the effect of exercise on BMD in HIV-positive patients, with one study showing no association between progressive resistance training and BMD at the lumbar spine [230].

BMI is a modifiable risk factor, which has been positively associated with BMD at both the spine and the hip in several studies in the general population [221]. In one longitudinal study of older men and women, men who lost $\geq 1\%$ of their baseline weight per year were twice as likely to lose BMD compared to those who gained weight or whose weight remained stable [231]. A meta-analysis found that low BMI is a risk factor for osteoporotic fractures, which was independent of age and sex, but dependent on BMD [232]. Numerous studies suggest low weight before initiating ART or low BMI is the main cause of reduced BMD in HIV-positive patients [146,193,194,208,209,233]. A

meta-analysis of 1371 HIV-positive patients and 1644 HIV-negative controls found that HIV-positive patients have a lower BMI than HIV-negative patients, and suggested that low BMI accounted for the high prevalence of reduced BMD [198].

VDD is another modifiable risk factor for reduced BMD [234-236]. Vitamin D plays a major role in calcium and bone homeostasis [234] and is needed to efficiently absorb calcium and phosphate from the diet [237]. Although the active metabolite is 1,25-dihydroxyvitamin D [1,25(OH)₂D], it is difficult to measure as it has a short half-life (approximately four hours), and therefore the US Endocrine Society recommends measuring the circulating form 25-hydroxyvitamin D [25(OH)D] (which has a half-life of two to three weeks) to assess vitamin D status [235]. VDD is defined as 25(OH)D levels <50 nmol/L [234]. In one study in the general population, vitamin D levels were positively correlated with BMD [238]. But in other studies, a lower BMD was only associated with severe VDD (<25 nmol/L) [239,240]. Additionally, via its effects on calcium, PTH is inversely correlated with vitamin D [241]. The 1 α -hydroxylation of 25(OH)D is regulated by PTH. PTH acts on the kidneys to increase tubular reabsorption of calcium and to produce 1,25(OH)₂D [237]. PTH activates osteoblasts to convert preosteoclasts into mature osteoclasts, which are involved in bone resorption [234]. If resorption occurs at a faster rate than bone formation, low BMD ensues, which can then lead to an increased risk of fragility fractures.

Although many studies in HIV-positive patients have reported a high prevalence of both VDD and reduced BMD [242-244], they have not determined whether a direct association exists. Of those that have, a few have shown an association between VDD and reduced BMD [159,194], with the first study showing an association at the hip only. However, the majority have shown that there is no direct relationship between vitamin D and BMD [211,245-248]. Interestingly, one study showed an association between BMD and 1,25(OH)₂D, but they did not measure 25(OH)D levels [151]. Higher PTH levels have been associated with an increased loss of BMD [249], as well as higher PTH levels in HIV-positive men on TDF with VDD [41]. Although several longitudinal studies have shown that TDF has an effect on BMD [250-252], none have shown a direct relationship between vitamin D and TDF.

3.1.2.3 Secondary causes of reduced BMD

There are numerous secondary causes that have an effect on lowering BMD in HIV-positive patients. However, diseases commonly associated with low BMD in the general population, such as rheumatoid arthritis and hyperthyroidism [253,254], are not common in HIV-positive patients.

Low BMD is highly prevalent in patients with chronic liver disease awaiting liver transplantation, with men and women equally affected [255]. In HIV-positive MSM, who formed the majority of my cohort, liver dysfunction is common, caused by a combination of HBV and/or HCV co-infection and ART-related toxicity [256,257]. Additionally, as HIV-positive patients are at risk of excess consumption of alcohol, alcohol-related liver disease is another pathway which may lead to low BMD in these patients.

A well-documented secondary cause of low BMD in men is hypogonadism [75]. It can be due to primary testicular disorders or be secondary to pituitary or hypothalamic disease. Initially, the mechanism of action was thought to be solely due to androgen deficiency, as androgen receptors are found on osteoblasts and androgens affect osteoblast function [70]. Androgen deficiency can also cause increased bone remodelling and lead to rapid bone loss [258]. However, studies have shown that BMD is more strongly correlated with oestradiol than testosterone [259]. More recently, a study in older men has shown that testosterone or oestradiol deficiency is associated with reduced BMD [260]. Although both are likely to play an important role, the relative roles of androgens and oestrogens on BMD are not fully known. HIV-positive men are at risk of early andropause, and although the exact mechanism is not completely understood, it is likely to be a combination of primary and secondary hypogonadism [261]. Studies have shown that higher testosterone levels are protective against loss of BMD in HIV-positive patients [199].

Low BMD can occur in diabetes mellitus secondary to low bone turnover, with reduced bone formation leading to loss of bone [262]. Although different mechanisms are involved, patients with both Type 1 and Type 2 diabetes mellitus are at increased risk [263,264]. In HIV-positive patients, there were no studies investigating the association between diabetes mellitus and BMD. However, there are studies which have shown a variable association between diabetes mellitus and fractures [265,266].

There is a complex relationship between the kidneys and bone, involving vitamin D and PTH, with patients with CKD at risk of developing renal osteodystrophy. The prevalence of low BMD in patients with CKD is higher than in the general population [267]. Patients with CKD are at risk of developing a number of complications related to bone loss, including osteoporosis and chronic kidney disease-mineral and bone disorder (CKD-MBD) [268]. In fact, some argue that osteoporosis should be included in the definition of CKD-MBD [269]. Although studies investigating an association between CKD and lower BMD are lacking in HIV-positive patients, many have shown

that they are at increased risk of renal disease, including RTD [270,271]. RTD is discussed in more detail in Chapters 6 and 7.

3.1.2.4 Drugs associated with BMD

Several classes of drugs are associated with reduced BMD. In the general population, exposure to long-term, high-dose steroids is an established risk factor [272,273]. Several studies in HIV-positive patients, who may be exposed to steroids for treating a number of opportunistic infections [274], have shown an association with lower BMD and an increased risk of fractures [192,193].

Other drugs associated with reduced BMD are anticonvulsants, antacids, chemotherapeutic agents, aromatase inhibitors, anticoagulants (e.g. heparin) and proton pump inhibitors (Table 1.4.3.1). Both clinical and recreational use of opiates (e.g. heroin, methadone) has been implicated in reducing BMD in the general population [275,276]. In HIV-positive patients, studies have shown a similar association with opiate use [153,156,195,201]. Other recreational drugs (e.g. cannabis) have also been associated with altered bone metabolism [277], but data in the HIV population are lacking.

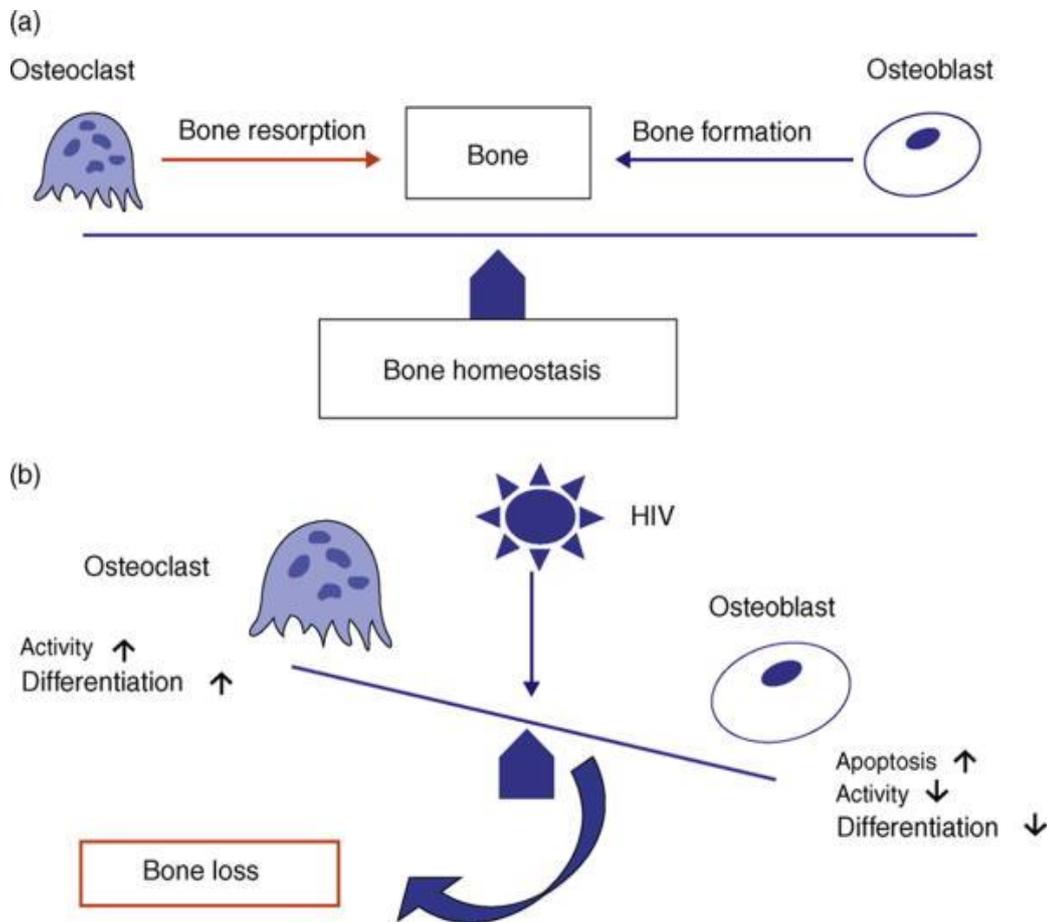
There are also drugs that may protect against reduced BMD. These include drugs used in the treatment of low BMD, such as calcium and vitamin D supplementation, as well as bisphosphonates.

3.1.3 Effect of HIV infection on BMD

As mentioned in Chapter 1, bone remodelling involves a close balance between bone resorption (via osteoclasts) and bone formation (via osteoblasts) (Figure 3.1.3.1a). Although the exact mechanisms involved are still not fully known, the pro-inflammatory state induced by HIV infection is thought to cause dysregulation of osteoclast and osteoblast function, leading to uncoupling of the normal bone remodelling process (Figure 3.1.3.1b) [22]. HIV infection is thought to induce a high bone turnover state, where bone resorption occurs faster than bone formation, leading to bone loss. This uncoupling may be mediated through a number of pathways causing persistent T-cell activation and release of cytokines. IL-6, interleukin-11 (IL-11), osteoprotegerin and RANKL stimulate osteoclasts, thereby activating bone resorption, whilst osteoblasts and bone formation are inhibited by IL-1 and TNF [278].

Figure 3.1.3.1 Mechanism of low BMD in HIV-positive patients [22]

Bone homeostasis is maintained by the coupled action of bone resorption and bone formation (a). In HIV infection, this process is disrupted (uncoupled), which causes a reduction in BMD (b).



HIV viral proteins can contribute to changes in the balance of bone formation and resorption. Osteoblasts are derived from mesenchymal cells in bone marrow and studies have shown that HIV infection can inhibit mesenchymal stem cells leading to reduced proliferation and survival [279,280]. An *in vitro* study has shown that the HIV viral proteins tat and nef can decrease the number of mesenchymal cells available to differentiate into osteoblasts [281]. They do this by activating and inhibiting RANKL and osteoprotegerin respectively, which leads to senescence of mesenchymal cells, thereby reducing the number available to differentiate into osteoblasts. Additionally, by activating peroxisome proliferator-activated receptor gamma (PPAR γ), the HIV viral protein gp120 can also shift mesenchymal cell differentiation from osteoblasts to adipocyte formation [280,282]. HIV gp120 can also increase apoptosis in primary osteoblasts [283] and can affect osteoblast activity by reducing calcium deposition, ALP activity and bone-specific RUNX-2 expression [282]. The viral proteins p55-gag and rev have also been found to interfere with osteogenesis *in vitro* [282,284].

HIV infection can also affect osteoclasts. The HIV viral proteins vpr and gp120 can upregulate RANKL and M-CSF in T-lymphocytes and macrophages, respectively, both of which are involved in osteoclast maturation [285-287]. This can lead to increased osteoclast differentiation and activity either directly via M-CSF or indirectly via an increase in the RANKL/osteoprotegerin ratio [22]. Additionally, the HIV viral proteins tat and rev can increase the number of monocytes differentiating into osteoclasts [288]. They can also increase reactive oxygen species and the production of TNF in osteoclast precursors, which can lead to an increase in the resorption function of osteoclasts.

HIV infection is associated with B-cell dysfunction, and this mechanism has been suggested as being involved in the loss of BMD in HIV-positive patients [289]. Titanji *et al* have demonstrated that HIV-positive patients have a higher frequency of RANKL-expressing B cells and a lower frequency of osteoprotegerin-expressing B cells compared to HIV-negative patients, and that this change in the RANKL/osteoprotegerin ratio is correlated with total hip BMD in HIV-positive patients [289].

Studies have investigated an independent role of HIV infection itself by assessing the effect of HIV viral load, CD4 count and duration of infection. A number of studies have reported an association between low nadir CD4 count and reduced BMD [210,215] and fractures [265]. Fausto *et al* showed an association between high HIV viral loads and reduced BMD [209]. Interestingly, one study reported an association between a higher risk of low BMD and an undetectable HIV viral load [210]. The authors suggested that this may be due to an indirect effect of ART exposure, but when they investigated the effect of ART, unadjusted for HIV viral load, this did not appear to be the case. Additionally, duration of HIV infection has also been linked to low BMD [146,193,194]. An American study comparing HIV-positive ART-naïve patients to HIV-negative controls reported that BMD at the total hip and trochanter decreased over 48 weeks in the HIV-positive patients but not in the controls, suggesting that the chronic inflammation of HIV infection may have an effect on BMD [290].

Primary HIV infection is associated with high levels of viraemia [291]. There appears to be a disruption of bone homeostasis during seroconversion, which leads to reduced BMD [292,293]. This further suggests that HIV infection itself has an effect on BMD, possibly by inducing an inflammatory state which accelerates bone resorption. Interestingly, a study investigating pre-exposure prophylaxis (PrEP) reported a low BMD in HIV-negative MSM [294].

3.1.4 ART and low BMD

There have been many studies investigating the effects of ART on BMD. The data are conflicting, with some studies showing an association between ART and low BMD, whilst others have shown no relationship. This may be due to the heterogeneity of the published studies, which include cross-sectional versus longitudinal studies, ART-naïve versus ART-experienced patients, lack of control groups for comparison, different definitions of reduced BMD and measurement of BMD at different sites. The Strategies for Management of Antiretroviral Therapy (SMART) sub-study provided useful insight into the effect of ART on BMD [11]. In this study, patients on continuous ART had a significantly greater decrease in BMD at both the hip and the spine compared to those in the intermittent arm. However, the rate of change in the continuous ART group was similar to what would have been expected as age-related changes in the general population and the study was also terminated early due to increased opportunistic infections and death in the intermittent arm.

3.1.4.1 ART-naïve versus ART-exposed patients

The majority of cross-sectional studies comparing ART-naïve to ART-exposed patients were conducted in the early 2000s (Table 3.1.4.1). Most of these have shown no significant difference in BMD between the two groups. However, one study did show an association between low HIV viral load and reduced BMD, which indirectly suggests that exposure to ART may have an effect on lowering BMD [210]. Additionally, a meta-analysis comparing 202 ART-naïve patients to 824 ART-exposed patients found that those on ART had significantly higher odds of reduced BMD (odds ratio [OR] 2.5, 95% CI 1.8, 3.7) and osteoporosis (OR 2.4, 95% CI 1.2, 4.8) compared to ART-naïve patients [136]. A more recent large cross-sectional study from South Africa with 77% women has also shown that BMD at the hip (total hip and femoral neck) was significantly lower in those who were on ART compared to ART-naïve patients, with VDD and EFV exposure being risk factors for lower BMD [295]. However, a cross-sectional analysis from a longitudinal study did not find any association between ART and lower BMD [193].

Table 3.1.4.1 Cross-sectional data comparing BMD in ART-naïve versus ART-exposed patients

Studies comparing ART-naïve to ART-exposed patients have shown no significant difference in BMD between the two groups.

Author	Year	Location	N	% male	Mean age (years)	Number ART-naïve	Number ART-exposed	% reduced BMD in ART-naïve	% reduced BMD in ART-exposed	P-value	Associations with low BMD
Carr [208]	2001	Australia	221	100	43	32	189	6	25	NS	Older age, low BMI, lactic acidemia and stavudine duration
Knobel [144]	2001	Spain	80	73	41	26	54	69	98	NS	Low BMI
Nolan* [233]	2001	Australia	183	100	-	28	155	46 ^a	55 ^a	-	Low BMI
Bruera [146]	2003	Argentina	111	80	34	33	78	70 ^b	74 ^b	NS	Duration of HIV infection
Fernandez-Rivera* [296]	2003	Spain	89	80	37	11	78	27	44	NS	Male sex, low albumin level and PI exposure
Vescini [297]	2003	Italy	70	49	41	4	66	50 ^a , 50 ^c	50 ^a , 56 ^d	-	Male sex
Amiel [148]	2004	France	148	100	40	48	100	8 ^c	18 ^c	NS	Low BMI
Madeddu* [151]	2004	Italy	172	65	38	20	152	30 ^a , 30 ^e	54 ^a , 49 ^e	NS	Male sex, intravenous drug use and more advanced HIV infection
Landonio [298]	2004	Italy	59	75	41	15	44	40	34	NS	None
Fausto [209]	2006	Italy	161	64	39	48	113	46	51	NS	Female sex, older age, low BMI and high HIV viral load
Garcia Aparicio [243]	2006	Spain	30	100	38	13	17	69	65	NS	Low testosterone level (lumbar spine)
Bongiovanni* [299]	2006	Italy	89	63	38	47	42	49	55	NS	Older age and low BMI
Cazanave [210]	2008	France	492	73	43 ^f	34	458	-	-	NS	Low HIV viral load

ART: antiretroviral therapy; BMI: body mass index; BMD: bone mineral density; NS: not significant; PI: protease inhibitor

*Longitudinal studies reporting cross-sectional baseline data

^aLumbar spine; ^bfemoral neck; ^costeoporosis only; ^dtotal hip; ^efemoral head; ^fmedian age

In summary, ART-naïve patients may be at risk of low BMD just as much as those exposed to ART, but as the aetiology of low BMD is multifactorial, the risk factors are likely to differ for each group. As HIV infection is an inflammatory condition, the cause of reduced BMD in ART-naïve patients may be related to this inflammatory state. In contrast, patients exposed to ART may be at risk of reduced BMD relating to the drugs they are exposed to. The difference in BMD between ART-naïve and ART-exposed patients has been extensively studied in longitudinal studies and is discussed in Chapter 4.

3.1.4.2 Effect of ART on BMD

Most of the data assessing the effect of ART on BMD come from longitudinal studies and these are discussed in detail in Chapter 4.

3.1.4.3 Individual classes of ART

When assessing individual classes of ART, again the data are conflicting, with some studies showing an association, but others not. Most early studies concentrated on exposure to boosted PIs. However, only half of these studies showed that there was a significant difference in BMD when boosted PIs were compared to an ART regimen not containing a boosted PI [142,144,151,199,233,296,300]. In a meta-analysis of 14 studies (n=410; 8 which showed an independent association between boosted PIs and BMD, and 6 studies that did not), the overall pooled data showed that exposure to boosted PIs was associated with reduced BMD (OR 1.5, 95% CI 1.1, 2.0) compared to patients who had never been exposed to boosted PIs [136]. Additionally, in the 12 studies with available data (n=666), the odds of osteoporosis was higher (OR 1.6, 95% CI 1.1, 2.3) in those on a boosted PI compared to those who were not [136]. However, the findings from this meta-analysis suggest that looking at the number of studies with an association in one direction or another may not be reliable. In ART-naïve patients followed prospectively, some studies have shown an association between loss of BMD and PIs [252,301,302], whilst others have not [215].

With regards to exposure to NRTIs, one study showed that duration of exposure to NRTIs was associated with a reduced BMD [194], whilst another has shown an association with zidovudine/lamivudine [249]. In a cross-sectional study, which investigated the effect of exposure to TDF, there was no association with either current exposure or duration of exposure and BMD [199]. However, most prospective studies in ART-naïve cohorts have shown an association between BMD loss and TDF use [29,251,252]. The Assessment of Safety and Efficacy of Abacavir/Lamivudine and Tenofovir/Emtricitabine (ASSERT) study is a longitudinal study investigating the effects

of TDF/emtricitabine against abacavir/lamivudine in ART-naïve patients. This study showed that there were reductions in BMD in both groups, but that there was a greater decrease in BMD patients on TDF/emtricitabine compared to those on abacavir/lamivudine [251]. In the metabolic sub-study of AIDS Clinical Trials Group (ACTG) 5202, which also compared TDF/emtricitabine against abacavir/lamivudine, there was a greater loss of BMD at both the lumbar spine and the hip with TDF/emtricitabine compared to abacavir/lamivudine [252]. Longitudinal studies in ART-experienced patients have also investigated the effect of antiretroviral classes. In the Simplification of Antiretroviral Therapy with Tenofovir-Emtricitabine or Abacavir-Lamivudine (STEAL) study, ART-experienced patients who switched to TDF/emtricitabine had a loss of BMD, whilst those randomised to abacavir/lamivudine had an increase in BMD [250]. Similar results were reported by Bonjoch *et al*, who noted that loss of BMD was associated with the length of time on TDF or a boosted PI, and the current use of a boosted PI [303], whilst another reported that the greatest loss was associated with concomitant use of TDF and a boosted PI [304].

There are fewer studies investigating the effect of NNRTIs, and the results have been variable. One study showed no difference in BMD compared to ART-naïve patients [299], whilst another showed that exposure to NNRTIs was actually protective [300].

Although the above results suggest that different classes of ART may have different effects on BMD, further work is needed to clarify the role of ART on BMD, as well as the effects of individual drugs.

3.1.5 Summary

With the high prevalence of a number of 'traditional' factors, plus the additional risk conferred by HIV infection itself and the exposure to ART, it is not surprising that HIV-positive patients are at a higher risk of developing reduced BMD. Numerous studies have identified potential risk factors for reduced BMD in HIV-positive patients. This chapter explores the baseline characteristics of the 422 HIV-positive men recruited into the study and investigates the factors associated with reduced BMD in this cohort.

3.2 Aims and objectives

Below are the aims for this chapter:

1. To investigate ascertainment bias by comparing the baseline characteristics of patients who participated in the pilot study with those who were randomly selected.
2. To describe the distribution of the following variables at baseline:
 - a. Demographic characteristics
 - b. Lifestyle factors
 - c. Traditional osteoporosis-related factors
 - d. HIV parameters.
3. To calculate the prevalence of reduced BMD at the following sites:
 - a. Lumbar spine
 - b. Non-dominant total hip
 - c. Non-dominant femoral neck.
4. To identify factors associated with BMD at the following sites:
 - a. Lumbar spine
 - b. Non-dominant total hip
 - c. Non-dominant femoral neck.

Many cross-sectional and longitudinal studies in HIV-positive patients have identified factors associated with reduced BMD. I identified the risk factors of interest in this cohort *a priori* in order to assess for them in each participant. This chapter describes the distributions of these factors and identifies which factors were associated with reduced BMD in this cohort.

3.3 Methods

3.3.1 Study design

The detailed methods of the study are given in Chapter 2. In this chapter, the data from the study participants' baseline visit (Year 1) were analysed.

Variables were grouped into the following categories:

1. Demographic characteristics: age, ethnicity and HIV transmission risk
2. Lifestyle factors: smoking, alcohol, recreational drug use, walking and exercise
3. Traditional osteoporosis-related factors: BMI, co-morbidities and drugs associated with osteoporosis

4. HIV-related factors: duration and clinical stage of HIV infection, CD4 cell count (nadir and at recruitment), HIV viral load, HBV and HCV co-infection, and ART status.

Demographic and HIV-related details, including ART history, were obtained from the HIV clinic database. Risk factors for reduced BMD were obtained from self-reported questionnaires. Additional information relating to HIV, bone and renal parameters were obtained from fasted blood and urine samples.

The GE Healthcare Lunar iDXA bone densitometer (GE Healthcare, Madison, Wisconsin, USA) was used to measure absolute BMD (g/cm^2) at the lumbar spine, the total hip (left total hip and right total hip) and the femoral neck (left femoral neck and right femoral neck). Using hand dominance, BMD data were reported for the non-dominant total hip and non-dominant femoral neck.

3.3.2 Definitions

The WHO T-score definitions were used to define BMD as normal (T-score ≥ -1), osteopenia (T-score < -1.0 to > -2.5) and osteoporosis (T-score ≤ -2.5) in those ≥ 50 years old. In participants < 50 years old, the Z-score was used to define BMD as normal (Z-score > -2) or reduced (Z-score ≤ -2). A composite definition of low BMD was derived using men ≥ 50 years old with osteoporosis (T-score ≤ -2.5) and men < 50 years old with reduced BMD (Z-score ≤ -2).

In linear regression, steroid use combined both oral and inhaled steroids.

3.3.3 Statistical analysis

Patients recruited from the pilot study and those randomly selected from the clinic were compared to ascertain selection bias. The factors compared were demographic factors (age, ethnicity and BMI), co-infection with HBV or HCV, and HIV factors (duration and clinical stage of HIV infection, CD4, HIV viral load, ART status and duration of ART use).

The distribution frequency of each variable was calculated. Mean and SD were measured in those that were normally distributed and median and interquartile range (IQR) in those that had skewed distributions. For continuous data, comparisons

between the two groups were made using paired t-tests for normally distributed variables and Wilcoxon rank sum for non-parametric variables. For categorical variables, chi-squared tests were used to compare the two groups. Statistical significance was denoted as p-value ≤ 0.05 .

The frequency distribution of absolute BMD in g/cm^2 was checked at each site. Absolute BMD at the left and right total hip and left and right femoral neck were compared using paired t-tests and the mean within-individual difference reported. Statistical significance was denoted as p-value ≤ 0.05 .

Prevalence of reduced BMD was calculated for the whole cohort using a composite definition of T-score and Z-score, as well as by age (<50 years old and ≥ 50 years old). However, this chapter concentrates on absolute BMD measured as a continuous variable.

To investigate the factors associated with absolute BMD at each site, linear regression was performed. Patients on bisphosphonates were excluded from these analyses. All variables that had a frequency of $\geq 5\%$ were included, as well as those considered of importance (e.g. renal disease, rheumatoid arthritis). Factors that were significant at the 10% level in univariable analysis were tested in a multivariable model. The mean difference in absolute BMD (β) was reported with 95% confidence intervals. Statistical significance was denoted by p-value ≤ 0.05 .

Data were complete or near complete for the majority of cases.

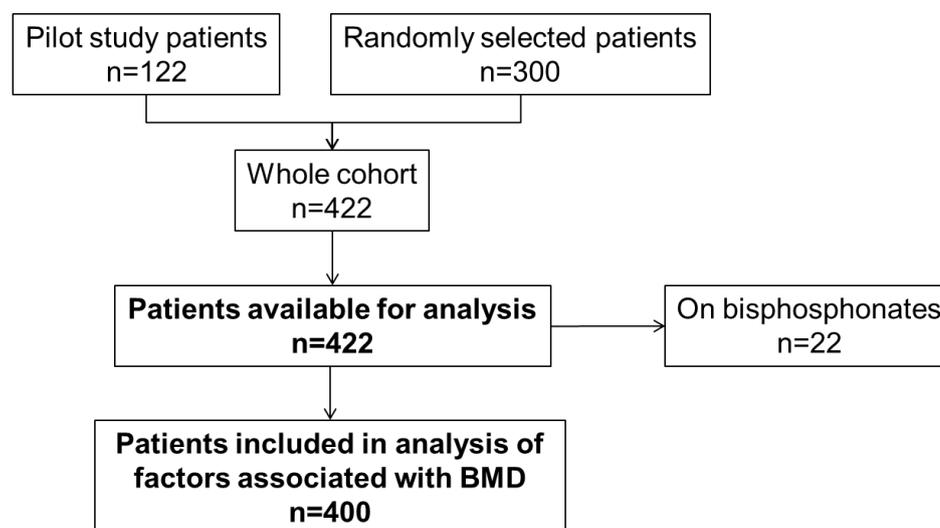
3.4 Results

3.4.1 Subject disposition

There were 422 HIV-positive men recruited into the study: 122 from the pilot study and 300 who were randomly selected (Figure 3.4.1.1). When analysing the factors associated with BMD at each site, 22 men on bisphosphonates were excluded because bisphosphonate use can have an effect on BMD.

Figure 3.4.1.1 Summary of subject disposition

This figure shows the number of patients recruited and analysed, including those on bisphosphonates that were excluded from the analysis of factors associated with low BMD. BMD: bone mineral density



3.4.2 Comparison of pilot study patients with randomly selected patients

In order to assess selection bias, patients from the pilot study were compared to those randomly selected to see if the two populations were different. There were no significant differences with regard to any demographic factors, including age, ethnicity, BMI, HIV transmission risk, duration and clinical stage of HIV infection, immune and virologic status, as well as ART use (Table 3.4.2.1). This indicates that the two populations were similar and that there was no significant ascertainment bias. Therefore, from henceforth, there will be no distinction between the two groups and all HIV-positive patients will be analysed as one group.

Table 3.4.2.1 Comparison of the baseline demographic and HIV-related characteristics between pilot study patients and randomly selected patients

There were no statistical differences in patients recruited from the pilot study and those that were randomly recruited, suggesting that there was no selection bias.

	N	Pilot study patients (N=122)	Randomly selected patients (N=300)	P-value
Age, years, mean (SD)	422	46 (9.1)	46 (10.0)	0.86
Ethnicity, n (%)	422			0.07
White		119 (97.5)	279 (93.0)	
Other		3 (2.5)	21 (7.0)	
BMI, kg/m ² , mean (SD)	422	25 (4.0)	25 (4.1)	0.76
HIV transmission risk, n (%)	422			0.48
MSM		115 (94.3)	277 (92.3)	
Other		7 (5.7)	23 (7.7)	
Duration of HIV infection, years, median (IQR)	422	8.1 (5.1, 13.6)	10.2 (4.9, 16.1)	0.39

	N	Pilot study patients (N=122)	Randomly selected patients (N=300)	P-value
HIV clinical stage, n (%)	422			0.78
Asymptomatic		59 (48.4)	134 (44.7)	
Symptomatic non-AIDS		32 (26.2)	83 (27.7)	
Symptomatic AIDS		31 (25.4)	83 (27.7)	
CD4, cells/ μ L, median (IQR)				
Nadir	422	205 (109, 277)	184 (92, 270)	0.21
At recruitment	422	547 (413, 719)	547 (411, 692)	0.92
HIV viral load <40, copies/mL, n (%)	422			0.88
Yes		106 (86.9)	259 (86.3)	
No		16 (13.1)	41 (13.7)	
HBV co-infection, n (%)	422			0.24
Yes		3 (2.5)	15 (5.0)	
No		119 (97.5)	285 (95.0)	
HCV co-infection, n (%)	422			0.74
Yes		16 (13.1)	43 (14.3)	
No		106 (86.9)	257 (85.7)	
ART status, n (%)	422			0.22
Never		11 (9.0)	23 (7.7)	
Previous		0 (0.0)	7 (2.3)	
Current		111 (91.0)	270 (90.0)	
Duration on ART, years, median (IQR)	422	5.8 (2.1, 11.3)	6.5 (2.3, 11.8)	0.23

ART: antiretroviral therapy; BMI: body mass index; HBV: hepatitis B; HCV: hepatitis C; IQR: interquartile range; MSM: men who have sex with men; SD: standard deviation

3.4.3 Baseline demographics

The baseline demographics are shown in Table 3.4.3.1. The majority were of white ethnicity (94.3%) with a mean age of 47 (SD 9.8) years. In total, 155 (36.7%) men were \geq 50 years old. Most had acquired HIV through sex with men (92.9%).

Table 3.4.3.1 Baseline demographics

The majority of men recruited into the study were young, white MSM.

	Total (N=422)
Age, years, mean (SD)	47 (9.8)
Ethnicity, n (%)	
White	398 (94.3)
Black	15 (3.6)
Other	9 (2.1)
HIV transmission risk, n (%)	
MSM	392 (92.9)
Heterosexual sex	26 (6.2)
IVDU/blood products	4 (0.9)

IVDU: intravenous drug use; MSM: men who have sex with men; SD: standard deviation

3.4.4 Lifestyle factors at baseline

Table 3.4.4.1 gives details relating to lifestyle factors, including smoking, alcohol and recreational drug use, walking and exercise.

Table 3.4.4.1 Lifestyle factors, including smoking, alcohol and recreational drug use, walking and exercise

One-third of the cohort comprised of current smokers, but only a few drank excessively (≥ 3 units/day). Many reported recreational drug use, but not intravenous use (see text). Surprisingly, for a young cohort of men, the majority did no weight-bearing or muscle-toning exercise, although 63% walked regularly.

	Total (N=422)
Smoking, n (%)	
Never smoked	132 (31.3)
Ex-smoker	137 (32.5)
Current smoker	153 (36.3)
Alcohol, n (%)	
Never	62 (14.7)
<3 units/day	298 (70.8)
≥ 3 units/day	62 (14.7)
Recreational drug use, n (%)	
Yes	244 (57.8)
No	178 (42.2)
Walk 30 minutes 3 times per week	
Never	31 (7.3)
Some weeks	63 (14.9)
Most weeks	62 (14.7)
Every week	266 (63.0)
Weight-bearing exercise	
Never	230 (54.5)
Some weeks	76 (18.0)
Most weeks	25 (5.9)
Every week	91 (21.6)
Muscle-toning exercise	
Never	215 (51.0)
Some weeks	73 (17.3)
Most weeks	35 (8.3)
Every week	99 (23.5)

3.4.4.1 Smoking, alcohol and recreational drug use

There were similar numbers of non-smokers (31.3%), ex-smokers (32.5%) and current smokers (36.3%). Of those who smoked at recruitment, 51 (33.3%) smoked <10 cigarettes per day, 72 (47.1%) smoked between 10 and 20 cigarettes per day and 30 (19.6%) smoked >20 cigarettes per day.

The majority (360, 85.3%) reported that they drank alcohol. Of these, most (70.8%) drank within the recommended allowance in the UK of <3 units per day, but 14.7% drank excessively i.e. ≥ 3 units per day.

Of those that stated they had ever used recreational drugs, some provided details of the drugs they had taken. These varied widely and included Class A, B and C drugs, as well as legalised forms of recreational drugs (e.g. meow meow). However, only 7 (2.9%) of these men reported that they had ever regularly injected drugs, with 2 (0.8%) having ever been on a methadone programme, although neither reported that they were taking methadone at recruitment.

3.4.4.2 Walking and exercise

The majority (63%) of men walked for 30 minutes three times per week, which was the minimum amount of exercise recommended by the NOS when the study was recruiting (note that this has now increased to 30 minutes five times per week) [305].

The majority stated that they did no weight-bearing (54.5%) or muscle-toning (51.0%) exercise, with 24 (5.7%) men reporting that they did no type of exercise at all. This was surprising considering the young age of the cohort.

3.4.5 Traditional factors associated with reduced BMD at baseline

A number of factors associated with reduced BMD were assessed, including BMI, self-reported co-morbidities and drugs (Table 3.4.5.1).

Table 3.4.5.1 Traditional factors associated with reduced BMD, including BMI, secondary causes and drugs

Nearly half had a healthy BMI. Men reported a range of co-morbidities, as well as being exposed to medications relating to low BMD. Although 16.6% and 14.0% were on calcium and vitamin D supplementation, respectively, only 5.2% were exposed to bisphosphonates.

	Total (N=422)
Height, cm, mean (SD)	177.1 (7.3)
Weight, kg, mean (SD)	79.2 (14.2)
BMI, kg/m ² , mean (SD)	25.2 (4.1)
BMI status, kg/m ² , n (%)	
Underweight (BMI <18.5)	11 (2.6)
Healthy (BMI 18.5–25)	206 (48.8)
Overweight (BMI 25–30)	154 (36.5)
Obese (BMI >30)	51 (12.1)
Secondary co-morbidities, n (%)	
Liver dysfunction	48 (11.4)
Hypogonadism	45 (10.7)
Diabetes	23 (5.5)
Renal disease	17 (4.0)
Rheumatoid arthritis	16 (3.8)
Hypothyroidism	12 (2.8)
Inflammatory bowel disease	12 (2.8)
Anorexia nervosa	8 (1.9)
Coeliac disease/malabsorption	3 (0.7)
Hyperthyroidism	3 (0.7)
Hyperparathyroidism/hypoparathyroidism	0 (0.0)

	Total (N=422)
Exposure ever to drugs associated with low BMD, n (%)	
a) Drugs associated with reduced BMD	
Antidepressants	144 (34.1)
Antacids	59 (14.0)
Steroid inhalers	56 (13.3)
Oral steroids	38 (9.0)
Chemotherapy	17 (4.0)
Anticonvulsants	16 (3.8)
b) Drugs associated with increased BMD	
Calcium tablets	70 (16.6)
Vitamin D tablets	59 (14.0)
Testosterone	38 (9.0)
Bisphosphonates	22 (5.2)
Thiazide diuretics	10 (2.4)

BMD: bone mineral density; BMI: body mass index; SD: standard deviation

3.4.5.1 Height, weight and BMI

Using the international classification for BMI, 2.6% were underweight (BMI <18.5 kg/m²), 48.8% had a normal BMI (BMI 18.5-25 kg/m²), 36.5% were overweight (BMI 25-30 kg/m²) and 12.1% were moderately obese (BMI >30 kg/m²). The mean BMI of 25.2 (SD 4.1) kg/m² was within the healthy range.

3.4.5.2 Secondary causes of reduced BMD

Of self-reported diagnoses relevant to secondary causes of reduced BMD, the commonest condition was liver dysfunction (11.4%), followed by hypogonadism (10.7%), diabetes (5.5%) and renal disease (4.0%). Other reported co-morbidities were rheumatoid arthritis (3.8%), hypothyroidism (2.8%), inflammatory bowel disease (2.8%) anorexia nervosa (1.9%), coeliac disease/malabsorption (0.7%) and hyperthyroidism (0.7%).

3.4.5.3 Drugs associated with reduced BMD

Participants provided a detailed history of ever being exposed to medications known to be associated with reduced BMD. Many were on antidepressants (34.1%). Other commonly used medications were antacids (13.7%), steroid inhalers (13.3%) oral steroids (9.0%), chemotherapy (4.0%) and anticonvulsants (3.8%). However, the collection of detailed information on dosage and duration of exposure were beyond the scope of this study.

Participants also provided details about medication used in the treatment of reduced BMD. There were 16.6% and 14.0% who reported taking calcium and vitamin D supplements, respectively. However, there were very few (5.2%) men taking bisphosphonates. All patients on bisphosphonates were also on calcium

supplementation and 16 were on vitamin D. There were also 9.0% on testosterone replacement (oral, topical or as a depot injection).

3.4.6 HIV parameters at baseline

Table 3.4.6.1 shows HIV-related factors.

Table 3.4.6.1 HIV-related factors

The duration of HIV infection was long and 27.0% had been diagnosed with AIDS. However, as the majority were on ART and had an undetectable HIV viral load, the median CD4 count at recruitment was high.

	Total (N=422)
Duration of HIV infection, years, median (IQR)	9.6 (5.0, 15.5)
HIV clinical stage, n (%)	
Asymptomatic	193 (45.7)
Symptomatic non-AIDS	115 (27.3)
Symptomatic AIDS	114 (27.0)
CD4, cells/ μ L, median (IQR)	
Nadir	191 (100, 277)
At recruitment	547 (411, 696)
HIV viral load <40, copies/mL, n (%)	
Yes	365 (86.5)
No	57 (13.5)
HBV co-infection, n (%)	
Yes	18 (4.3)
No	404 (95.7)
HCV co-infection, n (%)	
Yes	59 (14.0)
No	363 (86.0)
ART status, n (%)	
Naïve	34 (8.1)
Previous	7 (1.7)
Current	381 (90.3)

ART: antiretroviral therapy; HBV: hepatitis B; HCV: hepatitis C; IQR: interquartile range

3.4.6.1 Duration and clinical stage of HIV infection

The median duration of HIV infection was 9.6 (IQR 5.0, 15.5) years. Using the WHO staging classification, 27.0% had an AIDS-defining condition. Although the median nadir CD4 count was low at 191 (IQR 100, 277) cells/ μ l, the median CD4 count at recruitment was 547 (IQR 411, 696) cells/ μ l. This reflects the high number of participants on ART (90.3%) with an undetectable HIV viral load (86.5%).

3.4.6.2 HBV and HCV co-infection

HBV and HCV co-infection rates were 4.3% and 14.0%, respectively.

3.4.6.3 ART use

At recruitment, 381 (90.3%) participants were on ART. Of the seven men who were previous, but not current users of ART, five had stopped ART of their own choice,

including one who had developed gastrointestinal side effects from one of the drugs, which led to him stopping his entire regimen. The other two men had taken part in clinical trials where they had taken a short course of ART.

The ART regimens taken by participants are shown in Table 3.4.6.2. The majority were on a standard triple regimen with 2 NRTIs and either an NNRTI (48.3%) or a boosted PI (27.6%). The commonest drug in the NRTI backbone was TDF, which is 1st line therapy (usually in combination with emtricitabine and EFV), with 73.8% on a TDF-containing regimen. Patients not on TDF were on a variety of NRTIs, including 28 (7.3%) on a regimen containing abacavir and 2 (0.5%) on a regimen containing zidovudine.

Table 3.4.6.2 ART regimens of participants on ART

The majority of participants were on an ART regimen containing 2 NRTIs and an NNRTI and that 73.8% were on a TDF-containing regimen. Participants were exposed to a range of NNRTIs and boosted PIs, with very small numbers on an integrase or entry inhibitor.

	Total (N=381)
Duration on ART, years, median (IQR)	6.1 (2.2, 11.7)
Current Regimen, n (%)	
2 x NRTI + NNRTI	184 (48.3)
2 x NRTI + PI/ritonavir	105 (27.6)
PI/ritonavir only	37 (9.7)
Other	55 (14.4)
Regimen components, n (%)	
NRTI Backbone	
None	51 (13.4)
1 x NRTI	22 (5.8)
2 x NRTI (including TDF)	281 (73.8)
2 x NRTI (no TDF)	25 (8.9)
3 or 4 NRTI	2 (0.5)
NNRTI	
Any	218 (57.2)
EFV	135 (35.4)
Nevirapine	55 (14.4)
Other	28 (7.3)
PI/ritonavir	
Any	185 (48.6)
Darunavir/ritonavir	110 (28.9)
Atazanavir or atazanavir/ritonavir	44 (11.5)
Lopinavir/ritonavir	31 (8.1)
Other	1 (0.3)
Other	
Any	29 (7.6)
Raltegravir	28 (7.3)
Maraviroc	2 (0.5)
Cumulative exposure to antiretroviral drugs, years, median (IQR)*	
TDF	1.7 (0.5, 3.8)
EFV	0.5 (0.0, 3.4)
PI/ritonavir	0.7 (0.0, 4.4)

ART: antiretroviral therapy; EFV: efavirenz; IQR: interquartile range; NNRTI: non-nucleoside reverse inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

*Includes all patients who have ever been exposed

With regards to the third drug, 57.2% had been exposed to an NNRTI, with the commonest drug being EFV (35.4%), followed by nevirapine (14.4%). There were

48.6% men on a boosted PI, with the majority being on darunavir/ritonavir (28.9%), atazanavir or atazanavir/ritonavir (11.5%) or lopinavir/ritonavir (8.1%). There were 9.7% men on boosted PI monotherapy. There were 7.6% on other classes of ART, including raltegravir (7.3%) and maraviroc (0.5%).

3.4.7 Baseline BMD data

Table 3.4.7.1 shows absolute BMD at the lumbar spine, left, right and non-dominant total hip and left, right and non-dominant femoral neck.

Table 3.4.7.1 Absolute BMD by site

Absolute BMD was normally distributed at all sites. Although all 422 men had their lumbar spine scanned, only 405 men had L1–L4 composite measured (see text). Not all men had their total hip or femoral neck measured, mainly because they had metalwork in situ or the region of interest was not correctly scanned (see text).

	N	Absolute BMD, g/cm ² , mean (SD)
Lumbar spine	422	1.137 (0.155)
Left total hip	416	0.996 (0.139)
Right total hip	415	1.002 (0.138)
Non-dominant total hip	415	0.997 (0.139)
Left femoral neck	419	0.949 (0.142)
Right femoral neck	418	0.957 (0.133)
Non-dominant femoral neck	418	0.950 (0.143)

BMD: bone mineral density; SD: standard deviation

3.4.7.1 Lumbar spine

All men had their lumbar spine scanned, but 17 did not have an L1–L4 composite measurement. In the majority of cases, this was because there was a problem with L4, including incomplete scanning (n=7), completely missing (n=4) or an artefact overlying it (n=1). In all these cases, a composite measurement of L1–L3 was used. In two men, L1 was incompletely scanned, therefore a composite of L2–L4 was used. One man had a body piercing overlying the L2 vertebra, so a composite of L1, L3–L4 was used in this case. In the remaining two cases, a composite of only two vertebrae (L3–L4) were used because one man had a metal rod affecting L1–L2 and the other had severe scoliosis, so his L1 and L2 vertebrae were excluded as their anatomical landmarks were difficult to discern.

3.4.7.2 Left total hip, right total hip and non-dominant total hip

There were 416 and 415 patients with left and right total hip data, respectively. The reason for a patient not having a total hip result was because they had undergone surgery (e.g. a total hip replacement, a metal screw was in the ROI), because inadequate amounts of the femoral shaft were scanned within the ROI to enable the

DXA machine to calculate absolute BMD for the total hip or because the data were missing and could not be retrieved from the DXA scanner. In total, 12 patients had data missing for left (n=6) and right (n=7) total hips, with only one missing data for both sides as he had undergone a bilateral total hip replacement. Of the remaining five patients missing left total hip data, two had undergone a total hip replacement, one had a hip screw within the ROI, and two had insufficient amounts of the femoral shaft scanned. Of those with missing right total hip data, two patients had a hip screw within the ROI, three did not have an adequate amount of the femoral shaft scanned, and one had no data stored on the DXA machine, which was irretrievable.

There were 365 (86.5%) men who were right-handed and 57 (13.5%) who were left-handed. Of the 365 right-handed men, 360 had their left total hip (i.e. their non-dominant total hip) scanned, whilst 55 of the 57 left-handed men had their right total hip scanned. In total, there were 415 patients with data for the non-dominant total hip.

3.4.7.3 Left femoral neck, right femoral neck and non-dominant femoral neck

There were 419 and 418 patients with left and right total hip data, respectively. Femoral neck data were missing in six patients, with three and four patients missing data on the left and right sides, respectively. All three patients missing left femoral neck data had undergone a total hip replacement. Reasons for missing right femoral neck data were a total hip replacement (n=1), a hip screw within the ROI (n=2) and no data (n=1).

Of the 365 right-handed men, 363 had their left femoral neck (i.e. their non-dominant femoral neck) scanned, whilst 55 of the 57 left-handed men had their right femoral neck scanned. In total, 418 men had data for the non-dominant femoral neck.

3.4.7.4 Absolute BMD at baseline

Absolute BMD was normally distributed at all sites (Table 3.4.7.1). Absolute BMD at the lumbar spine was higher than at any of the hip sites. There was a significant difference between absolute BMD at the left and right total hip (left minus right: -0.006 [SD 0.040] g/cm², p=0.004), but not at the left and right femoral neck (left minus right: -0.006 [SD 0.074] g/cm², p=0.08).

3.4.7.5 T- and Z-scores at baseline

At all sites, the T- and Z-scores were also normally distributed and the mean T- and Z-scores were within the normal range (Table 3.4.7.2). There was a significant difference between T-scores at the left and right total hip (-0.73 [SD 1.06] vs. -0.69 [SD 1.06], p=0.01), but not between the right and left femoral neck (-0.93 [SD 1.09] vs. -0.88 [SD

1.02], $p=0.09$). This was also similar for the Z-score, where there was a difference between the left and right total hip (-0.40 [SD 0.96] vs. -0.35 [SD 0.96], $p=0.01$), but not between the left and right femoral neck (-0.44 [SD 1.00] vs. -0.39 [SD 0.91], $p=0.09$).

Table 3.4.7.2 Baseline T- and Z-scores

The mean T- and Z-scores were normally distributed and within the normal range at all sites.

	N	T-score, mean (SD)	Z-score, mean (SD)
Lumbar spine	422	-0.69 (1.29)	-0.58 (1.22)
Left total hip	416	-0.73 (1.06)	-0.40 (0.96)
Right total hip	415	-0.69 (1.06)	-0.35 (0.96)
Non-dominant total hip	415	-0.72 (1.07)	-0.38 (0.96)
Left femoral neck	419	-0.93 (1.09)	-0.44 (1.00)
Right femoral neck	418	-0.88 (1.02)	-0.39 (0.91)
Non-dominant femoral neck	418	-0.92 (1.10)	-0.43 (1.00)

SD: standard deviation

3.4.8 Prevalence of reduced BMD

Table 3.4.8.1 shows the prevalence of low BMD using a combination of T- and Z-scores, as well as the prevalence using T-score in all patients. The overall prevalence of reduced BMD at each site was small. In total, there were 64 (15.2%) men with low BMD at any site using the composite definition. The site with the highest percentage of reduced BMD was the lumbar spine (10.7%), followed by the non-dominant total hip (4.8%) and the non-dominant femoral neck (3.3%). When using the T-score in all patients, the majority had normal BMD at each site, but 192 (45.5%) had osteopenia and 36 (8.5%) had osteoporosis at any site. Again, the lumbar spine had the highest prevalence of osteoporosis (5.5%). Interestingly, a high proportion of men had osteopenia at all sites, although this group of men was not included in the composite definition of reduced BMD as there is ongoing debate as to the clinical significance of osteopenia.

Table 3.4.8.1 Prevalence of reduced BMD

The percentage of participants with low BMD (using composite of Z-score ≤ -2.0 in men <50 years old and T-score ≤ -2.5 in men ≥ 50 years old) was small at all sites. When using the T-score in all patients, the majority had normal BMD at all sites.

	N	Reduced BMD, n (%)	T-score in all patients, n (%)		
			Normal	Osteopenia	Osteoporosis
Lumbar spine	422	45 (10.7)	241 (57.1)	158 (37.4)	23 (5.5)
Non-dominant total hip	415	20 (4.8)	249 (60.0)	156 (37.6)	10 (2.4)
Non-dominant femoral neck	418	14 (3.3)	203 (48.6)	199 (47.6)	16 (3.8)
At any site	422	64 (15.2)	194 (46.0)	192 (45.5)	36 (8.5)

BMD: bone mineral density

Prevalence of low BMD at the left and right total hips and at the left and right femoral necks were also assessed (Table 3.6.1.1).

The prevalence of low BMD was also calculated according to age, using the Z-score in those <50 years old and the T-score in those ≥50 years old (Table 3.4.8.2). In those <50 years, the majority had normal BMD at all sites as would be expected in relatively young men. In those ≥50 years, the majority had normal BMD at the lumbar spine and the non-dominant total hip, but not at the non-dominant femoral neck. Interestingly, a high proportion of men ≥50 years old had osteopenia at all sites, although this group of men were not included in the composite definition of low BMD as there is ongoing debate as to the clinical significance of osteopenia.

Table 3.4.8.2 Prevalence of reduced BMD according to age

Most men <50 years old had normal BMD. The majority of men ≥50 years old had normal BMD at the lumbar spine and the non-dominant total hip, but at the non-dominant femoral neck, most had osteopenia.

	N	Z-score in <50 year olds, n (%)		T-score in ≥50 year olds, n (%)		
		Normal	Reduced BMD	Normal	Osteopenia	Osteoporosis
Lumbar spine	422	235 (88.0)	32 (12.0)	86 (55.5)	56 (36.1)	13 (8.4)
Non-dominant total hip	415	251 (95.4)	12 (4.6)	85 (55.9)	59 (38.8)	8 (5.3)
Non-dominant femoral neck	418	258 (98.1)	5 (1.9)	63 (40.6)	83 (53.5)	9 (5.8)

BMD: bone mineral density

3.4.9 Factors associated with BMD at baseline

Factors associated with absolute BMD at the lumbar spine (Table 3.4.9.1), the non-dominant total hip (Table 3.4.9.2) and the non-dominant femoral neck (Table 3.4.9.3) were compared. Absolute BMD at the left total hip (Table 3.6.2.1), the right total hip (Table 3.6.3.1), the left femoral neck (Table 3.6.4.1) and the right femoral neck (Table 3.6.5.1) were also compared and the data are presented in Section 3.6. For these analyses, 22 men on bisphosphonates were excluded.

3.4.9.1 Factors associated with BMD at the lumbar spine

Table 3.4.9.1 shows factors associated with BMD at the lumbar spine. In univariable analyses, factors associated with a lower BMD were current smoking, a history of rheumatoid arthritis, use of steroids and being on calcium and/or vitamin D supplementation, which are all traditional factors known to be associated with low BMD. The only HIV factors associated with a lower BMD were longer duration of TDF exposure and longer duration of exposure to a boosted PI. Factors associated with a higher BMD were non-white ethnicity, having a higher BMI and doing some exercise.

Table 3.4.9.1 Factors associated with BMD at the lumbar spine

In multivariable analyses, current smoking, exposure to calcium and/or vitamin D supplements and longer duration of exposure to a boosted PI remained associated with a lower BMD, whilst doing some exercise and having a higher BMI remained associated with a higher BMD.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	0.003 (-0.01, 0.02)	0.70		
Ethnicity				
White	0.00	-	0.00	-
Other	0.05 (-0.01, 0.12)	0.096	0.04 (-0.02, 0.10)	0.16
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.01 (-0.05, 0.03)	0.53	-0.01 (-0.04, 0.03)	0.68
Current smoker	-0.06 (-0.10, -0.02)	0.002	-0.04 (-0.08, -0.01)	0.02
Alcohol use				
Never	-0.02 (-0.07, 0.02)	0.32		
<3 units/day	0.00	-		
>3 units/day	-0.002 (-0.05, 0.04)	0.94		
Recreational drug use				
No	0.00	-		
Yes	-0.004 (-0.04, 0.03)	0.81		
Exercise				
Never	0.00	-	0.00	-
Some weeks	0.10 (0.02, 0.18)	0.01	0.08 (0.01, 0.15)	0.03
Most weeks	0.07 (-0.02, 0.14)	0.11	0.03 (-0.05, 0.10)	0.46
Every week	0.04 (-0.03, 0.10)	0.28	0.01 (-0.06, 0.07)	0.85
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.06, 0.12)	<0.0001	0.07 (0.04, 0.10)	<0.0001
>30	0.17 (0.07, 0.16)	<0.0001	0.10 (0.05, 0.14)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	-0.01 (-0.05, 0.04)	0.83		
Hypogonadism				
No	0.00	-		
Yes	-0.02 (-0.07, 0.03)	0.44		
Diabetes				
No	0.00	-		
Yes	-0.002 (-0.07, 0.07)	0.95		
Renal disease				
No	0.00	-		
Yes	0.01 (-0.07, 0.09)	0.80		
Rheumatoid arthritis				
No	0.00	-	0.00	-
Yes	-0.09 (-0.17, -0.01)	0.03	-0.08 (-0.16, 0.00)	0.05
Antidepressants				
No	0.00	-		
Yes	-0.01 (-0.04, 0.02)	0.48		
Antacids				
No	0.00	-		
Yes	0.02 (-0.03, 0.06)	0.41		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.08, 0.001)	0.06	-0.03 (-0.07, 0.01)	0.16
Calcium and/or vitamin D supplements				
No	0.00	-	0.00	-
Yes	-0.06 (-0.10, -0.02)	0.01	-0.07 (-0.11, -0.02)	0.002
Duration of HIV infection, per year	-0.001 (-0.004, 0.001)	0.21		
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.06, 0.02)	0.28		
Symptomatic AIDS	-0.03 (-0.07, 0.01)	0.13		

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
CD4 count, per 50 cells/ μ L				
Nadir	0.002 (-0.004, 0.01)	0.50		
Current	0.002 (-0.001, 0.01)	0.29		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.01 (-0.04, 0.05)	0.71		
Current ART regimen				
None	0.00	-		
ART including TDF	-0.02 (-0.07, 0.04)	0.57		
ART including PI/ritonavir	-0.02 (-0.08, 0.04)	0.52		
ART including TDF + PI/ritonavir	-0.039 (-0.095, 0.02)	0.18		
Other ART	0.04 (-0.05, 0.12)	0.36		
Cumulative ART exposure, per year**	-0.002 (-0.01, 0.001)	0.14		
Cumulative TDF exposure, per year**	-0.01 (-0.02, -0.002)	0.01	-0.004 (-0.01, 0.003)	0.22
Cumulative PI/ritonavir exposure, per year**	-0.01 (-0.01, -0.002)	0.004	-0.01 (-0.01, -0.001)	0.02
Cumulative NNRTI exposure, per year**	0.001 (-0.003, 0.01)	0.69		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

In multivariable analyses, factors that remained associated with a lower BMD were current smoking, exposure to calcium and/or vitamin D supplements and longer duration of exposure to a boosted PI. Doing some exercise and having a higher BMI remained associated with a higher BMD.

3.4.9.2 Factors associated with BMD at the non-dominant total hip

Factors associated with BMD at the non-dominant total hip are shown in Table 3.4.9.2. In univariable analyses, current smoking, a history of hypogonadism, rheumatoid arthritis, steroid use and supplementation with calcium and/or vitamin D were associated with a lower BMD. HIV factors associated with a lower BMD were an undetectable HIV viral load, an ART regimen containing a boosted PI or a combination of TDF and a boosted PI, longer exposure to ART and longer exposure to a boosted PI. Non-white ethnicity, doing some regular exercise and a higher BMI were associated with a higher BMD.

Table 3.4.9.2 Factors associated with BMD at the non-dominant total hip

In multivariable analyses, only current smoking and calcium and/or vitamin D supplementation remained associated with a lower BMD, whilst non-white ethnicity and a higher BMI remained associated with a higher BMD.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	-0.01 (-0.02, 0.01)	0.19		
Ethnicity				
White	0.00	-	0.00	-
Other	0.08 (0.02, 0.14)	0.01	0.06 (0.01, 0.11)	0.02
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.001 (-0.03, 0.03)	0.97	-0.002 (-0.03, 0.03)	0.91
Current smoker	-0.06 (-0.09, -0.02)	0.001	-0.04 (-0.07, -0.01)	0.01
Alcohol use				
Never	-0.004 (-0.04, 0.04)	0.85		
<3 units/day	0.00	-		
\geq 3 units/day	0.00 (-0.04, 0.04)	0.98		
Recreational drug use				
No	0.00	-		
Yes	-0.004 (-0.03, 0.02)	0.77		
Exercise				
Never	0.00	-	0.00	-
Some weeks	0.06 (-0.01, 0.13)	0.07	0.05 (-0.02, 0.11)	0.14
Most weeks	0.05 (-0.02, 0.13)	0.14	0.03 (-0.04, 0.09)	0.46
Every week	0.04 (-0.02, 0.10)	0.21	0.02 (-0.04, 0.07)	0.57
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.06, 0.12)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.14 (0.10, 0.18)	<0.0001	0.12 (0.08, 0.17)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	-0.003 (-0.05, 0.04)	0.91		
Hypogonadism				
No	0.00	-	0.00	-
Yes	-0.04 (-0.09, 0.004)	0.07	-0.03 (-0.08, 0.01)	0.17
Diabetes				
No	0.00	-		
Yes	-0.002 (-0.06, 0.06)	0.95		
Renal disease				
No	0.00	-		
Yes	-0.03 (-0.10, 0.04)	0.41		
Rheumatoid arthritis				
No	0.00	-	0.00	-
Yes	-0.07 (-0.14, 0.001)	0.05	-0.04 (-0.11, 0.03)	0.22
Antidepressants				
No	0.00	-		
Yes	-0.001 (-0.03, 0.03)	0.94		
Antacids				
No	0.00	-		
Yes	0.01 (-0.03, 0.05)	0.68		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.08, -0.01)	0.02	-0.03 (-0.07, 0.001)	0.06
Calcium and/or vitamin D supplements				
No	0.00	-	0.00	-
Yes	-0.04 (-0.08, 0.000)	0.05	-0.04 (-0.08, -0.004)	0.03
Duration of HIV infection, per year	-0.002 (-0.004, 0.000)	0.11		
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.05, 0.01)	0.23		
Symptomatic AIDS	-0.02 (-0.05, 0.01)	0.26		

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
CD4 count, per 50 cells/ μ L				
Nadir	0.003 (-0.002, 0.01)	0.28		
Current	0.000 (-0.002, 0.003)	0.72		
HIV viral load <40, copies/mL	0.00	-	0.00	-
Yes	0.04 (0.000, 0.08)	0.05	0.02 (-0.04, 0.08)	0.56
No				
Current ART regimen				
None	0.00	-	0.00	-
ART including TDF	-0.03 (-0.08, 0.01)	0.15	-0.03 (-0.10, 0.04)	0.44
ART including PI/ritonavir	-0.06 (-0.11, -0.002)	0.04	-0.03 (-0.11, 0.05)	0.51
ART including TDF + PI/ritonavir	-0.04 (-0.09, 0.01)	0.09	-0.02 (-0.09, 0.05)	0.59
Other ART	-0.03 (-0.10, 0.05)	0.51	-0.03 (-0.12, 0.06)	0.51
Cumulative ART exposure, per year**	-0.002 (-0.01, 0.000)	0.06	0.001 (-0.002, 0.01)	0.45
Cumulative TDF exposure, per year**	-0.003 (-0.01, 0.004)	0.41		
Cumulative PI/ritonavir exposure, per year**	-0.004 (-0.01, 0.000)	0.05	-0.003 (-0.01, 0.002)	0.27
Cumulative NNRTI exposure, per year**	0.000 (-0.003, 0.003)	0.94		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

In multivariable analyses, only current smoking and calcium and/or vitamin D supplementation remained associated with a lower BMD, although supplementation with calcium and/or vitamin D was not associated with BMD at either the left (Table 3.6.2.1) or right (Table 3.6.3.1) total hips. Non-white ethnicity and a higher BMI remained associated with a higher BMD. Interestingly, steroid exposure, which had been associated with lower BMD at both the left and right total hips, was not significantly associated with BMD at the non-dominant total hip.

3.4.9.3 Factors associated with BMD at the non-dominant femoral neck

Table 3.4.9.3 shows risk factors associated with BMD at the non-dominant femoral neck. In univariable analyses, factors associated with a lower BMD were older age, current smoking, hypogonadism, rheumatoid arthritis and steroid use. HIV factors associated with a lower BMD were longer duration of HIV infection, more advanced HIV infection, a detectable HIV viral load, an ART regimen containing TDF, a boosted PI or a combination of TDF and a boosted PI, longer duration of exposure to ART and longer duration of exposure to a boosted PI. Non-white ethnicity, doing some regular exercise, a higher BMI and a higher nadir CD4 count were associated with a higher BMD.

Table 3.4.9.3 Factors associated with BMD at the non-dominant femoral neck

In multivariable analysis, only older age was associated with a lower BMD, whilst non-white ethnicity and a higher BMI remained associated with a higher BMD.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	-0.02 (-0.03, -0.01)	0.002	-0.02 (-0.03, -0.001)	0.03
Ethnicity				
White	0.00	-	0.00	-
Other	0.09 (0.04, 0.14)	0.001	0.07 (0.01, 0.12)	0.01
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.000 (-0.03, 0.03)	0.99	0.000 (-0.03, 0.03)	1.00
Current smoker	-0.04 (-0.07, -0.01)	0.01	-0.03 (-0.06, 0.003)	0.07
Alcohol use				
Never	-0.01 (-0.04, 0.03)	0.74		
<3 units/day	0.00	-		
\geq 3 units/day	-0.01 (-0.05, 0.03)	0.61		
Recreational drug use				
No	0.00	-		
Yes	0.01 (-0.02, 0.03)	0.62		
Exercise				
Never	0.00	-	0.00	-
Some weeks	0.06 (-0.004, 0.13)	0.07	0.05 (-0.02, 0.11)	0.15
Most weeks	0.07 (0.002, 0.14)	0.05	0.04 (-0.03, 0.10)	0.23
Every week	0.04 (-0.01, 0.10)	0.13	0.02 (-0.04, 0.08)	0.48
BMI				
<25	0.00	-	0.00	-
25-30	0.08 (0.05, 0.10)	<0.0001	0.07 (0.04, 0.10)	<0.0001
>30	0.11 (0.07, 0.15)	<0.0001	0.10 (0.06, 0.14)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	-0.003 (-0.05, 0.04)	0.87		
Hypogonadism				
No	0.00	-	0.00	-
Yes	-0.05 (-0.09, -0.001)	0.04	-0.03 (-0.07, 0.02)	0.27
Diabetes				
No	0.00	-		
Yes	-0.03 (-0.09, 0.02)	0.25		
Renal disease				
No	0.00	-		
Yes	-0.02 (-0.09, 0.04)	0.49		
Rheumatoid arthritis				
No	0.00	-	0.00	-
Yes	-0.08 (-0.15, -0.01)	0.03	-0.04 (-0.11, 0.03)	0.23
Antidepressants				
No	0.00	-		
Yes	-0.001 (-0.03, 0.03)	0.95		
Antacids				
No	0.00	-		
Yes	0.01 (-0.03, 0.05)	0.56		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.07, -0.01)	0.02	-0.03 (-0.07, 0.001)	0.06
Calcium and/or vitamin D supplements				
No	0.00	-		
Yes	-0.03 (-0.07, 0.01)	0.14		
Duration of HIV infection, per year	-0.002 (-0.004, 0.000)	0.01	0.002 (-0.002, 0.005)	0.32
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.04 (-0.07, -0.004)	0.03	-0.03 (-0.06, 0.003)	0.08
Symptomatic AIDS	-0.03 (-0.06, 0.001)	0.06	-0.01 (-0.04, 0.02)	0.53

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
CD4 count, per 50 cells/ μ L				
Nadir	0.004 (0.000, 0.01)	0.08		
Current	-0.001 (-0.003, 0.002)	0.64	0.000 (-0.01, 0.01)	0.94
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.06 (0.02, 0.10)	0.003	0.02 (-0.05, 0.08)	0.62
Current ART regimen				
None	0.00	-	0.00	-
ART including TDF	-0.06 (-0.10, -0.01)	0.01	-0.03 (-0.10, 0.04)	0.45
ART including PI/ritonavir	-0.08 (-0.14, -0.03)	0.002	-0.01 (-0.09, 0.07)	0.72
ART including TDF + PI/ritonavir	-0.06 (-0.11, -0.01)	0.02	-0.01 (-0.08, 0.06)	0.80
Other ART	-0.05 (-0.12, 0.02)	0.18	-0.03 (-0.12, 0.06)	0.57
Cumulative ART exposure, per year**	-0.004 (-0.01, -0.002)	0.001	-0.001 (-0.01, 0.004)	0.79
Cumulative TDF exposure, per year**	-0.004 (-0.01, 0.002)	0.20		
Cumulative PI/ritonavir exposure, per year**	-0.01 (-0.01, -0.001)	0.01	-0.004 (-0.01, 0.002)	0.18
Cumulative NNRTI exposure, per year**	-0.003 (-0.01, 0.001)	0.12		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

In multivariable analysis, only older age was associated with a lower BMD, whilst non-white ethnicity and a higher BMI remained associated with a higher BMD. These same factors were associated with BMD at the left femoral neck (Table 3.6.4.1), which most likely reflects the right handedness of the cohort.

3.4.9.4 Summary of factors associated with BMD at different sites

Factors that were associated with BMD in multivariable analyses at the different sites are summarised in Table 3.4.9.4. Current smoking (versus being a non-smoker) was associated with a lower BMD at the lumbar spine and the total hip, but not at the femoral neck. Older age was associated with a lower femoral neck BMD but not with lumbar spine BMD. Supplementation with calcium and/or vitamin D was associated with a lower BMD at the lumbar spine and the non-dominant total hip only. Interestingly, steroid use was associated with lower BMD at the left and right total hips (Table 3.6.2.1 and Table 3.6.3.1), but not at the non-dominant total hip. Non-white ethnicity (compared to white ethnicity) was associated with a higher hip BMD (both non-dominant total hip and femoral neck). Doing some exercise as opposed to no exercise was associated with a higher BMD at the lumbar spine, but not at the hip. However, the effect was not dose-dependent. A higher BMI (both 25 – 30 kg/m² and >30 kg/m²) was associated with a higher BMD at all sites, and was the only factor found to be significantly associated at all sites. The only HIV-related factor that was associated with BMD was longer duration of a boosted PI, which was associated with

lower BMD at the lumbar spine but not at the total hip or the femoral neck. Although the above factors were all significantly associated with lower or higher BMD, the β coefficient, which measured the mean difference in absolute BMD, was small in all cases, suggesting that the changes that occurred were relatively small.

Table 3.4.9.4 Summary of factors associated with BMD in multivariable analyses by site

This table shows the significant factors associated at each site (lumbar spine, non-dominant total hip and non-dominant femoral neck) with BMD in multivariable linear regression.

Factor	Lumbar spine		Non-dominant total hip		Non-dominant femoral neck	
	Adjusted β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Older age vs. younger age	-	-	-	-	-0.02 (-0.03, -0.001)	0.03
Non-white vs. white ethnicity	-	-	0.06 (0.01, 0.11)	0.02	0.07 (0.01, 0.12)	0.01
Current vs. never smoking	-0.04 (-0.08, -0.01)	0.02	-0.04 (-0.07, -0.01)	0.01	-	-
Exercise in some weeks vs. no exercise	0.08 (0.01, 0.15)	0.03	-	-	-	-
BMI 25-30 vs. <25 kg/m ²	0.07 (0.04, 0.10)	<0.0001	0.08 (0.05, 0.11)	<0.0001	0.07 (0.04, 0.10)	<0.0001
BMI >30 vs. <25 kg/m ²	0.10 (0.05, 0.14)	<0.0001	0.12 (0.08, 0.17)	<0.0001	0.10 (0.06, 0.14)	<0.0001
Calcium and/or vitamin D supplementation vs. no supplementation	-0.06 (-0.10, -0.02)	0.01	-0.04 (-0.08, -0.004)	0.03	-	-
Longer vs. shorter duration of PI/ritonavir exposure**	-0.01 (-0.01, -0.001)	0.02	-	-	-	-

95% CI: 95% confidence interval; BMD: bone mineral density; BMI: body mass index; PI: protease inhibitor

Factors inserted into model: age, ethnicity, smoking, alcohol, recreational drug use, exercise, BMI, liver disease, hypogonadism, diabetes, renal disease, rheumatoid arthritis, antidepressants, antacids, steroids, calcium supplementation, vitamin D supplementation, duration of HIV infection, HIV clinical stage, CD4 count (nadir and current), HIV viral load, current ART (none, ART including TDF, ART including boosted PI, ART including TDF and boosted PI, other), cumulative ART exposure (including exposure to TDF, boosted PI or NNRTI).

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

3.5 Discussion

3.5.1 Summary

In total, 422 HIV-positive men were enrolled into my study. The cohort was homogeneous, with the majority of men being relatively young, white MSM, with good immune function and well-controlled HIV infection on ART, which reflected the demographics of the men attending the HIV outpatient clinic in Brighton. They had well established HIV infection, with a long duration of infection, as well as a significant percentage having had an AIDS-defining condition and a low nadir CD4 count. This suggests that many of the participants were diagnosed a number of years ago, when patients were more likely to present with opportunistic infections and AIDS-related conditions. However, most were on ART at the time of recruitment, and had well controlled HIV infection, reflecting the advances made in HIV treatment with the introduction of ART, including commencing ART at higher CD4 counts and the use of newer drug regimens [26]. Most patients in my cohort were on a regimen containing TDF. The different antiretroviral drugs used reflected the changes in practice over the years. Additionally, the prevalence of HCV co-infection was high, in keeping with rates reported in MSM populations [256], but the prevalence of HBV was lower than that reported in the UK CHIC (UK Collaborative HIV Cohort) study [257].

Although participants were recruited into the study in two different ways, there were no statistically significant differences in demographic and HIV-related factors between those who had taken part in the pilot study and those who were randomly recruited. This suggests that the two different methods did not lead to ascertainment bias and that the study cohort comprised a good representation of the entire cohort of patients attending the HIV outpatient clinic.

3.5.1.1 Prevalence of reduced BMD

In my cohort, the overall prevalence of reduced BMD at each site was relatively small. This probably reflects the relatively young mean age of the cohort, as well as good immune function and well-controlled HIV infection. Additionally, as many had a long duration of exposure to TDF, and most of the BMD loss associated with TDF has been reported to occur in the first 24 to 48 months after initiation [29,251], there may have been stabilisation of BMD in men in this cohort.

In total, the prevalence of reduced BMD using the composite definition of low BMD (T-score ≤ -2.5 in men ≥ 50 years old in men < 50 years old) was 15.2% at any site, with 45.5% having osteopenia and 8.5% having osteoporosis when using the T-score in all

participants. Even in men ≥ 50 years old, the prevalence of osteoporosis was low, with the lumbar spine having the highest prevalence (8.4%). This prevalence is lower than that reported in the pilot study of 58% and 12% for osteopenia and osteoporosis, respectively [160], of whom 72.6% were included in my cohort. My figures are also lower than that reported in a meta-analysis of 11 studies, which included 884 HIV-positive patients, and reported an overall prevalence of osteopenia and osteoporosis as 67% and 15%, respectively [136]. However, the pooled data were from studies in the early 2000s, and may reflect different risk factors for reduced BMD than in this cohort, which may partly explain the differences. The only other UK cohort investigating BMD in HIV-positive men is the Probono-1 study [306]. Compared to my study, the men in this study were of a similar age and a similar number of patients were on TDF. In the Probono-1 study, the prevalence of osteopenia and osteoporosis in HIV-positive men was 50% (66/133) and 14% (19/133), respectively, whilst the prevalence in HIV-negative male controls was 39% (17/44) and 14% (6/44), respectively, for osteopenia and osteoporosis [306]. They reported no difference in the rates between HIV-positive and HIV-negative men. The prevalence of osteopenia and osteoporosis in the Probono-1 study were slightly higher but similar to the rates in my cohort when calculated using the T-score in everyone.

Absolute BMD was normally distributed at all five sites, as well as at the non-dominant total hip and the non-dominant femoral neck. Absolute BMD at the lumbar spine was higher than that at any of the hip sites, which may reflect age-associated degenerative spondylotic changes [307]. There was a significant difference in absolute BMD at either side in relation to the total hips but not at and the femoral necks. As expected, the right side was the dominant side in most men. This meant that in the majority of cases, the non-dominant total hip or non-dominant femoral neck related to the left total hip or left femoral neck, respectively.

3.5.1.2 Bisphosphonate use

There were 22 patients on bisphosphonate treatment. This small number probably reflects the relatively young age of the cohort and the issues relating to long-term bisphosphonate use in younger patients, in whom there is debate as to whether bisphosphonate treatment leads to a reduction in micro-fractures, which are needed to improve bone strength [308]. As bisphosphonates are known to increase BMD and lead to fewer vertebral fractures [309], these 22 men were excluded from the analyses investigating factors associated with BMD. However, excluding these men may have had an impact on the results as these men clearly had osteoporosis prior to participating in the study.

3.5.1.3 Factors associated with reduced BMD

The questionnaire provided a detailed history of factors relating to reduced BMD. The secondary causes most commonly seen were liver dysfunction, hypogonadism and diabetes. Liver dysfunction can be a common finding in HIV-positive MSM, secondary to high rates of co-infection with HBV and HCV, as well as ART toxicity [256,257,310] and alcohol consumption. Additionally, HIV-positive patients are at increased risk of renal disease, in particular, RTD associated with certain antiretroviral drugs, such as TDF and boosted PIs [270,271,310]. However, other diseases that are commonly associated with reduced BMD in the general population, such as rheumatoid arthritis and thyroid disorders [253,254], were not common in my cohort.

Another risk factor for reduced BMD was smoking, which is highly prevalent in HIV-positive patients [216-219]. In one large American study, HIV-positive adults were more likely to smoke and less likely to quit smoking compared to the general population [219]. A high alcohol intake is a further risk factor occurring in high rates in MSM [311], who were the majority group in this cohort. Both smoking and alcohol are risk factors for osteoporotic fractures in the general population [61] and in HIV-positive patients [193,194,218]. Interestingly, the fracture risk with alcohol use is not linear, with the relationship being a U-shaped curve [224]. This suggests that abstinence, as well as excess alcohol consumption may lead to reduced BMD. However, with regards to moderate alcohol intake, the relationship with BMD appears to be linear [312,313], suggesting that some alcohol may actually be beneficial.

Study participants also reported a high rate of recreational drug use, but not injecting drug use. In the general population, most studies have reported an association between low BMD and opiate use [275,276]. This has also been the case in HIV-positive cohorts in both the pre-ART [195] and ART [153,156,201] eras. However, both heroin and methadone were not commonly used in my cohort, with only seven patients reporting that they had ever injected recreational drugs. Low BMD has also been associated with amphetamine and inhalant use in HIV-negative MSM using TDF for PrEP [294], as well as in the general population [314]. These recreational drugs may prove to be important risk factors for reduced BMD in HIV-positive MSM who engage in recreational drug use and/or chemsex, and require further investigation, although this study was planned and completed before the current epidemic of chemsex [315].

The results showed that the study participants had been exposed to a large number of prescribed medications associated with reduced BMD. Many were taking antidepressants, which reflected the high number of patients who reported a history of

depression. Patients with depression may have not been able to recall all the relevant details in their medical histories, which may have had an effect on the results from the questionnaires. However, I did not have information on whether participants were depressed at the time of their study visit. Other medications commonly used were antacids, steroid inhalers and oral steroids, with the latter frequently prescribed for the treatment of opportunistic infections in HIV-positive patients [26].

Although my cohort comprised a relatively young group of men with a mean age of 47 years, many led a sedentary lifestyle with 7.3% stating that they did not walk for at least 30 minutes three times a week, which was the minimum amount of exercise recommended by the NOS when I started this study. Interestingly, NOS has more recently increased their recommendations to suggest that 30 minutes of moderate-intensity weight-bearing exercise at least five days a week plus muscle strength training on at least two days a week should be performed by adults to maximise their bone health [305]. Additionally, the majority of patients never did any weight-bearing or muscle-toning exercise, further exacerbating their risks for developing reduced BMD. A meta-analysis in postmenopausal women has shown that prescribed exercise programmes including aerobic and resistance training are effective at slowing the loss of BMD at one year, although the duration, intensity and frequency of exercise have not been determined [229]. Another meta-analysis of published exercise intervention studies has also shown that exercise can improve or maintain BMD in men [228]. Data in HIV-positive patients are sparse. One study showed that progressive resistance training had no effect on BMD, although only BMD at the lumbar spine was assessed [230]. A study in HIV-positive patients with lipodystrophy has shown that strength training can increase BMD at the lumbar spine, the femoral neck and the radius [316]. However, it is likely that all HIV-positive patients, and not only those with lipodystrophy, would gain a benefit because weight-bearing exercise increases bone strength [317,318], whilst resistance training increases muscle strength, both of which are needed to prevent a reduction in BMD [319].

3.5.1.4 Factors associated with BMD in this cohort

I evaluated a number of risk factors found in the general population to see if they were of concern in my cohort. I found that lower BMD was associated with current smoking at the lumbar spine and the total hip (left, right and non-dominant). This is a traditional risk factor for osteoporotic fractures [222], and known to be prevalent in HIV-positive patients [193,194]. Steroid use (both oral and inhaled) showed a borderline association with lower BMD at the hip (both left and right), but not at the non-dominant total hip, probably due to small numbers and mostly due to past exposure. Steroid exposure is a

risk factor associated with reduced BMD and osteoporotic fractures in both the general [272,273] and HIV-positive populations [192,193]. Older age was also associated with a lower BMD at the femoral neck (left femoral neck and the non-dominant femoral neck). This is another well-documented risk factor for reduced BMD in the general population [69], but has also been described in numerous HIV cohorts [153,156,208-211].

Interestingly, in my cohort, calcium and/or vitamin D supplementation was associated with a lower BMD at the lumbar spine and the non-dominant total hip only, which was an unexpected finding, but probably reflected the fact that those at risk of or with lower BMD were already on preventative treatment. This is contrary to studies which have shown that calcium supplementation has a positive effect on BMD and reduces bone loss after two or more years of treatment [320]. However, my data may reflect the fact that men taking calcium and/or vitamin D were started on supplementation due to concerns about reduced BMD.

Although I investigated a number of HIV-related factors, including duration and clinical stage of HIV infection, immune suppression and the use of specific antiretroviral drugs, only longer cumulative exposure to a boosted PI remained significantly associated with a lower BMD, and this association was only seen at the lumbar spine. This finding has been previously reported in other studies, including several longitudinal ones [252,296,301,302,304]. A study from the UK has also shown a higher prevalence of reduced BMD in those on a PI-containing regimen compared to those not on a PI [306]. Interestingly, there did not appear to be an association between TDF and lower BMD, which has previously been implicated in reduced BMD [192,250-252,321,322]. Most data relating to ART use have been conducted in longitudinal studies and are discussed in detail in Chapter 4.

HIV infection has been shown to be independently associated with lower BMD [323], and has been postulated to cause a high bone turnover state, causing a 'catabolic window' [324]. However, lack of an association with HIV-related factors may be because this was a relatively healthy cohort of patients with good immune function and good virological control of their HIV infection. Additionally, lack of an HIV-negative control group meant that the effects of the virus alone could not be accounted for.

Higher BMI was strongly associated with a higher BMD at all sites. These findings are in keeping with results from studies in the general population [232], as well as in the HIV literature, which have reported that HIV-positive patients have low weight or a low BMI prior to initiating ART [146,193,194,208,209,233]. A study where 85% of patients

were on ART has also shown an association with low BMD and low BMI [306]. My findings are also similar to those reported by Arnsten *et al*, which found no association with BMD and HIV infection in overweight or obese HIV-positive men, suggesting that higher BMI may lessen the effect of HIV infection on BMD [156]. Additionally, a meta-analysis suggested that the high prevalence of low BMD was a result of low body weight [198]. The majority of patients in this cohort were on ART and had good virological control, and therefore, a normal or higher BMI was not unexpected.

Another factor associated with a higher BMD was non-white ethnicity at all sites except at the lumbar spine. This is in keeping with studies indicating that white ethnicity is associated with lower BMD compared to black ethnicity in children [212] and in adults [213,214]. Similar findings have also been reported in HIV-positive patients [153,156,215].

Finally, doing some regular exercise was associated with a higher BMD at the lumbar spine, but not at any of the hip sites. Weight-bearing and muscle-toning exercise has been shown to reduce loss of BMD in the general population [229], as well as maintaining or improving BMD in men [228,325]. In HIV-positive patients, physical inactivity has been associated with a number of parameters relating to fatigue, weight, BMI, subcutaneous fat and abdominal girth, all of which reduced with supervised aerobic exercise [326]. However, it is not clear why there was an effect at the spine but not at the hip, a finding that warrants further investigation.

3.5.1.5 Conclusions

In summary, there was a low prevalence of reduced BMD in this cohort. This may be due to the fact that the majority of participants were stable with well-controlled HIV infection having been on ART long-term. The factors associated with BMD in this cohort were the 'traditional' risk factors, such as older age, white ethnicity, smoking and exposure to steroids, suggesting that traditional factors may be of more importance than HIV-related factors in reducing BMD in men with well-controlled HIV infection. These findings are similar to many others that have reported a high prevalence of traditional risk factors in HIV-positive patients, including the Strategic Timing of AntiRetroviral Treatment (START) study, which assessed a large number of racially diverse ART-naïve HIV-positive patients with normal CD4 cell counts [327]. In this study, lower BMD was associated with traditional risk factors, but not with HIV-related factors, such as CD4 cell count or HIV viral load.

Although there was an association between longer duration of exposure to an ART regimen containing a boosted PI at the lumbar spine, there were no other associations

between lower BMD and HIV- or ART-related factors. There did not appear to be an association between ART use and a lower BMD with TDF, which has previously been implicated in the loss of BMD. This may be due to the fact that this cohort comprised of men with well-controlled HIV infection, a good immune function and long duration of exposure to TDF, and it may reflect stabilisation of BMD over time with TDF use.

3.5.2 Strengths and limitations

The main strengths of this study are the large sample size, the homogeneity within the subjects and a detailed study of numerous factors associated with low BMD, including traditional risk factors, as well as those relating to HIV infection and ART exposure.

However, this study has some limitations. I had planned to investigate the role of HIV infection in reducing BMD by comparing men with chronic HIV infection to those with recently acquired HIV infection (seroconverters) to those who were HIV-negative. However, both seroconverters and HIV-negative patients were difficult to recruit, and therefore, I was unable to conduct this analysis. I attempted to recruit HIV-negative men from the sexual health clinic. Another more successful way may have been to approach the HIV-negative MSM partner in a serodiscordant couple, especially if the HIV-positive partner was already recruited into my study. A longer period for recruitment may also have increased the number of HIV-negative controls and seroconverters recruited into my study.

Another limitation was that although the study participants provided a detailed history of factors associated with low BMD, this was mainly in the form of a self-reported questionnaire. This method of data gathering has limitations, the main one being recall bias, especially with regard to details which had occurred many years previously. Additionally, some of the questions were not detailed enough (e.g. those questions relating to steroid use did not ask for details of the dose and length of time exposed to steroids), and so this may have led to an over-estimation of risk. I was also unable to independently verify the details provided in the questionnaire against their medical records, so I had to rely solely on the participants' provision of the details. As general practitioners tend to have a patient's complete health records, obtaining consent from study participants to contact their general practitioner for further information may have been useful in verifying some of the results from the questionnaire.

3.5.3 Future work

Further recruitment of seroconverters and HIV-negative controls would enable a more in-depth investigation of the role of HIV infection in reducing BMD.

The findings from this Chapter, together with the results from Chapter 4, have been presented at the 21st Conference on Retroviruses and Opportunistic Infections, Boston, USA in March 2014 (poster) and at the 3rd Joint Conference of BHIVA with BASHH in Liverpool in April 2014 (poster):

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. Minimal change in bone density and no association with HIV factors over 12 months in HIV-infected men (Poster 777).

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. Minimal change in bone density over 12 months in cART-experienced HIV-infected men (Poster 163), HIV Med 2014;15(3):1–16.

3.6 Supplementary data

3.6.1 Prevalence of reduced BMD at the left and right total hips and at the left and right femoral necks

Table 3.6.1.1 Prevalence of low BMD at the left and right total hips and the left and right femoral necks

The percentage of participants with low BMD (using composite of Z-score ≤ -2.0 in men <50 years old and T-score ≤ -2.5 in men ≥ 50 years old) was small at all sites. When using the T-score in all patients, the majority had normal BMD at all 3 sites.

	N	Low BMD, n (%)	T-score in all patients, n (%)		
			Normal	Osteopenia	Osteoporosis
Left total hip	416	22 (5.3)	251 (60.3)	154 (37.0)	11 (2.6)
Right total hip	415	16 (3.9)	248 (59.8)	155 (37.3)	12 (2.9)
Left femoral neck	419	15 (3.6)	201 (48.0)	202 (48.2)	16 (3.8)
Right femoral neck	418	14 (3.4)	217 (51.9)	190 (45.5)	11 (2.6)

BMD: bone mineral density

3.6.2 Factors associated with BMD at the left total hip

Table 3.6.2.1 Factors associated with BMD at the left total hip

In multivariable analyses, current smoking and steroid use remained associated with a lower BMD. Higher BMD continued to be associated with non-white ethnicity and having a higher BMI.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	-0.01 (-0.02, 0.01)	0.21		
Ethnicity				
White	0.00	-	0.00	-
Other	0.08 (0.02, 0.13)	0.02	0.06 (0.01, 0.11)	0.02
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.001 (-0.03, 0.03)	0.97	-0.003 (-0.03, 0.03)	0.87
Current smoker	-0.05 (-0.08, -0.02)	0.002	-0.04 (-0.07, -0.01)	0.02
Alcohol use				
Never	-0.01 (-0.05, 0.03)	0.72		
<3 units/day	0.00	-		
\geq 3 units/day	0.002 (-0.04, 0.04)	0.93		
Recreational drug use				
No	0.00	-		
Yes	-0.01 (-0.04, 0.02)	0.58		
Exercise				
Never	0.00	-	0.00	-
Some weeks	0.07 (-0.01, 0.14)	0.07	0.05 (-0.02, 0.12)	0.13
Most weeks	0.05 (-0.02, 0.12)	0.15	0.03 (-0.04, 0.09)	0.44
Every week	0.04 (-0.02, 0.10)	0.20	0.02 (-0.04, 0.08)	0.50
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.07, 0.12)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.14 (0.10, 0.18)	<0.0001	0.13 (0.09, 0.17)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	-0.01 (-0.05, 0.04)	0.83		
Hypogonadism				
No	0.00	-	0.00	-
Yes	-0.05 (-0.09, 0.001)	0.05	-0.04 (-0.08, 0.01)	0.11
Diabetes				
No	0.00	-		
Yes	-0.003 (-0.06, 0.06)	0.93		
Renal disease				
No	0.00	-		
Yes	-0.03 (-0.10, 0.04)	0.42		
Rheumatoid arthritis				
No	0.00	-	0.00	-
Yes	-0.07 (-0.14, 0.001)	0.05	-0.04 (-0.11, 0.03)	0.24
Antidepressants				
No	0.00	-		
Yes	-0.004 (-0.03, 0.02)	0.76		
Antacids				
No	0.00	-		
Yes	0.01 (-0.03, 0.05)	0.76		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.08, -0.01)	0.02	-0.04 (-0.07, -0.001)	0.04
Calcium and/or vitamin D supplements				
No	0.00	-	0.00	-
Yes	-0.03 (-0.07, 0.01)	0.09	-0.04 (-0.07, 0.001)	0.06
Duration of HIV infection, per year	-0.001 (-0.003, 0.001)	0.18		

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.05, 0.01)	0.23		
Symptomatic AIDS	-0.02 (-0.05, 0.01)	0.21		
CD4 count, per 50 cells/ μ L				
Nadir	0.002 (-0.003, 0.01)	0.37		
Current	0.001 (-0.002, 0.003)	0.70		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.03 (-0.01, 0.07)	0.08	0.02 (-0.04, 0.08)	0.56
Current ART regimen				
None	0.00	-	0.00	-
ART including TDF	-0.03 (-0.07, 0.02)	0.26	-0.02 (-0.09, 0.05)	0.55
ART including PI/ritonavir	-0.05 (-0.11, 0.003)	0.06	-0.02 (-0.10, 0.06)	0.55
ART including TDF + PI/ritonavir	-0.04 (-0.09, 0.01)	0.14	-0.02 (-0.09, 0.06)	0.64
Other ART	-0.02 (-0.09, 0.06)	0.62	-0.02 (-0.12, 0.07)	0.60
Cumulative ART exposure, per year**	-0.002 (-0.01, 0.000)	0.08	0.001 (-0.002, 0.01)	0.41
Cumulative TDF exposure, per year**	-0.002 (-0.01, 0.01)	0.58		
Cumulative PI/ritonavir exposure, per year**	-0.004 (-0.01, 0.000)	0.05	-0.003 (-0.01, 0.002)	0.27
Cumulative NNRTI exposure, per year**	0.000 (-0.003, 0.003)	0.95		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

3.6.3 Factors associated with BMD at the right total hip

Table 3.6.3.1 Factors associated with BMD at the right total hip

As with the left total hip, in multivariable analysis, only current smoking and exposure to steroids continued to be associated with a lower BMD, whilst non-white ethnicity and a higher BMI remained associated with a higher BMD.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	-0.01 (-0.02, 0.01)	0.43		
Ethnicity				
White	0.00	-	0.00	-
Other	0.07 (0.02, 0.13)	0.01	0.06 (0.004, 0.11)	0.03
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.001 (-0.03, 0.03)	0.96	0.001 (-0.03, 0.03)	0.93
Current smoker	-0.06 (-0.09, -0.02)	0.001	-0.04 (-0.07, -0.01)	0.02
Alcohol use				
Never	-0.001 (-0.04, 0.04)	0.97		
<3 units/day	0.00	-		
\geq 3 units/day	-0.001 (-0.04, 0.04)	0.96		
Recreational drug use				
No	0.00	-		
Yes	-0.01 (-0.03, 0.02)	0.64		
Exercise				
Never	0.00	-		
Some weeks	0.05 (-0.02, 0.12)	0.13		
Most weeks	0.02 (-0.05, 0.09)	0.51		
Every week	0.02 (-0.04, 0.08)	0.42		

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.06, 0.12)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.14 (0.10, 0.18)	<0.0001	0.13 (0.09, 0.17)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	0.01 (-0.04, 0.05)	0.72		
Hypogonadism				
No	0.00	-	0.00	-
Yes	-0.04 (-0.09, 0.01)	0.08	-0.03 (-0.07, 0.02)	0.19
Diabetes				
No	0.00	-		
Yes	-0.01 (-0.07, 0.05)	0.78		
Renal disease				
No	0.00	-		
Yes	-0.03 (-0.10, 0.04)	0.47		
Rheumatoid arthritis				
No	0.00	-	0.00	-
Yes	-0.06 (-0.13, 0.01)	0.10	-0.03 (-0.10, 0.04)	0.41
Antidepressants				
No	0.00	-		
Yes	-0.003 (-0.03, 0.03)	0.84		
Antacids				
No	0.00	-		
Yes	0.02 (-0.02, 0.06)	0.40		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.08, -0.01)	0.02	-0.03 (-0.07, -0.001)	0.04
Calcium and/or vitamin D supplements				
No	0.00	-		
Yes	-0.03 (-0.07, 0.01)	0.17		
Duration of HIV infection, per year	-0.002 (-0.004, 0.000)	0.10		
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.03 (-0.06, 0.01)	0.23	-0.02 (-0.05, 0.01)	0.14
Symptomatic AIDS	-0.03 (-0.06, 0.004)	0.08	-0.02 (-0.05, 0.02)	0.35
CD4 count, per 50 cells/ μ L				
Nadir	0.003 (-0.001, 0.01)	0.15		
Current	0.000 (-0.003, 0.003)	0.96		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.04 (0.001, 0.08)	0.05	0.01 (-0.05, 0.08)	0.66
Current ART regimen				
None	0.00	-	0.00	-
ART including TDF	-0.03 (-0.08, 0.01)	0.17	-0.03 (-0.10, 0.05)	0.48
ART including PI/ritonavir	-0.07 (-0.12, -0.01)	0.02	-0.03 (-0.11, 0.05)	0.43
ART including TDF + PI/ritonavir	-0.04 (-0.09, 0.01)	0.10	-0.02 (-0.09, 0.06)	0.65
Other ART	-0.02 (-0.09, 0.05)	0.59	-0.03 (-0.12, 0.07)	0.59
Cumulative ART exposure, per year**	-0.003 (-0.01, 0.000)	0.04	0.001 (-0.002, 0.01)	0.43
Cumulative TDF exposure, per year**	-0.002 (-0.01, 0.004)	0.46		
Cumulative PI/ritonavir exposure, per year**	-0.004 (-0.01, -0.001)	0.02	-0.003 (-0.01, 0.003)	0.29
Cumulative NNRTI exposure, per year**	-0.001 (-0.004, 0.003)	0.75		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

3.6.4 Factors associated with BMD at the left femoral neck

Table 3.6.4.1 Factors associated with BMD at the left femoral neck

In multivariable analyses, only older age remained associated with a lower BMD, whilst non-white ethnicity and a higher BMI were associated with a higher BMD.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	-0.02 (-0.03, -0.01)	0.002	-0.02 (-0.03, -0.002)	0.03
Ethnicity				
White	0.00	-	0.00	-
Other	0.08 (0.03, 0.14)	0.002	0.06 (0.01, 0.11)	0.02
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.001 (-0.03, 0.03)	0.94	-0.002 (-0.03, 0.03)	0.88
Current smoker	-0.04 (-0.07, -0.01)	0.01	-0.03 (-0.06, 0.002)	0.07
Alcohol use				
Never	-0.01 (-0.05, 0.03)	0.71		
<3 units/day	0.00	-		
>3 units/day	0.002 (-0.04, 0.03)	0.71		
Recreational drug use				
No	0.00	-		
Yes	0.004 (-0.02, 0.03)	0.79		
Exercise				
Never	0.00	-	0.00	-
Some weeks	0.06 (-0.01, 0.13)	0.09	0.04 (-0.02, 0.11)	0.18
Most weeks	0.06 (-0.01, 0.13)	0.08	0.03 (-0.03, 0.09)	0.35
Every week	0.04 (-0.02, 0.10)	0.17	0.02 (-0.04, 0.07)	0.57
BMI				
<25	0.00	-	0.00	-
25-30	0.08 (0.05, 0.10)	<0.0001	0.07 (0.04, 0.10)	<0.0001
>30	0.11 (0.07, 0.15)	<0.0001	0.10 (0.06, 0.14)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	-0.01 (-0.05, 0.04)	0.75		
Hypogonadism				
No	0.00	-	0.00	-
Yes	-0.05 (-0.10, -0.01)	0.03	-0.03 (-0.08, 0.02)	0.19
Diabetes				
No	0.00	-		
Yes	-0.04 (-0.09, 0.02)	0.20		
Renal disease				
No	0.00	-		
Yes	-0.03 (-0.10, 0.04)	0.39		
Rheumatoid arthritis				
No	0.00	-	0.00	-
Yes	-0.08 (-0.15, -0.01)	0.03	-0.04 (-0.11, 0.03)	0.22
Antidepressants				
No	0.00	-		
Yes	-0.002 (-0.03, 0.03)	0.90		
Antacids				
No	0.00	-		
Yes	0.01 (-0.03, 0.05)	0.61		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.07, -0.01)	0.02	-0.03 (-0.07, 0.000)	0.05
Calcium and/or vitamin D supplements				
No	0.00	-		
Yes	-0.02 (-0.06, 0.02)	0.25		
Duration of HIV infection, per year	-0.002 (-0.004, 0.000)	0.02	0.002 (-0.001, 0.01)	0.27

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.04 (-0.07, -0.01)	0.02	-0.03 (-0.06, 0.000)	0.05
Symptomatic AIDS	-0.03 (-0.07, -0.002)	0.04	-0.02 (-0.05, 0.02)	0.37
CD4 count, per 50 cells/ μ L				
Nadir	0.004 (-0.001, 0.01)	0.11		
Current	-0.001 (-0.003, 0.002)	0.64		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.06 (0.02, 0.09)	0.004	0.02 (-0.04, 0.08)	0.58
Current ART regimen				
None	0.00	-	0.00	-
ART including TDF	-0.05 (-0.10, -0.01)	0.02	-0.02 (-0.09, 0.05)	0.58
ART including PI/ritonavir	-0.08 (-0.13, -0.03)	0.003	-0.01 (-0.09, 0.07)	0.78
ART including TDF + PI/ritonavir	-0.06 (-0.10, -0.01)	0.02	-0.01 (-0.08, 0.07)	0.86
Other ART	-0.04 (-0.11, 0.03)	0.23	-0.02 (-0.11, 0.07)	0.68
Cumulative ART exposure, per year**	-0.004 (-0.01, -0.002)	0.001	-0.001 (-0.01, 0.004)	0.80
Cumulative TDF exposure, per year**	-0.003 (-0.01, 0.003)	0.32		
Cumulative PI/ritonavir exposure, per year**	-0.01 (-0.01, -0.001)	0.008	-0.004 (-0.01, 0.002)	0.21
Cumulative NNRTI exposure, per year**	-0.002 (-0.01, 0.001)	0.16		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

3.6.5 Factors associated with BMD at the right femoral neck

Table 3.6.5.1 Factors associated with BMD at the right femoral neck

In multivariable analysis, only steroid use remained associated with a lower BMD. As at the left femoral neck, non-white ethnicity and a higher BMI remained associated with a higher BMD.

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, per 10 years	-0.02 (-0.03, -0.004)	0.01	-0.01 (-0.03, 0.003)	0.13
Ethnicity				
White	0.00	-	0.00	-
Other	0.09 (0.03, 0.14)	0.002	0.07 (0.01, 0.12)	0.01
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.01 (-0.02, 0.04)	0.51	0.01 (-0.02, 0.04)	0.55
Current smoker	-0.04 (-0.07, -0.01)	0.02	-0.02 (-0.06, 0.01)	0.22
Alcohol use				
Never	0.000 (-0.04, 0.04)	1.00		
<3 units/day	0.000	-		
\geq 3 units/day	-0.001 (-0.04, 0.04)	0.96		
Recreational drug use				
No	0.00	-		
Yes	0.003 (-0.02, 0.03)	0.84		
Exercise				
Never	0.00	-	0.00	-
Some weeks	0.06 (-0.01, 0.13)	0.07	0.05 (-0.01, 0.11)	0.12
Most weeks	0.05 (-0.02, 0.12)	0.16	0.03 (-0.04, 0.09)	0.42
Every week	0.04 (-0.01, 0.10)	0.13	0.03 (-0.03, 0.08)	0.29

	Univariable		Multivariable	
	β^* (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
BMI				
<25	0.00	-	0.00	-
25-30	0.07 (0.05, 0.10)	<0.0001	0.07 (0.04, 0.09)	<0.0001
>30	0.11 (0.07, 0.15)	<0.0001	0.11 (0.07, 0.15)	<0.0001
Liver dysfunction				
No	0.00	-		
Yes	0.01 (-0.036, 0.047)	0.79		
Hypogonadism				
No	0.00	-	0.00	-
Yes	-0.04 (-0.09, 0.003)	0.07	-0.02 (-0.06, 0.03)	0.42
Diabetes				
No	0.00	-		
Yes	-0.04 (-0.09, 0.02)	0.22		
Renal disease				
No	0.00	-		
Yes	-0.02 (-0.09, 0.05)	0.53		
Rheumatoid arthritis				
No	0.00	-		
Yes	-0.05 (-0.12, 0.02)	0.14		
Antidepressants				
No	0.00	-		
Yes	-0.003 (-0.03, 0.02)	0.82		
Antacids				
No	0.00	-		
Yes	0.03 (-0.01, 0.06)	0.16		
Steroids				
No	0.00	-	0.00	-
Yes	-0.04 (-0.07, -0.002)	0.04	-0.03 (-0.07, 0.000)	0.05
Calcium and/or vitamin D supplements				
No	0.00	-		
Yes	-0.02 (-0.05, 0.02)	0.37		
Duration of HIV infection, per year	-0.002 (-0.004, 0.000)	0.01	0.002 (-0.001, 0.01)	0.25
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.03 (-0.06, 0.000)	0.05	-0.02 (-0.05, 0.01)	0.15
Symptomatic AIDS	-0.03 (-0.06, 0.002)	0.07	-0.01 (-0.04, 0.03)	0.69
CD4 count, per 50 cells/ μ L				
Nadir	0.004 (0.000, 0.01)	0.07		
Current	-0.001 (-0.003, 0.002)	0.70	0.001 (-0.004, 0.01)	0.75
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.05 (-0.02, 0.09)	0.01	0.02 (-0.04, 0.08)	0.50
Current ART regimen				
None	0.00	-	0.00	-
ART including TDF	-0.05 (-0.09, -0.002)	0.04	-0.01 (-0.08, 0.06)	0.73
ART including PI/ritonavir	-0.08 (-0.13, -0.03)	0.003	-0.004 (-0.08, 0.08)	0.92
ART including TDF + PI/ritonavir	-0.05 (-0.10, 0.000)	0.05	0.01 (-0.07, 0.08)	0.87
Other ART	-0.03 (-0.10, 0.05)	0.48	0.01 (-0.08, 0.10)	0.88
Cumulative ART exposure, per year**	-0.004 (-0.01, -0.002)	0.0004	-0.002 (-0.01, 0.003)	0.46
Cumulative TDF exposure, per year**	-0.004 (-0.01, 0.003)	0.25		
Cumulative PI/ritonavir exposure, per year**	-0.01 (-0.01, -0.002)	0.004	-0.004 (-0.01, 0.002)	0.21
Cumulative NNRTI exposure, per year**	-0.003 (-0.01, 0.001)	0.11		

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

* β is the mean difference in absolute BMD

**Includes all patients who have ever been exposed

Chapter 4: A longitudinal analysis of risk factors associated with reduction in BMD at 12 months

4.1 Background

4.1.1 Introduction

As well as assessing the prevalence of reduced BMD in HIV-positive patients, others have investigated BMD prospectively. However, in many studies this was not the primary objective, they were looking for changes in BMD over time to identify risk factors associated with a reduction in BMD. These factors are the same as those mentioned in Chapter 3, and include traditional factors and HIV-related factors, in particular, those relating to ART exposure.

4.1.2 BMD data from longitudinal studies in HIV-positive cohorts

Most longitudinal studies that have investigated change in BMD over time have grouped cohorts according to ART use. Investigators have either assessed the effect of ART initiation on BMD in ART-naïve patients or investigated the effect of switching ART in patients who are ART-experienced. Some researchers have also investigated whether ART *per se* has an effect on BMD. In the SMART sub-study, the effects of continuous ART were compared with intermittent ART, although this study had to be terminated early due to an increase in opportunistic infections and mortality in patients in the intermittent arm [11]. This study showed that there was a significantly greater decrease in BMD in patients on continuous ART compared to those on intermittent ART, suggesting that ART may have an effect on BMD.

4.1.2.1 Effect of ART initiation on BMD

Several longitudinal studies in ART-naïve patients have provided a useful insight into the relationship between HIV and ART exposure on BMD (Table 4.1.2.1). Although the follow-up periods and ART regimens varied considerably, the results from different studies have been consistent. The majority have shown an initial reduction in BMD of between 2% and 6% in the first two years of initiating ART [29,215,249,251,252,301,302,328-332]. Some studies reported a rapid lowering of BMD on commencing ART (i.e. within the first two years), with rates varying depending on the ART combination and the site measured [249,252,330].

Table 4.1.2.1 Longitudinal studies investigating BMD in ART-naïve patients

Longitudinal studies in ART-naïve patients have shown an initial loss of BMD on initiating ART, which stabilises after 24–48 months, although usually lower than at baseline.

Author (name of study)	Year	Location	N	% male	Mean age (years)	Follow-up period (weeks)	ART regimens	Comment
Mallon [328]	2003	Australia	40	100	40 ^a	144	Variety	BMD decreased with ART initiation (24–48 weeks) and then stabilised, but was lower at week 144 than at baseline.
Gallant [29] (903)	2004	South America, Europe and USA	600	74	36	144	TDF/lamivudine/EFV vs. stavudine/lamivudine/EFV	BMD decreased with ART initiation (24–48 weeks) and then stabilised (48–144 weeks); greater decrease in lumbar spine BMD in TDF arm.
Cassetti [329] (903 open label extension)	2007	Brazil, Argentina and the Dominican Republic	86	62	33	288	TDF/lamivudine/EFV	Small BMD changes at lumbar spine and hip seen in the first 48 weeks were non-progressive, but BMD was lower at week 288 than at baseline.
Rivas [301]	2008	Spain	32	100	-	48	Zidovudine/lamivudine/abacavir vs. zidovudine/lamivudine/lopinavir/ritonavir	BMD decreased with ART initiation, with greater loss associated with lopinavir/ritonavir at the lumbar spine.
Brown [215] (613)	2009	USA	106	78	38	96	Zidovudine/lamivudine/EFV vs. zidovudine/lamivudine/lopinavir/ritonavir (24–48 weeks, followed by lopinavir/ritonavir only)	BMD decreased with ART initiation, with similar rates of loss in both arms.
Duvivier [302] (Hippocampe-ANRS 121)	2009	France	71	77	40 ^a	48	NNRTI/PI/ritonavir vs. NRTIs/PI/ritonavir vs. NRTIs/NNRTI	BMD decreased with ART initiation in both arms; decrease in lumbar spine BMD was greater in NNRTI/boosted PI and NRTIs/boosted PI arms than in NRTIs/NNRTI arm at week 48.
van Vonderen [249] (MEDICLAS)	2009	Netherlands, Spain, Finland and UK	50	100	41 ^a	48	Zidovudine/lamivudine/lopinavir/ritonavir vs. nevirapine/lopinavir/ritonavir	BMD decreased rapidly with ART initiation in both arms, but was greater in zidovudine/lamivudine/lopinavir/ritonavir arm.
Stellbrink [251] (ASSERT)	2010	13 European countries	385	81	37 ^a	48	TDF/emtricitabine/EFV vs. abacavir/lamivudine/EFV	BMD decreased with ART initiation in both arms, with greater decrease in TDF/emtricitabine/EFV arm at week 48.

Author (name of study)	Year	Location	N	% male	Mean age (years)	Follow-up period (weeks)	ART regimens	Comment
McComsey [252] [ACTG A5224s (ACTG A5202 sub-study)]	2011	USA	269	85	38 ^a	96	TDF/emtricitabine/EFV vs. abacavir/lamivudine/EFV vs. TDF/emtricitabine/atazanavir/ritonavir vs. abacavir/lamivudine/atazanavir/ritonavir	BMD decreased rapidly with ART initiation in both arms (0–24 weeks) and then stabilised; greater BMD loss in spine and hip BMD with TDF/emtricitabine compared with abacavir/lamivudine and in spine (but not hip) BMD with atazanavir/ritonavir compared to EFV.
Huang [330] (ACTG A5142)	2013	USA	687	81	38 ^a	96	NRTIs (lamivudine plus zidovudine, stavudine or TDF)/EFV vs. NRTIs/lopinavir/ritonavir vs. EFV/lopinavir/ritonavir	Total BMD decreased in all arms, but greatest decline with TDF-containing regimens (0–48 weeks) followed by similar losses in all arms (48–96 weeks).
Reynes [333] (PROGRESS)	2013	Canada, USA, Puerto Rico, Italy, Spain, Poland and France	206	85	40	96	TDF/emtricitabine/lopinavir/ritonavir vs. raltegravir/lopinavir/ritonavir	Significant decrease in mean percent BMD in TDF/emtricitabine/lopinavir/ritonavir arm at 96 weeks from baseline compared to TDF-sparing arm.
Bedimo [334] (RADAR)	2014	USA	85	93	39/44 ^{a*}	48	TDF/emtricitabine/darunavir/ritonavir vs. raltegravir/darunavir/ritonavir	Although raltegravir/ darunavir/ritonavir arm did not achieve similar virologic efficacy, there was an increase in BMD compared to TDF/emtricitabine/ darunavir/ritonavir.
Bernardino [331] (NEAT001/ANR S143 sub-study)	2015	15 European countries	146	88/89*	37/39*	48	TDF/emtricitabine/darunavir/ritonavir vs. raltegravir/darunavir/ritonavir	BMD decreased with ART initiation in both arms, with greater decrease in TDF/emtricitabine/darunavir/ritonavir arm than raltegravir/darunavir/ritonavir arm at week 48.
Brown [332] [ACTG A5260s (ACTG A5257 sub-study)]	2015	USA	328	82	37/35/36 ^{a*}	96	TDF/emtricitabine/atazanavir/ritonavir vs. TDF/emtricitabine/darunavir/ritonavir vs. TDF/emtricitabine/raltegravir	BMD decreased in all arms, with similar losses with either PI regimen but lowest loss with raltegravir.
Taiwo [335] (ACTG A5303)	2015	USA	259	91	33 ^a	48	TDF/emtricitabine/darunavir/ritonavir vs. maraviroc/emtricitabine/darunavir/ritonavir	Maraviroc associated with less bone loss at lumbar spine and hip compared with TDF at 48 weeks.

Author (name of study)	Year	Location	N	% male	Mean age (years)	Follow- up period (weeks)	ART regimens	Comment
Young [336]	2015	USA	30	85	38 ^a	104	TDF/emtricitabine/ raltegravir	Small (1.5%) but statistically significant decrease in BMD from baseline to week 104.

ACTG: AIDS Clinical Trials Group; ANRS: Agence Nationale de Recherche sur le Sida; ART: antiretroviral therapy; ASSERT: Assessment of Safety and Efficacy of Abacavir/Lamivudine and Tenofovir/Emtricitabine; BMD: bone mineral density; EFV: efavirenz; MEDICLAS: Metabolic Effects of Different Classes of AntiretroviralS; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; RADAR: Efficacy of RAltegravir combined with boosted DARunavir compared to tenofovir/emtricitabine combined with boosted darunavir in antiretroviral-naïve patients; TDF: tenofovir

^amedian

*Age for each arm

Most of these studies were RCTs. In one of these studies, patients were randomised to receive either zidovudine/lamivudine/EFV or zidovudine/lamivudine/lopinavir/ritonavir [215]. Although there was a decrease in total BMD in both arms after 48 weeks, there was no significant difference between the EFV and lopinavir/ritonavir groups. The ACTG 5202 metabolic sub-study was another study in ART-naïve patients which compared TDF/emtricitabine against abacavir/lamivudine in the backbone, as well as the effect of EFV against atazanavir/ritonavir as the third drug [252]. In this study, there was an initial loss of BMD in both arms, which stabilised after 48 weeks. Additionally, there was no significant difference in fracture rates between the different arms.

In the remaining studies, significant differences were noted between the different arms, and these differences related to both the type of ART regimens and the sites affected [29,249,301,302,330-332]. In a French study, patients were randomised into three groups (NNRTI/ PI/ritonavir vs. NRTIs/ PI/ritonavir vs. NRTIs/NNRTI) and changes were observed over 48 weeks [302]. Although there was a decrease in BMD in all three arms at the lumbar spine and the hip, there was a significantly greater loss of BMD at the lumbar spine in patients on a boosted PI. Similar results were observed in a Spanish study comparing abacavir with lopinavir/ritonavir and a backbone of zidovudine/lamivudine [301]. In another, where HIV-positive men were randomised to receive either zidovudine/lamivudine/lopinavir/ritonavir or nevirapine/lopinavir/ritonavir, there was a decrease in BMD in both arms, but a significantly greater loss of BMD at the femoral neck in the zidovudine/lamivudine arm [249].

In studies followed-up beyond two years, BMD tended to stabilise after the first two years, but was lower than at baseline [29,328]. In the study following patients for the longest period of six years, the small reduction in BMD at the lumbar spine and the hip was non-progressive after 48 weeks [329]. Similar results have been reported in a meta-analysis, which showed that there was an initial accelerated loss of BMD on commencing ART, which stabilised over time [337]. However, it is still unclear whether this reduction in BMD is due to ART or to the pro-inflammatory nature of HIV infection.

When investigating individual antiretroviral drugs, several studies have shown greater losses in BMD with regimens containing TDF [29,251,252,330,331,334,335]. In the Gilead 903 study, TDF was compared to stavudine in combination with lamivudine and EFV [29]. Although there was a reduction in lumbar spine BMD at 24 weeks and in hip BMD at 48 weeks, there was a significantly greater loss of BMD at the lumbar spine in patients on TDF. This study also showed that the increase in BMD was only partial, and that it did not reach baseline levels. The ASSERT study is a 96-week study, which

investigated the effects of TDF/emtricitabine against abacavir/lamivudine in ART-naïve patients [251]. It showed that there were reductions in BMD in both groups at 48 weeks, but a greater decrease occurred in patients on TDF/emtricitabine compared to those on abacavir/lamivudine. A study measuring total BMD also reported greater reductions in BMD with TDF in the first 48 weeks, while patients on EFV without TDF had lesser reductions even compared to the NRTI-sparing arm [330]. In this study, all arms showed similar losses from 48 to 96 weeks, suggesting that the effect of TDF is greatest in the first two years after initiating ART.

Interestingly, in the *Iniciativa Profilaxis Pre-Exposición (iPrEx)* study, a RCT of PrEP in MSM and transgender women, those randomised to TDF/emtricitabine had a small but significant reduction in BMD at week 24 compared to those on placebo [338]. This loss of BMD by 24 weeks was inversely correlated to intracellular TDF concentrations. On discontinuation of TDF/emtricitabine, BMD at the lumbar spine improved, with BMD being more stable after the first 24 weeks. This study demonstrated that MSM at risk of acquiring HIV have a reduction in BMD on initiating PrEP with TDF and suggests that TDF rather than HIV infection has an effect on loss of BMD. However, there was no difference in fracture rates between the two arms.

In contrast to TDF, raltegravir appears to have the least effect on reducing BMD [331,332,334]. In one study conducted across 15 European countries, there was less reduction in BMD with a raltegravir-based ART regimen compared to a standard regimen containing TDF [331]. In an American study using TDF/emtricitabine as the backbone and comparing two PIs (atazanavir/ritonavir vs. darunavir/ritonavir) against raltegravir, there were similar reductions in BMD with either PI arm, with those on raltegravir having the least loss of BMD [332]. In another small American study where ART-naïve patients were started on TDF/emtricitabine/raltegravir, there was a reduction in BMD from baseline to week 104 [336]. However, the loss of BMD was small (1.5%) and lower than the 2% to 6% quoted in many other studies. Interestingly, in the *Efficacy of RAltegravir combined with boosted DARunavir compared to tenofovir/emtricitabine combined with boosted darunavir in antiretroviral-naïve patients (RADAR)* study, there was an increase in BMD in the NRTI-sparing arm (raltegravir/darunavir/ritonavir), but patients in this arm failed to achieve virologic suppression that was similar to those on TDF/emtricitabine/darunavir/ritonavir [334]. In the *PROGRESS* study, there was a mean percent reduction in BMD in the TDF/emtricitabine/lopinavir/ritonavir arm at 96 weeks compared to the TDF-sparing arm that contained raltegravir [333].

4.1.2.2 Effect of ART switching on BMD

Prospective studies in ART-exposed patients are shown in Table 4.1.2.2. In many of these studies, BMD remained stable over time [151,159,194,233,296,303,339,340]. Two studies have shown that although the baseline BMD was lower in ART-experienced HIV-positive women compared to HIV-negative women, the rate of loss of BMD in the HIV-positive group was not significant and was no different to that seen in the HIV-negative group [159,194]. In the first study, loss of BMD was associated with NRTI use [194], whilst in the second study, VDD and opiate use were significantly associated [159]. Additionally, a Spanish study in mainly HIV-positive men has shown that although there was no significant change in BMD over time, loss of BMD was associated with exposure to boosted PIs [296]. One study in osteopenic HIV-positive men showed that BMD changed modestly over two years, although a loss of BMD was associated with exposure to TDF [322].

Table 4.1.2.2 Longitudinal studies investigating BMD in ART-exposed patients

The majority of longitudinal studies in ART-exposed patients have shown that BMD remained stable over time with ART exposure.

Author (name of study)	Year	Location	N	% male	Mean age (years)	Follow-up period (weeks)	ART regimens	Comment
Nolan [233] (Western Australian HIV Cohort)	2001	Australia	54	100	43	377 days	Variety of regimens containing 2 NRTIs (zidovudine/stavudine/lamivudine/didanosine) and PI (nelfinavir or indinavir)	BMD remained stable with ART over time, with an increase in BMD with indinavir.
Dube [341]	2002	USA	14	86	38	48	2 NRTIs (abacavir or didanosine/lamivudine or stavudine) and PI (amprenavir)	BMC increased from baseline.
Fernandez-Rivera [296]	2003	Spain	70	-	-	48	Variety of regimens	Low BMD associated with PIs, but remained stable with ART.
Mondy [193]	2003	USA	90	-	-	72	Variety of regimens	BMD increased over time.
Madeddu [151]	2004	Italy	27	-	-	56	NRTI/PI vs. NRTI/NNRTI	BMD remained stable with ART over time.
Dolan [194]	2006	USA	25	0	-	96	Variety of regimens; compared to HIV-negative controls	Although BMD remained stable with ART over time, loss of BMD was associated with NRTIs.
Bolland [339]	2007	New Zealand	23	100	47	96	Variety of regimens	BMD remained stable with ART over time.
McComsey [342]	2008	USA	24	79	45 ^a	48	Continue stavudine or reduce to half-dose	Significant loss of BMD in patients continuing on standard-dose stavudine but not in those on low-dose.
Martin [250] (STEAL)	2009	Australia	357	98	45	96	TDF/emtricitabine vs. abacavir/lamivudine and NNRTI or PI/ritonavir	TDF/emtricitabine associated with lower BMD than abacavir/lamivudine.
Yin [159]	2010	USA	168	0	38	120	Variety of regimens; compared to HIV-negative controls	BMD remained stable with ART over time.
Bonjoch [303] (Osteoporosis)	2010	Spain	391	-	-	120	Variety of regimens	Although BMD remained stable with ART over time, loss of BMD was associated with time on a boosted PI, time on TDF and current use of a boosted PI.
Bolland [340] (Follow-up study of Bolland 2007)	2012	New Zealand	44	100	49	288	Variety of regimens; compared to HIV-negative controls	BMD remained stable with ART over time.

Author (name of study)	Year	Location	N	% male	Mean age (years)	Follow-up period (weeks)	ART regimens	Comment
Curran [343] (BICOMBO)	2012	Spain	45	73	42 ^a	96	TDF/emtricitabine vs. abacavir/lamivudine	Total BMD significantly increased at 96 weeks from baseline in both arms, with no difference between arms.
Yin [304]	2012	USA	73	0	56	48	Variety of regimens containing 2 NRTIs and NNRTI or PI/ritonavir; compared to HIV-negative women	BMD loss was associated with TDF use and greatest in women on TDF/PI vs. other NRTI/PI.
Assoumou [322] (ANRS 120 Fosivir)	2013	France	94	100	46 ^a	96 - 144	Variety of regimens including TDF and PI/ritonavir	Osteopenia only changed modestly over 2 years, but a quarter of patients experienced a >SDD loss with TDF.
Cotter [344] (PREPARE)	2013	Europe	53	85	46	48	zidovudine/lamivudine vs. TDF/emtricitabine	Switching to TDF/emtricitabine was associated with BMD loss that was not statistically significant.
Martin [345] (Second Line)	2013	South Africa, India, Thailand, Malaysia and Argentina	97	48	39	48	NRTIs/lopinavir/ritonavir vs. raltegravir/lopinavir/ritonavir	Less BMD loss with NRTI-sparing regimen.
Bianco [346] (GUSTO)	2014	Italy	27	74	47 ^a	48	Switch from triple ART (NRTI/NNRTI or NRTI/PI/ritonavir) to maraviroc/darunavir/ritonavir	Significant improvement in femoral BMD after switching to maraviroc/darunavir/ritonavir.
Bloch [347] (TROP)	2014	Australia	37	97	49	48	Switch from TDF/PI/ritonavir + lamivudine/emtricitabine to raltegravir/PI/ritonavir + lamivudine/emtricitabine	Switching from TDF to raltegravir significantly improved hip and spine BMD at 48 weeks.
Negredo [348] (OsteoTDF)	2014	Spain	54	83	49	48	Switch from TDF to abacavir or continue on TDF	Slight improvement in femoral BMD when switching from TDF to abacavir, but no difference between arms.
Calza [349]	2016	Italy	46	78	45	48	Switch from TDF/emtricitabine/PI/ritonavir to nevirapine/raltegravir	Significant increase in lumbar spine and total hip BMD at 48 weeks.
Hamzah [350] (MIDAS)	2016	UK	64	86	43	48	TDF/emtricitabine/EFV vs. darunavir/ritonavir	Darunavir/ritonavir monotherapy associated with increase in BMD of 2-3% at lumbar spine and femoral neck at 48 weeks compared to triple ART.

ANRS: Agence Nationale de Recherche sur le Sida; ART: antiretroviral therapy; BMC: bone mineral content; BMD: bone mineral density; GUSTO: GUIded Simplification with Tropism Assay; MIDAS: Metabolic Impact of DARunavir/ritonavir maintenance monotherapy; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; SDD: smallest detectable difference; TDF: tenofovir; TROP: Switch from Tenofovir to Raltegravir for Low Bone Density

^aMedian

Some studies, however, have shown that BMD or BMC increases over time in HIV-positive patients established on ART [193,233,339-341,343,347-349]. Often the increases occur mainly at the lumbar spine but not at the hip [193,233,339,340]. Although an increase in BMD at the lumbar spine could be attributed to age-related spondylotic degenerative changes [307], in all but one study [193], which did not mention the mean age of the participants, the mean ages of the subjects were in the mid- to late forties.

The STEAL study investigated the effect of ART on BMD in ART-experienced patients switched to either TDF/emtricitabine or abacavir/lamivudine [250]. This showed that at week 96, patients switched to TDF/emtricitabine had a lowering of their T-score, compared to an increase in T-score in those on abacavir/lamivudine (change of -0.04 vs. +0.07 at the lumbar spine and -0.07 vs. +0.09 at the right hip), suggesting that TDF/emtricitabine was associated with a reduction in BMD, whilst abacavir/lamivudine was associated with an increase in BMD. Further analysis of this cohort revealed that treatment with TDF/emtricitabine, lower bone formation and lower fat mass were predictors of BMD loss, although there was no association between TDF/emtricitabine and increased risk of fracture [321]. Several other studies have demonstrated a reduction in BMD on switching to a regimen containing TDF [303,304,322,333,344].

Conversely, studies have also shown that switching from TDF to other regimens can improve BMD [345-350]. BMD was found to increase when switching to raltegravir [345,347,349], maraviroc [346] and with darunavir/ritonavir monotherapy [350]. One study showed a reduction in BMD with standard-dose stavudine, but this side effect was prevented by halving the dose [342].

Overall, these results suggest that long-term ART may have a beneficial effect on general health, and this includes bone health. A meta-analysis of longitudinal studies suggested that patients established on ART had stable BMD [337].

4.1.3 Methods for measuring change in BMD over time

DXA can be used to measure changes in BMD over time [102]. However, the precision of DXA measurements varies with skeletal site, varying between 1.0% to 1.2% at the lumbar spine, 0.9% to 1.3% at the total hip and 1.5% to 1.9% at the femoral neck [351]. This means that small changes in BMD on an individual level could be due to errors in precision rather than actual changes in BMD, even if the same densitometer is used.

There are also other factors that can cause variability in measurements, including inter-operator variations, device errors and patient movements during scanning [96,352].

Change in BMD can also be assessed by measuring changes in SD. The T-score is based on the number of SDs from the mean peak bone mass by which BMD is reduced when compared to a young, healthy population matched for sex and ethnicity [57]. The Z-score is similar to the T-score, but is calculated by comparing BMD to adults of a similar age. The Z-score has greater clinical utility in younger individuals. The Z-score is more useful in men <50 years old, and reduced BMD is defined as -2.0 SDs from peak bone mass.

To overcome precision error, a French group calculated a decrease in BMD greater than the smallest detectable difference (SDD) [352]. This group defined SDD as the smallest change that exceeded the variability that occurred when BMD was measured at two different time points. They further evaluated changes in SDD when different densitometers were used, and found that the SDD was greater for scans carried out employing two different devices compared to two scans obtained with the same device [353]. They calculated that SDDs were 0.034, 0.027 and 0.036 g/cm² for the lumbar spine, total hip and femoral neck, respectively, when measured using a single device, and 0.048, 0.047 and 0.046 g/cm² at the lumbar spine, total hip and femoral neck, respectively, when BMD was measured using two different densitometers [353]. Although differences less than SDD may be due to precision errors in individuals, when assessed at a group level, a difference less than SDD may actually represent a true difference.

4.1.4 Summary

Although studies have shown that there is a reduction in BMD over time in HIV-positive patients, results have mainly been from RCTs. In ART-naïve patients, studies have consistently shown an initial lowering of BMD between 2% and 6% on commencing ART, which is greater than that observed in the general population [69] and certainly higher than the 0.5% to 1% reduction reported in HIV-negative men [70]. However, the rate of loss of BMD stabilised over time. In ART-experienced cohorts, most studies have shown that BMD remained stable. Studies have also investigated the effect of different classes of ART on BMD, with TDF shown to have a significant effect. This chapter explores the longitudinal results of the men that returned for a follow-up visit at 12 months, investigates the changes in BMD that occurred and assesses the factors associated with a loss of BMD.

4.2 Aims and objectives

Below are the aims for this chapter:

1. To compare the characteristics of subjects who returned for a second visit at 12 months with those who did not, to assess attrition bias.
2. To describe the distribution of the following variables at 12 months:
 - a. Demographic characteristics
 - b. Lifestyle factors
 - c. Traditional osteoporosis-related factors
 - d. HIV parameters.
3. To quantify changes in BMD from baseline to follow-up at 12 months at the following sites:
 - a. Lumbar spine
 - b. Non-dominant total hip
 - c. Non-dominant femoral neck.
4. To determine the best method to measure change in BMD over time to overcome precision error.
5. To identify factors associated with loss of BMD over 12 months at the following sites:
 - a. Lumbar spine
 - b. Non-dominant total hip
 - c. Non-dominant femoral neck.

I hypothesised that there would be a reduction in BMD in this cohort over 12 months. In this chapter, I describe the change in BMD that occurred and the factors associated with this loss in a homogeneous group of HIV-positive men with longstanding HIV infection and well exposed to ART.

4.3 Methods

4.3.1 Study design

The detailed methods of the study are given in Chapter 2. In this chapter, the data from the study participants' baseline visit (Year 1) were compared with the data from their follow-up visit (Year 2).

The variables were grouped into the same categories mentioned in Chapter 3. Baseline and 12-month follow-up data of men with HIV infection were used in this longitudinal

analysis. Demographic and HIV-related details, including ART history, were obtained from the HIV clinic database. Risk factors for low BMD were evaluated using data from self-reported questionnaires. Additional information relating to HIV, bone and renal parameters were obtained from fasted blood and urine samples.

The GE Healthcare Lunar iDXA bone densitometer (GE Healthcare, Madison, Wisconsin, USA) was used to measure absolute BMD (g/cm^2) at the lumbar spine, the total hip (left total hip, right total hip and non-dominant total hip) and the femoral neck (left femoral neck, right femoral neck and non-dominant femoral neck). Using hand dominance, BMD data were also reported for the non-dominant total hip and non-dominant femoral neck.

4.3.2 Definitions

The SDD was defined as the smallest change that exceeded the variability that inherently occurred when measuring BMD [352]. I used the cut-offs for SDD derived by Kolta *et al* [353]. As a single densitometer was used to measure BMD in patients at baseline and at 12 months, the SDD was defined as a decrease in BMD of ≥ 0.034 , 0.027 and 0.036 g/cm^2 at the lumbar spine, total hip and femoral neck, respectively.

4.3.3 Statistical analysis

Patients who attended for their follow-up visit were compared to those that did not, to ascertain attrition bias. The factors compared were demographic factors (age, ethnicity and BMI), co-infection with HBV or HCV, and HIV factors (duration and clinical stage of HIV infection, nadir and current CD4 count, HIV viral load, ART status and duration of ART use).

The distribution frequency of each variable was calculated. Mean and standard deviation were measured in those that were normally distributed and median and interquartile range in those that had skewed distributions. For continuous data, comparisons between the two groups were made using paired t-tests for normally distributed variables and Wilcoxon rank sum for non-normally distributed variables. For categorical variables, chi-squared tests were used to compare the two groups. Statistical significance was denoted as $p \leq 0.05$.

The frequency distribution of absolute BMD in g/cm² was checked at each site. Absolute BMD at the left and right total hip and left and right femoral neck were compared using paired t-tests and the mean within-individual difference reported. Statistical significance was denoted as p-value ≤0.05.

Change in absolute BMD and percentage BMD was assessed using paired t-tests at each site. Patients on bisphosphonates were excluded prior to analysis. Change in absolute BMD was investigated by analysing 1% change in BMD, as well as a greater than SDD decrease in BMD at 12 months.

Logistic regression, which was only adjusted for baseline BMD due to small numbers, was performed to investigate the relationships between risk factors in patients with a greater than SDD decrease in BMD. All variables that had been significant in multivariable analysis at baseline (Chapter 3) were included, and statistical significance was denoted by p-value ≤0.05.

Data were complete or near complete for the majority of cases.

4.4 Results

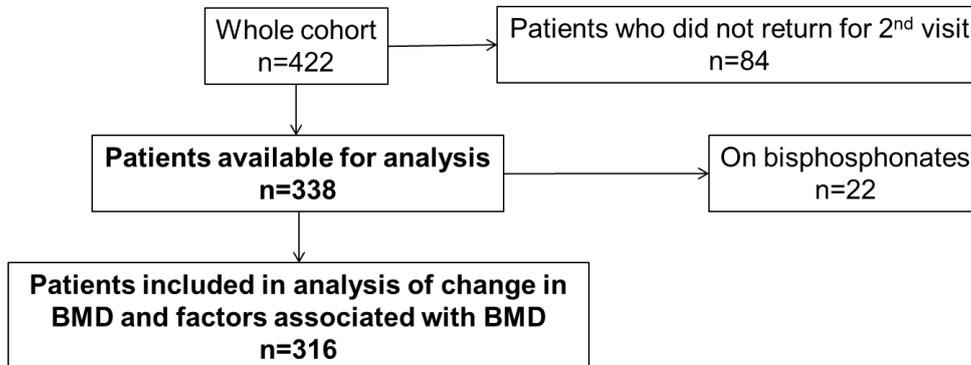
4.4.1 Subject disposition

All patients who enrolled into the study were invited for a follow-up visit at 12 months. In total, 338 patients (comprising 101 patients who had participated in the pilot study and 237 patients who had been randomly selected) returned for a follow-up visit at 12 months (Figure 4.4.1.1), constituting a 19.9% dropout rate. As established in Chapter 3, there was no significant difference at baseline related to how the patients were recruited into the study, indicating that there was no significant selection bias, and that the two populations were similar. Therefore, as in Chapter 3, from henceforth, this chapter will not distinguish between the two groups, with all HIV-positive men being analysed as one group. As bisphosphonates are known to have an effect on BMD, when analysing change in BMD and the factors associated with BMD at each site at 12 months, the same 22 men on bisphosphonates as in Chapter 3 were excluded.

Figure 4.4.1.1 Summary of subject disposition

This figure shows the number of patients who returned for a second visit at 12 months, including those on bisphosphonates who were excluded from the analysis of change in BMD and factors associated with low BMD.

BMD: bone mineral density



4.4.2 Participants who dropped out of the study

4.4.2.1 Reasons for dropping out of the study

Of the 84 men who did not re-attend for a second visit, 71 missed their appointment (some on more than one occasion having re-booked appointments), but in most cases, a specific reason could not be elicited, although some did state difficulty in booking appointments due to work commitments. One man died before his second visit due to a condition unrelated to his HIV infection. The remaining 12 men declined for a number of reasons, including too much going on (e.g. other medical diagnoses, family commitments; n=4), dislike of morning appointments due to tiredness or the stress of waking up early (n=2), mental health issues causing stress due to additional hospital appointments (n=2) and the baseline appointment (visit 1) had been too time-consuming (n=1). There were three men who declined to re-attend but did not give a reason.

4.4.2.2 Comparison of patients who returned at 12 months with those who dropped out of the study

In order to assess attrition bias, the baseline demographic and HIV-related characteristics of men who returned for a second visit at 12 months and those that did not were compared (Table 4.4.2.1). The only significant difference between the men who returned for a second visit and those that did not was that those who returned were older on average by four years.

Table 4.4.2.1 Comparison of the baseline and HIV-related characteristics between patients that returned for a second visit at 12 months and those that dropped out of the study

The only statistical difference between patients who returned for a second visit and those that did not is that those that returned were older. However, there was a weak association with duration of infection, with those returning for a second visit having a longer duration of infection than those that did not.

	N	Return visit		P-value
		Yes (N=338)	No (N=84)	
Age, years, mean (SD)	422	47 (9.6)	43 (9.7)	0.002
Ethnicity, n (%)	422			1.00
White		319 (94.4)	79 (94.0)	
Other		19 (5.6)	5 (6.0)	
BMI, kg/m ² , mean (SD)	422	25 (4.1)	25 (4.0)	0.79
HIV transmission risk, n (%)	422			0.34
MSM		316 (93.5)	76 (90.5)	
Other		22 (6.5)	8 (9.5)	
Duration of HIV infection, years, median (IQR)	422	10.1 (5.2, 16.3)	7.9 (4.4, 14.0)	0.09
HIV clinical stage, n (%)	422			0.69
Asymptomatic		158 (46.8)	35 (41.7)	
Symptomatic non-AIDS		91 (26.9)	24 (28.6)	
Symptomatic AIDS		89 (26.3)	25 (29.8)	
CD4, cells/ μ L, median (IQR)				
Nadir	422	184 (92, 267)	205 (113, 295)	0.21
At follow-up visit	422	547 (410, 689)	539 (427, 747)	0.53
HIV viral load <40, copies/mL, n (%)	422			0.34
Yes		295 (87.3)	70 (83.3)	
No		43 (12.7)	14 (16.7)	
HBV co-infection, n (%)	422			1.00
Yes		15 (4.4)	3 (3.6)	
No		323 (95.6)	81 (96.4)	
HCV co-infection, n (%)	422			0.29
Yes		44 (13.0)	15 (17.9)	
No		294 (87.0)	69 (82.1)	
ART status, n (%)	422			0.33
Never		24 (7.1)	10 (11.9)	
Previous		308 (91.1)	73 (86.9)	
Current		6 (1.8)	1 (1.2)	
Duration on ART, years, median (IQR)	422	6.3 (2.3, 11.7)	5.5 (1.7, 11.5)	0.26

ART: antiretroviral therapy; BMI: body mass index; HBV: hepatitis B; HCV: hepatitis C; IQR: interquartile range; MSM: men who have sex with men; SD: standard deviation

4.4.3 Demographic characteristics at 12 months

The demographics of the patients who returned are shown in Table 4.4.3.1 and are similar to the baseline characteristics.

Table 4.4.3.1 Demographic characteristics at baseline and at 12 months

As expected, the demographics are similar to those at baseline, with the majority of men being young, white, MSM.

	Baseline visit (N=422)	12 month visit (N=338)
Age, years, mean (SD)	47 (9.8)	49 (9.6)
Ethnicity, n (%)		
White	398 (94.3)	319 (94.4)
Black	15 (3.6)	11 (3.3)
Other	9 (2.1)	8 (2.4)
HIV transmission risk, n (%)		
MSM	392 (92.9)	317 (93.8)
Heterosexual sex	26 (6.2)	21 (6.2)
IVDU/blood products	4 (0.9)	0 (0.0)

IVDU: intravenous drug use; MSM: men who have sex with men; SD: standard deviation

4.4.4 Lifestyle factors at 12 months

Details of lifestyle factors, such as smoking, alcohol and recreational drug use, walking and exercise are shown in Table 4.4.4.1.

Table 4.4.4.1 Lifestyle factors, including smoking, alcohol and recreational drug use, walking and exercise at baseline and at 12 months

The results were similar to baseline, with one-third of the cohort being current smokers and only a few drinking excessively (≥ 3 units/day). Many reported recreational drug use, but not intravenous use (see text). Although most walked regularly, for a young cohort of men, the majority did no exercise at all.

	Baseline visit (N=422)	12 month visit (N=338)
Smoking, n (%)		
Never smoked	132 (31.3)	106 (31.4)
Ex-smoker	137 (32.5)	120 (35.5)
Current smoker	153 (36.3)	112 (33.1)
Alcohol, n (%)		
Never	62 (14.7)	56 (16.6)
<3 units/day	298 (70.8)	235 (69.5)
≥ 3 units/day	62 (14.7)	47 (13.9)
Recreational drug use, n (%)		
Yes	244 (57.8)	201 (59.5)
No	178 (42.2)	137 (40.5)
Walk 30 minutes 3 times per week		
Never	31 (7.3)	20 (5.9)
Some weeks	63 (14.9)	56 (16.6)
Most weeks	62 (14.7)	56 (16.6)
Every week	266 (63.0)	206 (60.9)
Weight-bearing exercise		
Never	230 (54.5)	205 (60.7)
Some weeks	76 (18.0)	55 (16.3)
Most weeks	25 (5.9)	19 (5.6)
Every week	91 (21.6)	59 (17.5)
Muscle-toning exercise		
Never	215 (51.0)	184 (54.4)
Some weeks	73 (17.3)	68 (20.1)
Most weeks	35 (8.3)	22 (6.5)
Every week	99 (23.5)	64 (18.9)

4.4.4.1 Smoking, alcohol and recreational drug use

Smoking, alcohol and recreational drug use were similar to those at baseline. Of the men who reported using recreational drugs, 4 (1.2%) gave a history of injecting drug use.

4.4.4.2 Walking and exercise

Compared to baseline, the number of men reporting walking 30 minutes three times a week had decreased (63.0% vs. 60.9%), although the number reporting walking some weeks (14.9% vs. 16.6%) and most weeks had increased (14.7% vs. 16.6%). The number of men reporting never doing any weight-bearing exercise (54.5% vs. 60.7%) or muscle-toning exercise (51.0% vs. 54.4%) had also increased.

4.4.5 Traditional factors associated with BMD at 12 months

Table 4.4.5.1 shows the traditional factors associated with BMD that were assessed, including BMI, self-reported co-morbidities and drugs.

Table 4.4.5.1 Traditional factors associated with reduced BMD, including BMI, secondary causes and drugs at baseline and at 12 months

A healthy BMI was reported in 45.3%. Men reported a range of co-morbidities, as well as being exposed to medications relating to low BMD. Although 22.8% and 21.6% were on calcium and vitamin D supplementation, respectively, only 6.5% were exposed to bisphosphonates.

	Baseline visit (N=422)	12 month visit (N=338)
Height, cm, mean (SD)	177.1 (7.3)	176.8 (7.4)
Weight, kg, mean (SD)	79.2 (14.2)	78.9 (14.4)
BMI, kg/m ² , mean (SD)	25.2 (4.1)	25.2 (4.1)
BMI status, kg/m ² , n (%)		
Underweight (BMI <18.5)	11 (2.6)	4 (1.2)
Healthy (BMI 18.5–25)	206 (48.8)	153 (45.3)
Overweight (BMI 25–30)	154 (36.5)	139 (41.1)
Obese (BMI >30)	51 (12.1)	42 (12.4)
Secondary co-morbidities, n (%)		
Liver dysfunction	48 (11.4)	44 (13.0)
Hypogonadism	45 (10.7)	42 (12.4)
Diabetes	23 (5.5)	18 (5.3)
Renal disease	17 (4.0)	14 (4.1)
Rheumatoid arthritis	16 (3.8)	24 (7.1)
Hypothyroidism	12 (2.8)	13 (3.8)
Inflammatory bowel disease	12 (2.8)	12 (3.6)
Anorexia nervosa	8 (1.9)	7 (2.1)
Coeliac disease/malabsorption	3 (0.7)	3 (0.9)
Hyperthyroidism	3 (0.7)	2 (0.6)
Hyperparathyroidism/hypoparathyroidism	0 (0.0)	1 (0.3)

	Baseline visit (N=422)	12 month visit (N=338)
Exposure ever to drugs associated with low BMD, n (%)		
a) Drugs associated with reduced BMD		
Antidepressants	144 (34.1)	134 (39.6)
Antacids	59 (14.0)	69 (20.4)
Steroid inhalers	56 (13.3)	61 (18.0)
Oral steroids	38 (9.0)	43 (12.7)
Chemotherapy	17 (4.0)	12 (3.6)
Anticonvulsants	16 (3.8)	18 (5.3)
b) Drugs associated with increased BMD		
Calcium tablets	70 (16.6)	77 (22.8)
Vitamin D tablets	59 (14.0)	73 (21.6)
Testosterone	38 (9.0)	36 (10.7)
Bisphosphonates	22 (5.2)	22 (6.5)
Thiazide diuretics	10 (2.4)	11 (3.3)

BMD: bone mineral density; BMI: body mass index; SD: standard deviation

4.4.5.1 Height, weight and BMI

Height and weight were unchanged from baseline, and 45.3% had a normal BMI.

4.4.5.2 Secondary causes of reduced BMD

The commonest conditions remained liver dysfunction (13.0%) and hypogonadism (12.4%). The numbers reporting rheumatoid arthritis had increased from baseline (3.8% vs. 7.1%). There was also one man (0.3%) who had developed hyperparathyroidism since his baseline visit.

4.4.5.3 Drugs associated with reduced BMD

Compared to the results at baseline, the numbers of men reporting using antidepressants (34.1% vs. 39.6%), antacids (14.0% vs. 20.4%), steroid inhalers (13.3% vs. 18.0%), oral steroids (9.0% vs. 12.7%) and anticonvulsants (3.8% vs. 5.3%) had increased.

With regards to medications used in the treatment of reduced BMD and conditions that cause secondary osteoporosis, the numbers of men reporting being on supplementation for calcium (16.6% vs. 22.8%) and vitamin D (14.0% vs. 21.6%) had increased from baseline. The same 22 (6.5%) patients were on bisphosphonates.

4.4.6 HIV parameters at 12 months

Table 4.4.6.1 shows HIV-related factors at 12 months.

Table 4.4.6.1 HIV-related factors at baseline and at baseline and at 12 months

The duration of HIV infection was long and 26.6% had been diagnosed with AIDS. However, as the majority were on ART and had an undetectable HIV viral load, the median CD4 count at recruitment was high.

	Baseline visit (N=422)*	12 month visit (N=338)*
Duration of HIV infection, years, median (IQR)	9.6 (5.0, 15.5)	11.2 (6.2, 17.3)
HIV clinical stage, n (%)		
Asymptomatic	193 (45.7)	158 (46.8)
Symptomatic non-AIDS	115 (27.3)	90 (26.6)
Symptomatic AIDS	114 (27.0)	90 (26.6)
CD4, cells/ μ L, median (IQR)		
Nadir	191 (100, 277)	184 (91, 261)
At follow-up visit	547 (411, 696)	576 (444, 772)
HIV viral load <40, copies/mL, n (%)		
Yes	365 (86.5)	304 (89.9)
No	57 (13.5)	34 (10.1)
HBV co-infection, n (%)		
Yes	18 (4.3)	15 (4.4)
No	404 (95.7)	323 (95.6)
HCV co-infection, n (%)		
Yes	59 (14.0)	44 (13.0)
No	363 (86.0)	294 (87.0)
ART status, n (%)		
Naïve	34 (8.1)	16 (4.7)
Previous	7 (1.7)	2 (0.6)
Current	381 (90.3)	320 (94.7)
Duration on ART, years, median (IQR) ^a	7.2 (3.2, 12.7) ^a	8.0 (3.9, 13.0) ^b

ART: antiretroviral therapy; HBV: hepatitis B; HCV: hepatitis C; IQR: interquartile range

*Unless otherwise stated

^aTotal number of patients = 381; ^btotal number of patients = 320

4.4.6.1 Duration and clinical stage of HIV infection

As would be expected, the median duration of HIV infection had increased. The clinical stage of HIV infection was similar to baseline for all categories. At their second visit, patients had a higher median CD4 count (547 [IQR 411, 696] vs. 576 [IQR 444, 772] cells/ μ L) than at baseline, and a greater proportion had an undetectable HIV viral load (86.5% vs. 89.9%).

4.4.6.2 HBV and HCV co-infection

There were 15 and 44 men with HBV and HCV co-infection, respectively. Although the number of patients with co-infection was lower than at baseline, the rates were similar for both HBV (4.3% vs. 4.4%) and HCV (14.0% vs. 13.0%).

4.4.6.3 ART use

At 12 months, there were more men on ART (90.3% vs. 94.7%). Amongst those receiving ART, there was a longer median duration of ART exposure at 12 months.

4.4.7 BMD at 12 months

The absolute BMD at the lumbar spine, left, right and non-dominant total hip and left, right and non-dominant femoral neck are shown in Table 4.4.7.1. There were four patients who did not have a DXA scan, so data were available for 334 patients.

Table 4.4.7.1 Absolute BMD by site at 12 months

Absolute BMD was normally distributed at all sites in the 334 patients that had a DXA scan. There were 328 patients that had L1–L4 composite measured (see text). Not all men had their total hip or femoral neck measured because they had metal work in situ.

	N	Absolute BMD, g/cm ² , mean (SD)
Lumbar spine	334	1.143 (0.162)
Left total hip	331	0.998 (0.140)
Right total hip	328	1.003 (0.137)
Non-dominant total hip	330	0.998 (0.140)
Left femoral neck	331	0.942 (0.134)
Right femoral neck	328	0.953 (0.130)
Non-dominant femoral neck	330	0.943 (0.134)

BMD: bone mineral density; DXA: dual-energy x-ray absorptiometry; SD: standard deviation

4.4.7.1 Lumbar spine

Although all 334 patients underwent a scan of their lumbar spine, six patients did not have an L1–L4 composite measurement. This was because L1 was missing (n=2) or incompletely scanned (n=1); in these patients, a composite of L2–L4 was used. In one patient, L4 was missing, and therefore, a composite of L1–L3 was used. There were two patients in whom a composite of only two vertebrae were used because one patient had a metal rod involving L1 and L2 and the other had a severe scoliosis, which led to his L1 and L2 vertebrae being excluded as the anatomical landmarks were unclear.

4.4.7.2 Left total hip, right total hip and non-dominant total hip

For the left and right total hip, there were data for 331 and 328 patients, respectively. All three patients with missing data for the left total hip had undergone a left total hip replacement. For the right total hip, there were six patients with missing data because they had either a right total hip replacement (n=4) or a screw in their right hip (n=2). In total, there were two patients who had no hip data as they had undergone bilateral total hip replacements, one of whom had undergone his operation since his baseline visit. In total, there were 330 patients with data for the non-dominant total hip.

4.4.7.3 Left femoral neck, right femoral neck and non-dominant femoral neck

There were 331 and 328 patients with data for the left and right femoral neck, respectively. The same patients as mentioned in Chapter 3 were missing data, and there were 330 patients in total with data for the non-dominant femoral neck.

4.4.7.4 Absolute BMD at 12 months

Absolute BMD was normally distributed at all sites (Table 4.4.7.1). Absolute BMD at the lumbar spine was higher than at any of the hip sites. There was a significant difference between mean absolute BMD at the left and right total hip (left minus right: -0.005 [SD 0.040] g/cm², p=0.032). There was a similar difference in mean absolute BMD at the left and right femoral neck (left minus right: -0.010 [SD 0.045] g/cm², p<0.0001).

4.4.7.5 T- and Z-scores at 12 months

The mean T- and Z-scores were normally distributed at all sites (Table 4.4.7.2). At the left and right total hip, there were significant differences in T-score (-0.71 [SD 1.07] vs. -0.67 [SD 1.06], p=0.032) and Z-score (-0.34 [SD 0.98] vs. -0.30 [SD 0.97], p=0.033). There were also significant differences in T-score (-0.98 [SD 1.02] vs. -0.90 [SD 1.00], p<0.0001) and Z-score (-0.44 [SD 0.92] vs. -0.36 [SD 0.90], p<0.0001) at the left and right femoral necks, respectively.

Table 4.4.7.2 T- and Z-scores at 12 months

The mean T- and Z-scores were normally distributed and within the normal range at all sites.

	N	T score, mean (SD)	Z score, mean (SD)
Lumbar spine	334	-0.64 (1.35)	-0.52 (1.27)
Left total hip	331	-0.71 (1.08)	-0.34 (0.98)
Right total hip	328	-0.67 (1.06)	-0.30 (0.97)
Non-dominant total hip	330	-0.71 (1.08)	-0.44 (0.98)
Left femoral neck	331	-0.98 (1.03)	-0.44 (0.92)
Right femoral neck	328	-0.90 (1.00)	-0.36 (0.90)
Non-dominant femoral neck	330	-0.98 (1.03)	-0.34 (0.93)

SD: standard deviation

4.4.8 Change in BMD over 12 months

From these analyses onwards, 22 men on bisphosphonates were excluded.

4.4.8.1 Change in absolute and percentage BMD

The change in absolute BMD over 12 months at each site is shown in Table 4.4.8.1. There was a significant increase in mean absolute BMD at the lumbar spine (1.144 [SD 0.16] vs. 1.149 [SD 0.16] g/cm², p=0.01). At the non-dominant femoral neck, there was a significant decrease in mean absolute BMD (0.951 [SD 0.13] vs. 0.946 [SD 0.13] g/cm², p=0.015). However, there was no difference in mean absolute BMD at the non-dominant total hip. Change in absolute and percentage BMD for the left and right total hips and the left and right femoral necks are shown in Table 4.6.1.1.

Table 4.4.8.1 Absolute BMD at baseline and at 12 months, including the change in absolute BMD and percentage change, at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck

There was a significant increase in absolute BMD at the lumbar spine and a significant decrease at the non-dominant femoral neck. However, the percentage change was small at each site.

Site	N	Absolute BMD at baseline, g/cm ² , mean (SD)	Absolute BMD at 12 months, g/cm ² , mean (SD)	Change in absolute BMD, g/cm ² , mean (SD)	Percentage change in BMD, %, mean (SD)	P-value*
Lumbar spine	312	1.144 (0.159)	1.149 (0.163)	0.005 (0.034)	0.46 (2.974)	0.01
Non-dominant total hip	306	1.003 (0.137)	1.003 (0.138)	0.000 (0.024)	0.06 (2.568)	0.79
Non-dominant femoral neck	308	0.951 (0.134)	0.946 (0.133)	-0.005 (0.032)	-0.42 (3.761)	0.01

BMD: bone mineral density; SD: standard deviation

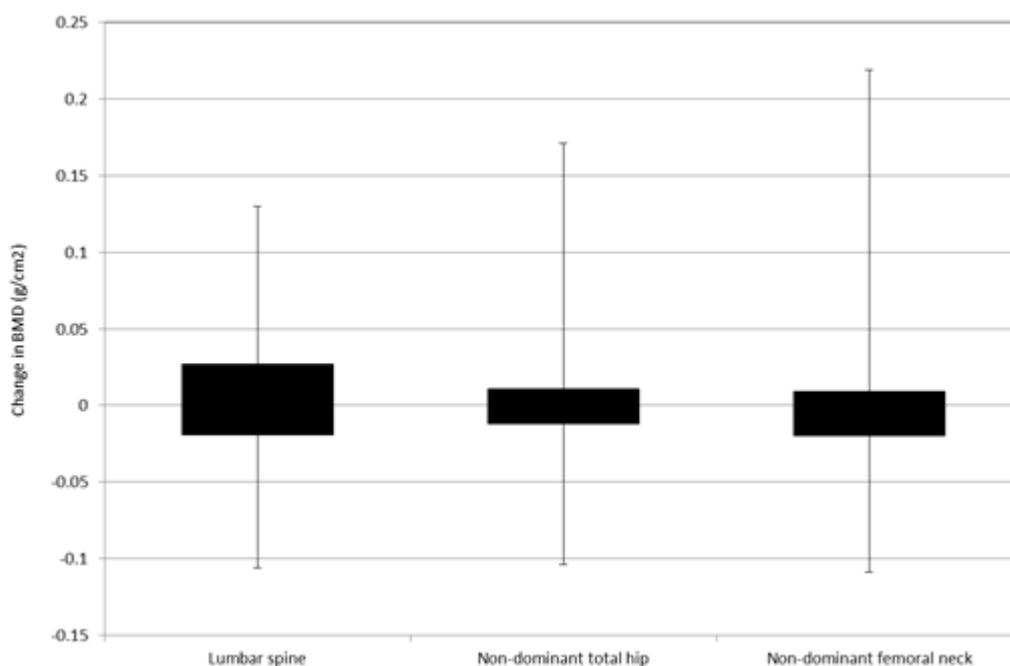
*P-value is for change in absolute BMD

Figure 4.4.8.1 shows the change in absolute BMD at 12 months at each site. This shows that although at some sites there was a significant change, the change in absolute BMD at each site was very small, which is also reflected by the small percentage change in BMD in Table 4.4.8.1.

Figure 4.4.8.1 Change in absolute BMD at 12 months by site

Although there was a significant change in BMD at the lumbar spine and the non-dominant femoral neck, the change in absolute BMD at each site was very small.

BMD: bone mineral density



4.4.8.2 Change in BMD by 1%

A change in BMD of 1% is shown in Table 4.4.8.2. A greater than 1% decrease in BMD at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck

were seen in 31.7%, 29.4% and 40.3%, respectively. Change in BMD by 1% at the left and right total hips and the left and right femoral necks are shown in Table 4.6.2.1.

Table 4.4.8.2 Change in BMD by 1%

There was a >1% decrease in BMD in 31.7%, 29.4% and 40.3% at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck, respectively.

Site	N	>1% decrease in BMD	No change in BMD (-1% to 1%)	>1% increase in BMD
Lumbar spine	312	99 (31.7)	77 (24.7)	136 (43.6)
Non-dominant total hip	306	90 (29.4)	132 (43.1)	84 (27.5)
Non-dominant femoral neck	308	124 (40.3)	111 (36.0)	73 (23.7)

BMD: bone mineral density

4.4.8.3 Smallest detectable difference (SDD)

The number of patients with a greater than SDD decrease in BMD at 12 months is shown in Table 4.4.8.3. The greatest number of patients were affected at the lumbar spine, with 42 (13.5%) men having a greater than SDD reduction in BMD at 12 months. A greater than SDD decrease in BMD at the left and right total hips and the left and right femoral necks are shown in Table 4.6.3.1.

Table 4.4.8.3 A greater than SDD decrease in BMD at 12 months

The site with the greatest number demonstrating a greater than SDD reduction in BMD at 12 months was the lumbar spine.

	N	>SDD decrease in BMD, n (%)
Lumbar spine	312	42 (13.5)
Non-dominant total hip	306	25 (8.2)
Non-dominant femoral neck	308	31 (10.1)

BMD: bone mineral density; SDD: smallest detectable difference

4.4.8.4 Best measure of change in BMD at 12 months

Although the changes in BMD at 12 months at the lumbar spine and the non-dominant femoral neck were significant, the best measure of change needed to be ascertained. The precision of DXA can vary [94]. Different machines produce different precision errors, with a 1.0% to 1.2% variation at the L1 to L4 lumbar spine, 0.9% to 1.3% at the total hip and 1.5% to 1.9% at the femoral neck [351]. A change in BMD of 1% could have been due to precision error, and therefore, was not used to analyse factors associated with change in BMD at 12 months. As the number of men with a greater than 2 SD decrease in BMD was very small at each site, no further analyses could be done with this measure. The options remaining were a greater than 3% change in BMD or a greater than SDD reduction in BMD. Apart from at the lumbar spine, there were more men with a greater than SDD decrease in BMD at 12 months compared to a 3% change, and also because this measure had already been published in other studies

[352,353], it was decided that the greater than SDD decrease in BMD was the best measure.

4.4.9 Factors associated with a greater than SDD decrease in BMD at 12 months

Factors associated with a greater than SDD decrease in BMD at 12 months at the lumbar spine (Table 4.4.9.1), the non-dominant total hip (Table 4.4.9.2) and the non-dominant femoral neck (Table 4.4.9.3) were compared. The factors associated at left total hip (Table 4.6.4.1), the right total hip (Table 4.6.5.1), the left femoral neck (Table 4.6.6.1) and the right femoral neck (Table 4.6.7.1) were also compared and the data are shown in Section 4.6

4.4.9.1 Factors associated with a greater than SDD decrease in BMD at the lumbar spine at 12 months

In univariable analyses, there were no factors associated with a greater than SDD decrease at the lumbar spine (Table 4.4.9.1).

Table 4.4.9.1 Factors associated with a greater than SDD decrease in BMD at the lumbar spine

There were no factors associated with a greater than SDD reduction in BMD at the lumbar spine at 12 months.

	OR* (95% CI)	P-value
Age, per 10 years	1.00 (0.72, 1.40)	0.98
Ethnicity		
White	1.00	-
Other	0.79 (0.17, 3.56)	0.75
Smoking		
Never	1.00	-
Ex-smoker	0.88 (0.40, 1.90)	0.74
Current smoker	0.70 (0.31, 1.58)	0.39
Exercise		
Never/some weeks	1.00	-
Most weeks	0.75 (0.21, 2.73)	0.66
Every week	0.79 (0.34, 1.85)	0.59
BMI		
<25	1.00	-
25-30	0.54 (0.25, 1.18)	0.12
>30	0.52 (0.16, 1.63)	0.26
Hypogonadism		
No	1.00	-
Yes	1.26 (0.41, 3.89)	0.69
Rheumatoid arthritis		
No	1.00	-
Yes	0.53 (0.07, 4.20)	0.55
Steroids		
No	1.00	-
Yes	1.13 (0.51, 2.51)	0.77
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	1.82 (0.79, 4.18)	0.16

	OR* (95% CI)	P-value
Duration of HIV infection, per year	1.00 (0.95, 1.05)	0.90
HIV clinical stage	1.00	-
Asymptomatic	0.65 (0.27, 1.54)	0.32
Symptomatic non-AIDS	1.40 (0.66, 2.96)	0.38
Symptomatic AIDS		
CD4 count, per 50 cells/ μ L		
Nadir	0.94 (0.80, 1.04)	0.18
HIV viral load <40, copies/mL		
Yes	1.00	-
No	0.94 (0.35, 2.56)	0.90
Current ART regimen		
None	1.00	-
ART including TDF	1.05 (0.28, 3.91)	0.94
ART including PI/ritonavir	0.53 (0.10, 2.85)	0.46
ART including TDF + PI/ritonavir	1.95 (0.52, 7.31)	0.32
Other ART	4.15 (0.83, 20.75)	0.08
Cumulative ART exposure, per year**	1.00 (0.94, 1.06)	0.98
Cumulative TDF exposure, per year**	1.00 (0.85, 1.16)	0.95
Cumulative PI/ritonavir exposure, per year**	0.99 (0.91, 1.09)	0.89
Cumulative NNRTI exposure, per year**	1.01 (0.93, 1.09)	0.89

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**Includes all patients who have ever been exposed

4.4.9.2 Factors associated with a greater than SDD decrease in BMD at the non-dominant total hip at 12 months

Factors associated with a greater than SDD decrease in BMD at the non-dominant total hip are shown in Table 4.4.9.2. The only factor associated with a greater than SDD reduction in BMD was a detectable HIV viral load, which was also associated with the left and right total hips (Table 4.6.4.1 and Table 4.6.5.1). Surprisingly, in univariable analyses, older age, BMI 25-30 kg/m², symptomatic non-AIDS disease and longer duration of exposure to TDF were associated with reduced likelihood of a greater than SDD decrease in BMD at 12 months. Current exposure to a boosted PI was also associated with reduced odds of a greater than SDD decrease in BMD at 12 months at both the left and right total hips (Table 4.6.4.1 and Table 4.6.5.1), but not at the non-dominant total hip. At the right total hip, there was also reduced risk of a greater than SDD decrease in BMD with respect to an AIDS diagnosis, and longer exposure to total ART, as well as to an NNRTI (Table 4.6.5.1).

Table 4.4.9.2 Factors associated with a greater than SDD decrease in BMD at the non-dominant total hip

The only factor positively associated with a greater than SDD reduction in BMD at 12 months was a detectable HIV viral load. Older age, BMI 25-30 kg/m², symptomatic non-AIDS disease and longer duration of exposure to TDF were associated with reduced odds of a greater than SDD decrease in BMD at 12 months in univariable analyses.

	OR* (95% CI)	P-value
Age, per 10 years	0.50 (0.32, 0.79)	0.003
Ethnicity		
White	1.00	-
Other	0.68 (0.09, 5.41)	0.72
Smoking		
Never	1.00	-
Ex-smoker	0.35 (0.11, 1.13)	0.08
Current smoker	0.88 (0.35, 2.19)	0.78
Exercise		
Never/some weeks	1.00	-
Most weeks	6.92 (0.73, 65.11)	0.09
Every week	4.44 (0.58, 33.96)	0.15
BMI		
<25	1.00	-
25-30	0.14 (0.03, 0.65)	0.01
>30	0.85 (0.25, 2.92)	0.80
Hypogonadism**		
No	-	-
Yes	-	-
Rheumatoid arthritis		
No	1.00	-
Yes	0.88 (0.11, 7.14)	0.91
Steroids		
No	1.00	-
Yes	1.01 (0.36, 2.84)	0.98
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	1.16 (0.37, 3.62)	0.80
Duration of HIV infection, per year	0.98 (0.93, 1.05)	0.59
HIV clinical stage		
Asymptomatic	1.00	-
Symptomatic non-AIDS	0.27 (0.08, 0.94)	0.04
Symptomatic AIDS	0.53 (0.19, 1.49)	0.23
CD4 count, per 50 cells/μL		
Nadir	1.12 (0.98, 1.28)	0.09
HIV viral load <40, copies/mL		
Yes	1.00	-
No	4.86 (1.96, 12.03)	0.001
Current ART regimen		
None	1.00	-
ART including TDF	0.44 (0.14, 1.38)	
ART including PI/ritonavir	0.09 (0.01, 0.80)	
ART including TDF + PI/ritonavir	0.27 (0.07, 1.02)	
Other ART	0.78 (0.13, 4.73)	
Cumulative ART exposure, per year***	0.93 (0.85, 1.01)	0.10
Cumulative TDF exposure, per year***	0.73 (0.55, 0.96)	0.02
Cumulative PI/ritonavir exposure, per year***	0.89 (0.76, 1.04)	0.13
Cumulative NNRTI exposure, per year***	0.92 (0.82, 1.03)	0.15

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**This could not be calculated as there were too few subjects in the control group

***Includes all patients who have ever been exposed

4.4.9.3 Factors associated with a greater than SDD decrease in BMD at the non-dominant femoral neck at 12 months

Table 4.4.9.3 shows the factors associated with a greater than SDD decrease in BMD at the non-dominant femoral neck at 12 months. As at the non-dominant total hip, the only factor associated with a greater than SDD reduction in BMD was a detectable HIV viral load, although the association was of borderline significance. The factors associated with a reduced risk of a greater than SDD reduction in BMD in univariable analyses were older age, longer duration of exposure to ART and longer duration of exposure to a boosted PI. At the left femoral neck, there was also reduced odds of a greater than SDD reduction in BMD with current simultaneous exposure to TDF and a boosted PI (Table 4.6.6.1), whilst at the right femoral neck, there was reduced risk of a greater than SDD reduction in BMD with BMI 25–30 kg/m² (Table 4.6.7.1).

Table 4.4.9.3 Factors associated with a greater than SDD decrease in BMD at the non-dominant femoral neck

There was a borderline association between a detectable HIV viral load and a greater than SDD reduction in BMD at 12 months. Older age, longer duration of exposure to ART and longer duration of exposure to a boosted PI were associated with reduced odds of a greater than SDD decrease in BMD at 12 months in univariable analyses.

	OR* (95% CI)	P-value
Age, per 10 years	0.62 (0.41, 0.94)	0.03
Ethnicity		
White	1.00	-
Other	0.37 (0.05, 3.07)	0.36
Smoking		
Never	1.00	-
Ex-smoker	0.59 (0.23, 1.50)	0.27
Current smoker	0.85 (0.35, 2.07)	0.72
Exercise		
Never/some weeks	1.00	-
Most weeks	1.68 (0.31, 8.98)	0.54
Every week	1.97 (0.57, 6.87)	0.29
BMI		
<25	1.00	-
25-30	0.51 (0.20, 1.27)	0.15
>30	0.82 (0.26, 2.54)	0.73
Hypogonadism		
No	1.00	-
Yes	0.97 (0.21, 4.40)	0.96
Rheumatoid arthritis		
No	1.00	-
Yes	0.88 (0.11, 7.15)	0.91
Steroids		
No	1.00	-
Yes	2.06 (0.88, 4.82)	0.10
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	1.43 (0.51, 4.01)	0.50
Duration of HIV infection, per year	0.94 (0.89, 1.01)	0.07
HIV clinical stage		
Asymptomatic	1.00	-
Symptomatic non-AIDS	0.68 (0.27, 1.73)	0.42
Symptomatic AIDS	0.66 (0.25, 1.75)	0.40

	OR* (95% CI)	P-value
CD4 count, per 50 cells/ μ L Nadir	1.06 (0.93, 1.20)	0.36
HIV viral load <40, copies/mL Yes	1.00	-
No	2.47 (1.00, 6.11)	0.05
Current ART regimen None	1.00	-
ART including TDF	0.80 (0.26, 2.45)	0.69
ART including PI/ritonavir	0.11 (0.01, 1.06)	0.06
ART including TDF + PI/ritonavir	0.35 (0.09, 1.37)	0.13
Other ART	1.54 (0.30, 7.92)	0.61
Cumulative ART exposure, per year**	0.89 (0.81, 0.97)	0.01
Cumulative TDF exposure, per year**	0.84 (0.68, 1.03)	0.10
Cumulative PI/ritonavir exposure, per year**	0.82 (0.69, 0.98)	0.03
Cumulative NNRTI exposure, per year**	0.94 (0.85, 1.04)	0.26

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**Includes all patients who have ever been exposed

4.4.9.4 Summary of factors associated with a greater than SDD decrease in BMD at the different sites at 12 months

In summary, there were no associated factors at the lumbar spine. The only factor associated with a greater than SDD reduction in BMD was a detectable HIV viral load (vs. an undetectable HIV viral load) at both the non-dominant total hip and the non-dominant femoral neck. Surprisingly, all other factors showing a significant association also showed reduced odds of a greater than SDD decrease, including traditional factors, such as older age (vs. younger age) and a higher BMI (vs. BMI <25 kg/m²). A number of HIV-related factors, most of which related to ART exposure, were associated with reduced risk of a greater than SDD decrease, suggesting that control of HIV infection may be beneficial in reducing BMD loss. These included more advanced HIV clinical stage, as well as longer duration of exposure (vs. shorter duration) to ART, TDF and a boosted PI.

However, it is important to note that a greater than SDD decrease in BMD occurred in only a small number of participants, which may have affected the results. For example, an effect was seen with BMI at the lumbar spine and the non-dominant femoral neck, with BMI <25 kg/m² halving the risk of a greater than SDD decrease in BMD. Conversely, the presence of rheumatoid arthritis also halved the risk of a greater than SDD reduction in BMD, but the confidence intervals were wide due to the small numbers.

4.5 Discussion

4.5.1 Summary

In total, 338 of the 422 HIV-positive men enrolled into the study returned for a second visit at 12 months. Compared to baseline, as would be expected, participants were older, had a longer duration of HIV infection and a longer duration of exposure to ART. When comparing lifestyle factors, more men had stopped smoking but alcohol consumption was similar to baseline. There was also a decrease in those who never walked, with an increase in both the some weeks and most weeks categories. There was also a reduction in weight-bearing exercise (all categories) and in muscle-toning exercise (all categories except some weeks). However, BMI was the same as at baseline, and the majority had a normal BMI. There was an increase in those with liver dysfunction, hypogonadism and rheumatoid arthritis, but rates of diabetes and renal disease were similar to baseline. Participants also reported an increase in the use of drugs associated with reduced BMD, including antidepressants, antacids, steroid inhalers, oral steroids and anticonvulsants. There was an increase in the use of drugs associated with increased BMD, including calcium and vitamin D supplements, testosterone replacement and thiazide diuretics. The same 22 men on bisphosphonates were still taking them at 12 months. With regards to HIV-related factors, CD4 count was higher than at baseline, which reflected the increased number of men on ART and an undetectable HIV viral load. Similar to baseline, this cohort remained a group of men with well-controlled HIV infection and good immune function.

There were 84 men who were lost to follow-up, representing a dropout rate of 19.9%. The only significant difference between the men who returned for a second visit and those that did not was that those who returned were older. This suggests that, apart from age, there was no attrition bias between those that returned and those that did not.

4.5.1.1 Change in BMD over time

Absolute BMD at the lumbar spine was higher than that at any of the hip sites, which was the case at baseline. Lumbar spine BMD also increased over the 12 months. These findings may reflect development of age-associated osteoarthritis in the lumbar spine [307], although intra-individual variability may also be a factor. In a study in the general population, an initial increase in BMD at the lumbar spine disappeared once participants with severe arthritis were excluded [207]. However, age-related degenerative changes are more problematic in patients >65 years old [307].

In HIV-positive patients, some longitudinal studies have shown an increase in BMD at the lumbar spine in comparison to HIV-negative controls [340]. Some have also demonstrated an increase in BMD [193,233,339] and in BMC [341] in patients on ART. In one of these studies, the increase in BMD was related to a rise in CD4 count and a decrease in HIV viral load [193]. These are both indicators of successful viral suppression, suggesting that BMD may be improved by good virologic control. Therefore, although age-related degenerative changes could be present, the rise in lumbar spine BMD seen in this cohort may be a reflection of good viral suppression, especially as the mean age was 49 years at the follow-up visit.

Conversely, there was a decrease in BMD over the 12 months at the non-dominant femoral neck, with no significant change at the non-dominant total hip. Unlike at baseline, there was a significant difference in BMD between the left and right total hip, and the left and right femoral neck, with the left-side having a lower BMD at both the total hip and the femoral neck. Although studies have not found a difference in hip BMD related to hand dominance [354,355], further investigation is needed to ascertain why such a difference occurred in this cohort.

As men and women age, they experience a loss in BMD [69]. In women, there is a rapid loss of bone mass, especially trabecular bone, in the 5 to 10 years after the menopause [69]. As men do not have an equivalent hormonal stage to the menopause, they undergo a more gradual loss of both trabecular and cortical bone [202]. In the general population, several early cross-sectional studies estimated bone loss to occur at a fairly slow rate, between 1% and 3% per decade [356-358]. However, longitudinal studies have reported a higher rate of BMD loss, between 5% and 10% per decade [359-361]. These differences may be due to longitudinal studies being able to estimate changes in BMD better than cross-sectional studies, but also suggest that men may have a higher rate of BMD loss than originally thought.

Longitudinal studies in HIV-positive patients have also investigated loss of BMD over time. These have been divided into ART-naïve studies and ART-exposed studies. Although the follow-up period is considerably variable, studies in ART-naïve patients have consistently shown a 2% to 6% reduction in BMD in the first 24 months of commencing ART [29,249,215,251,252,301,302,328]. In some cases, this initial loss of BMD was rapid, varying between 2% and 6% per year [249,252]. BMD then stabilised over time, although it did not reach pre-ART levels [29,328]. In a study with six years of follow-up, an initial small reduction in BMD at both the lumbar spine and the hip did not progress after 12 months [329]. A meta-analysis also showed similar results [337]. Another study which pooled data from three ACTG trials in ART-naïve patients has

reported a mean lowering in BMD of 2% over 96 weeks [362]. In this meta-analysis, low baseline CD4 count (but not a greater increase in CD4 count) was associated with greater BMD loss after initiating ART.

In ART-exposed patients, most studies have shown that BMD remained stable over time [151,159,194,233,296,303,339,340]. Some have also shown either no change in BMD [159,194,303,340] or an increase in lumbar spine BMD [193,233,339]. Stable BMD was also confirmed in a meta-analysis of prospective studies in ART-exposed subjects [337]. As my cohort was comprised mainly of ART-experienced men, it is unsurprising then that the change in BMD over 12 months was small. This is because most had been on ART for many years, and any initial reduction in BMD should have stabilised by the time they were recruited into this study.

With increasing use of TDF/emtricitabine for PrEP, investigators have been able to report on the effect of TDF on BMD in HIV-negative men and women who are at risk of acquiring HIV. In a large study in HIV-uninfected young adults in Botswana comparing daily TDF/emtricitabine with placebo, there was a small but significant reduction in BMD at the lumbar spine, hip and forearm in those on PrEP with TDF/emtricitabine [363]. However, it was noted in this study that a high percentage of healthy young adults in Botswana had abnormal BMD at baseline. In another study assessing PrEP in MSM and transgender women, there was an initial loss of BMD on starting PrEP which was inversely correlated to intracellular TDF levels [338].

4.5.1.2 Methods for measuring change in BMD

Although DXA is the gold-standard investigation for measuring BMD and for measuring change in BMD over time, it does have limitations. Its main limitation in calculating BMD change is precision, although this can be improved by using the same DXA machine for baseline and subsequent visits [94]. However, different machines can produce precision errors that vary between 1.0% to 1.2% at the lumbar spine, 0.9% to 1.3% at the total hip and 1.5% to 1.9% at the femoral neck [351]. Therefore, it is important to consider that a small change in BMD may not reflect an actual change. With this in mind, I investigated different methods of assessing change in BMD to ascertain the best method in this cohort.

Another way of investigating change is to calculate the percentage change in BMD over 12 months. I categorised patients by 1% change in BMD at the different sites. Reflecting the changes in absolute BMD, most men had an increase in lumbar spine BMD of greater than 1%, a decrease in non-dominant femoral neck BMD of greater than 1% and no change at the non-dominant total hip. A 1% decrease is at the upper

limit of normal for what has been reported in the general population [359-361]. However, as there are no reference ranges in HIV-positive patients (either men or women), what is normal for this group of patients is not known. A reference range based on HIV-positive patients would be helpful in determining the clinical significance of a 1% change in BMD in HIV-positive men.

Measuring changes in SD is an alternative method for assessing change in BMD [57]. As BMD was normally distributed in this cohort, I was able to calculate a greater than 2 SD reduction in BMD, which equated to a Z-score of -2 and a definition of reduced BMD. However, the number of participants who experienced a greater than 2 SD decrease in BMD was very small at each site, and no further analyses could be done.

The final method I investigated was a greater than SDD decrease in BMD at 12 months which was devised by Ravaud *et al* to help overcome precision error [352]. The group then investigated changes to SDD obtained from different densitometers, and concluded that the SDD was greater when scans were performed on different machines as opposed to the same densitometer being used for first and second scans [353]. This group have recently applied their principles of SDD to investigate change in BMD over two years in HIV-positive men on ART diagnosed with osteopenia [322]. Although the mean change in absolute BMD was modest at both the lumbar spine ($-0.5 \pm 1.7\%$ per year) and the total hip ($-0.4 \pm 1.8\%$ per year), there was a reduction in BMD by greater than the SDD in 25.5% and 27.7% at the lumbar spine and the total hip, respectively [322]. Additionally, they found that TDF use for <2.5 years was significantly associated with a greater reduction in BMD at both the lumbar spine (OR 2.4 [95% CI 1.2 – 4.9], $p=0.016$) and the total hip (OR 2.8 [95% CI 1.3 – 5.9], $p=0.010$), compared to patients not exposed to TDF, and that this reduction was greater than in patients on TDF for >2.5 years [322].

In summary, the changes in absolute BMD and percentage change seen in this cohort were small, and may reflect precision error rather than true change in BMD [94,351]. Although the number of men with a greater than SDD reduction in BMD in this cohort was not high, and was not as high as those that experienced a greater than 1% change in absolute BMD, I felt that this was a more robust measure of actual changes as it attempted to overcome variability that could happen due to precision error. Therefore, this measure was used to assess factors associated with a change in BMD at 12 months.

4.5.1.3 Factors associated with change in BMD over 12 months in this cohort

All factors associated with BMD that were significant in multivariable analyses at baseline at any site were assessed at 12 months. At the lumbar spine, there were no factors associated with a greater than SDD decrease in BMD. This was unexpected as the lumbar spine was the site which had the highest number of men affected by a greater than SDD reduction in BMD, plus a number of factors were associated with BMD at baseline (Chapter 3). However, as there was a significant increase in BMD at the lumbar spine, and BMD was not measured as a continuous variable like at baseline, these may account for there being no significant factors associated with a greater than SDD reduction on BMD.

Interestingly, the only factor associated with a greater than SDD decrease in BMD was a detectable HIV viral load (vs. an undetectable HIV viral load) at both the non-dominant total hip and the non-dominant femoral neck. This was the case at all hip sites except for the right femoral neck. A study has shown an association between a high viral load and lower BMD [209], which suggests that HIV infection may have an effect on BMD. My results also suggest that detectable viraemia has an effect on reducing BMD, although it would be interesting to see if this association remained in multivariable analysis.

All the other factors that had a significant association showed reduced odds of a greater than SDD decrease in BMD at 12 months at either the non-dominant total hip, the non-dominant femoral neck or both. These included the only two traditional factors found to have an association, including older age and higher BMI. A higher BMI being associated with a higher BMD has been reported in the general population [232]. These results are in keeping with studies in HIV-positive patients that have shown no association between BMD and a higher BMI, suggesting that a higher BMI may reduce the impact of HIV infection on BMD [156]. Conversely, most associations seen between BMD and BMI have occurred at low BMIs, usually seen prior to ART initiation [146,193,194,208,209,233]. Lower BMI has also been reported as an independent risk factor for bone loss in ART-experienced patients [364]. A higher BMD was associated with a higher BMI at baseline in this cohort (Chapter 3), reflecting the well nature of this group of men with good viraemic control.

Surprisingly, older age was associated with a reduced risk of a greater than SDD decrease in BMD. This is contrary to what was found at baseline, and also to what has been reported in both the general population [69] and in HIV-positive patients [153,156,208-211]. However, this association needs further investigation before any conclusions can be drawn.

There were several HIV-related factors that were also associated with reduced odds of a greater than SDD reduction in BMD. Most of these were ART-related, including longer duration of exposure to ART (at the non-dominant femoral neck, right total hip and the left femoral neck), TDF (at the non-dominant total hip, and the left and right total hips), a boosted PI (at the non-dominant femoral neck and the left femoral neck), TDF and a boosted PI concurrently (at the left femoral neck) and an NNRTI (at the right total hip). Several longitudinal studies have shown stable BMD in ART-experienced patients [151,159,194,233,296,303,339,340]. My cohort comprised of mainly ART-experienced patients, and lack of an association may be due to well-controlled HIV infection, suggesting that ART may be helpful in reducing loss of BMD. It would be interesting to see if this association remained with a longer follow-up period.

Surprisingly, in this cohort, more advanced HIV infection was also associated with reduced risk of a greater than SDD reduction in BMD. Most studies have investigated the effect of HIV infection by studying CD4 count and/or HIV viral load. Details relating to HIV viral load are mentioned above. Several studies have shown an association between low nadir CD4 count and lower BMD [210,215,362], as well as fractures [265]. One study has reported a negative correlation between more advanced HIV infection and BMD [151]. In this study, patients with CDC HIV clinical stages B (symptomatic non-AIDS) and C (symptomatic AIDS) had lower BMD than those with stage A (asymptomatic). It is difficult to find a logical explanation for more advanced HIV infection having an association with reduced odds of a greater than SDD decrease in this cohort. One suggestion is that although these patients may have had an AIDS-defining condition in the past, they were currently well with good viraemic control, and a greater than SDD decrease was an indirect marker of HIV viral load. One study previously reported an association between lower BMD and an undetectable HIV viral load [210]. In this study, the authors suggested the association may have been an indirect effect of ART, but were unable to qualify this when further investigations were conducted.

4.5.1.4 Conclusions

In summary, 338 men returned for a second visit at 12 months, which led to a 19.9% loss to follow-up rate. Although the change in absolute BMD over the 12 months was small, there was an increase in BMD at the lumbar spine, a decrease at the non-dominant femoral neck and no change at the non-dominant total hip. I investigated a number of methods to assess change in BMD. In order to take into account precision error caused by DXA, I decided the best method was to use a greater than SDD decrease in BMD.

Interestingly, unlike at baseline, no traditional factors were associated with a greater than SDD reduction in BMD. The only factor that was associated with a greater than SDD reduction in BMD was a detectable HIV viral load, which suggests that uncontrolled HIV infection may have an effect on loss of BMD. This concurs with published data, and it has been postulated that the inflammatory nature of HIV infection leads to a catabolic state where the rate of bone resorption is greater than that of bone formation. Surprisingly, older age was associated with a reduced risk of a greater than SDD decrease in BMD and needs to be further investigated. A number of HIV-related factors were also found to be associated with lower odds of a greater than SDD reduction in BMD, including several related to ART exposure. These suggest that patients on ART have returned to health. By maintaining good immunologic and virologic control, ART may actually be important in reducing BMD loss, especially in ART-experienced patients, where the initial loss of BMD has stabilised. Although more work is needed, these results suggest that there is no need to alter ART regimens in patients with reduced BMD, but to primarily concentrate on ensuring HIV-positive patients are started on ART.

4.5.2 Strengths and limitations

As 338 men returned for a second visit at 12 months, there was a good sample size for conducting analyses. Although the dropout rate was 19.9%, the power calculation done prior to the study took into account a dropout rate of 20% (Chapter 2). However, the power calculation was done for follow-up at 24 months, so this dropout rate at 12 months may have been too high, and a larger sample size might have enabled more meaningful conclusions to be made.

The main limitation of this study was the relatively short period of follow-up. In general, men experience a reduction in BMD of 0.5% to 1% per year [70]. Therefore, the follow-up period of one year was most likely too short to reveal significant changes in BMD. I tried to overcome this problem by categorising patients into those with a greater than SDD loss in BMD over the 12 months as changes in absolute BMD were too small to show any differences. However, as only a small number of participants were affected, most likely due to the short follow-up period, I was only able to conduct univariable logistic regression with adjustment for BMD only.

Additionally, the SDD cut-offs I used had been derived by Kolta *et al* using the QDR 4500 densitometer (Hologic, Bedford, Massachusetts, USA) [353], which was different to the Lunar iDXA densitometer that was used in my study. Hence, the cut-offs may not

have been as accurate as if I had used the same make of densitometer. However, another study used the same SDD cut-offs, and although they did not specify the exact model of the densitometers they used, they scanned patients using either a GE Healthcare Lunar or a Hologic densitometer [322].

4.5.3 Future work

Although beyond the scope of this thesis, following these study participants over a longer period of time (e.g. 5 years, 10 years) would be useful to further investigate BMD, in particular, the rate of loss of BMD over time and the factors associated with a reduction in BMD, in a relatively young, homogeneous group of HIV-positive men in whom the prevalence of reduced BMD would be expected to be low. A follow-up period of 10 years would allow FRAX[®] scores to be compared with the actual rate of fragility fractures. However, a change in BMD over 5 to 10 years may be difficult to interpret as newer ART is introduced and there are changes in other factors, including cohort composition and potential increase in the lost to follow-up rate.

Another option is to calculate SDD cut-off values for the Lunar iDXA densitometer that was used in this study. If the values are different to those published by Kolta *et al* [353], then the analyses could be repeated to see if the results varied from those obtained thus far.

Finally, a validated reference range based on data from HIV-positive patients (ideally HIV-positive men similar in characteristics to this cohort) would be extremely useful in interpreting reduced BMD results in HIV-positive men, and further analysing the clinical significance of a loss of BMD.

The findings from this Chapter, together with the results from Chapter 3, have been presented at the 21st Conference on Retroviruses and Opportunistic Infections, Boston, USA in March 2014 (poster) and at the 3rd Joint Conference of BHIVA with BASHH in Liverpool in April 2014 (poster):

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. Minimal change in bone density and no association with HIV factors over 12 months in HIV-infected men (Poster 777).

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. Minimal change in bone density over 12 months in cART-experienced HIV-infected men (Poster 163), HIV Med 2014;15(3):1–16.

4.6 Supplementary data

4.6.1 Change in absolute and percentage BMD at the left and right total hips and the left and right femoral necks at 12 months

Table 4.6.1.1 Absolute BMD at baseline and at 12 months, including the change in absolute BMD and percentage change, at the left and right total hips and the left and right femoral necks

There was a significant decrease in mean absolute BMD at the left femoral neck, but not at the right femoral neck, nor at the left or right total hips. The percentage change at each site was very small overall.

Site	N	Absolute BMD at baseline, g/cm ² , mean (SD)	Absolute BMD at 12 months, g/cm ² , mean (SD)	Change in absolute BMD, g/cm ² , mean (SD)	Percentage change in BMD, %, mean (SD)	P-value*
Left total hip	307	1.003 (0.14)	1.003 (0.14)	0.001 (0.02)	0.11 (2.57)	0.56
Right total hip	302	1.007 (0.14)	1.007 (0.14)	0.001 (0.03)	0.12 (2.91)	0.65
Left femoral neck	309	0.950 (0.13)	0.946 (0.13)	-0.005 (0.03)	-0.40 (3.77)	0.01
Right femoral neck	308	0.960 (0.13)	0.957 (0.13)	-0.003 (0.03)	-0.17 (3.66)	0.15

BMD: bone mineral density; SD: standard deviation

* P-value is for change in absolute BMD

4.6.2 Change in BMD by 1%

Table 4.6.2.1 Change in BMD by 1% at the left and right total hips and the left and right femoral necks

A >1% decrease in BMD was only seen at the left femoral neck, while there was no change at the other sites.

Site	N	>1% decrease in BMD	No change in BMD (-1% to 1%)	>1% increase in BMD
Left total hip	307	91 (29.6)	129 (42.0)	87 (28.3)
Right total hip	302	89 (29.5)	115 (38.1)	98 (32.5)
Left femoral neck	309	128 (41.4)	110 (35.6)	71 (23.0)
Right femoral neck	305	115 (37.7)	101 (33.1)	89 (29.2)

BMD: bone mineral density

4.6.3 Smallest detectable difference (SDD)

Table 4.6.3.1 A greater than SDD decrease in BMD at 12 months at the left and right total hips and the left and right femoral necks 12 months

There were a similar number of men affected at the left and right total hip, and at the left and right femoral necks.

	N	>SDD decrease in BMD, n (%)
Left total hip	307	25 (8.1)
Right total hip	302	29 (9.6)
Left femoral neck	309	32 (10.4)
Right femoral neck	305	25 (8.2)

BMD: bone mineral density; SDD: smallest detectable difference

4.6.4 Factors associated with a greater than SDD decrease in BMD at the left total hip at 12 months

Table 4.6.4.1 Factors associated with a greater than SDD decrease in BMD at the left total hip

The only factor associated with a greater than SDD reduction in BMD at 12 months was a detectable HIV viral load. Older age, BMI 25-30 kg/m², symptomatic non-AIDS disease, current exposure to a boosted PI and longer duration of exposure to TDF were associated with reduced odds of a greater than SDD decrease in BMD at 12 months in univariable analyses.

	OR* (95% CI)	P-value
Age, per 10 years	0.50 (0.32, 0.79)	0.003
Ethnicity		
White	1.00	-
Other	0.68 (0.09, 5.39)	0.72
Smoking		
Never	1.00	-
Ex-smoker	0.35 (0.11, 1.13)	0.08
Current smoker	0.87 (0.35, 2.18)	0.77
Exercise**		
Never/some weeks	-	-
Most weeks	-	-
Every week	-	-
BMI		
<25	1.00	-
25-30	0.14 (0.03, 0.64)	0.01
>30	0.81 (0.24, 2.78)	0.74
Hypogonadism**		
No	-	-
Yes	-	-
Rheumatoid arthritis		
No	1.00	-
Yes	0.89 (0.11, 7.21)	0.91
Steroids		
No	1.00	-
Yes	1.02 (0.36, 2.86)	0.97
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	1.14 (0.37, 3.55)	0.82
Duration of HIV infection, per year	0.98 (0.93, 1.05)	0.59
HIV clinical stage		
Asymptomatic	1.00	-
Symptomatic non-AIDS	0.27 (0.08, 0.95)	0.04
Symptomatic AIDS	0.53 (0.19, 1.50)	0.23

	OR* (95% CI)	P-value
CD4 count, per 50 cells/ μ L		
Nadir	1.12 (0.98, 1.28)	0.09
HIV viral load <40, copies/mL		
Yes	1.00	-
No	4.84 (1.96, 11.95)	0.001
Current ART regimen		
None	1.00	-
ART including TDF	0.44 (0.14, 1.38)	0.16
ART including PI/ritonavir	0.09 (0.01, 0.81)	0.03
ART including TDF + PI/ritonavir	0.27 (0.07, 1.03)	0.06
Other ART	0.79 (0.13, 4.78)	0.80
Cumulative ART exposure, per year***	0.93 (0.85, 1.01)	0.10
Cumulative TDF exposure, per year***	0.73 (0.56, 0.96)	0.02
Cumulative PI/ritonavir exposure, per year***	0.89 (0.77, 1.04)	0.14
Cumulative NNRTI exposure, per year***	0.92 (0.82, 1.03)	0.15

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**This could not be calculated as there were too few subjects in the control group

***Includes all patients who have ever been exposed

4.6.5 Factors associated with a greater than SDD decrease in BMD at the right total hip at 12 months

Table 4.6.5.1 Factors associated with a greater than SDD decrease in BMD at the right total hip

The only factor associated with a greater than SDD reduction in BMD at 12 months was a detectable HIV viral load. However, BMI 25-30 kg/m², an AIDS diagnosis, current exposure to a boosted PI, and longer duration of exposure to ART, TDF and an NNRTI were associated with reduced odds of a greater than SDD decrease in BMD at 12 months in univariable analyses.

	OR* (95% CI)	P-value
Age, per 10 years	0.66 (0.43, 1.01)	0.06
Ethnicity		
White	1.00	-
Other	1.10 (0.24, 5.12)	0.90
Smoking		
Never	1.00	-
Ex-smoker	0.39 (0.13, 1.16)	0.09
Current smoker	1.08 (0.45, 2.57)	0.86
Exercise		
Never/some weeks	1.00	-
Most weeks	3.61 (0.31, 41.81)	0.30
Every week	6.72 (0.89, 50.97)	0.07
BMI		
<25	1.00	-
25-30	0.30 (0.10, 0.86)	0.03
>30	0.81 (0.26, 2.57)	0.72
Hypogonadism**		
No	-	-
Yes	-	-
Rheumatoid arthritis		
No	1.00	-
Yes	0.90 (0.11, 7.27)	0.92
Steroids		
No	1.00	-
Yes	1.47 (0.59, 3.66)	0.41
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	0.73 (0.21, 2.54)	0.62

	OR* (95% CI)	P-value
Duration of HIV infection, per year	0.95 (0.90, 1.01)	0.12
HIV clinical stage		
Asymptomatic	1.00	-
Symptomatic non-AIDS	0.37 (0.13, 1.03)	0.06
Symptomatic AIDS	0.25 (0.07, 0.87)	0.03
CD4 count, per 50 cells/ μ L		
Nadir	1.12 (0.99, 1.26)	0.07
HIV viral load <40, copies/mL		
Yes	1.00	-
No	5.27 (2.26, 12.30)	0.0001
Current ART regimen		
None	1.00	-
ART including TDF	0.42 (0.14, 1.23)	0.12
ART including PI/ritonavir	0.08 (0.01, 0.71)	0.02
ART including TDF + PI/ritonavir	0.41 (0.13, 1.32)	0.14
Other ART	0.29 (0.03, 2.69)	0.28
Cumulative ART exposure, per year***	0.85 (0.77, 0.94)	0.001
Cumulative TDF exposure, per year***	0.66 (0.50, 0.88)	0.01
Cumulative PI/ritonavir exposure, per year***	0.88 (0.76, 1.02)	0.09
Cumulative NNRTI exposure, per year***	0.79 (0.68, 0.93)	0.003

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**This could not be calculated as there were too few subjects in the control group

***Includes all patients who have ever been exposed

4.6.6 Factors associated with a greater than SDD decrease in BMD at the left femoral neck at 12 months

Table 4.6.6.1 Factors associated with a greater than SDD decrease in BMD at the left femoral neck

The only factor associated with a greater than SDD reduction in BMD at 12 months was a detectable HIV viral load. Older age, current simultaneous exposure to TDF and a boosted PI, and longer duration of exposure to ART and a boosted PI were associated with reduced odds of a greater than SDD decrease in BMD at 12 months in univariable analyses.

	OR* (95% CI)	P-value
Age, per 10 years	0.64 (0.42, 0.97)	0.04
Ethnicity		
White	1.00	-
Other	0.31 (0.04, 2.61)	0.28
Smoking		
Never	1.00	-
Ex-smoker	0.76 (0.31, 1.84)	0.54
Current smoker	0.78 (0.31, 1.95)	0.59
Exercise		
Never/some weeks	1.00	-
Most weeks	1.30 (0.27, 6.32)	0.75
Every week	1.47 (0.48, 4.51)	0.50
BMI		
<25	1.00	-
25-30	0.52 (0.21, 1.27)	0.15
>30	0.68 (0.22, 2.15)	0.51
Hypogonadism		
No	1.00	-
Yes	1.03 (0.22, 4.73)	0.97
Rheumatoid arthritis		
No	1.00	-
Yes	0.92 (0.11, 7.54)	0.94

	OR* (95% CI)	P-value
Steroids		
No	1.00	-
Yes	2.08 (0.89, 4.90)	0.09
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	1.73 (0.65, 4.59)	0.27
Duration of HIV infection, per year	0.95 (0.89, 1.01)	0.08
HIV clinical stage		
Asymptomatic	1.00	-
Symptomatic non-AIDS	0.68 (0.27, 1.73)	0.42
Symptomatic AIDS	0.66 (0.25, 1.75)	0.40
CD4 count, per 50 cells/ μ L		
Nadir	1.09 (0.96, 1.23)	0.18
HIV viral load <40, copies/mL		
Yes	1.00	-
No	2.77 (1.14, 6.73)	0.02
Current ART regimen		
None	1.00	-
ART including TDF	0.66 (0.23, 1.94)	0.45
ART including PI/ritonavir	0.20 (0.04, 1.11)	0.07
ART including TDF + PI/ritonavir	0.24 (0.06, 0.96)	0.04
Other ART	1.32 (0.26, 6.66)	0.73
Cumulative ART exposure, per year**	0.88 (0.80, 0.96)	0.01
Cumulative TDF exposure, per year**	0.83 (0.68, 1.02)	0.08
Cumulative PI/ritonavir exposure, per year**	0.82 (0.69, 0.98)	0.03
Cumulative NNRTI exposure, per year**	0.93 (0.83, 1.03)	0.16

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**Includes all patients who have ever been exposed

4.6.7 Factors associated with a greater than SDD decrease in BMD at the right femoral neck at 12 months

Table 4.6.7.1 Factors associated with a greater than SDD decrease in BMD at the right femoral neck

There were no factors associated with a greater than SDD reduction in BMD at the lumbar spine at 12 months. Only BMI 25-30 kg/m² was associated with reduced odds of a greater than SDD decrease in BMD at 12 months in univariable analyses.

	OR* (95% CI)	P-value
Age, per 10 years	0.78 (0.49, 1.25)	0.30
Ethnicity		
White	1.00	-
Other	0.42 (0.05, 3.51)	0.42
Smoking		
Never	1.00	-
Ex-smoker	0.57 (0.19, 1.65)	0.30
Current smoker	1.08 (0.41, 2.85)	0.88
Exercise**		
Never/some weeks	-	-
Most weeks	-	-
Every week	-	-
BMI		
<25	1.00	-
25-30	0.30 (0.10, 0.88)	0.03
>30	0.54 (0.15, 1.98)	0.36
Hypogonadism		
No	1.00	-
Yes	0.56 (0.07, 4.42)	0.58

	OR* (95% CI)	P-value
Rheumatoid arthritis		
No	1.00	-
Yes	1.24 (0.15, 10.30)	0.84
Steroids		
No	1.00	-
Yes	1.63 (0.60, 4.38)	0.33
Calcium and/or vitamin D supplements		
No	1.00	-
Yes	1.44 (0.46, 4.53)	0.53
Duration of HIV infection, per year	1.00 (0.94, 1.06)	0.94
HIV clinical stage		
Asymptomatic	1.00	-
Symptomatic non-AIDS	0.69 (0.24, 2.04)	0.51
Symptomatic AIDS	0.89 (0.32, 2.46)	0.82
CD4 count, per 50 cells/ μ L		
Nadir	1.00 (0.87, 1.16)	0.96
HIV viral load <40, copies/mL		
Yes	1.00	-
No	1.57 (0.54, 4.58)	0.41
Current ART regimen		
None	1.00	-
ART including TDF	1.13 (0.29, 4.44)	0.86
ART including PI/ritonavir	0.47 (0.07, 3.11)	0.43
ART including TDF + PI/ritonavir	0.84 (0.19, 3.77)	0.82
Other ART	0.79 (0.07, 8.60)	0.84
Cumulative ART exposure, per year***	0.98 (0.90, 1.07)	0.67
Cumulative TDF exposure, per year***	0.94 (0.77, 1.16)	0.57
Cumulative PI/ritonavir exposure, per year***	1.00 (0.89, 1.13)	0.98
Cumulative NNRTI exposure, per year***	1.01 (0.92, 1.12)	0.78

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TDF: tenofovir

*Adjusted for baseline BMD

**This could not be calculated as there were too few subjects in the control group

***Includes all patients who have ever been exposed

Chapter 5: Fracture assessment, fracture risk and screening tools

5.1 Background

5.1.1 Introduction

BMD has been discussed in detail in Chapters 3 and 4. In this Chapter, I concentrate on fracture assessment and fracture risk in the entire cohort at baseline. I investigate factors associated with fragility fractures, including family history of osteoporosis and/or fractures, past history of fragility fractures, and reduced mobility and falls. I also calculate FRAX[®] scores in all participants and investigate how FRAX[®] scores vary when BMD is or is not included, as well as with or without HIV as a secondary risk factor. Finally, I compare pDXA to cDXA and assess the utility of both FRAX[®] and pDXA as screening tools in calculating future fracture risk.

5.1.2 Risk factors for fractures in epidemiological studies

5.1.2.1 Fragility fractures

A past history of a fragility fracture is a non-modifiable risk factor for future fracture risk. Cross-sectional studies on the general population have found a prevalent fracture is associated with reduced BMD [204,365-367]. A longitudinal study in the general population has also shown an association between prevalent fracture and bone loss at the lumbar spine, but not at the hip, although a lack of association at the hip may have been due to lack of power [368]. A meta-analysis has demonstrated that a past history of fracture was associated with an increased risk of future osteoporotic or hip fractures in both men and women which was greater than that explained by measurement of BMD alone [369].

A number of studies comparing HIV-positive patients (both men and women) have found no difference in the rates of past fracture history compared to HIV-negative controls [157,323]. However, in these studies, the sites of fracture were not mentioned, and 'any' history of a fracture has been used. Similar results have also been reported in studies comparing HIV-positive to HIV-negative women [153,370,371]. In the study by Arnsten *et al*, fractures were specifically categorised at the wrist and lumbar spine [153], whilst in the latter two studies from the same cohort, previous fracture has been

defined as a fracture at the hip, the wrist or the lumbar spine ever, or in the preceding six months [370,371]. Two RCTs in HIV-positive cohorts have also compared past history of fractures in patients on different combinations of ART and found no difference [251,252]. In the first study, past fractures were classified as vertebral or non-vertebral [251], whilst in the second study, the sites included were not mentioned [252].

One of the main issues in comparing fragility fractures is that the classification of fractures varies between studies. In my study, fragility fractures were classified according to the sites associated with osteoporotic fractures [372] and this is discussed in more detail in Section 5.3.2.

5.1.2.2 Family history of osteoporosis

A positive family history of fracture and/or osteoporosis is another non-modifiable risk factor. It is thought to lead to thinner cortices and lower BMC in both men and women, which in turn leads to a higher risk of sustaining a femoral neck fracture [373]. However, some cross-sectional studies in the general population have shown an association with reduced BMD [204,373], whilst others have not [365,374]. A lack of an association may be due to poor recall, or because most studies do not specifically inquire about maternal or paternal history of fractures and/or osteoporosis. A meta-analysis found that a history of a hip fracture in a parent was associated with a higher risk of all osteoporotic fractures, including hip fractures [375].

Very few studies of HIV-positive patients have investigated the association between family history and risk of osteoporosis and/or fragility fractures. Two studies have shown no difference in rates between HIV-positive and HIV-negative patients [158,306], although in the first study there was borderline significance for a higher rate of family history in HIV-positive women who were not on ART compared to those who were [158]. The pilot study for my study also found no association between family history of osteoporosis and reduced BMD [160].

5.1.2.3 Falls and reduced mobility

Fall rates increase with age. Studies in the general population have reported that 30% of people ≥ 65 years and 40% to 50% of those ≥ 80 years have had a fall in the preceding year [376,377]. Falls can be associated with serious injury, resulting in fractures in 5% to 10% of white women and hip fractures in 1% [378]. Additionally, the primary risk factor for a hip fracture is a fall, with over 90% of fractures occurring after a fall [378,379]. The majority of fractures occur in people ≥ 65 years old [380]. As the

population continues to grow older, the worldwide incidence of hip fractures is expected to rise by 240% in women and 310% in men by 2050 [80].

BMD alone does not determine the risk of a fracture, with the mechanics of a fall also being important [378,381]. The circumstances and the direction of a fall help to determine fracture type, whilst the energy of the fall and factors offering protection (e.g. soft tissues over the bone), as well as BMD, help to determine whether or not a fracture occurs [378,381]. For example, hip fractures usually occur indoors in less active people who fall from a standing height and tend to fall sideways or straight down on to their hip [381]. In contrast, distal forearm fractures tend to occur outdoors in more active older people who display a greater forward momentum when they fall [382]. Immobility can also lead to fractures due to restriction of movement and activities, and can occur in people who have fallen once and then develop a fear of falling, leading to a restricted lifestyle [383].

There are numerous risk factors for falls. These include older age, increased frailty, co-morbidities (e.g. arthritis, diabetes), physical impairments (e.g. visual impairment, gait instability, cognitive impairment, neuropathy, strength), medication (e.g. antihypertensives, antidepressants, anticonvulsants, benzodiazepines) especially polypharmacy, excess alcohol use, VDD, home hazards or a combination of several risk factors [384-386]. Further evidence for other factors involved in fracture risk is shown in people with osteoarthritis of the weight-bearing joint, who are still at risk of fracture, despite osteoarthritis causing an increase in BMD [387].

Although studies have shown an association between falls and fracture risk, and there are plausible mechanisms involved, fall assessment has not been standardly assessed in RCTs or epidemiologic datasets [388]. This has led to falls not being included in fracture prediction tools, which is discussed further in Section 5.1.4.

HIV-positive patients are at risk of accelerated ageing, which has led to them being susceptible to age-related co-morbidities at a younger age than in the general population [389]. HIV-positive patients have a high prevalence of risk factors and co-morbidities associated with ageing [390]. These risk factors include those associated with falling, such as multiple co-morbidities, medications and functional impairment [391,392]. In Erlandson *et al's* study in American HIV-positive patients, co-morbidities associated with falling were cardiovascular disease, hypertension, dementia, neuropathy, arthritis, chronic pain, psychiatric disease, frailty or disability [391]. Additionally, certain drugs were independently associated with falling and included beta-blockers, antidepressants, antipsychotics, sedatives, and opiates. They found that

female gender, diabetes, antidepressants, sedatives, opiates, didanosine, exhaustion, weight loss and difficulty with balance were the most significant predictors of falls [391]. In another smaller American study, falls were associated with being on more than five medications, having more than three co-morbidities and non-compliance with medications [392].

5.1.3 pDXA

Although cDXA remains the gold standard investigation for measuring BMD [90,95], pDXA of the distal forearm can be used to screen high-risk populations [111]. Scans can be performed with portable equipment, are quick and use very low levels of radiation. The UK National Osteoporosis Society recommends using pDXA as an adjunct to cDXA [114]. The main advantage of pDXA is that it can be performed by non-radiographers, making it suitable for screening in an outpatient setting.

The concern with reduced BMD is that it can lead to hip and vertebral fractures [58]. BMD at the distal forearm has been shown to be a good predictor of future hip fracture risk [113,393]. A population-based American study reported that the cumulative incidence of any fracture following a distal forearm fracture was 55% at 10 years and 80% at 20 years [394]. In this study, there was an increased risk of a future hip fracture in both men and women when compared to expected fracture rates in the community. Women >70 years old had a higher risk of a hip fracture following the distal forearm fracture than those ≤70 years old. In contrast, the risk of a future vertebral fracture was increased in all age groups, with men having the highest risk.

Most studies evaluating pDXA have been performed in post-menopausal women and older institutionalised adults [117,118]. A pilot study for this cohort showed good correlation between pDXA and cDXA and that pDXA was acceptable in HIV-positive men [395]. Data suggest that a combination of pDXA and assessment of risk factors can reduce the need for cDXA [115]. Additionally, this approach has been shown to be cheaper than cDXA alone [116]. If pDXA is to be used as a screening tool, it needs to correlate with BMD at the lumbar spine and hip (total hip and femoral neck) without over- or under-predicting BMD. If this was the case, it could be used as a screening test in the HIV outpatient clinic, with cDXA reserved for those with proven low BMD on pDXA.

5.1.4 Assessment of fracture risk

Fracture is a potentially fatal outcome of reduced BMD [58]. Although reduced BMD is important, there are a number of other risk factors that contribute to fracture risk that are independent of BMD [61,388]. Therefore, assessment tools to predict the risk of fracture and treat it accordingly are invaluable. The WHO has approved the FRAX[®] score, a fracture assessment tool based on key risk factors, as well as femoral neck BMD (if available), which calculates the 10-year absolute risk of a major osteoporotic fracture, as well as that of a hip fracture (<http://www.shef.ac.uk/frax>, Figure 5.1.4.1). It has primarily been validated in women >50 years old and in men. Important risk factors used in the model include past history of fracture, family history of hip fracture, current smoking, corticosteroid use, rheumatoid arthritis, the presence of other causes of secondary osteoporosis and excess alcohol intake (>3 units/day) [61]. The FRAX[®] tool can be used to assess fracture risk in men and women using risk factors only and/or by combining with BMD data [396]. FRAX[®] scores use National Osteoporosis Guideline Group (NOGG) guidance to recommend management options including the need for specific pharmacologic intervention (e.g. bisphosphonates) [397].

Figure 5.1.4.1 A screen shot of the WHO FRAX[®] tool (<http://www.shef.ac.uk/frax>, accessed 30th January 2016)

The FRAX[®] tool predicts the 10-year probability of a major osteoporotic fracture, as well as that of a hip fracture. It provides guidance on management using NOGG.

FRAX[®]: Fracture Risk Assessment Tool; NOGG: National Osteoporosis Guideline Group

FRAX[®] Fracture Risk Assessment Tool

Home Calculation Tool Paper Charts FAQ References English

Calculation Tool

Please answer the questions below to calculate the ten year probability of fracture with BMD.

Country: UK Name/ID: About the risk factors

Questionnaire:

1. Age (between 40 and 90 years) or Date of Birth
Age: Date of Birth: Y: M: D:

2. Sex Male Female

3. Weight (kg)

4. Height (cm)

5. Previous Fracture No Yes

6. Parent Fractured Hip No Yes

7. Current Smoking No Yes

8. Glucocorticoids No Yes

9. Rheumatoid arthritis No Yes

10. Secondary osteoporosis No Yes

11. Alcohol 3 or more units/day No Yes

12. Femoral neck BMD (g/cm²)
GE-Lunar T-score: -0.3

BMI: 21.1
The ten year probability of fracture (%)

Major osteoporotic	5.8
Hip Fracture	0.5

If you have a TBS value, click here:

Weight Conversion
Pounds → kg

Height Conversion
Inches → cm

04570276
Individuals with fracture risk assessed since 1st June 2011

www.nos.org.uk
National Osteoporosis Society

Although FRAX[®] includes a number of important risk factors for future osteoporotic fractures, a previous history of falls is not one of them. This is because fall assessment has not been standardised in large studies [388]. Additionally, falls have historically been assessed retrospectively, and are heavily subject to recall bias, with those that do not cause significant injury and those with a longer follow-up period being forgotten [398]. However, the Garvan normogram (<https://www.garvan.org.au/promotions/bone-fracture-risk/calculator/>), another fracture assessment tool which has been developed in Australia as an alternative to FRAX[®], does include fall as a risk factor for future fracture. When the two fracture prediction tools were compared, they reported similar accuracy rates in post-menopausal women, with the Garvan normogram being better in men [399,400]. The similar accuracy of both, although fall history was included in the Garvan normogram, may be due to the longer length of fall assessment in the Garvan normogram [398].

Currently, screening for reduced BMD in the UK general population is not recommended [401]. However, strategies combining the use of a case-finding approach with fracture assessment, and the additional testing of BMD, are likely to be beneficial. Many guidelines on the management of reduced BMD and fracture risk recommend the use of FRAX[®] scores in HIV-positive patients [402,403].

Although HIV-positive patients are at risk of many chronic co-morbidities associated with ageing, few risk calculators have been adjusted to take into account the additional risk conferred by HIV infection [404]. This is also true for the FRAX[®] tool, which has not been validated in HIV-positive patients, which therefore may lead to under-estimation of predicted scores [405]. Several studies have investigated whether FRAX[®] scores calculated using classic risk factors only are sufficiently discriminatory to determine which HIV-positive patients would benefit from DXA screening. In the first study that evaluated the use of FRAX[®] in HIV-positive patients, Calmy *et al* compared the FRAX[®] score with and without BMD [199]. They investigated whether classic risk factors alone would be sufficient in identifying HIV-positive patients with low BMD. However, they found that the FRAX[®] score based only on classic risk factors was not able to distinguish between HIV-positive patients with osteopenia to those without, and that the FRAX[®] score was more sensitive when calculated using femoral neck BMD [199]. Another similar study also reported that the FRAX[®] score using classic risk factors alone was insensitive in HIV-positive patients, with the addition of HIV as a secondary risk factor increasing sensitivity [406]. However, the specificity of the FRAX[®] score with classic risk factors in patients with normal BMD was 83% [406]. Pepe *et al* also reported similar results (sensitivity 23%, specificity 100%) in HIV-positive men for

detecting 'bone fragility' (defined as T-score <-2.5 or T-score between -2.5 and -1.0 with fracture) when the FRAX[®] score was set at a 7% threshold [407].

Additionally, two studies have investigated the utility of DXA screening guidelines instead of using the FRAX[®] tool. In the French ANRS-120 FOSIVIR study, screening all patients >50 years old with DXA led to a sensitivity and specificity of 52% and 65%, respectively [408]. However, they found that using a combination of age, BMI and CD4 cell count increased sensitivity and specificity to 65% and 67%, respectively. In the second study, which followed Italian guidelines on DXA screening, using two risk factors other than HIV infection to detect a Z-score of ≤ -2 only resulted in sensitivity and specificity of 32% and 81%, respectively [409]. The differences seen in these two studies are most likely attributable to the use of very different criteria for screening. The Italian study included patients with two risk factors (but HIV infection was not one of them) [409], whilst the French study took into account immunosuppression [408]. The results from the French study suggest that the inclusion of HIV-related criteria in screening guidelines may increase the sensitivity of DXA in HIV-positive patients.

Finally, a study has investigated the accuracy of a modified-FRAX[®] tool (history of secondary osteoporosis and parental hip fracture were not included) in predicting fracture risk in HIV-positive individuals [410]. Yin *et al* included 24,451 HIV-positive and HIV-negative men aged 50 to 70 years old from the Veterans Aging Study Virtual Cohort (VACS-VC). They compared the modified-FRAX[®] score without BMD data against the 10-year observational data for incident fragility fracture. They found that although the accuracy improved when HIV infection was included as a risk factor for secondary osteoporosis, the modified-FRAX[®] score still under-estimated fracture rates in HIV-positive men compared to those that were HIV-negative, and that the tool was worse in older men [410]. However, using a FRAX[®] score with complete risk factors may have improved the sensitivity and specificity.

In summary, these studies suggest that a FRAX[®] score based on classic risk factors in HIV-positive patients is not accurate enough to identify patients at risk of fracture and therefore cannot replace DXA screening. This was also the case even when HIV infection was included as a secondary cause of osteoporosis. The results suggest that the FRAX[®] tool's utility is in identifying 'at risk' patients who would benefit from DXA screening to assess BMD, although more studies are needed to further evaluate this. It is also worth noting that direct comparison between studies can be difficult due to the differences in patient populations, the use of different screening criteria and the inclusion of varied risk factors.

5.1.5 Fractures in HIV-positive patients

Most studies investigating fractures in HIV-positive patients have been conducted in Europe, North America and Australia. There was contradictory evidence from earlier studies, although latter ones have shown a sharp rise in fracture rates in the first few years after diagnosis of HIV infection [411]. Some studies have compared fractures with those in the general population, whereas others have investigated fracture rates in HIV-positive populations only. In a meta-analysis of incident fractures in HIV-positive individuals, Shiau *et al* analysed data from 13 eligible studies [162]. In seven studies, fracture rates in HIV-positive patients were compared to those in the general population. Nine studies reported data on fracture incidence relating to all fractures, whilst ten presented data on fragility fractures only. The pooled incidence rate ratio was 1.58 (95% CI 1.25, 2.00) for all fractures and 1.35 (95% CI 1.10, 1.65) for fragility fractures [162]. Factors associated with fragility fractures were older age, white ethnicity and smoking. Additionally, cross-sectional studies have reported low rates of new fractures following a diagnosis of HIV infection [145,147,208].

5.1.5.1 Fracture rates compared to HIV-negative patients

Despite less data relating to fractures than BMD, there is increasing evidence that HIV-positive patients may have a modest increase in fracture risk compared to the general population for both fragility and non-fragility fractures (Table 5.1.5.1). Fracture rates are likely to increase as HIV-positive patients become older. However, early studies showed that there was no increased risk compared to HIV-negative populations [156,370]. In an observational longitudinal study, comparing 328 HIV-positive men to 231 HIV-negative men, there was a higher rate of fractures in the HIV-positive group (hazard ratio [HR] 1.38; 95% CI 0.63, 3.01), although this did not reach statistical significance [156]. There was also no increased fracture risk seen in 1728 American HIV-positive women compared to HIV-negative women who were followed for five years in a multicentre study [370].

Table 5.1.5.1 Studies investigating fracture prevalence and incidence in HIV-positive patients compared to HIV-negative patients

Despite earlier studies showing contradictory evidence, several more have consistently shown that HIV-positive patients have a moderate increase in fracture risk compared to HIV-negative individuals.

Author (name of study)	Year	Location	Study design	N	HIV-positive, n (%)	% male	Mean age (years)	% ART-exposed	Fractures, n	Fracture prevalence/incidence
Arnsten [156] (CHAMPS)	2007	USA	Prospective	559	328 (59)	100	55 ^a	87	21 in HIV-positive vs. 12 in HIV-negative	Fracture incidence rates/100 person-years 1.4, 3.6 and 6.5 for HIV-positive men with normal BMD, osteopenia or osteoporosis. No difference in rates between HIV-positive vs. HIV-negative men (3.1 vs. 2.6/100 person-years, p=0.69).
Prior [412]	2007	Canada	Case-control	540	138 (26)	0	38	72	-	OR 1.7 (95% CI 1.1, 2.6) but no difference in BMD between HIV-positive women and those in general population.
Triant [413]	2008	USA	Population-based	2,217,317	8525 (0.4)	44	-	-	-	Overall fracture prevalence 2.87 (95% CI 2.52, 3.23)/100 persons in HIV-positive vs. 1.77 (95% CI 1.75, 1.79)/100 persons in HIV-negative patients.
Yin [370] (WIHS)	2010	USA	Prospective	2391	1728 (72)	0	40	66	148 in HIV-positive vs. 47 in HIV-negative	No increase in fracture risk in HIV-positive women.
Womack [414] (VACS-VC)	2011	USA	Case-control	119,318	40115 (33)	100	-	75	1615	HR 1.24 (95% CI 1.11, 1.39), with risk attenuated by BMI.
Young [265] (HOPS)	2011	USA	Prospective	224,485,500	5826 (0.003)	79	40 ^a	73	233	Age-adjusted fracture rates higher than in general population during 2000-2006.
Hansen [415]	2012	Denmark	Population-based	31,836	5306 (17)	76	37 ^a	78	806 in HIV-positive vs. 3312 in HIV-negative	IRR 1.5 (95% CI 1.4, 1.7) for all fractures in HIV-positive patients. Increased fracture risk associated with 'low energy' (vs. 'high energy') fractures and ART.
Torti [416]	2012	Italy	Cross-sectional	323	160 (50)	100	53 ^a	78	43 in HIV-positive vs. 21 in HIV-negative	Prevalence of vertebral fractures higher in HIV-positive patients vs. controls (26.9% vs. 12.9%; p=0.002).

Author (name of study)	Year	Location	Study design	N	HIV-positive, n (%)	% male	Mean age (years)	% ART-exposed	Fractures, n	Fracture prevalence/incidence
Guerra-Fernandez [417]	2013	Spain	Population-based	1,118,156	2489 (0.2)	-	50	-	49 in HIV-positive vs. 24,408 in HIV-negative	Adjusted HR 2.7 (95% CI 2.01, 3.5) for major fractures and 6.2 (95% CI 3.5, 10.9) for hip fractures.
Peters [306]	2013	UK	Cross-sectional	444	222 (50)	60	46	85	45 in HIV-positive vs. 16 in HIV-negative	Fracture prevalence higher (OR 3.27, p=0.0001) and RLFP higher (OR 1.22 [95% CI 1.07, 1.40], p=0.003) in HIV-positive vs. HIV-negative.
Prieto-Alhambra [418]	2014	Denmark	Case-control	498,617	102 (0.02)	48	43	-	50	Increased risk of any fracture (OR 2.89 [95% CI 1.99, 4.18])
Byrne [419]	2015	USA	Retrospective cohort	849,256	100,409 (12)	32	41	100	-	HIV/HBV co-infected patients had higher cumulative hip fracture incidence vs. HIV-mono-infected (adjusted HR, 1.37 [95% CI, 1.03, 1.83]) and HIV/HBV-negative (adjusted HR, 1.35 [95% CI, 1.03, 1.84]) patients.
Sharma [371] (WIHS)	2015	USA	Prospective	2375	1713 (72)	0	40 ^a	63	300 in HIV-positive vs. 90 in HIV-negative	Unadjusted incidence rates (any fracture) higher in HIV-positive vs. HIV-negative women (2.19/100 vs. 1.54/100 person-years, p=0.002).

95% CI: 95% confidence interval; ART: antiretroviral therapy; BMI: body mass index; BMD: bone mineral density; CHAMPS: Cohort of HIV at-risk Aging Men's Prospective Study; HBV: hepatitis B; HOPS: HIV Outpatient Study; HR: hazard ratio; IRR: incidence rate ratio; OR: odds ratio; RLFP: remaining lifetime fracture probability; VACS-VC: Veterans Aging Study Virtual Cohort; WIHS: Women's Interagency HIV Study

^amedian age

In a large population-based American study using data registry records from a secondary care setting, an increased prevalence of fractures was seen in HIV-positive patients compared to HIV-negative individuals [413]. Fracture rates in 8,525 HIV-positive patients were compared to 2.2 million HIV-negative patients with at least one inpatient or outpatient episode over an 8-year period. The total fracture prevalence was 2.87 (95% CI 2.52, 3.23) per 100 persons in HIV-positive patients compared to 1.77 (95% CI 1.75, 1.79) per 100 persons in HIV-negative individuals ($p < 0.0001$) [413]. Rates of fracture were higher in black and white HIV-positive females, and in white HIV-positive males. HIV-positive women had a higher prevalence of vertebral and wrist fractures compared to HIV-negative women. HIV-positive men had the highest prevalence of fractures (3.08 per 100 persons, 95% CI 2.62, 3.53 vs. 1.83 per 100 persons, 95% CI 1.81, 1.86; $p < 0.0001$) at all three of the commonest sites (lumbar spine, femoral neck and wrist) [413]. However, there were no BMD data to compare fracture prevalence against and the data did not allow adjustments to be made for differences in risk factors between groups. A study of 138 younger HIV-positive Canadian women found a 70% increased likelihood of fragility fracture compared to HIV-negative controls [412]. However, there was no BMD difference between HIV-positive and HIV-negative women and the results were confounded by the high prevalence of secondary risk factors in HIV-positive women, including intravenous drug use (IVDU) and ART exposure.

Results from longitudinal studies have been mixed. Arnsten *et al* showed no difference in American men aged ≥ 49 years old who either had HIV infection or high-risk behaviour for acquiring HIV infection [156]. In contrast, two other large prospective observational studies have shown an increased rate of fractures compared to HIV-negative individuals in male and female HIV-positive American patients [265] and in HIV-positive American male veterans [414]. In the study by Young *et al*, age-standardised rates of fracture were higher in HIV-positive patients compared to the general population for both fragility fractures and total fractures [265]. Furthermore, nadir CD4 < 200 cells/ μL was associated with increased all-fracture incidence [265]. In the other study, which compared HIV-positive to HIV-negative men, HIV infection was associated with an increased risk of fracture (HR 1.24; 95% CI 1.11, 1.39), but the association was weakened when adjusted for BMI (HR 1.10; 95% CI 0.97, 1.25) [414].

Studies since 2012 have consistently shown an increased risk of fracture in HIV-positive patients. Two Danish studies have shown that HIV infection and ART were both associated with an increased risk [415,418]. HIV infection was associated with an almost 3-fold increase in fracture risk compared with that of age- and gender-matched

uninfected patients [418]. ART-exposed patients had a 60% higher risk of fragility ('low-energy') fractures compared to the general population, even after adjusting for co-morbidities, although there was no such association for non-fragility ('high-energy') fractures [415]. An increased risk has also been reported in Spanish [417] and American studies [371]. A cross-sectional study from the UK also reported a higher fracture prevalence and a higher remaining lifetime fracture probability (RLFP) in HIV-positive patients compared to HIV-negative ones [306]. A study investigating vertebral fractures only also reported a higher prevalence in HIV-positive patients, and a correlation between BMI, diabetes and vertebral fractures [416]. Another study in HIV/HBV co-infected patients has reported a higher cumulative incidence of hip fracture compared to HIV-mono-infected and HIV/HBV-negative controls [419].

5.1.5.2 Fracture rates in HIV-positive cohorts

A number of studies in HIV-positive cohorts have investigated fracture prevalence and incidence (Table 5.1.5.2). Several studies have reported varying rates of fracture incidence [420-424]. Although one study reported an incidence of 3.3 (95% CI 2.0, 4.6)/1000 person-years, the authors found that this rate was similar to the general population of the same age [420]. However, higher rates were seen in patients with excessive alcohol consumption and HCV co-infection [420]. In the VACS-VC cohort, fracture incidence was 2.6/1000 person-years and fragility fractures were associated with frailty [423]. In a large study from the USA, low BMD at baseline was associated with a significantly increased risk of incident fracture [424].

Table 5.1.5.2 Studies investigating fracture prevalence and incidence in HIV-positive patients

Variable fracture incidence reported in studies in HIV-positive cohorts, including studies investigating vertebral fractures and the effect of ART on fractures.

Author (name of study)	Year	Location	Study design	N	HIV-positive, n (%)	% male	Mean age (years)	% ART-exposed	Fractures, n	Fracture prevalence/incidence
Collin [420] (ANRS CO8 APROCO-COPILOTE)	2009	France	Prospective	1281	1281 (100)	77	36 ^a	100	27	Fracture incidence 3.3 (95% CI 2.0, 4.6)/1000 person-years.
Grund* [11] (SMART)	2009	Australia, Spain, USA	Prospective	5473	5473 (100)	73	43 ^a	95 (ever)	10/2753 in continuous ART vs. 2/2720 in intermittent ART	HR 4.9 (95% CI 1.1, 22.5).
Hasse [421] (Swiss HIV Cohort)	2011	Switzerland	Prospective	8444	8444 (100)	71	45 ^a	85	37	Fracture incidence 1.64 (95% CI 1.19, 2.26)/1000 person-years.
Yong [422]	2011	Australia	Case-control	183	183 (100)	89	50	84	73	Fracture incidence 0.53 (95% CI 0.43, 0.65)/100 person-years.
Bedimo [266] (VHA CCR)	2012	USA	Retrospective	56,660	56,660 (100)	98	46 (fractures) vs. 44 (no fractures)	-	951	Fracture rates significantly higher in ART era (after 1996). Exposure to TDF or boosted PIs associated with increased risk, with highest risk when TDF and boosted PIs concomitantly prescribed.
Yin [425] (ACTG A5001 [ALLRT])	2012	USA	Retrospective	4640	4640 (100)	83	39 ^a	26	116	Fracture incidence 0.40 (95% CI 0.33, 0.48)/100 person-years among all participants and 0.38 (95% CI 0.30, 0.49)/100 person-years in 3398 ART-naive participants.
Womack [423] (VACS-VC)	2013	USA	Prospective	40,115	40,115 (100)	100	46	-	588	Fracture incidence 2.6/1000 person-years.
Borderi [426]	2014	Italy	Cross-sectional	202	202 (100)	65	51 ^a	86	47	Fracture prevalence 23.3%. Prevalence in naive vs. ART-experienced was 18% vs. 24%.
Porcelli [427]	2014	Italy	Cross-sectional	131	131 (100)	71	51 ^a	100	35	Vertebral fractures occurred more frequently in patients with low BMD vs. normal BMD (88.5% vs. 11.4%; p < 0.001).

Author (name of study)	Year	Location	Study design	N	HIV-positive, n (%)	% male	Mean age (years)	% ART-exposed	Fractures, n	Fracture prevalence/incidence
Gazzola [428]	2015	Italy	Cross-sectional	194	194 (100)	73	49 ^a	71	24	Vertebral fracture prevalence 12.4%.
Mazzotta [409]	2015	Italy	Prospective**	163	163 (100)	70	44	80	49	Fracture prevalence of non-traumatic bone fractures 27.0%.
Battalora [424] (HOPS-DIDC and SUN)	2016	USA	Prospective	1006	1006 (100)	83	43 ^a	96	85	Fracture incidence 85/4068 person-years.
Stephens [429]	2016	USA	Retrospective	232	232 (100)	98	49 ^a	82	108	Subclinical vertebral fracture prevalence 46.6%.

95% CI: 95% confidence interval; ACTG: AIDS Clinical Trials Group; ALLRT: ACTG Longitudinal-Linked Randomized Trial; ANRS: Agence Nationale de Recherche sur le Sida; ART: antiretroviral therapy; BMD: bone mineral density; HOPS-DIDC: HIV Outpatient Study-Denver Infectious Diseases Consultants; HR: hazard ratio; PI: protease inhibitor; SMART: Strategies for Management of Antiretroviral Therapy; SUN: Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy; TDF: tenofovir; VACS-VC: Veterans Aging Study Virtual Cohort; VHA CCR: Veterans Health Administration's Clinical Case Registry

*Results for parent study

**Cross-sectional data from a prospective study are presented

^aMedian age

Several studies have investigated the effect of ART on fracture incidence [11,266,425]. In the SMART study, fractures were reported in 10 patients (out of 2753) on the continuous ART arm and two (out of 2720) in the intermittent arm, leading to a HR of 4.9 (95% CI 1.1, 22.5) [11]. Although fracture incidence was 0.40 (95% CI 0.33, 0.48)/100 person-years in all participants and 0.38 (95% CI 0.30, 0.49)/100 person-years in 3398 ART-naïve patients, one study found that fracture rates were higher within the first two years after starting ART (0.53/100 person-years) than subsequent years (0.30/100 person-years) [425]. However, they were unable to establish which specific ART drugs were implicated. In a retrospective study of different ART regimens and their association with fracture risk, cumulative exposure to TDF was independently predictive of increased risk of osteoporotic fracture (12% higher per year of exposure) after controlling for traditional osteoporotic risk factors and concomitant ART [266]. The highest risk was seen when TDF and boosted PIs were co-prescribed. In another analysis from the same cohort, fracture rates were significantly higher in HIV/HCV co-infected patients compared to those with HIV-mono-infection (2.57 vs. 2.07/1000 patient-years, $p < 0.0001$), and the increased risk of osteoporotic fractures seen in HIV/HCV co-infected patients was partly explained by the severity of liver disease [430]. An Italian study also found that HIV/HCV co-infection was associated with non-traumatic bone fractures in multivariate analyses [409].

Most studies have investigated clinical fractures, with few reporting on morphometric vertebral fractures. Those that have investigated vertebral fractures have reported prevalences that vary greatly [426-429]. One study used lateral x-rays to identify vertebral fractures and reported a prevalence of 23.3% overall, with the prevalence in ART-naïve patients being 18% and that in ART-experienced patients being 24% [426]. An Italian study reported a prevalence of 12.4%, but found that 70% of fractures occurred in patients that were not osteoporotic [428]. In a retrospective American study in HIV-positive patients with VDD, the prevalence of subclinical vertebral fractures was 46.6% [429]. In this study, those with fractures had a similar prevalence of osteoporosis, low BMD and FRAX[®] scores to patients without fractures. Another study that also investigated BMD found that vertebral fractures occurred more frequently in patients with low compared to normal BMD (88.5% vs. 11.4%; $p < 0.001$) [427]. In three of these studies, vertebral fractures were associated with older age [426-428], and in two, steroid exposure [426,428].

5.1.6 Summary

Most studies in HIV-positive populations have used BMD as a surrogate marker of fracture risk, but it is its relationship with fragility fractures that makes reduced BMD clinically important. In older patients, there is a strong relationship between low BMD and fractures, especially as these patients are at increased risk of falls [379]. An increase in the age of HIV-positive patients is likely to lead to an increased rate of fracture. However, there is uncertainty as to whether reduced BMD leads to an increased risk of fractures in younger, HIV-positive men. It is also unclear whether high rates of reduced BMD in HIV-positive men <50 years old will lead to increased rates of fracture at an older age.

Although there are many studies showing a link between HIV infection, ART and reduced BMD (as well as lower BMD and loss of BMD), those showing an increased risk of fracture and fracture incidence in HIV-positive patients are far fewer. However, it is likely that the rates will increase as life expectancy in HIV-positive patients continues to increase. This coupled with data suggesting that fracture rates are higher in HIV-positive patients compared to HIV-negative individuals, may mean that the consequence of reduced BMD becomes more important in HIV-positive patients. There is growing interest in estimating the future risk of fracture in HIV-positive patients using the FRAX[®] algorithm.

In this chapter, I concentrate on describing the data relating specifically to future fractures (e.g. past history of fragility fractures, family history of osteoporosis, falls and reduced mobility) [369,378,379,383,396]. I also analyse the utility of FRAX[®], pDXA of the non-dominant wrist and a combination of both tests as screening tools in predicting future fracture risk.

5.2 Aims and objectives

Below are the aims for this Chapter:

1. To describe the distribution of the following variables:
 - a. Previous history of fracture and fragility fracture
 - b. Past medical history of osteoporosis
 - c. Family history of osteoporotic fractures
 - d. Mobility and falls.
2. To assess agreement of non-dominant wrist BMD measured by pDXA against BMD of the non-dominant wrist using cDXA.

3. To assess correlation of non-dominant wrist BMD measured by pDXA against BMD at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck measured by cDXA.
4. To calculate FRAX[®] scores, and to compare the difference with and without BMD, as well as with and without HIV as a secondary risk factor.
5. To assess the utility of FRAX[®] and pDXA as screening tools for identifying reduced BMD.

The concern with reduced BMD is that it can lead to hip and vertebral fractures [58]. I hypothesised that BMD at the forearm (using pDXA) would correlate with BMD at the lumbar spine and hip (total hip and femoral neck). Hence, I aimed to assess the utility of pDXA as a screening tool for identifying patients who need further assessment of BMD. I will examine whether wrist BMD on pDXA correlates with BMD at the lumbar spine and hip (total hip and femoral neck). Additionally, I will investigate the usefulness of combining pDXA and FRAX[®] to assess whether they work better as screening tools when used in combination.

5.3 Methods

5.3.1 Study design

The detailed methods of the study are given in Chapter 2. In this Chapter, the data from the study participants' baseline visit (Year 1) were analysed.

The data relating to past medical history of osteoporosis, family history of osteoporosis, previous fracture history, mobility and falls were obtained using self-reported questionnaires.

The GE Healthcare Lunar iDXA bone densitometer (GE Healthcare, Madison, Wisconsin, USA) was used to measure absolute BMD (g/cm^2) at the lumbar spine, the total hip (left total hip and right total hip), the femoral neck (left femoral neck and right femoral neck) and the non-dominant wrist. BMD data were also reported for the non-dominant total hip and non-dominant femoral neck. Additionally, the Lunar PIXI pDXA densitometer (GE Healthcare, Madison, Wisconsin, USA) was used to measure absolute BMD (g/cm^2) at the non-dominant wrist.

FRAX[®] scores were calculated using the WHO FRAX[®] tool (<http://www.shef.ac.uk/frax>) for the 10-year risk of a major osteoporotic fracture and the 10-year risk of a hip

fracture. FRAX[®] scores were calculated with and without BMD data, as well as using non-dominant femoral neck BMD and T-score data. NOGG guidance was assessed for each FRAX[®] score.

5.3.2 Definitions

In this study, I defined fragility fractures as below. As I was interested in fractures sustained as an adult, all fractures sustained prior to 20 years of age were classified as childhood fractures and were excluded. Using Kanis *et al's* definitions, sites associated with osteoporotic fractures were defined as those involving the spine, ribs, pelvis, humerus, hip, clavicle, scapula and sternum [372]. Fractures not thought to be associated with osteoporosis were classified as those involving the skull and face, tibia and fibula, hands and fingers, feet and toes, ankle and patella. All of these were excluded. Finally, multiple fractures sustained in a single episode and which included sites not usually associated with osteoporotic fractures were deemed to be trauma-related, and were also excluded.

FRAX[®] scores were calculated using secondary risk factors as reported (defined as standard secondary risk factors), as well as with no secondary risk factors and HIV as a secondary risk factor in all patients. Intermediate threshold was defined using the yellow and/or red guidance under NOGG.

Reduced BMD was defined as a T-score ≤ -2.5 in men ≥ 50 years old and a Z-score ≤ -2 in men < 50 years old. As the clinical significance of osteopenia was uncertain and there was debate as to whether osteopenia signified reduced BMD in people ≥ 50 years old, men ≥ 50 years old diagnosed with osteopenia (T-score < -1.0 to > -2.5) were excluded.

5.3.3 Statistical analysis

The distribution frequency of each variable was calculated. Mean and SD were measured in those that were normally distributed and median and IQR in those that had skewed distributions.

For the three variations in secondary risk factors, FRAX[®] scores were calculated in three ways for each participant: without BMD data, with non-dominant femoral neck

BMD data and with non-dominant femoral neck T-score data. This was done for both a major osteoporotic fracture and a hip fracture.

A Bland-Altman plot was drawn to compare agreement between BMD at the non-dominant wrist using pDXA and cDXA. Student's t-test was used to compare differences in BMD obtained using pDXA and cDXA at the non-dominant wrist. The relationship between BMD at the non-dominant wrist measured using pDXA and BMD at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck (all measured using cDXA) were examined using Pearson correlation coefficients.

The effectiveness of FRAX[®] as a screening tool comparing patients with normal BMD to those with reduced BMD was assessed. Men without absolute BMD or T-score data at the non-dominant femoral neck were excluded. In total, 83 participants ≥ 50 years old diagnosed with osteopenia were also excluded. This was in keeping with the analyses conducted in patients from the pilot study to ensure the results could be directly compared between them [395]. The results from the FRAX[®] scores were translated into intervention thresholds which corresponded to a 10-year probability of a major osteoporotic fracture of 7.5% as set by NOGG [431]. A low intervention threshold meant lifestyle advice and reassurance, an intermediate intervention threshold meant further assessment of BMD (cDXA scan) and a high intervention threshold meant treatment was indicated.

In order to assess the effectiveness of the FRAX[®] score as a screening tool, as well as the effectiveness of combining both pDXA and FRAX[®] scores, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. A FRAX[®] score above the intermediate threshold would indicate a positive result for reduced BMD, whereas a FRAX[®] score below the threshold would signify a negative result. Sensitivity is the proportion of HIV-positive men with reduced BMD who are correctly identified by FRAX[®], whilst specificity is the proportion of men without low BMD who are identified as such by FRAX[®]. PPV relates to the proportion of HIV-positive patients who had a FRAX[®] score above the intermediate threshold who had reduced BMD, whilst NPV referred to the proportion of men with a FRAX[®] score below the intermediate threshold that did not have low BMD.

The likelihood ratio for a positive and negative result was also calculated when assessing the effectiveness of FRAX[®] alone, as well as the utility of combining both FRAX[®] and pDXA. In this scenario, the likelihood ratio for a positive result is the ratio of the probability of an HIV-positive man who has reduced BMD testing positive on FRAX[®] (sensitivity) to the probability of a man without low BMD who tested positive (1-

specificity). The likelihood ratio for a negative result is the probability of an HIV-positive man who has low BMD testing negative (1-sensitivity) to the probability of a man without low BMD testing negative (specificity). A high likelihood ratio for a positive result is an indicator of how well a positive result from the FRAX[®] tool increases the likelihood that an individual truly has reduced BMD. Conversely, a low likelihood ratio for a negative result indicates how the test result decreases an individual's post-test probability i.e. after getting a negative result, it becomes less likely that a person has the disease than their hypothetical probability before the test (pre-test probability).

To assess the effectiveness of pDXA of the non-dominant wrist as a diagnostic tool, a table of thresholds was constructed displaying the sensitivity and specificity of different pDXA thresholds. A receiver-operating characteristic (ROC) curve was constructed and the area under the receiver-operating characteristic curve (AUROC) was calculated to assess whether pDXA was useful as a screening test in diagnosing reduced BMD in this population. AUROC is the probability that a random patient from the group with reduced BMD has a higher predicted probability of having reduced BMD compared to a random person from those with normal BMD. A test which is perfect at discriminating between normal and reduced BMD would have an AUROC of 1.0, whilst a test that is no better than chance at discriminating between the two would have an AUROC of 0.5.

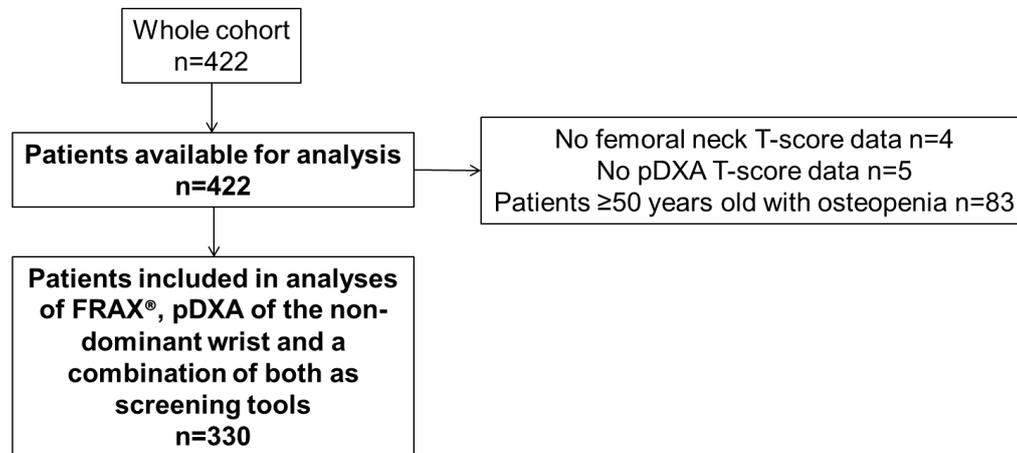
5.4 Results

5.4.1 Subject disposition

All 422 men were included in the analyses in this chapter. When assessing FRAX[®], pDXA of the non-dominant wrist and a combination of both as screening tools, men without non-dominant femoral neck T-score (n=4) or pDXA (n=5) data and those aged ≥ 50 years old with osteopenia (n=83) were excluded (Figure 5.4.1.1).

Figure 5.4.1.1 Summary of subject disposition

Although all 422 men in the study were analysed, 92 were excluded when FRAX[®], pDXA of the non-dominant wrist and a combination of both were assessed as screening tools.
 FRAX[®]: Fracture Risk Assessment Tool; pDXA: peripheral dual-energy x-ray absorptiometry

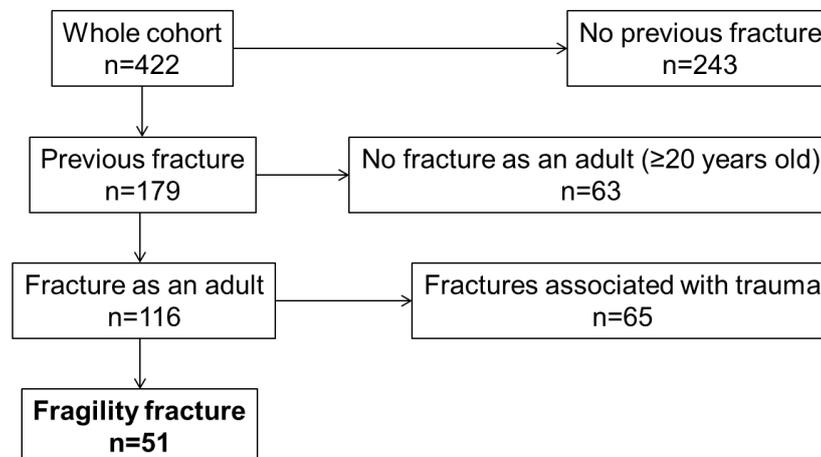


5.4.2 Previous fracture history

There were 179 (42.4%) men who reported ever having had a fracture (Figure 5.4.2.1). Of these, 116 (27.5%) reported that they had sustained a fracture in adulthood (age ≥ 20 years old). In total, 51 (12.1%) men were categorised as ever having had a fragility fracture in adult life. Of patients with a past history of a fracture in adulthood, 65 (15.4%) had fractures that were related to trauma (e.g. road traffic accidents, sporting injuries).

Figure 5.4.2.1 Previous history of fracture

This figure shows the number of patients who reported a past history of fracture, and how other fractures were excluded to identify those with fragility fractures



5.4.3 Past medical and family history of osteoporosis and osteoporotic fractures

Study participants provided information as to whether they or a member of their family had ever been affected by osteoporosis.

5.4.3.1 Past medical history of osteoporosis

In total, 248 (58.8%) reported that they had no past medical history of osteoporosis, whilst 76 (18.0%) reported that they had. However, 98 (23.2%) men reported that they were unsure as to whether they or a member of their family had ever been affected by osteoporosis.

5.4.3.2 Family history of osteoporotic fractures

Regarding family history of osteoporotic fracture, the question specifically inquired about hip fractures in the study participant's mother or maternal grandmother. In response, 303 (71.8%) stated that there was no family history of hip fractures, whilst 55 (13.0%) said that there was a positive family history. Again, 64 (15.2%) participants were unsure of their family history.

5.4.4 Mobility and falls

The questionnaire asked participants to provide details about their mobility, including walking and falling. The majority (285, 67.5%) reported no problems with walking. Of the remainder, 125 (29.6%) reported having some difficulty with walking, whilst 12 (2.8%) required assistance, either with a walking aid or from someone. However, no one reported not being able to walk. There were 63 (14.9%) men who reported a positive falls history, with the median number of falls being three (IQR 2, 5) in the year preceding recruitment.

5.4.5 pDXA

5.4.5.1 Agreement of BMD at the non-dominant wrist measured by pDXA and cDXA

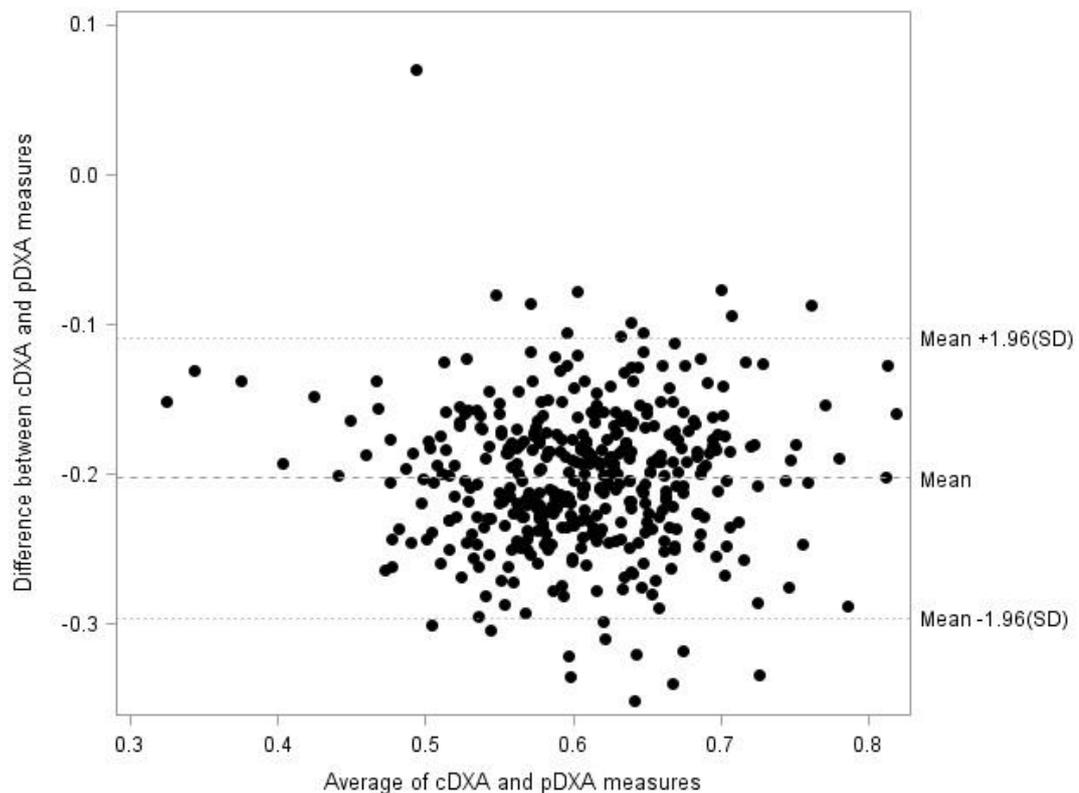
The Bland-Altman plot of pDXA BMD against cDXA BMD at the non-dominant wrist is shown in Figure 5.4.5.1. When adjusting for the mean difference, there was a systematic error of -0.2. Therefore, 0.2 g/cm² needed to be added to the pDXA results to obtain a similar result to that from cDXA. Furthermore, a student's t-test of the differences in pDXA and cDXA at the non-dominant wrist showed they were

significantly different from 0 ($p < 0.001$), which suggests a systematic bias between the two methods.

Figure 5.4.5.1 Bland-Altman plot of pDXA BMD against cDXA BMD at the non-dominant wrist

This figure shows that there is a significant difference in BMD at the non-dominant wrist when measured using pDXA compared to cDXA.

cDXA: central dual energy x-ray absorptiometry; pDXA: peripheral dual energy x-ray absorptiometry



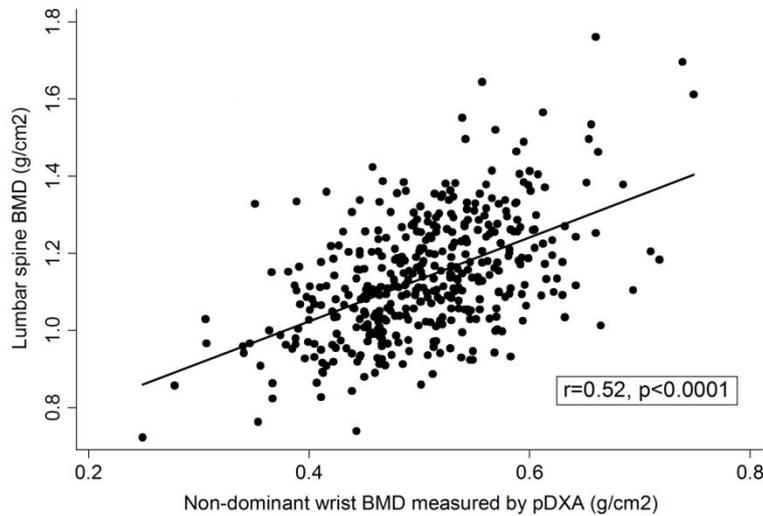
5.4.5.2 Comparing BMD measured by pDXA against BMD measured by cDXA at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck

5.4.5.2.1 Lumbar spine

There was a weak correlation between BMD at the non-dominant wrist measured using pDXA and BMD at the lumbar spine measured using cDXA. ($r=0.52$, $p < 0.0001$, Figure 5.4.5.2).

Figure 5.4.5.2 Correlation between pDXA non-dominant wrist BMD and cDXA lumbar spine BMD

Plot of absolute BMD at the non-dominant wrist measured using pDXA against absolute BMD at the lumbar spine measured using cDXA showing a significant positive correlation ($r=0.52$, $p<0.0001$).
BMD: bone mineral density; pDXA: peripheral dual energy x-ray absorptiometry



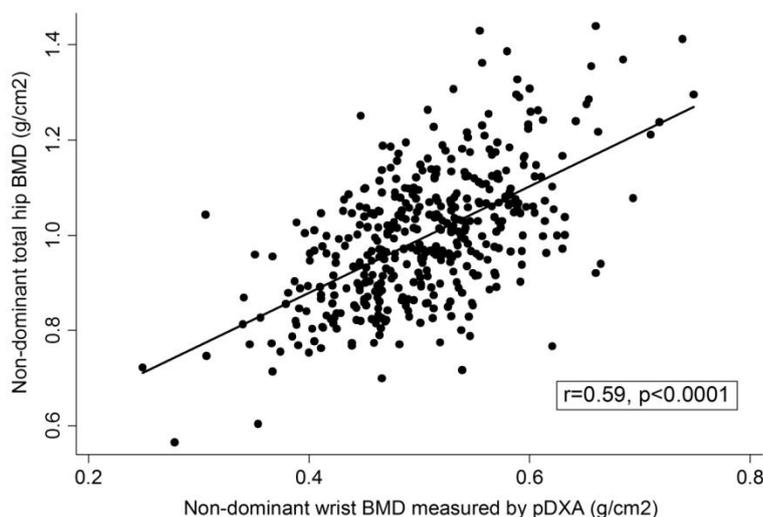
5.4.5.2.2 Non-dominant total hip

As with the results obtained for the lumbar spine, there was a positive but weak correlation ($r=0.59$, $p<0.0001$, Figure 5.4.5.3) between non-dominant wrist BMD (measured by pDXA) and BMD at the non-dominant total hip (measured by cDXA).

Figure 5.4.5.3 Correlation between pDXA non-dominant wrist BMD and cDXA non-dominant total hip BMD

Plot of absolute BMD at the non-dominant wrist measured using pDXA against absolute BMD at the non-dominant total hip measured using cDXA showing a significant but weak positive correlation ($r=0.59$, $p<0.0001$).

BMD: bone mineral density; pDXA: peripheral dual energy x-ray absorptiometry



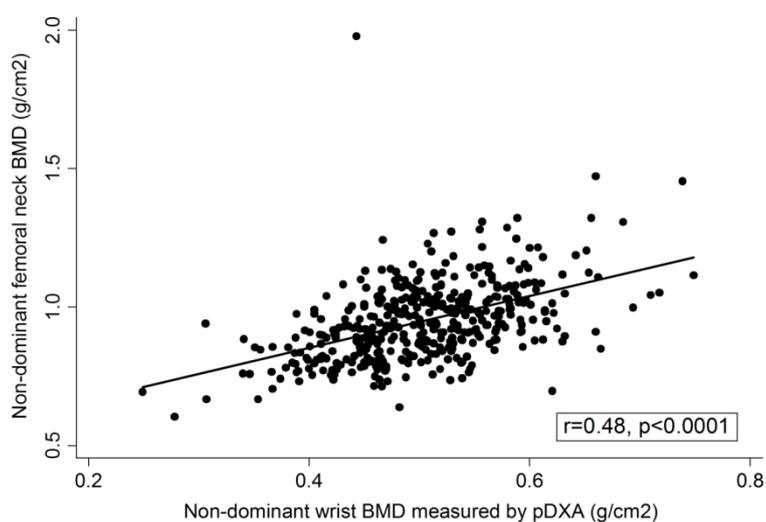
5.4.5.2.3 Non-dominant femoral neck

There was also a weak correlation between non-dominant wrist BMD measured using pDXA and non-dominant femoral neck BMD measured using cDXA ($r=0.48$, $p<0.0001$, Figure 5.4.5.4).

Figure 5.4.5.4 Correlation between pDXA non-dominant wrist BMD and cDXA non-dominant femoral neck BMD

Plot of absolute BMD at the non-dominant wrist measured using pDXA against absolute BMD at the non-dominant total hip measured using cDXA showing a significant positive but weak correlation ($r=0.48$, $p<0.0001$).

BMD: bone mineral density; pDXA: peripheral dual energy x-ray absorptiometry



5.4.6 FRAX[®] scores and estimated fracture risk

5.4.6.1 Distribution of FRAX[®] scores

The distribution of FRAX[®] scores using risk factors as standard, without any secondary risk factors and HIV as a secondary risk factor in all participants are shown in Table 5.4.6.1. In general, the 10-year probability of fracture was low, with median scores for osteoporotic fractures ranging from 3.0% to 4.4%, and those for a hip fracture ranging between 0.2% and 0.6%.

Table 5.4.6.1 Distribution of FRAX® scores

This table shows that the distribution of FRAX® scores were similar for both a major osteoporotic and a hip fracture when non-dominant femoral neck BMD or T-score data were included, irrespective of whether standard, none or HIV infection was used as a secondary risk factor. Using T-score data led to the highest FRAX® scores.

	Risk factors as standard, median (IQR)	No secondary risk factors, median (IQR)	HIV infection as a secondary risk factor, median (IQR)
Not including BMD			
Osteoporotic fracture	3.3 (2.6, 5.3)	3.0 (2.5, 4.9)	4.0 (3.4, 6.5)
Hip fracture	0.2 (0.2, 0.5)	0.2 (0.1, 0.5)	0.4 (0.2, 0.8)
Including non-dominant femoral neck BMD			
Osteoporotic fracture	3.9 (2.8, 5.7)	3.9 (2.8, 5.7)	3.9 (2.8, 5.7)
Hip fracture	0.5 (0.2, 1.1)	0.4 (0.2, 1.1)	0.5 (0.2, 1.1)
Including non-dominant femoral neck T-score			
Osteoporotic fracture	4.4 (3.0, 6.6)	4.4 (3.0, 6.5)	4.4 (3.0, 6.6)
Hip fracture	0.6 (0.2, 1.8)	0.6 (0.2, 1.8)	0.6 (0.2, 1.8)

BMD: bone mineral density; IQR: interquartile range

When non-dominant femoral neck BMD or T-score data were included, the distribution of FRAX® scores appeared to be the same or similar for both a major osteoporotic and a hip fracture, irrespective of whether risk factors were recorded as standard, none or with HIV infection included as a secondary risk factor. When BMD was not included in the calculation, the median FRAX® scores ranged between 3.0% and 4.0% for a major osteoporotic fracture and between 0.2% and 0.4% for a hip fracture depending on how risk factors were classified. For both a major osteoporotic and a hip fracture, when BMD data were not included, the highest FRAX® scores were obtained with HIV included as a secondary risk factor, which was as expected. Additionally, including BMD data led to higher FRAX® scores for both a major osteoporotic fracture and a hip fracture, with T-score data providing the highest FRAX® scores.

5.4.6.2 Comparison of FRAX® scores with and without BMD and T-score data

In order to compare whether including BMD or T-score data had an effect on the FRAX® scores, the difference in medians was calculated (Table 5.4.6.2). When BMD data were not included, there were differences in the median FRAX® scores for both a major osteoporotic and hip fracture when compared to inclusion of BMD or T-score data for both standard secondary risk factors and no secondary risk factors. In these cases, use of BMD or T-score data led to a higher FRAX® score. However, when HIV was considered as a secondary risk factor, the results were more variable. For a major osteoporotic fracture, the FRAX® scores were lower when BMD data were used, with no significant difference when T-score data were used. For a hip fracture, higher FRAX® scores were obtained when T-score data were included, but there were no differences in FRAX® scores when BMD data were used.

Table 5.4.6.2 Difference in median FRAX[®] scores with and without BMD data according to inclusion of standard, none and HIV as a secondary risk factor

Use of BMD or T-score data led to a higher FRAX[®] score when secondary risk factors were included as standard or none. When HIV was included as a secondary risk factor, the results were more variable.

		Median (IQR)
Standard secondary risk factors	Osteoporotic fracture	
	Difference: FRAX [®] without BMD - FRAX [®] with BMD	-0.2 (-1.0, 0.3)
	Difference: FRAX [®] without BMD - FRAX [®] with T-score	-0.5 (-1.7, 0.1)
	Hip fracture	
No secondary risk factors	Osteoporotic fracture	
	Difference: FRAX [®] without BMD - FRAX [®] with BMD	-0.5 (-1.3, 0.0)
	Difference: FRAX [®] without BMD - FRAX [®] with T-score	-0.9 (-2.1, -0.1)
	Hip fracture	
HIV infection as a secondary risk factor	Osteoporotic fracture	
	Difference: FRAX [®] without BMD - FRAX [®] with BMD	0.6 (-0.2, 1.2)
	Difference: FRAX [®] without BMD - FRAX [®] with T-score	0.3 (-0.9, 1.0)
	Hip fracture	
	Difference: FRAX [®] without BMD - FRAX [®] with BMD	0.0 (-0.4, 0.2)
	Difference: FRAX [®] without BMD - FRAX [®] with T-score	-0.1 (-0.8, 0.1)

BMD: bone mineral density; FRAX[®]: Fracture Risk Assessment Tool; IQR: interquartile range

5.4.6.3 Comparison of FRAX[®] scores with standard, none and HIV as secondary risk factors

To investigate whether FRAX[®] scores varied with the inclusion of secondary risk factors as standard, none and HIV, the difference in medians was calculated. When BMD data were not included, use of HIV as a secondary risk factor led to lower median FRAX[®] scores for a major osteoporotic fracture (1.0 [IQR 0.8, 1.6] vs. 0.9 [IQR 0.0, 1.2]), but not for a hip fracture (0.1 [IQR 0.1, 0.3] vs. 0.1 [IQR 0.0, 0.2]). When BMD or T-score data were included, there were no differences in the median FRAX[®] scores.

5.4.7 FRAX[®] and pDXA as diagnostic tools for diagnosing reduced BMD

5.4.7.1 FRAX[®] as a screening tool for diagnosing reduced BMD

FRAX[®] was used as a diagnostic tool to calculate its utility in diagnosing low BMD. The results from the FRAX[®] scores were translated into intervention thresholds which corresponded to a 10-year probability of a major osteoporotic fracture of 7.5% as set by NOGG (Kanis Osteoporosis Int 2008b), with an intermediate intervention threshold meaning further assessment with a DXA scan.

The FRAX[®] tool was assessed using standard secondary risk factors, as well as with no secondary risk factors or HIV as a secondary risk factor (Table 5.4.7.1). There were 14 men with reduced BMD and 316 with normal BMD, leading to a prevalence of low

BMD of 4.2%. The sensitivity of the FRAX[®] tool increased when HIV was used as a secondary risk factor, although specificity reduced from 55.1% (95% CI 0.50, 0.60) with standard secondary risk factors to 18.4% (95% CI 0.14, 0.23). The PPV was low for all risk factor categorisations, although it was lowest when no secondary risk factors were included, which was as expected. The NPV was similar irrespective of whether secondary risk factors were categorised as standard, none or as yes for all. Due to the extremely low sensitivity, the likelihood ratios for a positive result were <1 and the likelihood ratios for a negative result were >1 for all risk categorisations. These suggest that FRAX[®] is not useful as a diagnostic test in this cohort.

Table 5.4.7.1 Effectiveness of FRAX[®] as a screening tool with standard secondary risk factors, none and HIV as secondary risk factors

Although the sensitivity of FRAX[®] increased when HIV was included as a secondary risk factor, the specificity decreased. Additionally, the PPV, NPV and likelihood ratio results suggest that FRAX[®] is not useful as a diagnostic test in this cohort. The intermediate intervention threshold was set by NOGG.

	Reduced BMD (n=14)	Normal BMD (n=316)	Total
FRAX [®] score (computed using standard secondary risk factors)			
Above intermediate intervention threshold	6	142	148
Below intermediate intervention threshold	8	174	182
Sensitivity (95% CI)	42.9% (0.21, 0.67)	-	
Specificity (95% CI)	-	55.1% (0.50, 0.60)	
PPV (95% CI)	4.1% (0.02, 0.09)	-	
NPV (95% CI)	-	95.6% (0.91, 0.98)	
Likelihood ratio for positive result	0.95	-	
Likelihood ratio for negative result	-	1.04	
FRAX [®] score (computed using no secondary risk factors)			
Above intermediate intervention threshold	3	108	111
Below intermediate intervention threshold	11	208	219
Sensitivity (95% CI)	21.4% (0.07, 0.48)	-	
Specificity (95% CI)	-	65.8% (0.60, 0.71)	
PPV (95% CI)	2.7% (0.01, 0.08)	-	
NPV (95% CI)	-	95.0% (0.91, 0.97)	
Likelihood ratio for positive result	0.63	-	
Likelihood ratio for negative result	-	1.19	
FRAX [®] score (computed using HIV as secondary risk factors)			
Above intermediate intervention threshold	11	258	269
Below intermediate intervention threshold	3	58	61
Sensitivity (95% CI)	78.6% (0.52, 0.93)	-	
Specificity (95% CI)	-	18.4% (0.14, 0.23)	
PPV (95% CI)	4.1% (0.02, 0.07)	-	
NPV (95% CI)	-	95.1% (0.86, 0.99)	
Likelihood ratio for positive result	0.96	-	
Likelihood ratio for negative result	-	1.17	

95% CI: 95% confidence interval; BMD: bone mineral density; FRAX[®]: Fracture Risk Assessment Tool; NPV: negative predictive value; PPV: positive predictive value

5.4.7.2 pDXA as a screening tool for diagnosing reduced BMD

In order to determine the optimal cut-off obtained by pDXA at the non-dominant wrist, a table of thresholds was calculated (Table 5.4.7.2). In this case, the utility of pDXA as a screening test was to help correctly to identify as many people with low BMD as possible in order to further investigate them or to correctly identify those with normal BMD so that they do not require further tests. Further investigation in those with a positive result would involve cDXA scanning. As pDXA is non-invasive and exposes the patient to very low doses of radiation, making a correct diagnosis was important. Therefore, a cut-off with high sensitivity was considered to be optimal, although a trade-off with specificity was needed. The pDXA threshold of -0.8 gave the highest sensitivity, but specificity was at its lowest. When the pDXA threshold was lowered, sensitivity remained at 85.7% between -1.0 and -1.6. However, specificity was higher, and in turn the false positive rate was lower, when the pDXA threshold was -1.6. Therefore, this cut-off was deemed to be optimal.

Table 5.4.7.2 A table of thresholds for pDXA of the dominant wrist

Although a pDXA threshold of -0.8 gave the highest sensitivity (92.9%), specificity (36.4%) was low. Therefore, a threshold of -1.6 was deemed to be the optimal cut-off for pDXA in this population.

pDXA threshold	Sensitivity	Specificity	False positive rate	False negative rate
-2.0	71.4	76.6	23.4	28.6
-1.8	78.6	70.9	29.1	21.4
-1.6	85.7	63.9	36.1	14.3
-1.4	85.7	57.3	42.7	14.3
-1.2	85.7	49.1	50.9	14.3
-1.0	85.7	44.3	55.7	14.3
-0.8	92.9	36.4	63.6	7.1

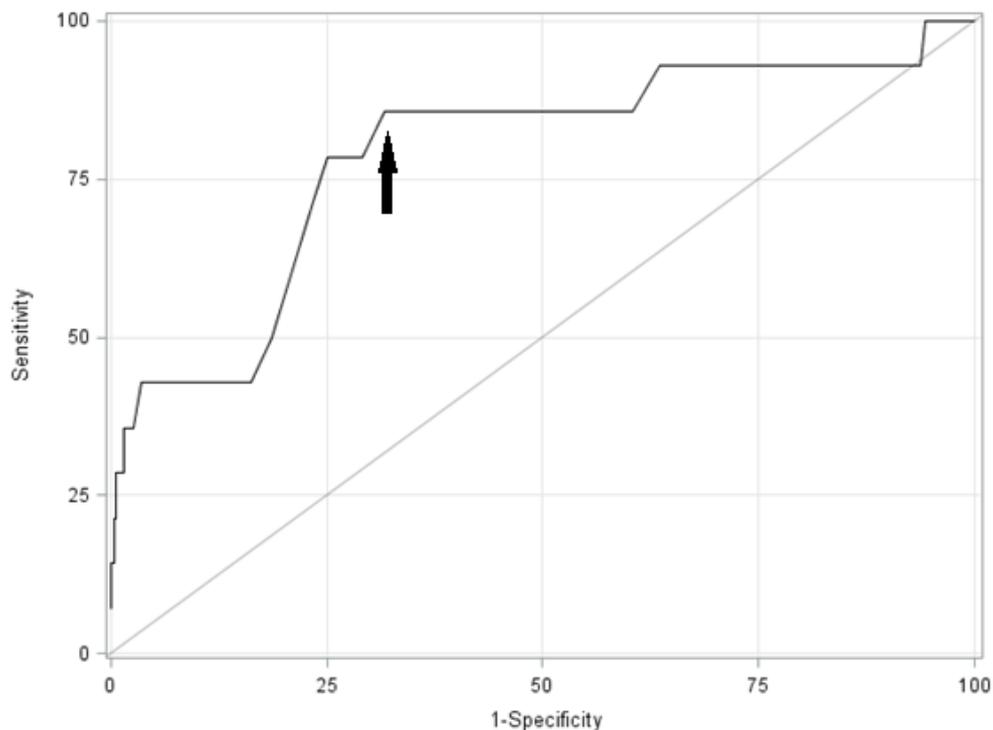
pDXA: peripheral dual-energy x-ray absorptiometry

To further assess the utility of pDXA of the non-dominant wrist, a ROC curve was constructed (Figure 5.4.7.1). The AUROC was 0.79 (95% CI 0.64, 0.93), which suggests that pDXA is reasonably accurate at discriminating between those who have reduced BMD and those that do not. The cut-off threshold of -1.6 is indicated on the graph with an arrow.

Figure 5.4.7.1 ROC curve for pDXA of the non-dominant wrist as a screening tool

The area under the ROC curve when assessing pDXA of the non-dominant wrist was 0.79, suggesting that pDXA is a useful screening tool in this cohort. The cut-off threshold of -1.6 is indicated on the graph with an arrow.

ROC: receiver-operating characteristic



The likelihood ratio for a positive test was 2.37, suggesting that a positive result is 2.37 times as likely to occur in an individual with low BMD than in one with normal BMD. The likelihood ratio for a negative test was 0.22. Overall, the likelihood ratios suggest that pDXA may be useful as a screening tool (which is similar to the results obtained from the ROC curve) and that pDXA is better as a screening tool than the FRAX[®] tool in this cohort of HIV-positive men.

5.4.7.3 Combining FRAX[®] and pDXA as screening tools for diagnosing low BMD

FRAX[®] and pDXA were combined to investigate whether the two tests together were better as screening tools than each on its own (Table 5.4.7.3). This shows that the sensitivity of combining both tools was no different to using FRAX[®] by itself, but that the addition of pDXA improved specificity in all risk categorisations. PPV and NPV were largely unchanged at each risk categorisation. However, the likelihood ratios were different compared to using the FRAX[®] score alone. When risk factors were categorised as standard or HIV was computed as a secondary risk factor, the likelihood ratios for a positive result were >1 and the likelihood ratios for a negative result were <1, suggesting that combining the tests may be useful in identifying those with reduced BMD. Interestingly, the likelihood ratios indicated that when combining FRAX[®] and

pDXA, use of standard risk factors may make them better as screening tools than when HIV was included as a secondary risk factor in this population with a low prevalence of reduced BMD.

Table 5.4.7.3 Effectiveness of combining FRAX® and pDXA as screening tools with standard secondary risk factors, none and HIV as secondary risk factors

Although combining pDXA and FRAX® did not alter sensitivity, PPV or NPV, it did improve specificity. The likelihood ratios when risk factors were included as standard or with HIV as a risk factor suggest that combining pDXA and FRAX® may be useful in identifying men with reduced BMD, with the use of standard risk factors having the better result. The intermediate intervention threshold was set by NOGG.

	Reduced BMD (n=14)	Normal BMD (n=316)	Total
FRAX® score and pDXA (computed using standard secondary risk factors)			
Above intermediate intervention threshold	6	95	101
Below intermediate intervention threshold	8	221	229
Sensitivity (95% CI)	42.9% (0.21, 0.67)	-	
Specificity (95% CI)	-	69.9% (0.65, 0.75)	
PPV (95% CI)	5.9% (0.03, 0.13)	-	
NPV (95% CI)	-	96.5% (0.93, 0.98)	
Likelihood ratio for positive result	1.43	-	
Likelihood ratio for negative result	-	0.82	
FRAX® score and pDXA (computed using no secondary risk factors)			
Above intermediate intervention threshold	3	74	77
Below intermediate intervention threshold	11	242	253
Sensitivity (95% CI)	21.4% (0.07, 0.48)	-	
Specificity (95% CI)	-	76.6% (0.72, 0.81)	
PPV (95% CI)	3.9% (0.01, 0.11)	-	
NPV (95% CI)	-	95.7% (0.92, 0.98)	
Likelihood ratio for positive result	0.92	-	
Likelihood ratio for negative result	-	1.03	
FRAX® score and pDXA (computed using HIV as secondary risk factors)			
Above intermediate intervention threshold	10	168	178
Below intermediate intervention threshold	4	148	152
Sensitivity (95% CI)	71.4% (0.45, 0.89)	-	
Specificity (95% CI)	-	46.8% (0.41, 0.52)	
PPV (95% CI)	5.6% (0.03, 0.10)	-	
NPV (95% CI)	-	97.4% (0.93, 0.99)	
Likelihood ratio for positive result	1.34	-	
Likelihood ratio for negative result	-	0.61	

95% CI: 95% confidence interval; BMD: bone mineral density; FRAX®: Fracture Risk Assessment Tool; NPV: negative predictive value; pDXA: peripheral dual-energy x-ray absorptiometry; PPV: positive predictive value

5.5 Discussion

5.5.1 Summary

In the entire cohort of 422 HIV-positive men, the prevalence of a past history of a fragility fracture in adult life was 12.1%, which is high for a relatively young male cohort. However, as I used Kanis *et al's* definitions to identify the typical sites associated with a

fragility fracture [372], and as some details relating to fragility fractures were missing (e.g. mode of injury), this figure could be misrepresentative, with some traumatic fractures misclassified as fragility fractures and some fragility fractures missed because they did not occur at the typical sites.

The majority of men (58.8%) did not report a past history of osteoporosis, and 71.8% reported no family history of hip fractures. However, a large number were unsure as to whether they had a positive family history for either osteoporosis or osteoporotic fractures, so these rates may be an underestimation and may have been subject to recall bias. It would have been useful if these results could have been verified further with medical records, but this was beyond the scope of this study.

In relation to mobility and falls, most men (67.5%) had no problems with walking which was as expected as this was a relatively young cohort with a mean age of 47 (SD 9.8) years. In contrast, a high percentage (14.9%) reported a history of falls, with the median number of falls in the year prior to recruitment being 3 (IQR 2, 5). Although HIV-positive patients are at increased risk of accelerated ageing [389], and there are plausible mechanisms to explain how falls may lead to fractures (e.g. sporting injuries, road traffic accidents, alcohol and drug-related injuries), many studies have not assessed falls in a standard manner. In this cohort, although the rate of falling was high, the rate of fragility fractures was low. This may be because the men were relatively young with normal BMD, with falls not necessarily leading to fractures. The clinical impact of falling leading to fractures in this young cohort of HIV-positive men is not known and requires further evaluation.

As the clinical significance of osteopenia is widely debated, 83 men ≥ 50 years old with osteopenia (T-score < -1.0 to > -2.5) were excluded. This was to enable the results to be compared directly with those obtained from the pilot study [395]. However, the inclusion of these men would not have altered the low prevalence of reduced BMD (4.2%) defined as a T-score ≤ -2.5 in men ≥ 50 years old and a Z-score ≤ -2 in men < 50 years old.

5.5.1.1 Comparison of BMD using cDXA and pDXA

In this study, I assessed the correlation between absolute BMD measured at the non-dominant wrist using pDXA and absolute BMD at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck using cDXA. In all three comparisons, absolute BMD at the non-dominant wrist measured using pDXA correlated with that measured by cDXA. I also compared the measurement of BMD using pDXA against the gold standard method of cDXA. When assessing the agreement of pDXA and

cDXA at the non-dominant wrist, I found that there was a systematic bias between the two methods and that 0.2 g/cm² needed to be added to the results obtained using the pDXA densitometer. This means that a direct comparison of absolute BMD using the Lunar iDXA and PIXI densitometers cannot be made.

Studies in the general population have shown that pDXA may be useful as a screening tool [117,118]. The pilot study of this cohort also suggested that pDXA was useful as a screening tool [395]. My results also suggest that pDXA might be a helpful screening tool in my cohort.

5.5.1.2 FRAX[®] scores in this cohort

Although not recommended in the BHIVA guidelines [402], the European AIDS Clinical Society recommend including HIV infection as a secondary risk factor in the FRAX[®] calculator [403]. I therefore calculated FRAX[®] scores using risk factors as standard, as well as without any secondary risk factors and HIV as a secondary risk factor. The 10-year probability for either a major osteoporotic fracture or a hip fracture was low, ranging from 3.0% to 4.4% for a major osteoporotic fracture and from 0.2% to 0.6% for a hip fracture, regardless of whether risk factors were included as standard, not included or with HIV infection as a secondary risk factor. These results were not unexpected as this was a relatively young cohort of men. When non-dominant femoral neck BMD or T-score data were added, FRAX[®] scores were similar (but higher than when no BMD data were included) for both a major osteoporotic and a hip fracture, irrespective of how risk factors were categorised. Inclusion of T-score data led to the highest FRAX[®] scores, which was as expected as BMD data can enhance fracture risk prediction [396]. A study in HIV-positive patients has also shown that the FRAX[®] score was more sensitive when BMD data were included [199].

I investigated whether the inclusion of BMD or T-score data would have an effect on the FRAX[®] scores. I found that when secondary risk factors were included as standard or none, the use of BMD or T-score data led to higher FRAX[®] scores. However, when HIV was included as a secondary risk factor, the results were more variable. I also compared FRAX[®] scores using risk factors as standard, no risk factors and with HIV infection as a secondary risk factor. When HIV infection was included as a secondary risk factor, the FRAX[®] scores were lower when BMD data were not included for either a major osteoporotic fracture or a hip fracture. However, when BMD or T-score data were included, there were no differences in FRAX[®] scores, irrespective of whether HIV was included as a secondary risk factor or not. However, these results are all predictable based on how the FRAX[®] score is generated. Interestingly, Gazzola *et al* reported that

including HIV as a secondary risk factor increased sensitivity of the FRAX[®] tool [406]. It will therefore be useful to follow-up the men in my cohort for 10 years and see if these fracture predictions are indeed accurate.

5.5.1.3 Utility of FRAX[®] and/or pDXA as screening tools

I assessed the utility of FRAX[®] and/or pDXA as screening tools. When assessing FRAX[®] as a screening tool for identifying patients at risk of developing fragility fractures, I found that the addition of HIV infection as a secondary risk factor improved its sensitivity from 42.9% (95% CI 0.21, 0.67) to 78.6% (95% CI 0.52, 0.93). This is in keeping with results from an Italian study [406]. However, this was at the expense of specificity, which reduced from 55.1% (95% CI 0.50, 0.60) to 18.4% (95% CI 0.14, 0.23). Irrespective of whether HIV infection was included as a secondary risk factor, I obtained a higher sensitivity than when the same analysis was conducted in the pilot study [395]. However, the specificity I obtained was lower. The difference in results can probably be explained by the much lower prevalence of reduced BMD in my cohort (4.2%) compared to that in the pilot study (24.0%) [395]. Although Short *et al* did not calculate the likelihood ratios, they would have obtained results of 1.92 and 0.88 for the likelihood ratios for a positive and negative result, respectively, when the FRAX[®] score was calculated using standard risk factors, and 1.19 and 0.93 for the likelihood ratios for a positive and negative result, respectively, when the FRAX[®] score was calculated using HIV as a secondary risk factor. In contrast, the likelihood ratios I obtained for a positive result were <1 and the likelihood ratios for a negative result were >1 for all risk categorisations, which suggests that the FRAX[®] tool is not useful as a diagnostic test in my cohort. However, this is not surprising as the FRAX[®] tool was designed to identify patients at risk of osteoporosis-related fractures and not BMD.

When assessing pDXA as a screening tool, a pDXA threshold of -1.6 was considered to be the optimal cut-off because it produced a high sensitivity (85.7%), with the best specificity (63.9%) and a low false positive rate (36.1%). The utility of pDXA as a screening tool was further assessed using a ROC curve. The AUROC was 0.79, which suggests that pDXA of the non-dominant wrist was reasonably accurate in discriminating between reduced and normal BMD. The likelihood ratios of 2.37 for a positive test and 0.22 for a negative test further confirmed that pDXA may be useful as a screening tool in this population.

I also evaluated whether combining both FRAX[®] and pDXA as screening tools would be more sensitive than using either alone. My results showed that although the addition of pDXA led to a slight improvement in specificity at all risk categorisations, the

combined sensitivity of the two tests was no better than that obtained using FRAX[®] alone. However, when risk factors were computed as standard or as HIV, the likelihood ratios suggested that combining FRAX[®] and pDXA may be useful in discriminating those with reduced BMD compared to those without, with the use of standard risk factors having the better result.

My results are in keeping with other studies that have found that FRAX[®] scores based on classic risk factors were not sufficiently sensitive in accurately predicting fracture risk in HIV-positive patients, even when HIV infection was included as a secondary risk factor [199,406-410]. However, when pDXA and FRAX[®] were combined, the results were interesting as they suggested that the use of both may be useful as screening tools, with risk factors classified as standard yielding the better result. More work is needed to further assess these findings and see if they apply to all HIV-positive cohorts or whether certain high-risk patients could be identified using pDXA and FRAX[®] in order to decide which patients would benefit from evaluation of BMD using DXA.

5.5.1.4 Conclusions

In summary, the rates of risk factors for osteoporotic fracture, including past medical history of osteoporosis, family history of osteoporotic fractures, falls and reduced mobility, were low in this cohort. These findings were expected as this was a relatively young cohort of HIV-positive men with well-controlled HIV infection. The 10-year probability of a major osteoporotic fracture or a hip fracture using FRAX[®] was also low. The use of the FRAX[®] tool alone was not sensitive enough to detect men at risk of osteoporotic fractures. A pDXA threshold of -1.6 was considered the optimal cut-off and the likelihood ratios suggested that pDXA may be useful as a screening tool in this cohort. Furthermore, combining pDXA and FRAX[®] (when risk factors were recorded as standard or as HIV) may be useful as screening tools in this homogeneous cohort of young, white HIV-positive MSM. However, as I was unable to calculate incident fracture rates, in particular, the 10-year fracture prediction rates for a major osteoporotic or hip fracture, further work is needed before any firm conclusions can be made in this cohort.

5.5.2 Strengths and limitations

The strengths of this analysis were the large number of patients included and the homogeneity of the study population. However, as the incident rate of fractures in my study was low, I was not able to assess either FRAX[®] or pDXA of the non-dominant

wrist as screening tools against incident fracture rates. The incident fracture rate may have been low due to the relatively short follow-up period.

The definition of a fragility fracture varied between studies in the literature, with some identifying all fractures sustained in adulthood, whilst others specified certain sites that are classically associated with osteoporosis, such as the hip, the spine and the wrist. In the questionnaire, there was insufficient detail provided regarding the mode of injury of some fractures, and some true fragility fractures may have been excluded, whilst other non-fragility fractures may have been included. It was also not possible to verify the results against participants' medical records, and therefore the results may be subject to recall bias.

The FRAX[®] tool uses a history of hip fracture in either the patient's mother or father, whereas I enquired about a history of hip fracture in either the participant's mother or maternal grandmother. This was what was asked in the pilot study, and to maintain consistency, the same was asked in my study. However, this may have led to under-reporting of a significant family history. Additionally, this variable could also not be verified against participants' medical records, and therefore the results may be subject to further recall bias.

5.5.3 Future work

Yin *et al* has shown that a modified-FRAX tool underestimated fracture rates more in HIV-positive men >50 years old compared to HIV-negative men, with only 3% to 6% of men with incident fractures correctly identified [410]. They demonstrated that adding HIV as a cause of secondary osteoporosis increased its accuracy, although it did not completely overcome the underestimation. However, their study involved a retrospective analysis. To date, the FRAX[®] tool has not been validated in HIV-positive patients and follow-up of the participants prospectively in this study over 10 years would enable the actual fracture rate to be calculated and then compared with the predicted fracture risk calculated using FRAX[®].

As TDF has been implicated the most out of all ART with regards to loss of BMD, comparing FRAX[®] scores in those who have been on TDF long-term to those on ART but unexposed to TDF may be further useful in ascertaining its role in fracture risk. The FRAX[®] scores could be compared with incident fractures over a 10 year period, which would help assess whether TDF is associated with not only lower BMD, but increased fracture risk as well.

Another avenue for future studies might be to compare the prediction of FRAX[®] scores in HIV and HCV mono-infected patients to those that are HIV/HCV co-infected. HCV is associated with significant fracture risk [432,433]. As HCV co-infection can be prevalent in certain HIV-positive cohorts such as in Brighton, comparison of FRAX[®] scores in HIV/HCV co-infected men to those with HCV mono-infection and HIV mono-infection from within my cohort would be particularly interesting.

Chapter 6: Utility of APR to diagnose tubular proteinuria in HIV-positive patients

6.1 Background

6.1.1 Introduction

HIV-positive patients are at increased risk of developing renal disease [163]. These include a range of glomerular, tubulo-interstitial and vascular diseases [164-166]. CKD was first defined by the National Kidney Foundation (NKF) using the Kidney Disease Outcomes Quality Initiative (K/DOQI) in 2002 as an eGFR <60 ml/min/1.73m², the presence of structural abnormalities in the kidney or abnormal urinary findings for more than three months [434]. It was accepted worldwide by the Kidney Disease: Improving Global Outcomes (KDIGO) in 2005 [435]. The severity of CKD can be categorised using GFR, which is an estimate of the kidney's ability to filter fluid through the glomerulus (Table 6.1.1.1).

Table 6.1.1.1 CKD categories

The stages of CKD according to GFR cut-offs.

Stage	GFR, ml/min/1.73m ²	Description
1	≥ 90	Kidney damage with normal or \uparrow GFR
2	60-89	Kidney damage with mild \downarrow GFR
3	30-59	Moderate \downarrow GFR
4	15-29	Severe \downarrow GFR
5	<15 or dialysis dependent	Kidney failure (ESRD)

ESRD: end stage renal disease; GFR: glomerular filtration rate

The causes of HIV-associated CKD have dramatically changed with the introduction of ART. In the pre-ART era, HIVAN, HIVICK and FSGS predominated on renal biopsies [164,167,168]. With the introduction of ART, the predominant causes of CKD are likely to be related to conditions associated with CKD in the general population (e.g. diabetes, hypertension), as well as to the side effects of ART [31].

CKD is a risk factor for progression to end stage renal disease (ESRD) and is associated with increased mortality in HIV-positive patients [436] making its early identification especially important. Current BHIVA guidelines recommend screening for CKD using eGFR and urine dipstick testing and assessment for the presence of proteinuria [437].

Proteinuria can be classified as glomerular or tubular. Proteinuria is usually defined as PCR >30 mg/mmol, which is equivalent to >200 mg of protein to 1 g of creatinine [434].

As proteinuria can occur even with a normal eGFR, it is important to be able to diagnose the presence of proteinuria and characterise its type [438,439]. RTD (also known as TP, but referred to as RTD henceforth) mainly refers to dysfunction of the proximal tubule, which can be due to a number of pathological conditions, including acute tubular injury, tubulo-interstitial nephritis, interstitial fibrosis and tubular atrophy [440].

6.1.2 Prevalence of CKD and proteinuria in HIV-positive patients

The prevalence of HIV-associated CKD varies greatly, depending on geography, the definition used, the equations used to measure eGFR, genetic heterogeneity, reporting methods and use of ART [441]. In North America and Europe, the reported prevalence of CKD as defined by eGFR <60 mL/min/1.73 m² was 4.7% to 9.7% [442] but it can rise to as much as 33% if using reduced eGFR or proteinuria in the definition [443,444].

In the pre-ART era, the prevalence of proteinuria, as measured either by >1+ on dipstick or microalbuminuria >30 mg/g, was relatively high [443,445,446]. Subclinical RTD is common in HIV-positive patients with the prevalence varying between 12% and 81% [270]. With the use of ART, the incidence of CKD and associated mortality has reduced, but the prevalence of CKD and the use of renal replacement therapy have increased [447]. The EuroSIDA study has reported that only a very small proportion (0.64%) of patients developed CKD, ESRD or died as a result of kidney disease over a median follow-up period of five years [448]. In the large START study in ART-naïve patients, the prevalence of CKD (defined by eGFR and/or proteinuria) was low at 6.2% [449].

6.1.3 Risk factors for proteinuria in HIV-positive patients

The aetiology of CKD in HIV-positive patients is multifactorial [450]. As HIV-positive patients continue to grow older, traditional risk factors for CKD are becoming more common [449]. These include older age, ethnicity (in particular, black ethnicity), hypertension, diabetes, cardiovascular disease and acute kidney injury (AKI).

However, HIV-positive patients are at increased risk of CKD, with HIV infection itself being an independent risk factor for CKD [451]. Other risk factors associated with CKD in HIV infection are low CD4 cell counts, high HIV viral loads, IVDU and HCV co-

infection [443,452-454]. However, black ethnicity and impaired renal function at baseline are the strongest risk factors for developing CKD and ESRD [436,452,455].

6.1.4 ART and CKD

The association between ART and CKD is complex. ART can be beneficial and has been shown to protect against CKD by preserving immune function [456,457]. Initiation of ART can also reduce the incidence of HIVAN and reduce its progression [458]. Additionally, discontinuation of ART has been associated with progression to ESRD in certain CKD conditions [459].

However, ART can also have a detrimental effect on the kidneys. Certain antiretroviral drugs are nephrotoxic and have been associated with CKD and AKI [460-462]. Those that have been commonly implicated include TDF and several PIs, including atazanavir, indinavir and lopinavir/ritonavir [461,463].

TDF can cause dysfunction of the proximal renal tubular epithelial cells, leading to AKI [464]. Although the exact mechanisms are not known, it is thought that TDF has an adverse effect on mitochondrial function [36,271,465]. In its severest manifestation of RTD, TDF can cause Fanconi syndrome, which is characterised by glycosuria, renal phosphate wasting and increased urinary excretion of LMWPs [33]. This usually only occurs when TDF and boosted PIs are co-prescribed [33]. Ritonavir boosts TDF levels by approximately 30% [466]. Additionally, it is thought that gene polymorphisms in renal tubular transporters, including organic anion transporters and multi-drug resistant protein families, can lead to increased intracellular tenofovir levels which can cause susceptibility to TDF-induced RTD [163,467]. As TDF mainly causes RTD, and it is still used as a first-line drug in treating HIV infection [26], having a robust method of distinguishing RTD from GP is useful in managing patients and in monitoring the adverse effects of treatment. Non-albumin proteinuria may be able to help distinguish RTD due to TDF from other types of kidney disease causing GP in HIV-positive patients [468].

6.1.5 The utility of APR in distinguishing RTD and GP

The aetiology of GP and RTD is different [438]. GP is associated with albuminuria, and hence, can be easily detected by measuring ACR [469]. Typically, non-HIV-related causes, such as diabetes and hypertension, have been implicated in GP [470].

In patients with RTD or Fanconi syndrome, there is impaired reabsorption of phosphate, glucose, urate, amino acids and LMWPs in the proximal renal tubule [163]. Proximal tubular enzymes and proteins may also be released into the urine during or after damage to the proximal renal endothelium [471]. In RTD, the LMWPs that have failed to be reabsorbed in the proximal tubule are excreted in the urine [169]. Albuminuria is rare, and so ACR will fail to detect RTD. Although PCR may provide an indication of the presence of proteinuria, it is unable to distinguish between GP and RTD.

Our group has previously demonstrated that an APR, which is the ratio of urine ACR to PCR, was highly sensitive and specific for tubulo-interstitial disease in the general population [172]. Proteinuria was defined as PCR ≥ 30 mg/mmol. RTD was categorised as PCR ≥ 30 mg/mmol and APR < 0.4 and GP as PCR ≥ 30 mg/mmol and APR > 0.4 . A cut-off for APR of < 0.4 was shown to be 88% sensitive and 99% specific for RTD, and this cut-off correlated well with a nephrological diagnosis of a primary tubulo-interstitial disorder [172]. I have further demonstrated that the calculation of APR is useful in distinguishing between RTD and GP in HIV-positive patients [472]. Measuring APR may be a useful screening tool, especially when trying to distinguish proteinuria secondary to ART, in particular, RTD associated with TDF and/or boosted PI use. It may be helpful in differentiating patients with ART-associated toxicity from those requiring further nephrological input including biopsy, in patients in whom there is significant proteinuria.

Normally, LMWPs are freely filtered through the glomerulus and reabsorbed by the proximal tubule. In RTD, high levels of LMWPs (e.g. RBP, beta 2-microglobulin, NGAL, cystatin C) are excreted in the urine [170,171]. Quantification of LMWPs has emerged as a sensitive way of assessing renal tubular function in HIV-positive patients [473]. Increased concentrations of LMWPs have been reported in HIV-positive patients with RTD [474]. However, LMWPs are not usually routinely available, and may be more expensive compared to PCR and ACR, which are relatively cheap to measure (approximately £0.20 and £0.50, respectively) to measure and readily accessible.

6.1.6 Summary

HIV-positive patients are at risk of HIV-associated CKD, especially as the population is ageing due to increased survival related to ART use. The aetiology is multifactorial, with proximal tubular pathology being different to that causing GP. Therefore, it is

important to be able to distinguish between GP and RTD. This chapter investigates diagnosis of RTD and examines the ability of APR to differentiate RTD from GP.

6.2 Aims and objectives

Below are the aims for this chapter:

1. To describe the prevalence of proteinuria in this cohort.
2. To identify the risk factors associated with proteinuria in this cohort, and in particular, any association between ART and proteinuria.
3. To assess the utility of APR in distinguishing between RTD and GP.

The initial work evaluating APR was done on retrospective samples from the HIV outpatient clinic [472]. My aim in this chapter is to assess the same hypotheses in my cohort of HIV-positive men who were prospectively recruited.

6.3 Methods

6.3.1 Study design

The detailed methods for the study are given in Chapter 2. In the current chapter, the data from the baseline visit (Year 1) were analysed.

Demographic and HIV-related details, including ART history, were obtained from the HIV clinic database. Risk factors for renal disease, including pre-existing renal disease and diabetes were evaluated using data from self-reported questionnaires. Blood pressure was measured. Relevant biochemical markers of renal function were measured in fasted blood. Fasting urine samples were analysed for PCR, ACR and for quantification of urinary phosphate using creatinine and phosphate.

6.3.2 Definitions

eGFR, expressed in ml/min/1.73m², was calculated using both the MDRD and CKD-Epi formulae. Hypertension was defined as a blood pressure >140/90 mmHg. FePO₄ was calculated using the following equation:

$$\text{FePO}_4 = \frac{\text{urine phosphate} \times \text{serum creatinine} \times 100}{\text{serum phosphate} \times \text{urine creatinine}}$$

The significance of low-level proteinuria (PCR <30 mg/mmol) is currently not known, so I focussed on proteinuric samples (PCR ≥30 mg/mmol, equivalent to ~300 mg/day of urinary protein). I used the cut-offs that I had established in a retrospective analysis of patients from the same HIV outpatient clinic [472]. Proteinuric samples were categorised into two classes according to the calculated APR:

1. Predominantly RTD: PCR ≥30 mg/mmol and APR <0.4.
2. Predominantly GP: PCR ≥30 mg/mmol and APR >0.4.

6.3.3 Statistical analyses

Patients with significant proteinuria (PCR ≥30 mg/mmol) were identified, and APR calculated in these patients. Those who did not have APR results were excluded. Variables were transformed where appropriate, to approximate a normal distribution prior to analyses.

The distribution frequency of each variable was calculated. Mean and SD were measured in those that were normally distributed and median and IQR in those that had skewed distributions.

Differences between groups were assessed using an paired t-tests for normally distributed continuous variables, Wilcoxon rank sum test for non-parametric variables and a Fisher's exact test for categorical variables. Statistical significance was denoted as p-value ≤0.05.

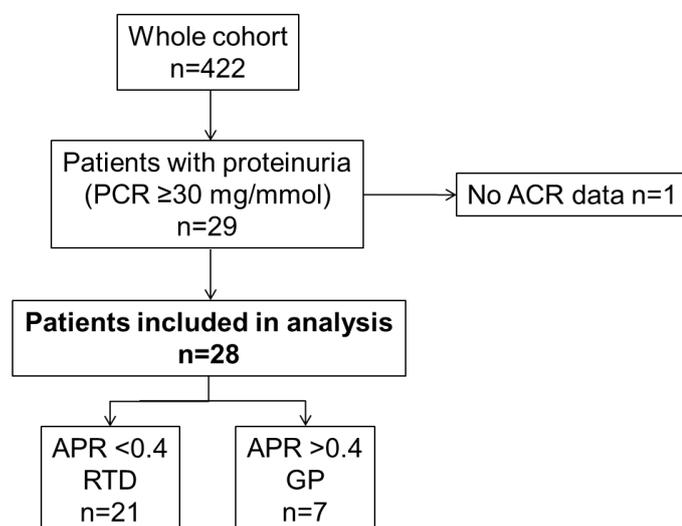
6.4 Results

6.4.1 Subject disposition and prevalence of proteinuria

Of 422 HIV-positive men recruited into the study, only 29 had proteinuria defined as PCR ≥30 mg/mmol (Figure 6.4.1.1). Of these, one patient was excluded as he had not had ACR measured, so it was not possible to calculate his APR. In total, 28 men were included in the analyses in this chapter, of whom 21 had RTD and seven had GP. The prevalence of proteinuria was 6.9% (29/422).

Figure 6.4.1.1 Summary of subject disposition

This figure shows the number of men with proteinuria included and analysed in this chapter. Using APR cut-offs, there were 21 men with RTD and 7 with GP.
 ACR: albumin/creatinine ratio; APR: albumin/protein ratio; GP: glomerular proteinuria; PCR: protein/creatinine ratio; RTD: renal tubular dysfunction



6.4.2 Demographics

Table 6.4.2.1 shows the demographic characteristics of patients with proteinuria. The majority were white (96.4%), MSM (96.4%) with a mean age of 53.9 (SD 8.4) years. There was no significant difference in demographics between patients with RTD and those with GP.

Table 6.4.2.1 Baseline demographics

The majority of men were young, white MSM.

	Total (N=28)	RTD (N=21)	GP (N=7)	P-value*
Age, years, mean (SD)	53.9 (8.4)	54.0 (8.1)	53.4 (10.0)	0.87
Ethnicity, n (%)				0.25
White	27 (96.4)	21 (100.0)	6 (85.7)	
Black	1 (3.6)	0 (0.0)	1 (14.3)	
HIV transmission risk, n (%)				1.00
MSM	27 (96.4)	20 (95.2)	7 (100.0)	
Heterosexual sex	1 (3.6)	1 (4.8)	0 (0.0)	

GP: glomerular proteinuria; MSM: men who have sex with men; RTD: renal tubular dysfunction; SD: standard deviation

*Comparison between RTD and GP

6.4.3 HIV parameters

HIV-related factors are shown in Table 6.4.3.1. The median duration of HIV infection was 13.8 (IQR 5.5, 23.3) years and 9 (32.1%) men had a previous AIDS-defining diagnosis. Although the median nadir CD4 count had been low at 171 (IQR 130, 235)

cells/ μ L, the median CD4 count at the time of recruitment was 481 (IQR 397, 639) cells/ μ L. All were on ART with 24 (85.7%) men having an undetectable HIV viral load. There were 19 (67.9%) and 15 (53.6%) men on TDF or a boosted PI, respectively, with 8 (28.6%) on both TDF and a boosted PI concurrently. The rates of HBV and HCV co-infection were 14.3% and 3.6%, respectively.

Table 6.4.3.1 HIV-related factors

The duration of HIV infection was long and 32.1% had been diagnosed with AIDS. However, as they were all on ART and had an undetectable HIV viral load, the median CD4 count at recruitment was high.

	Total (N=28)	RTD (N=21)	GP (N=7)	P- value*
Duration of HIV infection, years, median (IQR)	13.8 (5.5, 23.3)	10.2 (4.3, 23.7)	20.1 (8.8, 23.0)	0.30
HIV clinical stage, n (%)				0.74
Asymptomatic	6 (21.4)	5 (23.8)	1 (14.3)	
Symptomatic non-AIDS	13 (46.4)	10 (47.6)	3 (42.9)	
Symptomatic AIDS	9 (32.1)	6 (28.6)	3 (42.9)	
CD4, cells/ μ L, median (IQR)				
Nadir	171 (130, 235)	157 (123, 217)	239 (137, 355)	0.20
At recruitment	481 (397, 639)	421 (338, 612)	547 (442, 785)	0.19
HIV viral load <40, copies/mL, n (%)				1.00
Yes	24 (85.7)	18 (85.7)	6 (85.7)	
No	4 (14.3)	3 (14.3)	1 (14.3)	
HBV co-infection, n (%)				1.00
Yes	4 (14.3)	3 (14.3)	1 (14.3)	
No	24 (85.7)	18 (85.7)	6 (85.7)	
HCV co-infection, n (%)				1.00
Yes	1 (3.6)	1 (4.8)	0 (0.0)	
No	27 (96.4)	20 (95.2)	7 (100.0)	
ART status, n (%)				-
Naive	0 (0.0)	0 (0.0)	0 (0.0)	
Current	28 (100.0)	21 (100.0)	7 (100.0)	
TDF and PI/ritonavir exposure, n (%)				
On TDF at recruitment	19 (67.9)	16 (76.2)	3 (42.9)	0.17
On PI/ritonavir at recruitment	15 (53.6)	11 (52.4)	4 (57.1)	1.00
On TDF and PI/ritonavir at recruitment	8 (28.6)	7 (33.3)	1 (14.3)	0.63

ART: antiretroviral therapy; GP: glomerular proteinuria; HBV: hepatitis B; HCV: hepatitis C; IQR: interquartile range; PI: protease inhibitor; RTD: renal tubular dysfunction; SD: standard deviation

*Comparison between RTD and GP

6.4.4 Renal parameters

6.4.4.1 Renal risk factors

Table 6.4.4.1 shows renal risk factors. The rates of self-reported history of prior renal disease and diabetes were 28.6% and 14.3%, respectively. There were 7 (25.0%) men with hypertension and 9 (32.1%) were current smokers at recruitment.

Table 6.4.4.1 Renal risk factors

Participants had renal risk factors, including pre-existing renal disease (28.6%), hypertension (25%) and diabetes (14.3%). Smoking was common with only 25% having never smoked.

	Total (N=28)	RTD (N=21)	GP (N=7)	P-value*
Pre-existing renal disease, n (%)				0.37
Yes	8 (28.6)	5 (23.8)	3 (42.9)	
No	20 (71.4)	16 (76.2)	4 (57.1)	
Hypertension, mmHg, n (%)				1.00
Yes	7 (25.0)	5 (23.8)	2 (28.6)	
No	21 (75.0)	16 (76.2)	5 (71.4)	
Diabetes, n (%)				0.04
Yes	4 (14.3)	1 (4.8)	3 (42.9)	
No	24 (85.7)	20 (95.2)	4 (57.1)	
Smoking, n (%)				0.65
Never smoked	7 (25.0)	5 (23.8)	2 (28.6)	
Ex-smoker	12 (42.9)	10 (47.6)	2 (28.6)	
Current smoker	9 (32.1)	6 (28.6)	3 (42.9)	

GP: glomerular proteinuria; RTD: renal tubular dysfunction

*Comparison between RTD and GP

6.4.4.2 Renal function

Renal function and related blood and urine parameters are shown in Table 6.4.4.2. The median eGFR was 79.4 (IQR 65.8, 90.2) and 80.6 (IQR 65.1, 92.5) ml/min/1.73m² using the MDRD and CKD-Epi equations, respectively. The medians were within stage 2 of the KDOQI classification for CKD, denoting minimally reduced renal function (National Kidney Foundation Am J Kidney Dis 2002). There were only 3 (10.7%) men with albuminuria. There were 24 (85.7%) men with raised RBPCR (>2.93 µg/mmol). The median FePO₄ was raised (21.3% [IQR 15.3, 30.0]), and 10 (35.7%) men had FePO₄ >20% and serum phosphate <0.8 mmol/L.

Table 6.4.4.2 Renal function and related parameters

Median eGFR was classified as as K/DOQI stage 2 (minimally reduced) using both MDRD and CKD-Epi equations. Only 3 men had albuminuria (ACR >30 mg/mmol). Median FePO₄ was 21.3% and 10 men had FePO₄ >20% with phosphate <0.8 mmol/L.

	Total (N=28)	RTD (N=21)	GP (N=7)	P-value*
Creatinine, µmol/L, median (IQR)	98 (82, 108)	100 (83, 111)	87 (81, 108)	0.60
eGFR (MDRD), ml/min/1.73m ² , median (IQR)	79.4 (65.8, 90.2)	72.7 (63.6, 88.7)	85.6 (67.4, 91.0)	0.35
eGFR (CKD-Epi), ml/min/1.73m ² , median (IQR)	80.6 (65.1, 92.5)	75.4 (63.4, 91.6)	86.0 (68.1, 97.6)	0.41
PCR, mg/mmol, median (IQR)	40.7 (33.3, 56.5)	37.3 (32.5, 41.7)	60.5 (57.6, 90.9)	0.002
ACR, mg/mmol, median (IQR)	6.4 (3.9, 21.9)	4.8 (3.4, 7.3)	30.0 (26.8, 74.2)	0.0002
ACR, mg/mmol, n (%)				0.01
<3	5 (17.9)	5 (23.8)	0 (0.0)	
3 - 30	20 (71.4)	16 (76.2)	4 (57.1)	
>30	3 (10.7)	0 (0.0)	3 (42.9)	
RBPCR, µg/mmol, median (IQR)	13.5 (3.5, 127.2)	21.1 (6.6, 182.9)	3.5 (2.0, 12.9)	0.06
RBPCR, µg/mmol, n (%)				0.12
<2.93	4 (14.2)	2 (9.5)	2 (28.6)	
2.93 - 14.65	11 (39.3)	7 (33.3)	4 (57.1)	
>14.65	13 (46.4)	12 (57.1)	1 (14.3)	
Phosphate, mmol/L, median (IQR)	0.82 (0.60, 0.94)	0.79 (0.57, 0.92)	0.89 (0.67, 0.95)	0.30

	Total (N=28)	RTD (N=21)	GP (N=7)	P- value*
FePO ₄ , %, median (IQR)	21.3 (15.3, 30.0)	21.7 (15.7, 29.8)	21.0 (11.8, 30.7)	0.94
FePO ₄ >20% and phosphate <0.8 mmol/L, n (%)	10 (35.7)	9 (42.9)	1 (14.3)	0.18

ACR: albumin/creatinine ratio; APR: urine albumin/total protein ratio; CKD-Epi: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate; FePO₄: fractional excretion of phosphate; GP: glomerular proteinuria; K/DOQI: Kidney Disease Outcomes Quality Initiative; IQR: interquartile range; MDRD: Modification of Diet in Renal Disease equation; PCR: protein/creatinine ratio; RBPCR: retinol binding protein creatinine ratio; RTD: renal tubular dysfunction

*Comparison between RTD and GP

6.4.5 Comparison of RTD and GP

When comparing patients with RTD to those with GP, there were no differences in demographic (Table 6.4.2.1) or HIV-related (Table 6.4.3.1) characteristics, including ART. Those with GP were more likely to be diabetic, although the overall number of men with diabetes was small (Table 6.4.4.1). Additionally, men with GP were significantly more likely to have a higher PCR and ACR compared to those with RTD (Table 6.4.4.2). There was a borderline difference in median RBPCR levels, with those with RTD having higher values than those with GP (Table 6.4.4.2).

There were five patients with a combination of RTD, reduced eGFR and albuminuria. All seven patients with GP had albuminuria. Interestingly, 16 patients had both RTD and albuminuria.

6.5 Discussion

6.5.1 Summary

Out of 422 men recruited into the study, only 28 had proteinuria (PCR \geq 30 mg/mmol), as well as APR measurements. The low prevalence of proteinuria has limited the analyses that I was able to conduct, but likely reflects the fact that although the patients had well-established and longstanding HIV infection, they had good immune function. This was due to most having their HIV infection well-controlled with ART. However, in the retrospective study I conducted from the same clinic population, which included a much larger sample size, approximately 18% of this predominantly white, male, ART-experienced cohort had at least one measurement indicating significant proteinuria (PCR \geq 30 mg/mmol), which is of concern [472]. Thus, screening for proteinuria is likely to be useful in identifying patients at risk of renal dysfunction and vascular disease, although that is not a conclusion that can be made from this analysis.

6.5.1.1 ART and RTD

Although ART can improve some renal conditions, such as HIVAN, it can also cause some types of renal disease. Studies have suggested that TDF can cause RTD, especially when co-prescribed with a boosted PI [475]. Some ART, especially TDF and boosted PIs, are associated with worsening of renal function and progression of CKD [476]. Despite these concerns, TDF remains a safe and effective drug against HIV for many patients, and is a recommended drug in first-line regimens [26].

Of crucial interest then is the ability to identify when such drugs are becoming a problem. Although easily calculated, eGFR is often insensitive in early renal disease and does not correlate well with RTD [477]. In the previous study from this cohort, RTD was associated with the use of TDF or a boosted PI [472]. Patients with RTD, compared to those with GP, were also more likely to have been on, or to be taking, a regimen containing both TDF and a boosted PI at the time of sampling. This is consistent with other studies showing that TDF use may cause renal dysfunction, and that the dysfunction is greater when TDF and a boosted PI are prescribed simultaneously [478-480]. I had hoped to further investigate these findings in this cohort, but was limited by the small number of men with proteinuria.

6.5.1.2 Measurement of proteinuria

It is important to consider which screening tests are used to determine the source of proteinuria in HIV-positive patients. Using ACR or dipstick urinalysis alone as a screening test for proteinuria may not be sufficient for detecting RTD or for identifying ART-related problems. Previous results from this clinic have shown that measuring both PCR and ACR on a single sample (and hence calculating APR) may be both practical and helpful in evaluating proteinuria in selected HIV-positive patients, and may help to identify those in whom a more careful evaluation of RTD is warranted [472].

Since those data were published, other groups have further confirmed the utility of APR in detecting RTD in HIV-positive patients. A large cross-sectional study used the same cut-offs that we had used to evaluate the prevalence of proteinuria in HIV-positive patients with normal eGFR [450]. They found that TDF was associated with RTD and low APR [450]. Those who were on TDF co-prescribed with a boosted PI had two times higher odds of RTD than those that were on TDF without a boosted PI, but these odds were still higher than those on a TDF-sparing regimen [450]. However, in this study, the diagnoses of RTD and GP were not confirmed by biopsy. Recently, Sise *et al* further confirmed the association of RTD and low APR in patients with biopsy-proven TDF

nephrotoxicity [481]. They conducted a retrospective case note review and compared 43 patients with TDF nephrotoxicity to 11 HIV-positive patients who had never been exposed to TDF but who had undergone a renal biopsy. They found that those with TDF nephrotoxicity had a lower median APR compared to those not on TDF (0.17 [IQR 0.14 - 0.19] vs. 0.65 [IQR 0.55 - 0.79], $p < 0.001$) [481]. The histopathology from the biopsies was in agreement with the findings from APR as the majority of patients with TDF nephrotoxicity had mitochondrial abnormalities, which are associated with TDF-related kidney damage [465].

There are a number of tests which can identify specific LMWPs associated with RTD, such as RBP [473]. However, an advantage of APR is that PCR and ACR are more widely available and are also cheaper. They could therefore be used to test for RTD in all patients, whereas the more specific tests could be reserved for use in certain high-risk patients (e.g. those on TDF and/or a boosted PI) or in those in whom there is uncertainty (i.e. it could be used as a secondary confirmatory test in those who have an APR value close to the cut-off of 0.4).

6.5.1.3 Conclusions

Although HIV-positive patients are at risk of developing proteinuria and RTD, and patients on TDF and/or a boosted PI may be more susceptible, the prevalence of proteinuria in this ART-experienced cohort of mainly white MSM was low, despite their long-term exposure to TDF. There are a number of tests that can be used to determine RTD. These include measuring PCR and ACR to calculate APR, or the use of novel LMWPs. Although the latter have been shown to be highly sensitive and specific for RTD, they can be expensive and are not routinely available. In contrast, PCR and ACR are routinely available and are cheap, and could be measured in all patients. As all tests have advantages and disadvantages, it may be that some are better as screening tests, whilst others are reserved for special circumstances (e.g. those that are hard to diagnose).

6.5.2 Strengths and limitations

The main finding of these results is the lack of proteinuria in a cohort heavily exposed to ART, and in particular, RTD. However, the analyses for this chapter were limited by the small number of patients who had overall proteinuria, as well as those with RTD or GP, which led to the analyses being mainly descriptive. A larger cohort of patients with proteinuria would have enabled me to conduct more detailed analyses.

As in the published paper, I had planned to assess patients with heavy proteinuria (PCR >100 mg/mmol, equivalent to ~300 mg/day of urinary protein) [472]. In these patients, the cause of renal disease would have been identified using hospital notes and renal biopsy results examined where available. However, no participant had heavy proteinuria in my cohort and none had undergone a renal biopsy, so these analyses were not able to be performed. In the retrospective analysis, there were 18 patients with heavy proteinuria [472]. Of these, six patients had RTD, two of whom had TDF-related RTD which improved on switching off TDF, suggesting that TDF was the cause of the RTD [472].

A definitive diagnosis of renal disease is made by obtaining tissue from biopsy. In the retrospective analysis of patients from this clinic, eight patients with heavy proteinuria had undergone a renal biopsy [472]. In all cases, the biopsy results correlated with the definitions of proteinuria using APR. However, the number of patients with a biopsy result was small. Since I conducted these analyses, a study with a larger cohort of patients with TDF-associated renal toxicity confirmed on biopsy has found APR to be a reliable measure of proteinuria [481]. The authors reported that patients with TDF-associated renal toxicity had a mean APR <0.4 which is in keeping with RTD.

As the clinical significance of low-level proteinuria was unknown, I excluded patients with low-level proteinuria. However, a study has since reported a high prevalence (55%) of low-grade proteinuria, which was defined as PCR >70 mg/g [482]. In this study, low-level proteinuria was associated with older age, diabetes and exposure to an NRTI independent of TDF [482]. Their cohort was similar demographically to my cohort. If I had assessed APR in those with low-level proteinuria, I might have had a larger sample from which I could have drawn more meaningful conclusions. However, the clinical significance of these results is yet to be determined.

6.5.3 Future work

It would be interesting to apply the criteria used by Gravemann *et al* [482] to assess low-level proteinuria in this cohort. This would be best done in a prospective study with long-term follow-up to check whether low-level proteinuria occurs every time.

It would also be useful to follow the 28 patients with proteinuria long-term. With the emergence of more renal-friendly ART, some of these patients are likely to be switched off TDF in the future. TDF has been shown to cause an improvement in renal function on its discontinuation, with some studies suggesting complete reversibility of its effects

[453], whilst others have shown that the reversibility is incomplete [483]. It would thus be interesting to assess the effect of switching off TDF and to see to what extent any proteinuria improved.

Chapter 7: Effects of RTD on bone in HIV-positive patients

7.1 Background

7.1.1 Introduction

In RTD, proximal tubular dysfunction leads to an impaired ability to reabsorb LMWPs and phosphate in the proximal tubule [169]. This leads to increased excretion of LMWPs in the urine and renal phosphate wasting. RTD is typically asymptomatic, non-progressive and non-treatment limiting, and characterised by mild to moderate increases in urinary LMWP concentration and variable manifestations of impaired reabsorption of phosphate, urate, glucose and other solutes [473]. In its severest form, RTD can lead to Fanconi syndrome [35,271]. RTD may also have an effect on bone mineralisation, which can contribute to bone pain, osteomalacia, reduced BMD and an increased risk of fragility fractures [33,35,37].

7.1.2 LMWPs and measurement of RTD

As mentioned in Chapter 6, RTD can be measured using PCR, ACR and calculating APR. It can also be measured using a range of LMWPs. LMWPs (e.g. β 2-microglobulin, RBP, NGAL, cystatin C) are small molecules that are freely filtered at the glomerulus and reabsorbed in the proximal tubule [473]. Although there are small amounts of LMWPs in the urine in people with normal tubular function, in RTD, increased levels are excreted. Consequently, quantification of LMWPs has emerged as a sensitive way of assessing renal tubular function and a measure of the severity of RTD [473].

RBP is a 21 kDa protein that circulates in plasma bound to transthyretin, with the unbound fraction (~10%) being freely filtered by the glomerulus and then reabsorbed in the proximal tubule [170]. RTD leads to an increase in RBP levels [174].

7.1.3 Burden of RTD in HIV-positive patients

Kidney disease can be caused by the HIV infection itself, other pathologies, and ART [163,166]. Proteinuria and albuminuria are common in HIV-positive patients, especially in those on ART [443,484].

RTD is one form of kidney disease commonly seen in HIV-positive patients. It has been reported in 12% to 81% of patients depending on the definition used [270]. Studies have shown that HIV-positive patients have higher levels of urinary RBP in both the pre-ART [438] and ART [485] eras. Kabanda *et al* reported an elevation of at least one LMWP in 74% of stable HIV-positive patients with normal renal function and normal urinalysis on dipstick testing [438]. The authors reported that older age and male sex were associated with higher levels of LMWPs [438]. In the post-ART era, the prevalence of RTD has remained high, with ART also implicated as a potential risk factor. In one UK study, the prevalence of RTD was 53% in ART-naïve patients compared to 80% in those on ART [485].

The main antiretroviral that has been implicated is TDF, and Hall *et al* found higher levels of urinary RBP in patients on TDF compared to those that were ART-naïve or to those on a regimen not containing TDF [474]. However, the risk is greater when TDF and boosted PIs are co-administered [271]. Other risk factors include older age and lower BMI [271,467], as well as the presence of genetic polymorphisms in the tubular transporter proteins, in particular, MRP2 and MRP4, which are involved in the transport of both TDF and boosted PIs [486,487].

7.1.4 ART and renal function

There is a complex association between ART and CKD. ART can have a beneficial effect on the kidneys. It can reduce the incidence and progression of HIVAN [458], as well as help to preserve immune function and thereby protect against CKD [456,457]. ART discontinuation has been shown to lead to ESRD progression in some types of CKD [459]. Conversely, exposure to ART can also have a negative impact on the kidneys. Some antiretroviral drugs are nephrotoxic and can cause AKI [460-462]. Those that have been shown to cause kidney damage are TDF and several PIs, including atazanavir, indinavir and lopinavir/ritonavir [461,463].

7.1.5 TDF and renal function

TDF is a preferred treatment option for HIV infection and one of the most widely prescribed antiretroviral drugs [26]. Although TDF was not associated with increased renal toxicity in RCTs [29-32], there are case reports and studies of renal tubular injury in patients taking TDF [33,36]. A small study showed that HIV-positive patients developed severe metabolic disease associated with hypophosphataemia and

increased urinary phosphate excretion whilst on TDF [33]. Several cohort studies have also shown an association between TDF exposure and a reduction in GFR [478,488-490].

TDF can cause or worsen RTD [491,492]. Although the exact mechanisms are not known, it has been postulated that TDF adversely affects mitochondrial function [36,271,465]. TDF exposure can lead to dysfunction of the proximal renal tubular epithelial cells, in turn causing AKI [464]. In a study of 19 cases with RTD, features seen on renal biopsy included severe acute tubular necrosis (ATN) with interstitial fibrosis and oedema [493]. Interestingly, many studies have noted partial or full reversal of abnormalities on stopping TDF [33,467,493,494].

Several studies have reported an association between TDF and Fanconi syndrome [33-38,271]. Fanconi syndrome more commonly occurs when boosted PIs are co-prescribed with TDF [33,493], with ritonavir shown to boost TDF levels by approximately 30% [466]. It is also postulated that gene polymorphisms in renal tubular transporters (e.g. organic anion transporters and multi-drug resistant protein families) can increase intracellular TDF levels which can lead to susceptibility to RTD [163,467].

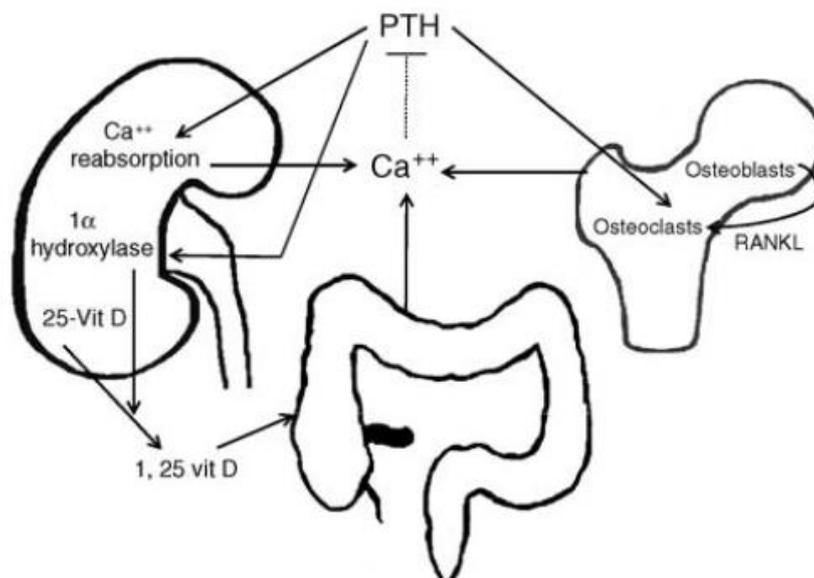
7.1.6 Relationship between bone and renal disease

There is a complex relationship between the bones and the kidneys, which is linked by calcium and phosphate homeostasis (Figure 7.1.6.1) [141,173]. Firstly, the proximal tubules are directly involved in calcium and phosphate regulation by altering how much is reabsorbed. Secondly, the kidneys are involved in the metabolism of vitamin D, which is linked to PTH release, which in turn has an effect on calcium and phosphate levels. On the other hand, there is release of calcium stored within the bones in response to hypocalcaemia. Bone also responds to an increase in PTH by activating osteoclastic reabsorption, which leads to the release of more calcium, as well as phosphate. Any alteration in these normal mechanisms can cause renal and/or bone disease.

Figure 7.1.6.1 Calcium homeostasis and the relationship between bones and kidneys
[141]

There is a complex relationship between the kidneys and the bones, which involve the regulation of calcium, phosphate, PTH and vitamin D.

1,25 vit D: 1,25-dihydroxyvitamin D; 25-Vit D: 25-hydroxyvitamin D; Ca⁺⁺: calcium; PTH: parathyroid hormone; RANKL: receptor activator for nuclear factor κB ligand



The kidneys can have an indirect effect on bone by causing acid/base disturbances, electrolyte imbalances and tubular defects. The kidneys may also have a direct effect by producing substances that alter bone (e.g. calcitriol and bone morphogenic protein 7 [BMP-7]) [173]. Additionally, bone can produce substances (e.g. fibroblast growth factor-23 [FGF-23]) that can have an effect on the kidneys [173]. Vitamin D is involved in promoting calcium and phosphate absorption from the gut [234]. The parathyroid glands are activated to release PTH to restore serum calcium levels in certain situations, which include hypocalcaemia, hyperphosphataemia and VDD [234,235].

The bone changes that occur as a result of glomerular-related kidney disease are termed CKD-MBD. This is the term defined by KDIGO to replace renal osteodystrophy and refers to a systemic disorder of mineral and bone metabolism due to CKD [495]. It involves abnormalities of calcium, phosphate, PTH and vitamin D [496]. It leads to changes in bone turnover, bone mineralisation, bone volume, linear growth or strength, or vascular and soft tissue calcification. Although CKD-MBD only occurs in the presence of a reduced GFR ($<60 \text{ ml/min/1.73m}^2$), in patients with pre-existing bone disease or risk factors for bone disease, it can co-exist with other pathologies and lead to osteoporosis.

Bone disease occurring secondary to RTD is usually associated with low bone turnover and abnormal mineralisation, which can cause osteomalacia [173]. Other forms of

kidney disease can also have an effect on bone. This includes renal tubular acidosis, where a rise in hydrogen ions directly leads to dissolved bone mineral. It can also cause osteomalacia indirectly by upregulating PGE2 and RANKL, increased collagen synthesis and osteoclast differentiation. Finally, tubular defects of calcium and phosphate transport can cause hypophosphataemia and hypercalcaemia, which can lead to osteomalacia and rickets.

7.1.7 TDF and its association with RTD and reduced BMD

TDF can also adversely affect BMD in a number of ways. It can cause a direct effect by its actions on osteoclasts and osteoblasts due to altered gene metabolism which have been seen *in vitro* [39,40]. TDF can also have an indirect effect on BMD by causing RTD or Fanconi syndrome, both of which can lead to renal phosphate wasting and osteomalacia [33]. Excessive renal phosphate wasting is a concern because phosphate loss can stimulate compensatory bone resorption, which can cause a reduction in BMD over time [141]. TDF can also affect the vitamin D/PTH axis (Figure 7.1.6.1). This can lead to secondary hyperparathyroidism and increased bone turnover, which are worse in patients with VDD [41].

Patients initiating TDF-containing regimens experience greater reductions in BMD compared to those starting regimens not containing TDF [251,252]. Additionally, switching to a TDF-containing regimen has been associated with reductions in BMD, whilst discontinuing TDF has been shown to improve BMD [250]. Preliminary evidence suggests that TDF-associated BMD reductions may translate into increased fracture risk [266]. Therefore, measuring RBP may be useful in diagnosing RTD and identifying HIV-positive patients most at risk of low or declining BMD, especially in those on TDF.

7.1.8 Summary

HIV-positive patients are at risk of developing RTD, which has been associated with ART, in particular, TDF. Although not used routinely in clinical practice, LMWPs, such as RBP, can be used to identify and quantify RTD. This chapter explores the relationship between RTD (measured using RBP as well as phosphate wasting) and bone (bone turnover and BMD) in the entire cohort, as well as specifically in patients on TDF.

7.2 Aims and objectives

The aims for this Chapter are:

1. To describe the distribution of the following variables:
 - a. Demographic characteristics
 - b. HIV parameters
 - c. Renal parameters
 - d. Bone parameters.
2. To investigate the relationship between RBPCR and FePO₄.
3. To identify the factors associated with RTD as defined by RBPCR and phosphate wasting.
4. To assess the relationship between bone turnover and RTD.
5. To assess the relationship between BMD and RTD.

Fux *et al* [497] reported that RTD was associated with increased bone turnover. Data have shown that some antiretroviral drugs, in particular TDF, are associated with low BMD [250-252]. There are also case reports which show that TDF is associated with renal phosphate wasting and Fanconi syndrome [35], which can lead to RTD and osteomalacia [33,37]. However, it remains unclear whether sub-clinical RTD is associated with altered bone homeostasis and/or reductions in BMD. I hypothesised that RTD is associated with changes in BMD and bone turnover. In this chapter, I examine the relationship between RTD (measured using RBP and phosphate wasting), bone turnover and BMD.

7.3 Methods

7.3.1 Study design

The detailed methods of the study are given in Chapter 2. In this chapter, the data from the study participants' baseline visit (Year 1) were used.

Demographic and HIV-related details, including ART history, were obtained from the HIV clinic database. Additional data on medical history and lifestyle factors associated with reduced BMD and renal disease were evaluated using data from self-reported questionnaires. Biometrics, including height, weight and blood pressure, were measured. BMI was calculated.

Fasting blood samples were measured for creatinine, phosphate, ALP, 25(OH)D and PTH. Bone turnover was measured using CTX and P1NP from frozen fasted blood samples. Fasting urine samples were analysed for PCR and for quantification of urinary phosphate using creatinine and phosphate. Urinary RBP was measured using a frozen aliquot of urine stored at -80° using the DELFIA[®] monoclonal antibody assay. The lower limit of detection was $<1.0 \mu\text{g/L}$ [498] and 98% of samples were within measuring range of the assay.

The GE Healthcare Lunar iDXA bone densitometer (GE Healthcare, Madison, Wisconsin, USA) was used to measure absolute BMD (g/cm^2) at the lumbar spine, the total hip (left total hip and right total hip) and the femoral neck (left femoral neck and right femoral neck). Using hand dominance, BMD data was reported for the non-dominant total hip and non-dominant femoral neck.

7.3.2 Definitions

eGFR, expressed in $\text{ml/min}/1.73\text{m}^2$, was calculated using both the MDRD and CKD-Epi formulae, with eGFR categorised using standard CKD definitions of eGFR >90 , 75-89 and $<75 \text{ ml/min}/1.73\text{m}^2$ (due to a small number of patients with eGFR $<60 \text{ ml/min}/1.73\text{m}^2$). Hypertension was defined as a blood pressure $>140/90 \text{ mmHg}$. RBP, expressed as a ratio with urine creatinine (RBPCR), was measured using an established reference range of 0.12-2.93 $\mu\text{g}/\text{mmol}$. FePO_4 was calculated using the following equation:

$$\text{FePO}_4 = \frac{\text{urine phosphate} \times \text{serum creatinine} \times 100}{\text{serum phosphate} \times \text{urine creatinine}}$$

RTD was assessed using both RBPCR and phosphate wasting. Patients were considered to have RTD if their RBPCR was above the laboratory upper limit of normal (ULN, $>2.93 \mu\text{g}/\text{mmol}$) and were arbitrarily stratified into those with mild to moderate RTD (RBPCR 1-5 times ULN i.e. 2.93-14.65 $\mu\text{g}/\text{mmol}$) and severe RTD (RBPCR >5 times ULN i.e. $>14.65 \mu\text{g}/\text{mmol}$). Participants were also considered to have RTD if they had evidence of phosphate wasting as defined by a $\text{FePO}_4 >20\%$ in the presence of hypophosphataemia (serum phosphate $<0.8 \text{ mmol/L}$).

7.3.3 Statistical analysis

Only patients with data on RBP and phosphate wasting were included in the analyses. Prior to conducting analyses, variables were transformed where appropriate to approximate to a normal distribution.

The correlation between RBPCR and FePO_4 was assessed. Logistic regression was performed to investigate the factors associated with RTD as defined by RBPCR and phosphate wasting. All factors that were significant at the 10% level in univariable analyses were tested in a multivariable model. Statistical significance was denoted by p-value ≤ 0.05 .

The relationship between bone turnover and BMD with RTD was examined using correlation coefficients, and where possible, multivariable regression. RBPCR was analysed as both a continuous and a categorical variable. Assumptions were tested graphically. Exposures, confounders and interactions were chosen *a priori* on the basis of biological plausibility as well as a significant association in univariable analysis with $p \leq 0.1$. In multivariable models, statistical significance was denoted by p-value ≤ 0.05 .

A sensitivity analysis involving all of the above statistical analyses was undertaken in patients on TDF.

Data were complete or near complete for the majority of cases.

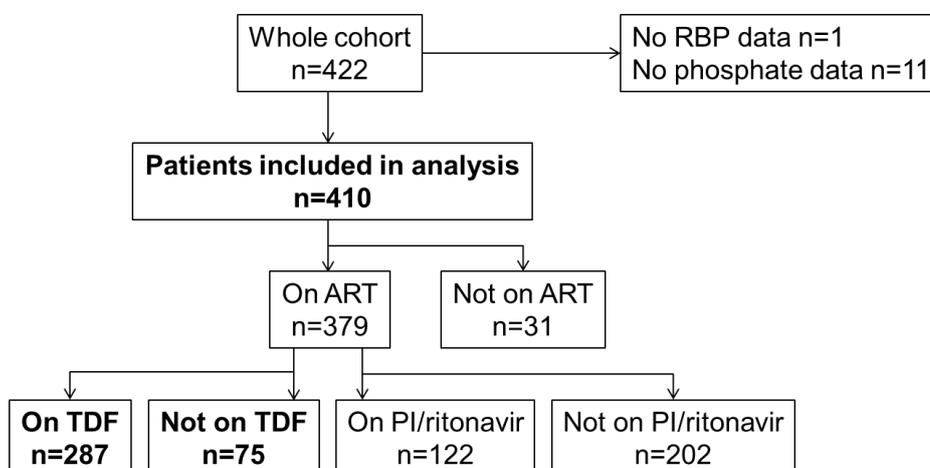
7.4 Results

7.4.1 Subject disposition

Of 422 HIV-positive men recruited into the study, 12 were excluded from the analyses (Figure 7.4.1.1). One did not have RBP measured, and due to missing creatinine or phosphate measurements, it was not possible to calculate renal phosphate wasting in 11 patients. In total, 410 subjects were included in the analyses in this chapter.

Figure 7.4.1.1 Summary of subject disposition

This figure shows the number of patients included in the analysis, as well as the breakdown by the use of TDF and boosted PI.
 ART: antiretroviral therapy; PI: protease inhibitor; RBP: retinol binding protein; TDF: tenofovir



7.4.2 Demographics

The baseline demographics of all patients included in the analyses (n=410) are shown in Table 7.4.2.1. The majority were white (94.1%), with a mean age of 47 (SD 9.5) years, and had acquired HIV through sex with men (92.9%).

Table 7.4.2.1 Baseline demographics

The majority of men were white MSM with a mean age of 47 years.

	Total (N=410)
Age, years, mean (SD)	47 (9.5)
Ethnicity, n (%)	
White	386 (94.1)
Black	15 (3.7)
Other	9 (2.2)
HIV transmission risk, n (%)	
MSM	381 (92.9)
Heterosexual sex	25 (6.1)
IVDU/blood products	4 (1.0)

IVDU: intravenous drug use; MSM: men who have sex with men; SD: standard deviation

7.4.3 HIV parameters

HIV parameters are shown in Table 7.4.3.1. The median duration of HIV infection was long (9.8 [IQR 5.0, 15.5] years). Although 27.3% had been diagnosed with an AIDS-defining condition, the majority (92.4%) were on ART with a good immune function (median CD4 count [IQR] 538 [408, 693] cells/μl) and an undetectable HIV viral load (86.8%). The rates of HBV and HCV co-infection were 4.2% and 14.2%, respectively.

Most had been exposed to TDF (70.0%), with 43.7% on a boosted PI and 27.8% on TDF and a boosted PI concurrently at recruitment.

Table 7.4.3.1 HIV parameters

The duration of HIV infection was long and 27.3% had been diagnosed with AIDS. However, as 92.4% were on ART, most (86.2%) had an undetectable HIV viral load and a high median CD4 count at recruitment. The majority (70.0%) of men had been exposed to TDF.

	N	Total
Duration of HIV infection, years, median (IQR)	410	9.8 (5.0, 15.5)
HIV clinical stage, n (%)	410	
Asymptomatic		188 (45.9)
Symptomatic non-AIDS		110 (26.8)
Symptomatic AIDS		112 (27.3)
CD4, cells/ μ L, median (IQR)	410	
Nadir		186 (95, 274)
At recruitment		538 (408, 693)
HIV viral load <40, copies/mL, n (%)	410	
Yes		356 (86.8)
No		54 (13.2)
HBV co-infection, n (%)	410	
Yes		17 (4.2)
No		393 (95.9)
HCV co-infection, n (%)	410	
Yes		58 (14.2)
No		352 (85.9)
ART status, n (%)	410	
Naïve		31 (7.6)
Current		379 (92.4)
TDF and PI/ritonavir exposure		
On TDF at recruitment, n (%)	410	287 (70.0)
Cumulative exposure to TDF, years, median (IQR)*	364	2.0 (1.0, 4.2)
On PI/ritonavir at recruitment, n (%)	410	179 (43.7)
Cumulative exposure to PI/ritonavir, years, median (IQR)*	220	3.7 (1.3, 6.4)
On TDF and PI/ritonavir at recruitment, n (%)	410	114 (27.8)
Cumulative exposure to TDF and PI/ritonavir, years, median (IQR)*	179	1.9 (0.6, 4.0)

ART: antiretroviral therapy; HBV: hepatitis B; HCV: hepatitis C; IQR: interquartile range; PI: protease inhibitor; TDF: tenofovir

*Includes all patients who have ever been exposed

7.4.4 Renal parameters

7.4.4.1 Renal risk factors

The rates of self-reported history of prior renal disease and diabetes were 4.1% and 5.1%, respectively (Table 7.4.4.1). There were 32 (7.8%) men with hypertension (blood pressure >140/90 mmHg).

Table 7.4.4.1 Renal risk factors

Only a few men had renal risk factors, including previous renal disease (4.1%), diabetes (5.1%) and hypertension (7.8%).

	Total (N=410)
Prior renal disease, n (%)	
Yes	17 (4.1)
No	393 (95.9)
Diabetes, n (%)	
Yes	21 (5.1)
No	389 (94.9)
Hypertension, n (%)	
Yes	32 (7.8)
No	378 (92.2)

7.4.4.2 Renal function

The majority of HIV-positive men had well preserved renal function (Table 7.4.4.2). The median eGFR calculated using the MDRD and CKD-Epi equations were 91.2 (IQR 80.2, 105.8) and 95.6 (IQR 82.5, 106.1) ml/min/1.73m² using the MDRD and CKD-Epi equations, respectively. Both these medians are normal using the K/DOQI classification for CKD [434]. As MDRD and CKD-Epi have been shown to have a high degree of agreement in both the general population [499,500] and in HIV-positive patients [501], only CKD-Epi will be used from this point forwards.

Table 7.4.4.2 Renal function

Median eGFR was normal using both MDRD and CKD-Epi equations. Only 29 men had proteinuria (PCR >30 mg/mmol) and 3 had albuminuria (ACR >30 mg/mmol). Using RBPCR, 85 (20.7%) men had RTD, whereas 52 (12.7%) men had RTD defined using FePO₄ >20% in those with phosphate <0.8 mmol/L.

	N	Total
eGFR (MDRD), ml/min/1.73m ² , median (IQR)	410	91.2 (80.2, 105.8)
eGFR (MDRD), ml/min/1.73m ² , n (%)	410	
>90		216 (52.7)
60-90		181 (44.1)
<60		13 (3.2)
eGFR (CKD-Epi), ml/min/1.73m ² , median (IQR)	410	95.6 (82.5, 106.1)
eGFR (CKD-Epi), ml/min/1.73m ² , n (%)	410	
>90		241 (58.8)
60-90		156 (38.1)
<60		13 (3.2)
PCR, mg/mmol, median (IQR)	409	11.2 (8.3, 16.6)
PCR, mg/mmol, n (%)	409	
<30		380 (92.9)
>30		29 (7.1)
ACR, mg/mmol, median (IQR)	265	0.6 (0.3, 1.8)
ACR, mg/mmol, n (%)	265	
<3		216 (81.5)
3-30		44 (16.6)
>30		5 (1.9)
RBPCR, µg/mmol, median (IQR)	410	1.3 (0.8, 2.4)
RBPCR, µg/mmol, n (%)	410	
<2.93		325 (79.3)
2.93-14.65		63 (15.4)
>14.65		22 (5.4)

	N	Total
Phosphate, mmol/L, median (IQR)	410	0.87 (0.76, 0.97)
FePO ₄ , %, median (IQR)	410	15.3 (10.7, 20.5)
FePO ₄ >20% and phosphate <0.8 mmol/L, n (%)	410	52 (12.7)

ACR: albumin/creatinine ratio; CKD-Epi: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate; FePO₄: fractional excretion of phosphate; IQR: interquartile range; MDRD: Modification of Diet in Renal Disease equation; PCR: protein/creatinine ratio; RBPCR: retinol binding protein creatinine ratio

There were PCR measurements in all but one patient. The median PCR was normal (11.2 [IQR 8.3, 16.6] mg/mmol) with only 29 (7.1%) men having proteinuria as defined by PCR >30 mg/mmol. ACR was measured in 265 men and the median ACR (0.6 [IQR 0.3, 1.8] mg/mmol) was normal, with 5 (1.9%) men having albuminuria (ACR >30 mg/mmol). The median RBPCR (1.3 [IQR 0.8, 2.4] µg/mmol) and FePO₄ (15.3 [IQR 10.7, 20.5] %) were both normal. Overall, 85 (20.7%) men had an abnormal RBPCR, with 63 (15.4%) having mild to moderate RTD (RBPCR 2.93 - 14.65 µg/mmol) and 22 (5.2%) having severe RTD (RBPCR >14.65 µg/mmol). Using FePO₄ >20% in patients with hypophosphataemia (serum phosphate <0.8 mmol/L), there were 52 (12.7%) men with RTD, which was a smaller number than when defining RTD using RBPCR.

7.4.5 Bone parameters

7.4.5.1 Bone risk factors

Table 7.4.5.1 shows bone-related risk factors. There were equal numbers of men who had never smoked (32.0%) and ex-smokers (32.0%). Of the 148 (36.1%) current smokers, 50 (12.2%) smoked <10 cigarettes per day, 70 (17.1%) smoked between 10 and 20 cigarettes per day and 28 (6.8%) smoked >20 cigarettes per day. The majority (85.6%) reported that they drank alcohol, with 14.4% stating that they were teetotal. Of those who drank alcohol, 332 (81.0%) drank within the recommended allowance in the UK (<3 units per day), whilst 19 (4.6%) drank excessively (i.e. ≥3 units per day). Exercise was defined as any weight-bearing or muscle-toning exercise, and the majority (235, 57.3%) did some exercise, although 175 (42.7%) men reported doing none at all. The mean BMI was 25.3 (SD 4.1) kg/m² and the majority (51.0%) had a normal BMI (<25 kg/m²). With regards to steroids, 12.7% and 8.8% reported being ever exposed to inhaled steroids and oral steroids, respectively. In total, 12.0% reported a past history of fragility fractures and 15.6% reported a history of a previous family history of a hip fracture in their mother or maternal grandmother.

Table 7.4.5.1 Bone risk factors

There were 36.1% current smokers, the majority drank alcohol and most did regular exercise. Mean BMI was normal. A few men were exposed to steroids. In total 12.0% reported a previous history of a fragility fracture and 13.2% had a positive family history of a hip fracture.

	Total (N=410)
Smoking, n (%)	
Never smoked	131 (32.0)
Ex-smoker	131 (32.0)
Current smoker	148 (36.1)
Alcohol, n (%)	
Never	59 (14.4)
<3 units/day	332 (81.0)
≥3 units/day	19 (4.6)
Exercise, n (%)	
Never	175 (42.7)
Some weeks	100 (24.4)
Most weeks	33 (8.0)
Every week	102 (24.9)
BMI, kg/m ² , mean (SD)	25.3 (4.1)
BMI, kg/m ² , n (%)	
<25	209 (51.0)
25–30	151 (36.8)
>30	50 (12.2)
Ever exposed to steroids, n (%)	
Steroid inhalers	52 (12.7)
Oral steroids	36 (8.8)
Previous fragility fracture, n (%)	
Yes	49 (12.0)
No	361 (88.0)
Family history of hip fracture, n (%)	
Yes	54 (13.2)
No	293 (71.5)
Not known	63 (15.4)

BMI: body mass index; SD: standard deviation

7.4.5.2 Bone biomarkers

Apart from vitamin D levels, all other standard bone biomarkers (including calcium, phosphate, ALP and PTH) were within the normal range (Table 7.4.5.2). The median vitamin D (47 [IQR 35, 62] nmol/L) indicated a level consistent with VDD. The median CTX was 2.0 (IQR 0.9, 5.2) ng/mL and the median P1NP was 13.6 (IQR 5.6, 33.5) ng/mL.

Table 7.4.5.2 Bone biomarkers

Median levels of corrected calcium, phosphate, ALP and PTH were in the normal range, but median levels of vitamin D were consistent with VDD.

	N	Median (IQR)	Normal range
Corrected calcium, mmol/L	408	2.17 (2.13, 2.22)	2.15-2.55
Phosphate, mmol/L	410	0.87 (0.76, 0.97)	0.87-1.45
ALP, IU/L	407	78 (64, 96)	40-129
Vitamin D, nmol/L	409	47 (35, 62)	>75
PTH, ng/L	410	50 (37, 66)	15-65
CTX, ng/mL	410	2.1 (0.9, 5.3)	No reference range available
P1NP, ng/mL	410	13.6 (5.6, 33.5)	No reference range available

ALP: alkaline phosphatase; CTX: C-terminal cross-linking telopeptides of type I collagen; IQR: interquartile range; P1NP: N-terminal propeptide of type I procollagen; PTH: parathyroid hormone

7.4.5.3 BMD

The mean absolute BMD measurements were 1.141 (SD 0.155) g/cm², 1.000 (SD 0.138) g/cm² and 0.953 (SD 0.142) g/cm² at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck, respectively (Table 7.4.5.3).

Table 7.4.5.3 Absolute BMD

Absolute BMD was normally distributed at all sites. Although all 410 men had lumbar spine results, only 403 and 406 had measurements at the non-dominant total hip and the non-dominant femoral neck, respectively, mainly because they had metalwork in situ or the region of interest was not correctly scanned (Chapter 3).

	N	Mean (SD)
Lumbar spine BMD, g/cm ²	410	1.141 (0.155)
Non-dominant total hip BMD, g/cm ²	403	1.000 (0.138)
Non-dominant femoral neck BMD, g/cm ²	406	0.953 (0.142)

BMD: bone mineral density; SD: standard deviation

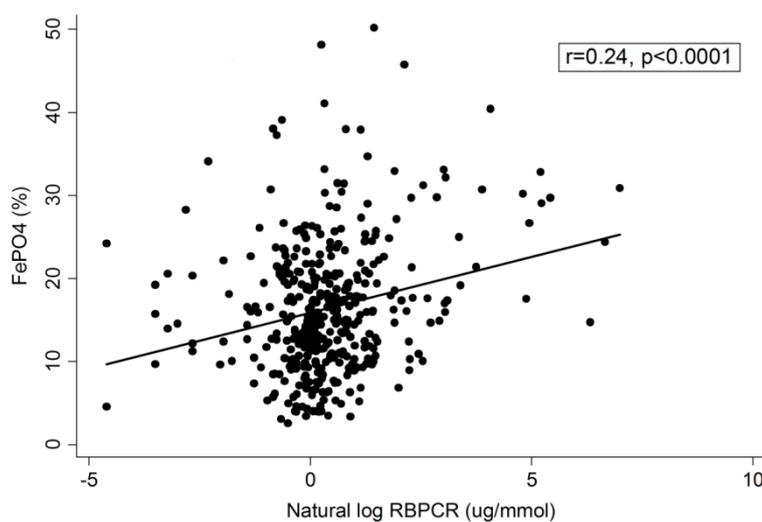
7.4.6 Relationship between RBPCR and FePO₄

The association between RBPCR and FePO₄ is shown in Figure 7.4.6.1. There was a positive correlation between FePO₄ and RBPCR ($r=0.24$, $p<0.0001$), indicating that RBPCR correlated with renal phosphate wasting.

Figure 7.4.6.1 Relationship between RBPCR and FePO₄

Plot of natural log RBPCR (x-axis) against FePO₄ (y-axis) showing a significant positive correlation ($r=0.24$, $p<0.0001$).

FePO₄: fractional excretion of phosphate; RBPCR: retinol binding protein creatinine ratio



7.4.7 Relationship between RTD, vitamin D and PTH

There was no significant correlation between RBPCR and vitamin D ($r=-0.04$, $p=0.44$, Figure 7.4.7.1) or PTH ($r=-0.07$, $p=0.15$, Figure 7.4.7.2).

Figure 7.4.7.1 Relationship between vitamin D and RBPCR

Plot of vitamin D (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=-0.04$, $p=0.44$).
RBPCR: retinol binding protein creatinine ratio

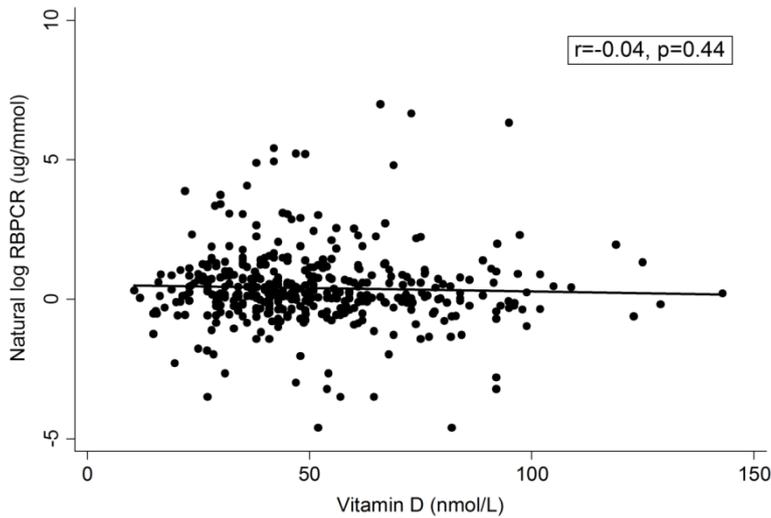
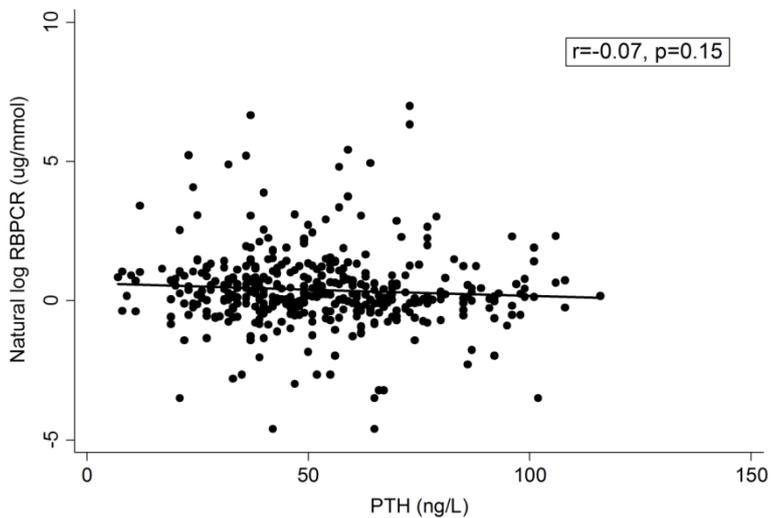


Figure 7.4.7.2 Relationship between PTH and RBPCR

Plot of PTH (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=-0.07$, $p=0.15$).
PTH: parathyroid hormone; RBPCR: retinol binding protein creatinine ratio



There was also no correlation between FePO_4 and vitamin D ($r=-0.04$, $p=0.43$, Figure 7.4.7.3) or PTH ($r=-0.07$, $p=0.14$, Figure 7.4.7.4).

Figure 7.4.7.3 Relationship between vitamin D and FePO₄

Plot of vitamin D (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.04$, $p=0.43$).
FePO₄: fractional excretion of phosphate

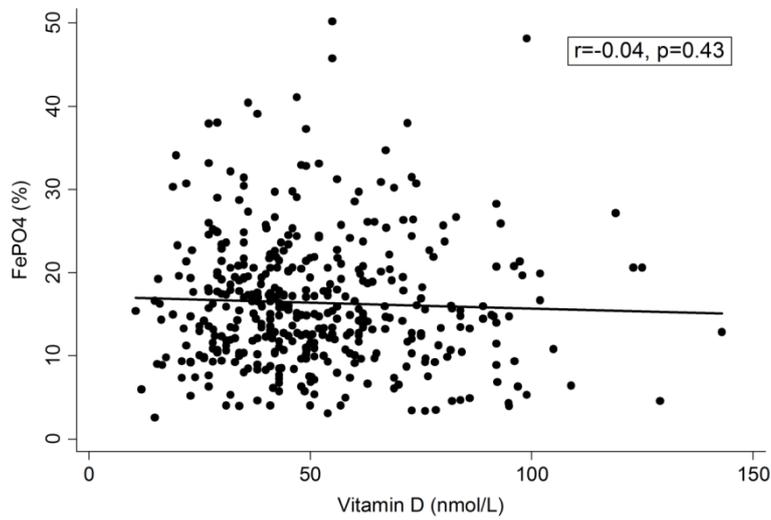
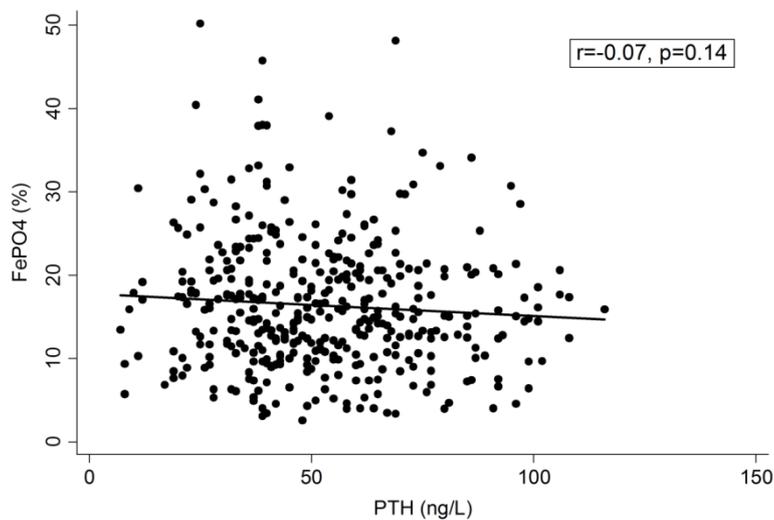


Figure 7.4.7.4 Relationship between PTH and FePO₄

Plot of PTH (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.07$, $p=0.14$).
FePO₄: fractional excretion of phosphate; PTH: parathyroid hormone



7.4.8 Factors associated with RBPCR-defined RTD

The factors associated with RBPCR were evaluated for mild to moderate RTD (RBPCR 2.93-14.65 $\mu\text{g}/\text{mmol}$, Section 7.4.8.1) and severe RTD (RBPCR >14.65 $\mu\text{g}/\text{mmol}$, Section 7.4.8.2).

7.4.8.1 Factors associated with mild to moderate RTD (RBPCR 2.93-14.65 µg/mmol)

Table 7.4.8.1 shows the factors associated with mild to moderate RTD (RBPCR 2.93-14.65 µg/mmol) compared to no RTD (RBPCR <2.93 µg/mmol). Although in univariable analyses, RBPCR was associated with hypertension, eGFR and AIDS clinical stage, only hypertension and a lower eGFR remained significantly associated with mild to moderate RTD in multivariable analysis.

Table 7.4.8.1 Factors associated with mild to moderate RTD (RBPCR 2.93-14.65 µg/mmol)

Mild to moderate RTD (RBPCR 2.93-14.65 µg/mmol) was significantly associated with hypertension and a lower eGFR when compared to patients with no RTD.

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Age, years, per 10 years	1.17 (0.88, 1.55)	0.29		
Ethnicity				
White	1.00	-		
Other	1.47 (0.52, 4.13)	0.46		
Smoking				
Never	1.00	-		
Ex-smoker	0.86 (0.43, 1.70)	0.66		
Current smoker	1.04 (0.55, 1.99)	0.90		
BMI				
<25	1.00	-		
25-30	0.64 (0.35, 1.18)	0.15		
>30	0.88 (0.38, 2.03)	0.76		
Diabetes				
No	1.00	-		
Yes	1.40 (0.45, 4.38)	0.56		
Hypertension				
No	1.00	-	0.00	-
Yes	3.22 (1.39, 7.43)	0.004	3.06 (1.32, 7.10)	0.01
eGFR**, per 10 ml/min decrease	0.80 (0.68, 0.94)	0.01	0.82 (0.69, 0.97)	0.02
Duration of HIV infection, years, per year	1.00 (0.96, 1.04)	0.88		
HIV clinical stage				
Asymptomatic	1.00	-	-	-
Symptomatic non-AIDS	1.20 (0.61, 2.37)	0.60	1.08 (0.54, 2.16)	0.83
Symptomatic AIDS	1.73 (0.91, 3.27)	0.09	1.55 (0.81, 2.96)	0.18
CD4 count, per 50 cells/µl				
Nadir	1.01 (0.92, 1.11)	0.90		
Current	0.98 (0.93, 1.04)	0.51		
HIV viral load <40, copies/mL				
Yes	1.00	-		
No	0.76 (0.33, 1.77)	0.52		
HBV co-infection				
No	1.00	-		
Yes	0.86 (0.19, 3.93)	0.84		
HCV co-infection				
No	1.00	-		
Yes	1.12 (0.53, 2.35)	0.77		
Current TDF				
No	1.00	-		
Yes	1.14 (0.63, 2.07)	0.66		

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Current PI/ritonavir				
No	1.00	-		
Yes	1.49 (0.87, 2.57)	0.15		

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for hypertension, eGFR and HIV clinical stage

**eGFR defined using CKD-Epi formula

7.4.8.2 Factors associated with severe RTD (RBPCR >14.65 µg/mmol)

The factors associated with RBPCR >14.65 µg/mmol are shown in Table 7.4.8.2. In univariable analyses, severe RTD was associated with increasing age, hypertension, a lower eGFR, longer duration of HIV infection, HIV clinical stage (both symptomatic non-AIDS and symptomatic AIDS), a lower nadir CD4 count, HBV co-infection, and current use of TDF or a boosted PI. In multivariable analysis, the factors that remained associated with severe RTD were a lower eGFR and current TDF use. There was a borderline association with hypertension and symptomatic non-AIDS HIV clinical stage.

Table 7.4.8.2 Factors associated with severe RTD (RBPCR >14.65 µg/mmol)

Factors associated with severe RTD were a lower eGFR and current TDF use, with hypertension and symptomatic non-AIDS HIV clinical stage showing a borderline association.

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Age, years, per 10 years	1.85 (1.18, 2.92)	0.01	0.92 (0.50, 1.67)	0.77
Ethnicity				
White	1.00	-		
Other	0.76 (0.10, 5.89)	0.79		
Smoking				
Never	1.00	-		
Ex-smoker	1.64 (0.52, 5.17)	0.39		
Current smoker	1.63 (0.53, 5.02)	0.39		
BMI				
<25	1.00	-		
25-30	1.41 (0.57, 3.49)	0.45		
>30	0.83 (0.18, 3.92)	0.81		
Diabetes				
No	1.00	-		
Yes	1.94 (0.42, 8.95)	0.39		
Hypertension				
No	1.00	-	-	-
Yes	2.86 (0.90, 9.08)	0.06	3.74 (0.98, 14.24)	0.05
eGFR**, per 10 ml/min decrease	0.50 (0.38, 0.64)	<0.0001	0.50 (0.36, 0.70)	<0.0001
Duration of HIV infection, years, per year	1.06 (0.99, 1.12)	0.09	1.00 (0.94, 1.08)	0.91
HIV clinical stage				
Asymptomatic	1.00	-	-	-
Symptomatic non-AIDS	4.10 (1.21, 13.84)	0.01	3.57 (0.93, 13.79)	0.06
Symptomatic AIDS	4.02 (1.19, 13.56)	0.02	2.92 (0.75, 11.42)	0.12
CD4 count, per 50 cells/µl				
Nadir	0.86 (0.74, 1.01)	0.06	0.95 (0.78, 1.16)	0.63
Current	1.01 (0.93, 1.10)	0.78		

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
HIV viral load <40, copies/mL				
Yes	1.00	-		
No	0.30 (0.04, 2.30)	0.22		
HBV co-infection				
No	1.00	-	-	-
Yes	4.22 (1.10, 16.10)	0.02	1.95 (0.40, 9.57)	0.41
HCV co-infection				
No	1.00	-		
Yes	0.28 (0.04, 2.11)	0.18		
Current TDF				
No	1.00	-	-	-
Yes	2.84 (0.82, 9.82)	0.09	6.12 (1.36, 27.61)	0.02
Current PI/ritonavir				
No	1.00	-	-	-
Yes	2.37 (0.96, 5.80)	0.05	1.54 (0.53, 4.44)	0.42

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for age, hypertension, eGFR, duration and clinical stage of HIV infection, nadir CD4 count, HBV co-infection and current TDF use

**eGFR defined using CKD-Epi formula

7.4.9 Factors associated with phosphate-defined RTD

Table 7.4.9.1 shows the factors associated with phosphate-defined RTD ($\text{FePO}_4 >20\%$ in patients with phosphate <0.8 mmol/L). In univariable analyses, RTD was associated with older age, current smoking, a lower eGFR and a lower nadir CD4 count. In multivariable analysis, a lower eGFR was the only factor that remained significantly associated with phosphate-defined RTD.

Table 7.4.9.1 Factors associated with phosphate-defined RTD ($\text{FePO}_4 >20\%$ and plasma phosphate <0.8 mmol/L)

The only factor associated with phosphate-defined RTD in multivariable analysis was a lower eGFR.

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Age, years, per 10 years	1.63 (1.20, 2.21)	0.002	1.08 (0.75, 1.56)	0.67
Ethnicity				
White	1.00	-		
Other	0.61 (0.14, 2.68)	0.51		
Smoking				
Never	1.00	-	-	-
Ex-smoker	0.79 (0.40, 1.55)	0.49	0.97 (0.48, 1.97)	0.94
Current smoker	0.44 (0.21, 0.93)	0.03	0.61 (0.28, 1.33)	0.22
BMI				
<25	1.00	-		
25-30	1.53 (0.81, 2.86)	0.18		
>30	1.38 (0.55, 3.46)	0.48		
Diabetes				
No	1.00	-		
Yes	0.71 (0.16, 3.16)	0.66		
Hypertension				
No	1.00	-		
Yes	1.67 (0.65, 4.27)	0.28		
eGFR**, per 10 ml/min decrease	0.65 (0.55, 0.77)	<0.0001	0.70 (0.57, 0.86)	0.001

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Duration of HIV infection, years, per year	1.03 (0.99, 1.08)	0.15		
HIV clinical stage				
Asymptomatic	1.00	-		
Symptomatic non-AIDS	0.96 (0.47, 1.99)	0.92		
Symptomatic AIDS	1.20 (0.60, 2.38)	0.61		
CD4 count, per 50 cells/ μ l				
Nadir	1.00 (1.00, 1.00)	0.05	0.94 (0.84, 1.07)	0.36
Current	1.00 (1.00, 1.00)	0.41		
HIV viral load <40, copies/mL				
Yes	1.00	-		
No	0.67 (0.25, 1.77)	0.42		
HBV co-infection				
No	1.00	-		
Yes	1.50 (0.42, 5.44)	0.53		
HCV co-infection				
No	1.00	-		
Yes	0.47 (0.16, 1.36)	0.15		
Current TDF				
No	1.00	-		
Yes	1.50 (0.76, 2.97)	0.24		
Current PI/ritonavir				
No	1.00	-		
Yes	1.23 (0.68, 2.20)	0.49		

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; OR: odds ratio; PI: protease inhibitor; TDF: tenofovir

*Adjusted for age, smoking status, eGFR and nadir CD4 count

**eGFR defined using CKD-Epi formula

7.4.10 Relationship between bone turnover and RTD

7.4.10.1 Bone resorption

When investigating the association between bone resorption (as measured by CTX) and RTD, there was no correlation with either RBPCR ($r=0.01$, $p=0.86$, Figure 7.4.10.1) or FePO_4 ($r=-0.01$, $p=0.80$, Figure 7.4.10.2).

Figure 7.4.10.1 Relationship between CTX and RBPCR

Plot of square root CTX (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=0.01$, $p=0.86$).

CTX: C-terminal cross-linking telopeptides of type I collagen; RBPCR: retinol binding protein creatinine ratio

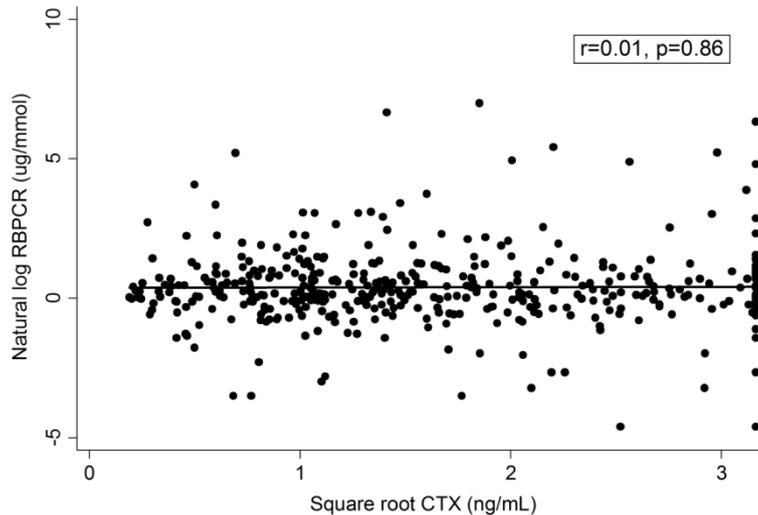
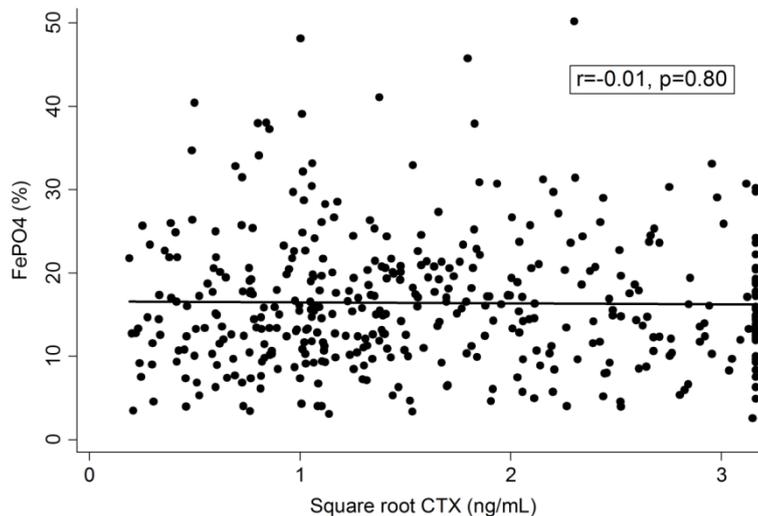


Figure 7.4.10.2 Relationship between CTX and FePO₄

Plot of square root CTX (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.01$, $p=0.80$).

CTX: C-terminal cross-linking telopeptides of type I collagen; FePO₄: fractional excretion of phosphate



7.4.10.2 Bone formation

Although there was no association between bone formation measured by P1NP and RTD measured by RBPCR ($r=-0.03$, $p=0.56$, Figure 7.4.10.3), there was a significant but weak negative correlation between P1NP and FePO₄ ($r=-0.10$, $p=0.04$, Figure 7.4.10.4).

Figure 7.4.10.3 Relationship between P1NP and RBPCR

Plot of natural log P1NP (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=-0.03$, $p=0.56$).

P1NP: N-terminal propeptide of type I procollagen; RBPCR: retinol binding protein creatinine ratio

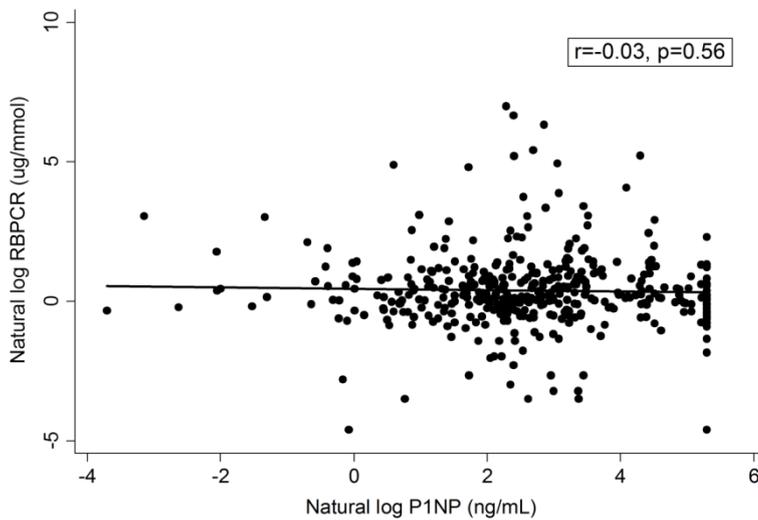
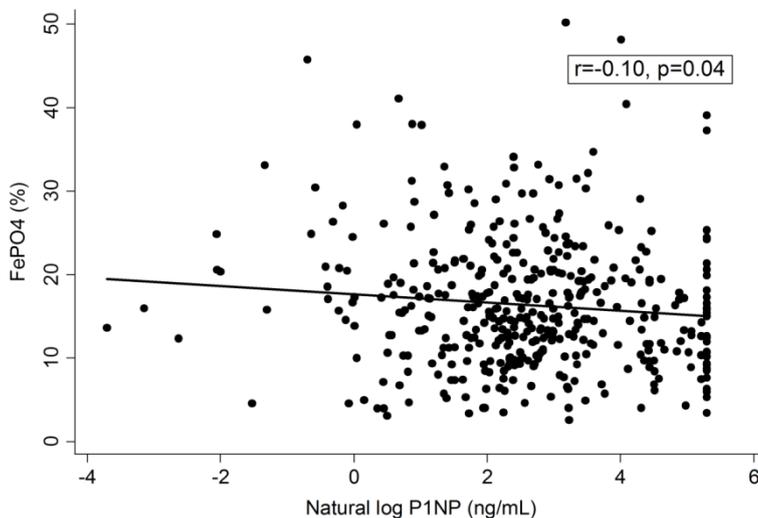


Figure 7.4.10.4 Relationship between P1NP and FePO₄

Plot of natural log P1NP (x-axis) against FePO₄ (y-axis) showing a significant but weak negative correlation ($r=-0.10$, $p=0.04$).

FePO₄: fractional excretion of phosphate; P1NP: N-terminal propeptide of type I procollagen



7.4.11 Relationship between BMD and RTD

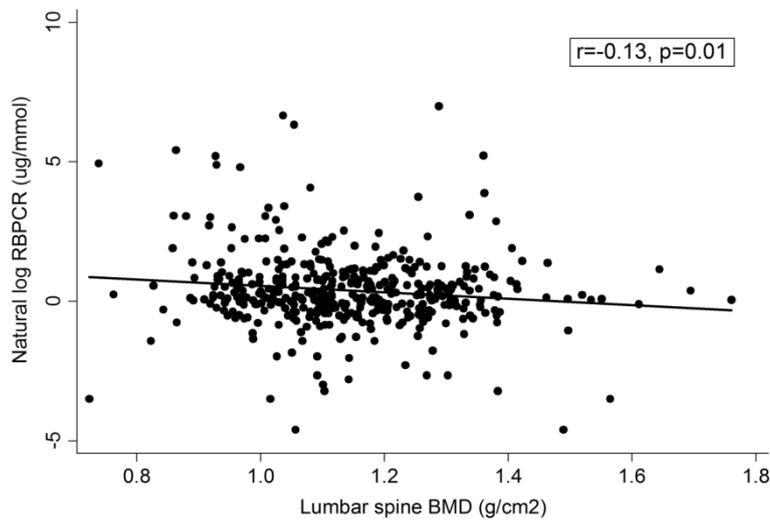
7.4.11.1 Lumbar spine

When investigating the relationship between BMD and RBPCR, there was a correlation at the lumbar spine ($r=-0.13$, $p=0.01$, Figure 7.4.11.1).

Figure 7.4.11.1 Relationship between lumbar spine BMD and RBPCR

Plot of lumbar spine BMD (x-axis) against natural log RBPCR (y-axis) showing a significant negative correlation ($r=-0.13$, $p=0.01$).

BMD: bone mineral density; RBPCR: retinol binding protein creatinine ratio

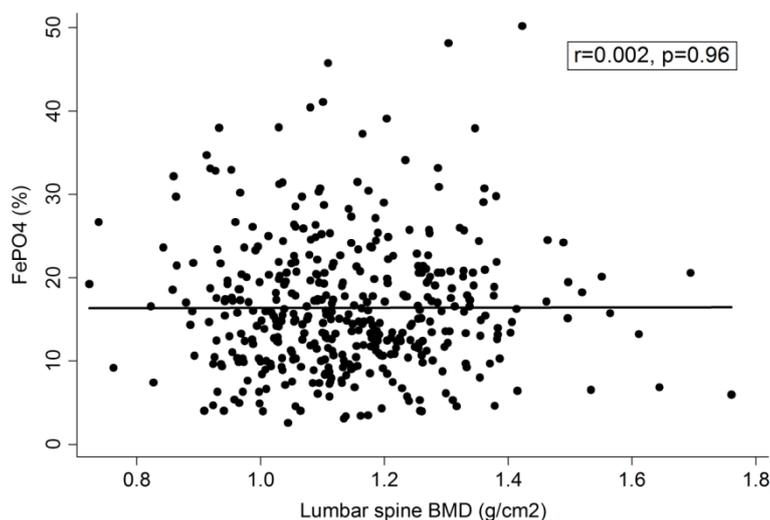


However, there was no correlation between BMD at the lumbar spine and FePO_4 ($r=0.002$, $p=0.96$, Figure 7.4.11.2).

Figure 7.4.11.2 Relationship between lumbar spine BMD and FePO_4

Plot of lumbar spine BMD (x-axis) against FePO_4 (y-axis) showing no significant correlation ($r=0.002$, $p=0.96$).

BMD: bone mineral density; FePO_4 : fractional excretion of phosphate



As there was a significant correlation between BMD at the lumbar spine and RBPCR, this association was further assessed in regression models. When RBPCR was considered as a continuous variable, there was a significant association with lumbar spine BMD in univariable analyses, but this association disappeared when adjusted for ethnicity, smoking status, BMI, duration of HIV infection, HIV clinical stage and current

exposure to a boosted PI (Table 7.4.11.1). When RBPCR was included as a categorical variable, there was a borderline association with severe RTD (RBPCR >14.65 µg/mmol, Table 7.4.11.2). Regardless of whether RBPCR was included as a continuous or a categorical variable, current smoking was associated with a lower BMD whilst normal or high BMI was associated with a higher BMD at the lumbar spine.

Table 7.4.11.1 Factors associated with lumbar spine BMD when RBPCR was measured as a continuous variable

Although there was a significant association in univariable analyses between higher RBPCR and lower BMD at the lumbar spine, this association did not remain in the multivariable model. Factors that remained significantly associated with BMD at the lumbar spine were current smoking and a normal or high BMI.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β* (95% CI)	P-value
Age, years, per 10 years	0.001 (-0.01, 0.02)	0.88		
Ethnicity				
White	0.00	-	0.00	-
Other	0.06 (-0.01, 0.12)	0.08	0.04 (-0.03, 0.12)	0.28
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.01 (-0.05, 0.03)	0.56	-0.02 (-0.06, 0.02)	0.28
Current smoker	-0.06 (-0.10, -0.03)	0.001	-0.04 (-0.08, -0.01)	0.01
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.06, 0.18)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.11 (0.06, 0.15)	<0.0001	0.09 (0.04, 0.15)	0.001
Diabetes				
No	0.00	-		
Yes	-0.001 (-0.07, 0.07)	0.97		
Hypertension				
No	0.00	-		
Yes	0.04 (-0.01, 0.10)	0.11		
eGFR**, per 10 ml/min decrease	-0.003 (-0.01, 0.01)	0.47		
Duration of HIV infection, years, per year	-0.002 (-0.004, 0.0001)	0.06	-0.001 (-0.003, 0.002)	0.67
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.02 (-0.06, 0.02)	0.26	-0.01 (-0.05, 0.03)	0.62
Symptomatic AIDS	-0.03 (-0.07, 0.002)	0.06	-0.02 (-0.06, 0.02)	0.29
CD4 count, per 50 cells/µl				
Nadir	0.0005 (-0.001, 0.002)	0.37		
Current	0.0003 (-0.0003, 0.001)	0.38		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.02 (-0.03, 0.06)	0.50		
HBV co-infection				
No	0.00	-		
Yes	-0.04 (-0.11, 0.04)	0.34		
HCV co-infection				
No	0.00	-		
Yes	-0.02 (-0.07, 0.02)	0.28		
Current TDF				
No	0.00	-		
Yes	-0.02 (-0.06, 0.01)	0.14		

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.04 (-0.07, -0.01)	0.02	-0.02 (-0.05, 0.01)	0.15
Log RBPCR, $\mu\text{g}/\text{mmol}$	-0.01 (-0.03, -0.004)	0.01	-0.01 (-0.02, 0.003)	0.13

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for ethnicity, smoking status, BMI, duration and clinical stage of HIV infection, current boosted PI use and RBPCR measured as a continuous variable

**eGFR defined using CKD-Epi formula

Table 7.4.11.2 Factors associated with lumbar spine BMD when RBPCR was measured as a categorical variable

When RBPCR was included in the multivariable model as a categorical variable, severe RTD (RBPCR >14.65 $\mu\text{g}/\text{mmol}$) only showed a borderline association with lower BMD at the lumbar spine. Factors that remained associated were current smoking and normal or high BMI.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	0.001 (-0.01, 0.02)	0.88		
Ethnicity				
White	0.00	-	0.00	-
Other	0.06 (-0.01, 0.12)	0.08	0.04 (-0.04, 0.12)	0.27
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.01 (-0.05, 0.03)	0.56	-0.02 (-0.05, 0.02)	0.37
Current smoker	-0.06 (-0.10, -0.03)	0.001	-0.04 (-0.07, -0.01)	0.01
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.06, 0.18)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.11 (0.06, 0.15)	<0.0001	0.10 (0.04, 0.15)	0.001
Diabetes				
No	0.00	-		
Yes	-0.001 (-0.07, 0.07)	0.97		
Hypertension				
No	0.00	-		
Yes	0.04 (-0.01, 0.10)	0.11		
eGFR**, per 10 ml/min decrease	-0.003 (-0.01, 0.01)	0.47		
Duration of HIV infection, years, per year	-0.002 (-0.004, 0.0001)	0.06	-0.001 (-0.003, 0.002)	0.67
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.02 (-0.06, 0.02)	0.26	-0.01 (-0.05, 0.03)	0.62
Symptomatic AIDS	-0.03 (-0.07, 0.002)	0.06	-0.02 (-0.06, 0.02)	0.31
CD4 count, per 50 cells/ μl				
Nadir	0.0005 (-0.001, 0.002)	0.37		
Current	0.0003 (-0.0003, 0.001)	0.38		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.02 (-0.03, 0.06)	0.50		
HBV co-infection				
No	0.00	-		
Yes	-0.04 (-0.11, 0.04)	0.34		
HCV co-infection				
No	0.00	-		
Yes	-0.02 (-0.07, 0.02)	0.28		

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Current TDF				
No	0.00	-		
Yes	-0.02 (-0.06, 0.01)	0.14		
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.04 (-0.07, -0.01)	0.02	-0.02 (-0.05, 0.01)	0.15
RBPCR, $\mu\text{g}/\text{mmol}$				
<2.93	0.00	-	0.00	-
2.93-14.65	0.002 (-0.04, 0.04)	0.92	0.01 (-0.03, 0.05)	0.51
>14.65	-0.09 (-0.16, -0.02)	0.01	-0.08 (-0.16, 0.002)	0.06

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for ethnicity, smoking status, BMI, duration and clinical stage of HIV infection, current boosted PI use and RBPCR measured as a categorical variable

**eGFR defined using CKD-Epi formula

7.4.11.2 Non-dominant total hip

At the non-dominant total hip, there was a significant negative correlation between BMD and RTD measured by RBPCR ($r=-0.11$, $p=0.03$, Figure 7.4.11.3), but not with FePO_4 ($r=0.02$, $p=0.67$, Figure 7.4.11.4).

Figure 7.4.11.3 Relationship between non-dominant total hip BMD and RBPCR

Plot of non-dominant total hip BMD (x-axis) against natural log RBPCR (y-axis) showing a significant negative correlation ($r=-0.11$, $p=0.03$).

BMD: bone mineral density; RBPCR: retinol binding protein creatinine ratio

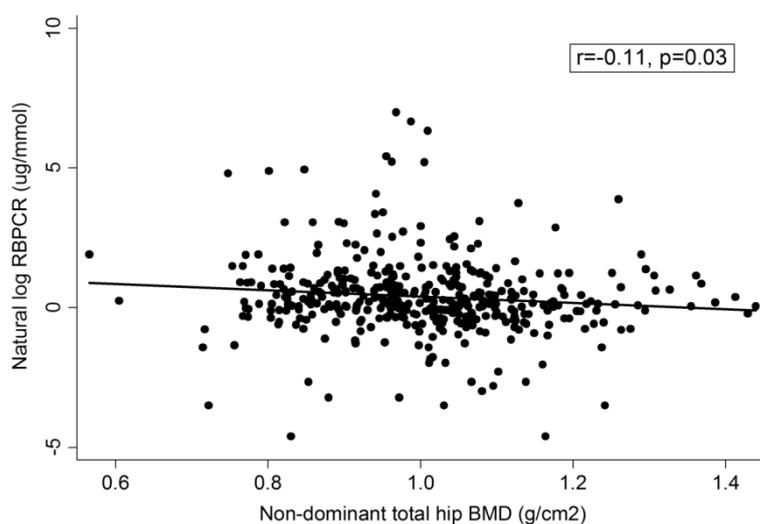
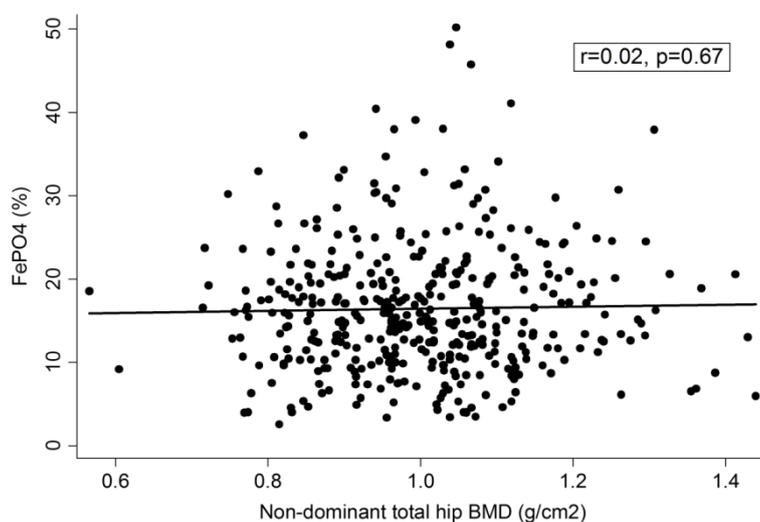


Figure 7.4.11.4 Relationship between non-dominant total hip BMD and FePO₄

Plot of non-dominant total hip BMD (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=0.02$, $p=0.67$).

BMD: bone mineral density; FePO₄: fractional excretion of phosphate



The relationship between RBPCR and non-dominant total hip BMD was further investigated. In multivariable models, non-dominant total hip BMD was not associated with RBPCR when measured as either a continuous (Table 7.4.11.3) or a categorical (Table 7.4.11.4) variable. Current smoking was associated with a lower BMD at the non-dominant total hip, whilst non-white ethnicity and BMI (normal and high BMI) were associated with a higher BMD.

Table 7.4.11.3 Factors associated with non-dominant total hip BMD when RBPCR was measured as a continuous variable

Factors associated with BMD at the non-dominant total hip were non-white ethnicity, current smoking and a normal or high BMI. There was no association between RBPCR measured linearly and BMD at the non-dominant total hip.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	-0.01 (-0.03, 0.003)	0.12		
Ethnicity				
White	0.00	-	0.00	-
Other	0.08 (0.03, 0.14)	0.01	0.06 (0.01, 0.12)	0.03
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.004 (-0.03, 0.04)	0.82	-0.004 (-0.03, 0.03)	0.81
Current smoker	-0.06 (-0.09, -0.03)	<0.0001	-0.03 (-0.06, -0.003)	0.03
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.07, 0.12)	<0.0001	0.08 (0.06, 0.11)	<0.0001
>30	0.14 (0.10, 0.18)	<0.0001	0.12 (0.08, 0.17)	<0.0001
Diabetes				
No	0.00	-		
Yes	0.004 (-0.06, 0.06)	0.90		

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Hypertension				
No	0.00	-	0.00	-
Yes	0.05 (-0.004, 0.10)	0.07	0.02 (-0.02, 0.07)	0.32
eGFR**, per 10 ml/min decrease	-0.001 (-0.01, 0.01)	0.81		
Duration of HIV infection, years, per year	-0.002 (-0.004, -0.0003)	0.02	-0.001 (-0.003, 0.001)	0.49
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.02 (-0.05, 0.01)	0.22	-0.01 (-0.04, 0.02)	0.51
Symptomatic AIDS	-0.03 (-0.07, -0.001)	0.04	-0.02 (-0.05, 0.02)	0.28
CD4 count, per 50 cells/ μ l				
Nadir	0.004 (-0.001, 0.008)	0.14		
Current	0.001 (-0.002, 0.003)	0.55		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.05 (0.01, 0.09)	0.02	0.02 (-0.02, 0.06)	0.28
HBV co-infection				
No	0.00	-		
Yes	-0.02 (-0.09, 0.04)	0.47		
HCV co-infection				
No	0.00	-		
Yes	-0.003 (-0.04, 0.04)	0.87		
Current TDF				
No	0.00	-		
Yes	-0.01 (-0.04, 0.02)	0.47		
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.03 (-0.06, -0.004)	0.03	-0.02 (-0.04, 0.01)	0.25
Log RBPCR, μ g/mmol	-0.01 (-0.02, -0.001)	0.03	-0.01 (-0.01, 0.003)	0.20

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for ethnicity, smoking status, BMI, hypertension, duration and clinical stage of HIV infection, HIV viral load, current boosted PI use and RBPCR measured as a continuous variable

**eGFR defined using CKD-Epi formula

Table 7.4.11.4 Factors associated with non-dominant total hip BMD when RBPCR was measured as a categorical variable

RBPCR measured as a categorical variable was not associated with non-dominant total hip BMD. However, factors that remained significantly associated were non-white ethnicity, current smoking and a normal or high BMI.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	-0.01 (-0.03, 0.003)	0.12		
Ethnicity				
White	0.00	-	0.00	-
Other	0.08 (0.03, 0.14)	0.01	0.07 (0.01, 0.13)	0.03
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.004 (-0.03, 0.04)	0.82	-0.002 (-0.03, 0.03)	0.89
Current smoker	-0.06 (-0.09, -0.03)	<0.0001	-0.03 (-0.06, -0.003)	0.03
BMI				
<25	0.00	-	0.00	-
25-30	0.09 (0.07, 0.12)	<0.0001	0.09 (0.06, 0.11)	<0.0001
>30	0.14 (0.10, 0.18)	<0.0001	0.12 (0.08, 0.17)	<0.0001

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Diabetes				
No	0.00	-		
Yes	0.004 (-0.06, 0.06)	0.90		
Hypertension				
No	0.00	-	0.00	-
Yes	0.05 (-0.004, 0.10)	0.07	0.02 (-0.02, 0.07)	0.35
eGFR**, per 10 ml/min decrease	-0.001 (-0.01, 0.01)	0.81		
Duration of HIV infection, years, per year	-0.002 (-0.004, -0.0003)	0.02	-0.001 (-0.003, 0.001)	0.48
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.02 (-0.05, 0.01)	0.22	-0.01 (-0.04, 0.02)	0.48
Symptomatic AIDS	-0.03 (-0.07, -0.001)	0.04	-0.02 (-0.05, 0.02)	0.30
CD4 count, per 50 cells/ μ l				
Nadir	0.004 (-0.001, 0.008)	0.14		
Current	0.001 (-0.002, 0.003)	0.55		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.05 (0.01, 0.09)	0.02	0.02 (-0.02, 0.07)	0.26
HBV co-infection				
No	0.00	-		
Yes	-0.02 (-0.09, 0.04)	0.47		
HCV co-infection				
No	0.00	-		
Yes	-0.003 (-0.04, 0.04)	0.87		
Current TDF				
No	0.00	-		
Yes	-0.01 (-0.04, 0.02)	0.47		
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.03 (-0.06, -0.004)	0.03	-0.02 (-0.04, 0.01)	0.25
RBPCR, μ g/mmol				
<2.93	0.00	-	0.00	-
2.93-14.65	0.002 (-0.04, 0.04)	0.92	-0.004 (-0.04, 0.03)	0.82
>14.65	-0.09 (-0.16, -0.02)	0.01	-0.03 (-0.09, 0.02)	0.26

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for ethnicity, smoking status, BMI, hypertension, duration and clinical stage of HIV infection, HIV viral load, current boosted PI use and RBPCR measured as a categorical variable

**eGFR defined using CKD-Epi formula

7.4.11.3 Non-dominant femoral neck

As with the lumbar spine and the non-dominant total hip, there was a significant negative correlation between absolute BMD at the non-dominant femoral neck and RBPCR ($r=-0.13$, $p=0.01$, Figure 7.4.11.5), but not with FePO₄ ($r=-0.06$, $p=0.25$, Figure 7.4.11.6).

Figure 7.4.11.5 Relationship between non-dominant femoral neck BMD and RBPCR

Plot of non-dominant femoral neck BMD (x-axis) against natural log RBPCR (y-axis) showing a significant negative correlation ($r=-0.13$, $p=0.01$).

BMD: bone mineral density; RBPCR: retinol binding protein creatinine ratio

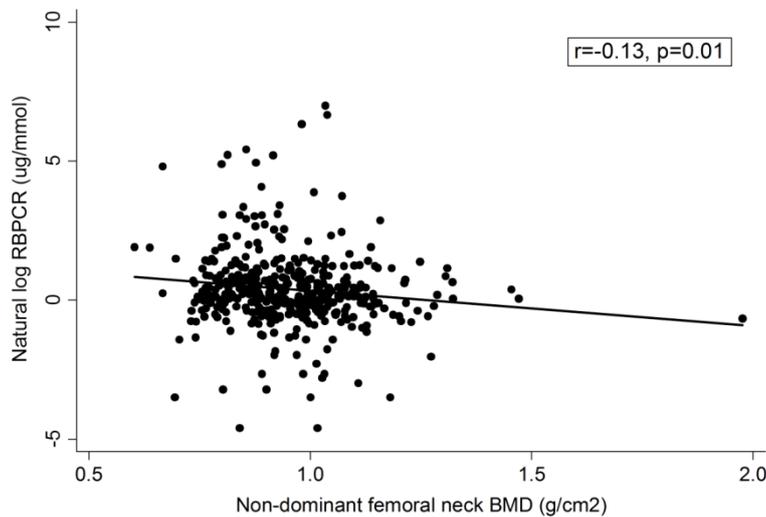
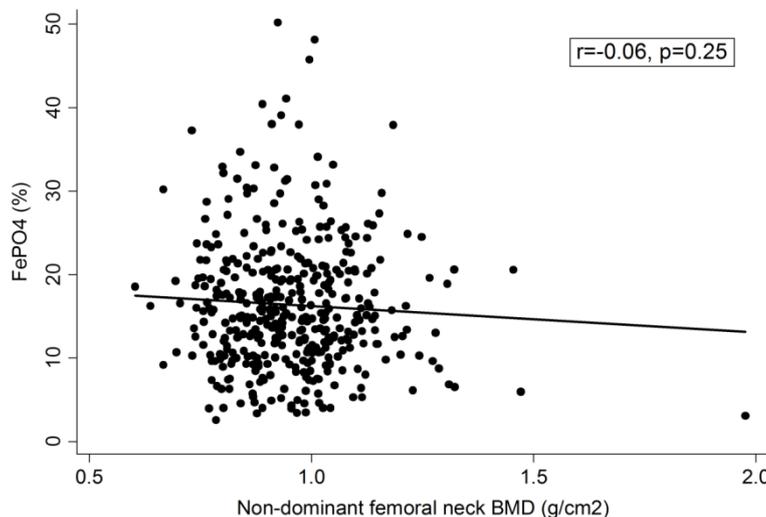


Figure 7.4.11.6 Relationship between non-dominant femoral neck BMD and FePO₄

Plot of non-dominant femoral neck BMD (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.06$, $p=0.25$).

BMD: bone mineral density; FePO₄: fractional excretion of phosphate



Although there was an association in univariable analyses between BMD at the non-dominant femoral neck and RBPCR (measured continuously [Table 7.4.11.5] or with severe RTD when measured as a categorical variable [Table 7.4.11.6]), this association did not remain in the multivariable models when adjusted for age, ethnicity, smoking status, BMI, duration of HIV infection, HIV clinical stage, HIV viral load and current boosted PI use. Increasing age was associated with a lower BMD, whilst non-white ethnicity and BMI (normal or high) were associated with a higher BMD. Current

smoking showed a borderline association with a lower BMD. The results were the same irrespective of whether RBPCR was included in multivariable analyses as a continuous or categorical variable.

Table 7.4.11.5 Factors associated with non-dominant femoral neck BMD when RBPCR was measured as a continuous variable

Factors associated with BMD at the non-dominant femoral neck were age, non-white ethnicity, and normal or high BMI, with current smoking having a borderline association. There was no association between RBPCR measured linearly and BMD at the non-dominant femoral neck.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	-0.02 (-0.03, -0.01)	0.01	-0.01 (-0.03, -0.0002)	0.047
Ethnicity				
White	0.00	-	0.00	-
Other	0.09 (0.03, 0.15)	0.003	0.06 (0.0005, 0.13)	0.048
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.004 (-0.04, 0.03)	0.82	-0.01 (-0.05, 0.02)	0.49
Current smoker	-0.05 (-0.08, -0.02)	0.003	-0.03 (-0.06, 0.0003)	0.05
BMI				
<25	0.00	-	0.00	-
25-30	0.08 (0.06, 0.11)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.11 (0.07, 0.15)	<0.0001	0.10 (0.05, 0.14)	<0.0001
Diabetes				
No	0.00	-		
Yes	-0.03 (-0.09, 0.03)	0.32		
Hypertension				
No	0.00	-		
Yes	0.02 (-0.03, 0.07)	0.42		
eGFR**, per 10 ml/min decrease	0.004 (-0.004, 0.01)	0.37		
Duration of HIV infection, years, per year	-0.003 (-0.005, -0.001)	0.002	-0.001 (-0.003, 0.001)	0.40
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.03 (-0.07, 0.0001)	0.05	-0.01 (-0.05, 0.02)	0.37
Symptomatic AIDS	-0.03 (-0.07, 0.002)	0.06	-0.01 (-0.05, 0.03)	0.62
CD4 count, per 50 cells/ μ l				
Nadir	0.004 (-0.001, 0.008)	0.12		
Current	-0.0004 (-0.003, 0.002)	0.76		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.06 (0.01, 0.10)	0.01	0.02 (-0.02, 0.07)	0.28
HBV co-infection				
No	0.00	-		
Yes	-0.004 (-0.07, 0.07)	0.91		
HCV co-infection				
No	0.00	-		
Yes	-0.004 (-0.04, 0.04)	0.86		
Current TDF				
No	0.00	-		
Yes	-0.02 (-0.05, 0.01)	0.15		

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.03 (-0.06, -0.004)	0.03	-0.01 (-0.04, 0.02)	0.39
Log RBPCR, $\mu\text{g}/\text{mmol}$	-0.01 (-0.02, -0.003)	0.01	-0.01 (-0.02, 0.003)	0.20

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for age, ethnicity, smoking status, BMI, duration and clinical stage of HIV infection, HIV viral load, current boosted PI use and RBPCR measured as a continuous variable

**eGFR defined using CKD-Epi formula

Table 7.4.11.6 Factors associated with non-dominant femoral neck BMD when RBPCR was measured as a categorical variable

When RBPCR was measured as a categorical variable, the results were no different to when RBPCR was included in the multivariable model as a continuous variable, with no association between RBPCR and non-dominant femoral neck BMD.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	-0.02 (-0.03, -0.01)	0.01	-0.01 (-0.03, -0.001)	0.04
Ethnicity				
White	0.00	-	0.00	-
Other	0.09 (0.03, 0.15)	0.003	0.07 (0.003, 0.13)	0.04
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.004 (-0.04, 0.03)	0.82	-0.01 (-0.04, 0.02)	0.53
Current smoker	-0.05 (-0.08, -0.02)	0.003	-0.03 (-0.06, 0.0002)	0.05
BMI				
<25	0.00	-	0.00	-
25-30	0.08 (0.06, 0.11)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.11 (0.07, 0.15)	<0.0001	0.10 (0.06, 0.14)	<0.0001
Diabetes				
No	0.00	-		
Yes	-0.03 (-0.09, 0.03)	0.32		
Hypertension				
No	0.00	-		
Yes	0.02 (-0.03, 0.07)	0.42		
eGFR**, per 10 ml/min decrease	0.004 (-0.004, 0.01)	0.37		
Duration of HIV infection, years, per year	-0.003 (-0.005, -0.001)	0.002	-0.001 (-0.003, 0.001)	0.40
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.03 (-0.07, 0.0001)	0.05	-0.01 (-0.05, 0.02)	0.35
Symptomatic AIDS	-0.03 (-0.07, 0.002)	0.06	-0.01 (-0.05, 0.03)	0.68
CD4 count, per 50 cells/ μl				
Nadir	0.004 (-0.001, 0.008)	0.12		
Current	-0.0004 (-0.003, 0.002)	0.76		
HIV viral load <40, copies/mL				
Yes	0.00	-	0.00	-
No	0.06 (0.01, 0.10)	0.01	0.02 (-0.02, 0.07)	0.27
HBV co-infection				
No	0.00	-		
Yes	-0.004 (-0.07, 0.07)	0.91		
HCV co-infection				
No	0.00	-		
Yes	-0.004 (-0.04, 0.04)	0.86		

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Current TDF				
No	0.00	-		
Yes	-0.02 (-0.05, 0.01)	0.15		
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.03 (-0.06, -0.004)	0.03	-0.01 (-0.04, 0.02)	0.41
RBPCR, $\mu\text{g}/\text{mmol}$				
<2.93	0.00	-	0.00	-
2.93-14.65	-0.03 (-0.07, 0.01)	0.13	-0.02 (-0.05, 0.02)	0.31
>14.65	-0.05 (-0.11, 0.01)	0.09	-0.03 (-0.09, 0.02)	0.21

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for age, ethnicity, smoking status, BMI, duration and clinical stage of HIV infection, HIV viral load, current PI/r use and RBPCR measured as a categorical variable

**eGFR defined using CKD-Epi formula

7.4.12 Patients on TDF

A sensitivity analysis of the 287 patients on TDF was conducted (Section 7.6). Their characteristics were similar to that of all 410 men (Table 7.6.1.1). As with the entire cohort, there was a significant positive correlation between RBPCR and FePO_4 ($r=0.24$, $p<0.0001$, Figure 7.6.2.1) in patients on TDF. Again, similar to the whole cohort, there was no correlation between RBPCR and vitamin D ($r=-0.05$, $p=0.44$, Figure 7.6.3.1) or PTH ($r=-0.09$, $p=0.13$, Figure 7.6.3.2), nor between FePO_4 and vitamin D ($r=-0.03$, $p=0.58$, Figure 7.6.3.3) or PTH ($r=-0.02$, $p=0.68$, Figure 7.6.3.4).

There was a borderline association between eGFR and mild to moderate RTD (RBPCR 2.93–14.65 $\mu\text{g}/\text{mmol}$, Table 7.6.4.1). Unsurprisingly, a lower eGFR was associated with severe RTD (RBPCR >14.65 $\mu\text{g}/\text{mmol}$, Table 7.6.4.2) and phosphate wasting (Table 7.6.4.3). Hypertension was associated with mild to moderate RTD, although this association was borderline in patients with severe RTD. An AIDS-defining illness also showed a borderline association with severe RTD measured by RBPCR.

There was no correlation between bone resorption (measured by CTX) and RBPCR ($r=0.004$, $p=0.94$, Figure 7.6.5.1) or FePO_4 ($r=0.004$, $p=0.94$, Figure 7.6.5.2), nor between bone formation (measured by P1NP) and RBPCR ($r=0.002$, $p=0.97$, Figure 7.6.5.3) or FePO_4 ($r=-0.07$, $p=0.26$, Figure 7.6.5.4).

As in the entire cohort, there was a significant negative correlation between BMD at the lumbar spine and RBPCR ($r=-0.18$, $p=0.003$, Figure 7.6.6.1), as well as at the non-dominant total hip ($r=-0.14$, $p=0.02$, Figure 7.6.6.3) and the non-dominant femoral neck BMD ($r=-0.13$, $p=0.02$, Figure 7.6.6.5). However, there was no correlation between FePO_4 and BMD at the lumbar spine ($r=-0.02$, $p=0.74$, Figure 7.6.6.2), the non-

dominant total hip ($r=-0.04$, $p=0.51$, Figure 7.6.6.4) or the non-dominant femoral neck ($r=-0.05$, $p=0.41$, Figure 7.6.6.6).

When considering RBPCR as a continuous variable, there was a significant association with BMD at the lumbar spine (Table 7.6.6.1) and the non-dominant total hip (Table 7.6.6.3), but this association was borderline at the non-dominant femoral neck (Table 7.6.6.5). When RBPCR was included in the multivariable model as a categorical variable, severe RTD (>14.65 $\mu\text{g}/\text{mmol}$) was associated with a lower BMD at the lumbar spine only (Table 7.6.6.2), with the association being borderline at both the non-dominant total hip (Table 7.6.6.4) and the non-dominant femoral neck (Table 7.6.6.6). Normal and high BMI was associated with a higher BMD at all three sites. Being a current or ex-smoker was significantly associated with a lower BMD at the lumbar spine, whilst current smoking was significantly associated at the non-dominant total hip. The association with current smoking was borderline at the non-dominant femoral neck.

7.5 Discussion

7.5.1 Summary

A total of 410 men with data on RBPCR and phosphate wasting were analysed in this chapter. The majority were white, MSM with a mean age of 47 years. They had long-standing HIV infection, with 92.4% on ART. Of these, 287 (70.0%) men were on TDF. Although 27.3% had been previously diagnosed with an AIDS-defining condition, the majority had well-controlled HIV infection with an HIV viral load <40 copies/mL and a good CD4 count. Although there was a high number of patients on TDF, only 7.1% had proteinuria (PCR ≥ 30 mg/mmol) at baseline.

7.5.1.1 Prevalence of and factors associated with RTD

In summary, 20.7% of HIV-positive men in this study had RBPCR-defined RTD, with 5.4% having severe RTD (RBPCR >5 times ULN). The presence of RTD in one-fifth of a relatively young and well cohort is of concern. As expected, RBPCR was associated with a lower eGFR. Mild to moderate RTD (RBPCR 2.93-14.65 $\mu\text{g}/\text{mmol}$) was also associated with hypertension, although this association was borderline in patients with severe RTD (>14.65 $\mu\text{g}/\text{mmol}$). The only HIV parameter associated with RBPCR was current TDF use, and this was only associated with severe RTD. However, there was no association with current use of a boosted PI. Although an association between RTD and TDF use has been reported in several studies, most of these have shown that

concurrent use of both TDF and a boosted PI lead to an increased risk of developing Fanconi syndrome [35,271,502,503]. A lack of association in my cohort may be due to the small number of patients on both TDF and a boosted PI (27.8%).

When assessing RTD using $\text{FePO}_4 >20\%$ in those with hypophosphataemia, 12.7% of the group were affected. The only factor associated with FePO_4 was eGFR. However, there was a positive correlation between RBPCR and FePO_4 , suggesting that there is an association between RBPCR and renal phosphate wasting.

7.5.1.2 RTD and bone

In the entire cohort, there was no association between RTD measured either by RBPCR or phosphate wasting and bone turnover (both resorption and formation). There was also no association between RTD (either RBPCR-defined or phosphate-defined) and vitamin D or PTH.

With BMD, although there was no correlation with FePO_4 , there was a negative correlation between RBPCR at all three sites. However, after adjusting for several traditional and HIV-related risk factors, there was no significant association found between RBPCR measured continuously and BMD at any site. When RBPCR was included as a categorical variable, there was a borderline association between severe RTD and BMD at the lumbar spine, with no such association at either the non-dominant total hip or the non-dominant femoral neck. Lower BMD was associated with current smoking at all three sites (as well as ex-smoking at the lumbar spine), whilst higher BMD was associated with a normal or high BMI. Additionally, non-white ethnicity was associated with higher BMD at the non-dominant total hip and the non-dominant femoral neck, whilst older age was also associated with lower BMD at the non-dominant femoral neck. Interestingly, no HIV-related factors were associated with BMD in my cohort, which probably reflects the well-controlled nature of HIV in the majority of the cohort.

7.5.1.3 RTD, bone and TDF

As I found an association between RBPCR and current TDF use, a sensitivity analysis was performed in patients on TDF only. The results obtained were similar to those seen in the whole cohort, with a lower eGFR associated with both RBPCR-associated and phosphate-defined RTD, although mild to moderate RTD measured using RBPCR only showed a borderline association. There was again no correlation between RTD (measured by either RBPCR or phosphate wasting) and vitamin D or PTH. There was also no correlation between bone turnover and RTD. Similar to the entire cohort, there

was no correlation found between BMD and FePO₄ at any site, with a significant negative correlation seen between RBPCR and BMD at the lumbar spine, the non-dominant total hip and the non-dominant femoral neck.

In contrast to the whole cohort, there was a significant association between a higher RBPCR and lower BMD at the lumbar spine (when RBPCR was measured as either a continuous or categorical variable) and the non-dominant total hip (with RBPCR as a continuous variable only), with a borderline association seen with BMD at the non-dominant femoral neck (when RBPCR was measured as both a continuous or a categorical variable). At the lumbar spine and the non-dominant total hip, lower BMD was associated with current smoking status (ex-smokers also had a lower BMD at the lumbar spine), whilst a normal or high BMI was associated with higher BMD at all three sites, all of which are factors known to be associated with BMD. Non-white ethnicity was associated with higher BMD at the non-dominant total hip and the non-dominant femoral neck, whilst older age also showed an association with a lower BMD at the non-dominant femoral neck. Interestingly, no HIV-related factors were found to be associated with BMD.

These results (which included 85 cases of RTD) suggest that RBPCR-defined RTD is associated with reduced BMD. This is in contrast to a previous study with 11 cases of RTD, which found no association between RTD and BMD [199]. However, the scatter plots of the correlations between RBPCR and BMD demonstrate a wide degree of scatter and low correlation coefficients. These results suggest that mild derangement of renal tubular function (RBPCR <5 times ULN) appears to have little, if any, effect on BMD. On the other hand, more severe RTD remained associated with reduced BMD after adjusting for confounders, and may potentially represent a sub-group of patients at increased risk of bone loss.

Other studies have demonstrated that BMD at the spine is more affected than at the hip in HIV-positive patients [337], which may explain why I found an association at the lumbar spine, but not at the non-dominant total hip or the non-dominant femoral neck. The strength of the relationship between RBPCR-defined RTD at the lumbar spine versus the hip may be due to differences in bone composition at the spine, which is predominantly trabecular [44], compared to the hip, which is predominantly cortical [45]. The metabolic rate per unit volume bone is higher in trabecular bone, which may make this type of bone more sensitive to endogenous insults [504]. Additionally, BMD changes may differ between bone sites, with high bone turnover states, such as HIV-associated osteoporosis, involving trabecular bone at an earlier stage than cortical bone [22].

The putative mechanism that links RTD to BMD is phosphate wasting. Although RBPCR was correlated with FePO_4 , I observed no association between this measure of RTD and BMD. However, the association between RBPCR and FePO_4 was weak ($r=0.24$). The lack of an association between FePO_4 and BMD may relate to the cut-offs used to define renal phosphate wasting, or may suggest that reduced fasting plasma phosphate concentrations are not reliable at reflecting total body phosphate depletion, which is a signal for tubular phosphate reabsorption. My data are consistent with a previous study which found superior specificity of LMWPs (β_2 - and α_1 -microglobulin) compared with FePO_4 to diagnose RTD [505]. This suggests that concurrent plasma and urine phosphate measurements may be of limited use in identifying patients at greatest risk of RTD-associated bone loss.

In keeping with Calmy *et al* [199], I found no correlation between RBPCR and markers of either bone resorption or bone formation in the whole cohort or in patients on TDF. This lack of association is somewhat puzzling, although the cross-sectional nature of this study may have limited my ability to detect such a relationship. The use of BTMs in clinical practise remains controversial, and changes in BTMs may not predict changes in BMD [506], so this may explain why I found no association.

In RCTs, reductions in BMD in patients on TDF have been associated with increased bone turnover [251,344,506]. However, it is possible that the mechanisms of TDF-associated initial bone loss are mediated through the PTH axis [507] or via a direct effect of TDF on bone [344]. In the ASSERT study, the initial reductions in BMD observed with TDF did not correlate with LMWP concentrations [251]. While these reductions in BMD tend to stabilise within 24 to 48 weeks of TDF exposure [250-252,506], the effects of persistent RTD may be masked by the magnitude of the initial BMD decline and may only become apparent with long-term TDF exposure. This may explain why I found associations between RBPCR and BMD in patients on TDF, which disappeared when investigating the entire cohort.

7.5.1.4 Conclusions

In conclusion, there was a negative correlation between RBPCR-defined RTD and BMD at all three sites in HIV-positive patients within the entire cohort and in those on TDF. Within the whole cohort, when RBPCR was investigated in multivariable models as either a continuous or a categorical variable, there was no association between BMD at any site, except for a borderline association between severe RTD and lumbar spine BMD. When the analyses were confined to patients on TDF only, there was a significant association between higher RBPCR (measured continuously) and lower

BMD at the lumbar spine and the non-dominant total hip, although this association was borderline at the non-dominant femoral neck. When RBPCR was measured categorically, severe RTD was associated with lower BMD at the lumbar spine, but the associations were borderline at the non-dominant total hip and the non-dominant femoral neck.

Although the clinical significance of these findings for patients with RTD and the impact on their bone health is yet to be fully elucidated, as TDF has been linked to a reduction in BMD, quantification of RBPCR may help identify patients who would benefit from TDF discontinuation to preserve BMD and reduce future fracture risk.

7.5.2 Strengths and limitations

The strengths of this study include the large, homogeneous and contemporaneous study population and the use of a robust assay to measure RBP. However, as the study population comprised of almost exclusively white men, these observations cannot necessarily be extrapolated to women or to those from other ethnicities.

One of the limitations of these data is the reliance on self-reported answers with regards to renal and bone risk factors, as variables determined on questionnaire were not checked against medical records and may therefore have been subject to recall and/or over-reporting bias. Patients who had permanently stopped TDF after developing RTD were not accounted for in the analyses, which may have underestimated the impact of TDF on renal tubular function.

RBP was used to measure RTD. Although RBP has been found to be a good marker of RTD [170] in the general population, as well as in HIV-positive patients [438], it is unclear whether other tubular biomarkers or measurements of phosphate excretion, such as 24-hour collections, would have yielded similar results. Although studies have examined the correlation between spot urine PCR and 24 hour urine phosphate excretion [508,509], the correlation between FePO_4 and these measurements are unknown.

The cross-sectional design of the study precludes inference of causality. Finally, all the correlations that were seen were weak, and therefore, the clinical significance of the observed BMD reductions remains to be defined.

7.5.3 Future work

Longitudinal studies are required to confirm the relationship between RBPCR-defined RTD and reduced BMD, and to investigate the improvement in both RTD and BMD on discontinuing TDF. Measuring a broader panel of tubular biomarkers of RTD would be useful in investigating whether this relationship remains. Finally, these findings should be confirmed in more ethnically diverse cohorts and in women.

Data relating to patients on TDF have been published in AIDS:

Hamzah L, Samarawickrama A*, Campbell L, Pope M, Burling K, Walker-Bone K, Gilleece Y, Fisher M, Post FA. Effects of renal tubular dysfunction on bone in tenofovir-exposed HIV-positive patients. AIDS. 2015 Sep 10;29(14):1785-92.*

**these authors contributed equally*

7.6 Supplementary data

7.6.1 Characteristics of patients on TDF

Table 7.6.1.1 Characteristics of patients on TDF

The characteristics of patients on TDF were similar to that of all 410 patients.

		N	Total
Demographics	Age, years, mean (SD)	287	48 (8.7)
	Ethnicity, n (%)	287	
	White		270 (94.1)
	Black		11 (3.8)
	Other		6 (2.1)
HIV transmission risk, n (%)	HIV transmission risk, n (%)	287	
	MSM		264 (92.0)
	Heterosexual sex		19 (6.6)
	IVDU/blood products		4 (1.4)
HIV parameters	Duration of HIV infection, years, median (IQR)	287	9.3 (5.0, 14.8)
	HIV clinical stage, n (%)	287	
	Asymptomatic		127 (44.3)
	Symptomatic non-AIDS		76 (26.5)
	Symptomatic AIDS		84 (29.3)
	CD4, cells/ μ L, median (IQR)	287	
	Nadir		186 (96, 263)
At recruitment		548 (418, 700)	
HIV viral load <40, copies/mL, n (%)	HIV viral load <40, copies/mL, n (%)	287	
	Yes		271 (94.4)
	No		16 (5.6)
HBV co-infection, n (%)	HBV co-infection, n (%)	287	
	Yes		15 (5.2)
	No		272 (94.8)

		N	Total
HIV parameters continued	HCV co-infection, n (%)	287	
	Yes		47 (16.4)
	No		240 (83.6)
	TDF and PI/ritonavir exposure		
	Cumulative exposure to TDF, years, median (IQR)*	287	2.1 (1.2, 4.2)
	On PI/ritonavir at recruitment, n (%)	287	114 (39.7)
Renal risk factors	Cumulative exposure to PI/ritonavir, years, median (IQR)*	148	3.3 (1.1, 5.2)
	On TDF and PI/ritonavir at recruitment, n (%)	287	136 (47.4)
	Cumulative exposure to TDF and PI/ritonavir, years, median (IQR)*	136	2.0 (0.7, 4.0)
	Prior renal disease, n (%)	287	
	Yes		7 (2.4)
	No		287 (97.6)
Renal function	Diabetes, n (%)	287	
	Yes		12 (4.2)
	No		275 (95.8)
Renal function	Hypertension, n (%)	287	
	Yes		17 (5.9)
	No		270 (94.1)
	eGFR**, ml/min/1.73m ² , median (IQR)	287	94.2 (82.0, 105.6)
	eGFR**, ml/min/1.73m ² , n (%)	287	
	>90		165 (57.5)
	60 – 90		114 (39.7)
	<60		8 (2.8)
	PCR, mg/mmol, median (IQR)	286	11.9 (8.9, 17.7)
	PCR, mg/mmol, n (%)	286	
	<30		266 (93.0)
	>30		20 (7.0)
	ACR, mg/mmol, median (IQR)	188	0.6 (0.4, 2.0)
	ACR, mg/mmol, n (%)	188	
	<3		154 (81.9)
3 - 30		30 (16.0)	
>30		4 (2.1)	
RBPCR, µg/mmol, median (IQR)	287	1.3 (0.9, 2.7)	
RBPCR, µg/mmol, n (%)	287		
<2.93		223 (77.7)	
2.93 - 14.65		45 (15.7)	
>14.65		19 (6.6)	
FePO ₄ , %, median (IQR)	287	15.9 (11.2, 20.6)	
Phosphate, mmol/L, median (IQR)	287	0.85 (0.75, 0.96)	
FePO ₄ , %, median (IQR)	287	15.9 (11.2, 20.6)	
FePO ₄ >20% and phosphate <0.8 mmol/L, n (%)	287	40 (13.9)	
Bone risk factors	Smoking, n (%)	287	
	Never smoked		84 (29.3)
	Ex-smoker		87 (30.3)
	Current smoker		116 (40.4)
	Alcohol, n (%)	287	
	Never		37 (12.9)
	<3 units/day		237 (82.6)
	≥3 units/day		13 (4.5)
	Exercise, n (%)	287	
	Never		120 (41.8)
Some weeks		64 (22.3)	
Most weeks		24 (8.4)	
Every week		79 (27.5)	
BMI, mean (SD)	287	25.4 (4.2)	
BMI, kg/m ² , n (%)	287		
<25		143 (49.8)	
25 – 30		107 (37.3)	
>30		37 (12.9)	

		N	Total
Bone risk factors continued	Ever exposed to steroids, n (%)	287	
	Steroid inhalers		30 (10.5)
	Oral steroids		23 (8.0)
	Anabolic steroids		5 (1.7)
	Previous fragility fracture, n (%)	287	
	Yes		33 (11.5)
	No		254 (88.5)
	Family history of hip fracture, n (%)	287	
	Yes		42 (14.6)
No		197 (68.6)	
Not known		48 (16.7)	
Bone biomarkers	Corrected calcium, mmol/L, median (IQR)	285	2.16 (2.12, 2.21)
	Phosphate, mmol/L, median (IQR)	287	0.85 (0.75, 0.96)
	ALP, IU/L, median (IQR)	284	81 (69, 103)
	Vitamin D, nmol/L, median (IQR)	286	46 (35, 62)
	PTH, ng/L, median (IQR)	287	48 (36, 65)
	CTX, ng/mL, median (IQR)	287	1.9 (0.9, 4.9)
	P1NP, ng/mL, median (IQR)	287	13.6 (5.4, 33.6)
BMD	Lumbar spine, g/cm ² , mean (SD)	287	1.134 (0.147)
	Non-dominant total hip, g/cm ² , mean (SD)	283	0.997 (0.132)
	Non-dominant femoral neck, g/cm ² , mean (SD)	283	0.947 (0.124)

ACR: albumin/creatinine ratio; ALP: alkaline phosphatase; ART: antiretroviral therapy; BMD: bone mineral density; BMI: body mass index; CTX: C-terminal cross-linking telopeptides of type I collagen; eGFR: estimated glomerular filtration rate; FePO₄: fractional excretion of phosphate; IQR: interquartile range; IVDU: intravenous drug use; MSM: men who have sex with men; P1NP: N-terminal propeptide of type I procollagen; PCR: protein/creatinine ratio; PI: protease inhibitor; PTH: parathyroid hormone; RBPCR: retinol binding protein creatinine ratio; SD: standard deviation; TDF: tenofovir

*Includes all patients who have ever been exposed

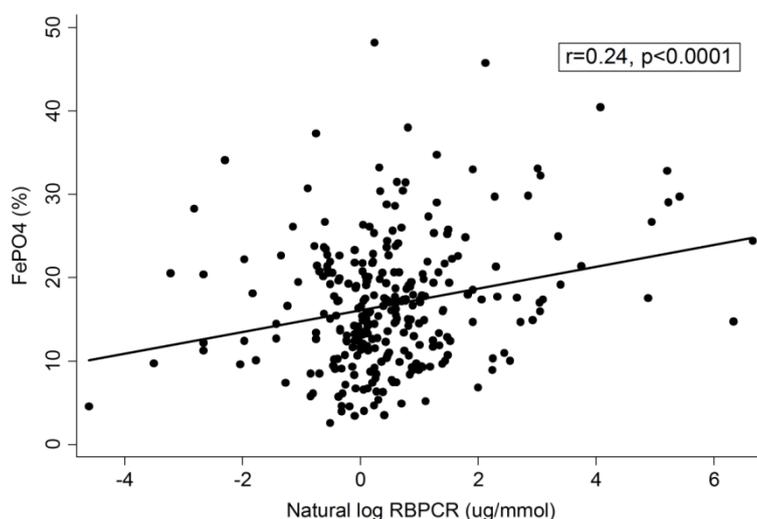
**eGFR defined using CKD-Epi formula

7.6.2 Relationship between RBPCR and FePO₄ in patients on TDF

Figure 7.6.2.1 Relationship between RBPCR and FePO₄ in patients on TDF

Plot of natural log RBPCR (x-axis) against FePO₄ (y-axis) showing a significant positive correlation ($r=0.24$, $p<0.0001$), which was exactly the same as in the entire cohort.

FePO₄: fractional excretion of phosphate; RBPCR: retinol binding protein creatinine ratio



7.6.3 Relationship between RTD, vitamin D and PTH in patients on TDF

Figure 7.6.3.1 Relationship between vitamin D and RBPCR in patients on TDF

Plot of vitamin D (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=-0.05$, $p=0.44$).

RBPCR: retinol binding protein creatinine ratio

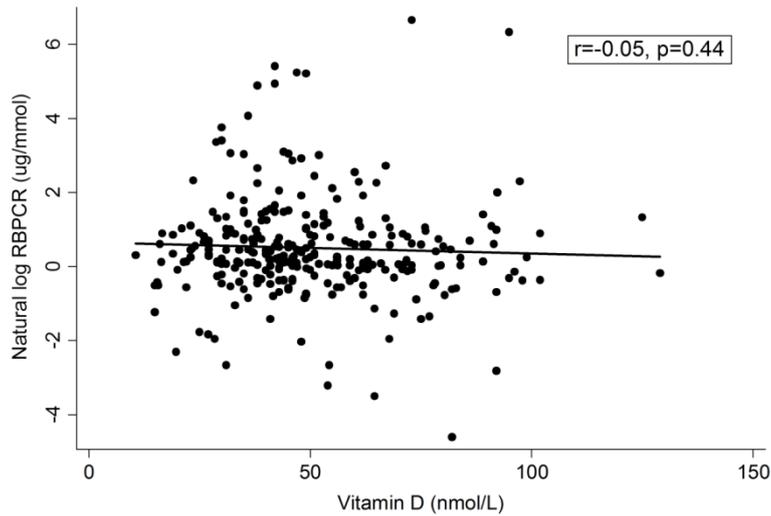


Figure 7.6.3.2 Relationship between PTH and RBPCR in patients on TDF

Plot of PTH (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=-0.09$, $p=0.13$).

PTH: parathyroid hormone; RBPCR: retinol binding protein creatinine ratio

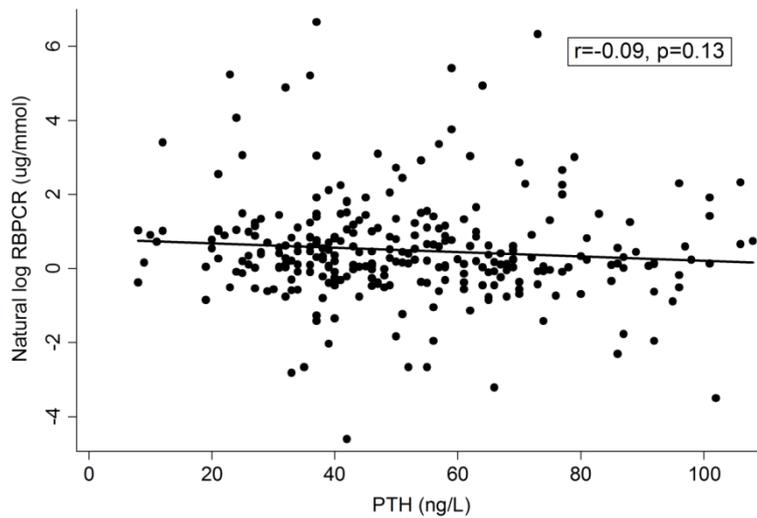


Figure 7.6.3.3 Relationship between vitamin D and FePO₄ in patients on TDF

Plot of vitamin D (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.03$, $p=0.58$).
FePO₄: fractional excretion of phosphate

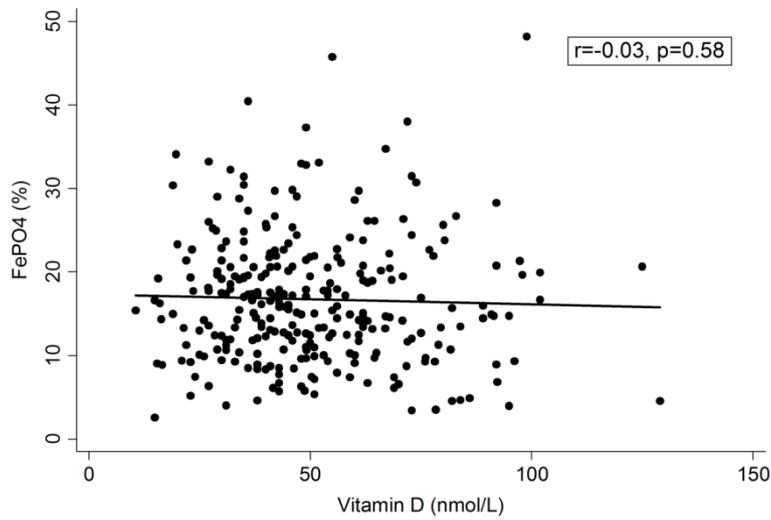
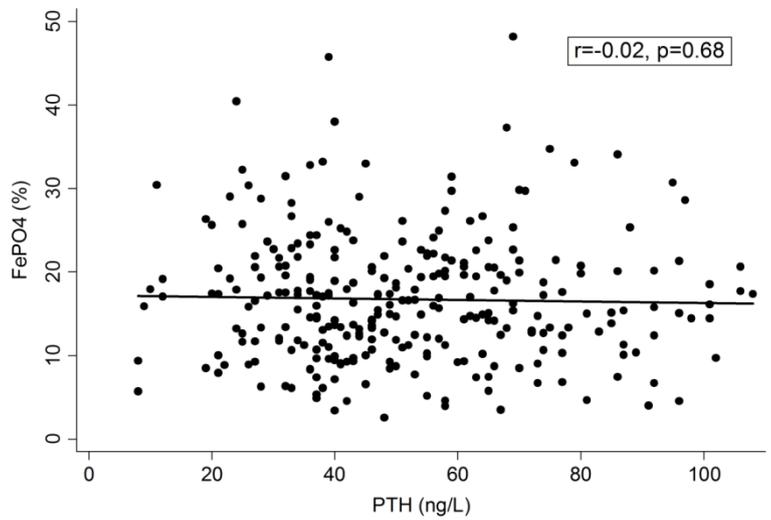


Figure 7.6.3.4 Relationship between PTH and FePO₄ in patients on TDF

Plot of PTH (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.02$, $p=0.68$).
FePO₄: fractional excretion of phosphate; PTH: parathyroid hormone



7.6.4 Factors associated with RTD in patients on TDF

7.6.4.1 RBPCR-defined RTD

Table 7.6.4.1 Factors associated with mild to moderate RTD (RBPCR 2.93-14.65 µg/mmol) in patients on TDF

Only hypertension was associated with mild to moderated RTD as measured by RBPCR, although a lower eGFR showed a borderline association.

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Age, years, per 10 years	1.00 (0.69, 1.44)	0.99		
Ethnicity				
White	1.00	-		
Other	1.72 (0.53, 5.61)	0.37		
Smoking				
Never	1.00	-		
Ex-smoker	0.76 (0.32, 1.81)	0.54		
Current smoker	1.06 (0.50, 2.26)	0.88		
Diabetes				
No	1.00	-		
Yes	1.11 (0.23, 5.31)	0.90		
Hypertension				
No	1.00	-	1.00	-
Yes	4.13 (1.34, 12.79)	0.01	4.18 (1.36, 12.79)	0.01
eGFR**, per 10 ml/min decrease	0.82 (0.67, 1.00)	0.05	0.81 (0.66, 1.00)	0.05
Duration of HIV infection, years, per year	0.99 (0.94, 1.03)	0.55		
HIV clinical stage				
Asymptomatic	1.00	-		
Symptomatic non-AIDS	0.95 (0.44, 2.06)	0.90		
Symptomatic AIDS	0.79 (0.36, 1.74)	0.56		
CD4 count, per 50 cells/µl				
Nadir	1.11 (0.98, 1.27)	0.10		
Current	0.98 (0.92, 1.05)	0.60		
HIV viral load <40, copies/mL				
Yes	1.00	-		
No	1.88 (0.57, 6.22)	0.29		
HBV co-infection				
No	1.00	-		
Yes	0.90 (0.19, 4.20)	0.89		
HCV co-infection				
No	1.00	-		
Yes	1.05 (0.45, 2.44)	0.90		
Current PI/ritonavir				
No	1.00	-		
Yes	1.35 (0.70, 2.58)	0.36		

95% CI: 95% confidence interval; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; OR: odds ratio; PI: protease inhibitor

*Adjusted for hypertension and eGFR

**eGFR defined using CKD-Epi formula

Table 7.6.4.2 Factors associated with severe RTD (RBPCR >14.65 µg/mmol) in patients on TDF

In multivariable analysis, only a lower eGFR remained significantly associated with severe RTD as measured by RBPCR, although hypertension and advanced HIV clinical stage showed borderline associations.

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Age, years, per 10 years	1.78 (1.04, 3.03)	0.03	1.01 (0.54, 1.88)	0.99
Ethnicity				
White	1.00	-		
Other	0.88 (0.11, 7.00)	0.90		
Smoking				
Never	1.00	-		
Ex-smoker	2.03 (0.58, 7.06)	0.26		
Current smoker	1.28 (0.36, 4.55)	0.70		
Diabetes				
No	1.00	-		
Yes	1.30 (0.16, 10.66)	0.81		
Hypertension				
No	1.00	-	1.00	-
Yes	3.40 (0.88, 13.20)	0.06	4.01 (0.93, 17.47)	0.06
eGFR**, per 10 ml/min decrease	0.53 (0.40, 0.71)	<0.0001	0.52 (0.37, 0.74)	<0.0001
Duration of HIV infection, years, per year	1.02 (0.96, 1.10)	0.50		
HIV clinical stage				
Asymptomatic	1.00	-	1.00	-
Symptomatic non-AIDS	2.64 (0.71, 9.76)	0.13	2.46 (0.61, 9.88)	0.21
Symptomatic AIDS	3.69 (1.08, 12.62)	0.03	3.65 (0.99, 13.48)	0.05
CD4 count, per 50 cells/µl				
Nadir	0.87 (0.73, 1.05)	0.15		
Current	1.01 (0.92, 1.10)	0.87		
HIV viral load <40, copies/mL				
Yes	1.00	-		
No	0.94 (0.12, 7.53)	0.95		
HBV co-infection				
No	1.00	-		
Yes	2.31 (0.48, 11.13)	0.28		
HCV co-infection				
No	1.00	-		
Yes	0.27 (0.03, 2.08)	0.18		
Current PI/ritonavir				
No	1.00	-	1.00	-
Yes	2.20 (0.85, 5.69)	0.09	1.55 (0.55, 4.38)	0.41

95% CI: 95% confidence interval; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; OR: odds ratio; PI: protease inhibitor

*Adjusted for age, hypertension, eGFR, HIV clinical stage and current boosted PI use

**eGFR defined using CKD-Epi formula

7.6.4.2 Phosphate-defined RTD

Table 7.6.4.3 Factors associated with phosphate-defined RTD (FePO_4 >20% and plasma phosphate <0.8 mmol/L) in patients on TDF

Only a lower eGFR remained significantly associated with phosphate-defined RTD in multivariable analyses.

	Univariable estimates		Multivariable estimates	
	OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Age, years, per 10 years	1.49 (1.02, 2.19)	0.04	1.03 (0.67, 1.58)	0.89
Ethnicity				
White	1.00	-		
Other	0.37 (0.05, 2.89)	0.32		
Smoking				
Never	1.00	-	1.00	-
Ex-smoker	0.96 (0.43, 2.11)	0.92	1.17 (0.51, 2.64)	0.71
Current smoker	0.43 (0.18, 1.03)	0.05	0.61 (0.25, 1.47)	0.27
Diabetes				
No	1.00	-		
Yes	1.25 (0.26, 5.93)	0.78		
Hypertension				
No	1.00	-		
Yes	0.37 (0.05, 2.89)	0.32		
eGFR**, per 10 ml/min decrease	0.67 (0.54, 0.81)	0.0001	0.69 (0.54, 0.87)	0.002
Duration of HIV infection, years, per year	1.02 (0.97, 1.07)	0.54		
HIV clinical stage				
Asymptomatic	1.00	-		
Symptomatic non-AIDS	0.59 (0.25, 1.42)	0.24		
Symptomatic AIDS	0.76 (0.34, 1.68)	0.50		
CD4 count, per 50 cells/ μ l				
Nadir	0.94 (0.82, 1.07)	0.34		
Current	0.99 (0.93, 1.06)	0.84		
HIV viral load <40, copies/mL				
Yes	1.00	-		
No	2.18 (0.66, 7.16)	0.19		
HBV co-infection				
No	1.00	-		
Yes	0.95 (0.21, 4.38)	0.94		
HCV co-infection				
No	1.00	-		
Yes	0.53 (0.18, 1.57)	0.24		
Current PI/ritonavir				
No	1.00	-		
Yes	1.01 (0.51, 2.01)	0.97		

95% CI: 95% confidence interval; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; OR: odds ratio; PI: protease inhibitor

*Adjusted for age, smoking status and eGFR

**eGFR defined using CKD-Epi formula

7.6.5 Relationship between bone turnover and RTD in patients on TDF

7.6.5.1 Bone resorption

Figure 7.6.5.1 Relationship between CTX and RBPCR in patients on TDF

Plot of square root CTX (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=0.004$, $p=0.94$).

CTX: C-terminal cross-linking telopeptides of type I collagen; RBPCR: retinol binding protein creatinine ratio

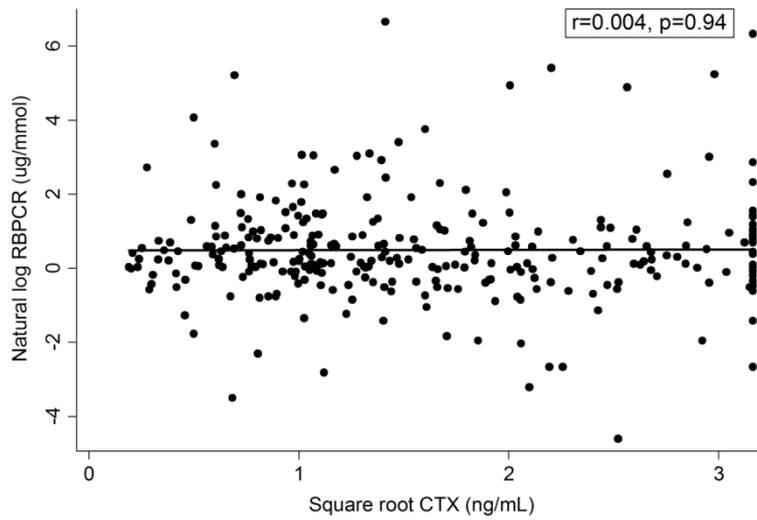
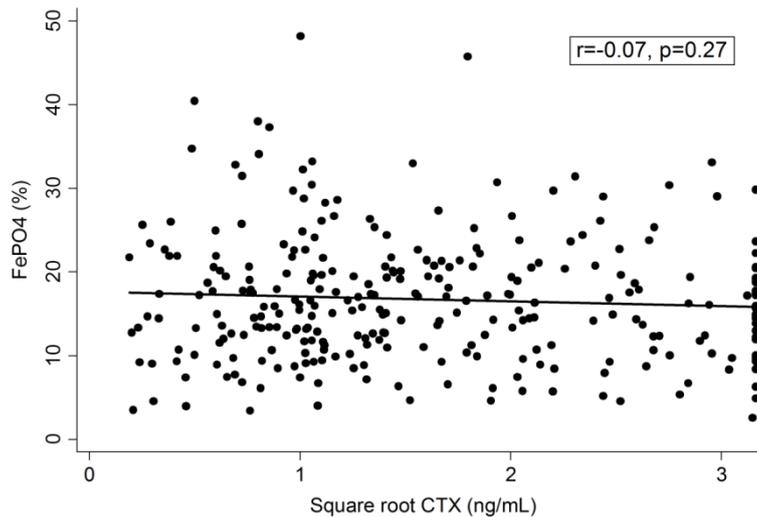


Figure 7.6.5.2 Relationship between CTX and FePO₄ in patients on TDF

Plot of square root CTX (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.07$, $p=0.27$).

CTX: C-terminal cross-linking telopeptides of type I collagen; FePO₄: fractional excretion of phosphate



7.6.5.2 Bone formation

Figure 7.6.5.3 Relationship between P1NP and RBPCR in patients on TDF

Plot of natural log P1NP (x-axis) against natural log RBPCR (y-axis) showing no significant correlation ($r=0.002$, $p=0.97$).

P1NP: N-terminal propeptide of type I procollagen; RBPCR: retinol binding protein creatinine ratio

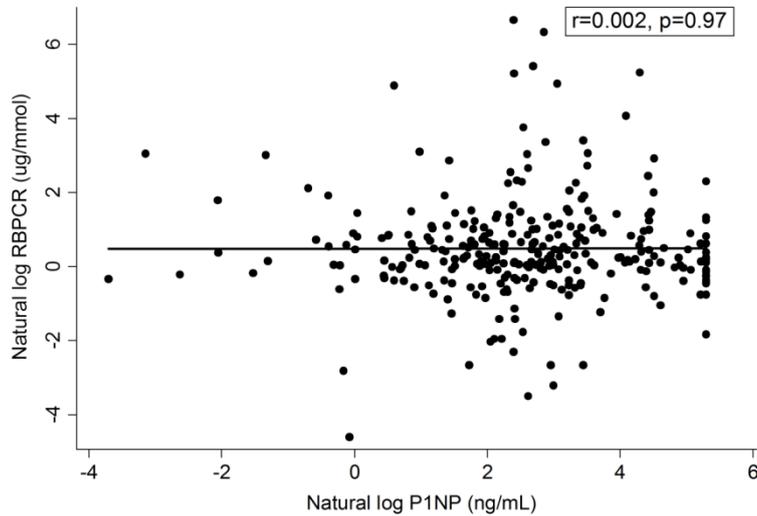
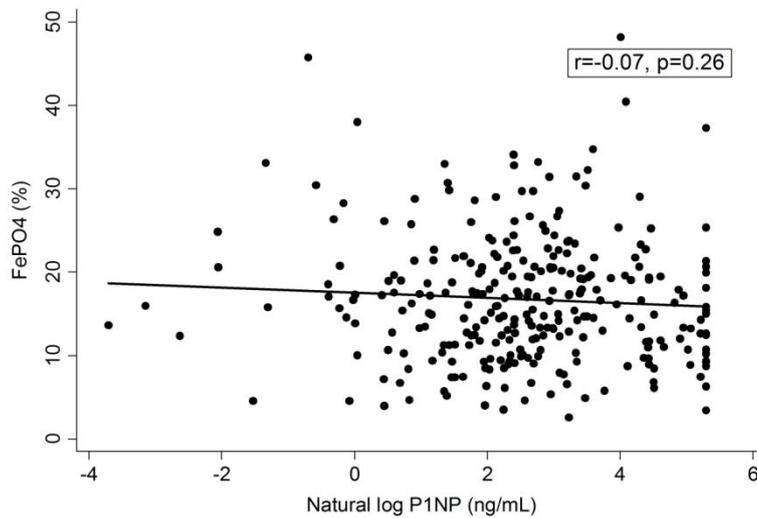


Figure 7.6.5.4 Relationship between P1NP and FePO₄ in patients on TDF

Plot of natural log P1NP (x-axis) against FePO₄ (y-axis) showing no significant negative correlation ($r=-0.07$, $p=0.26$).

FePO₄: fractional excretion of phosphate; P1NP: N-terminal propeptide of type I procollagen



7.6.6 Relationship between BMD and RTD in patients on TDF

7.6.6.1 Lumbar spine

Figure 7.6.6.1 Relationship between lumbar spine BMD and RBPCR in patients on TDF

Plot of lumbar spine BMD (x-axis) against natural log RBPCR (y-axis) showing a significant negative correlation ($r=-0.18$, $p=0.003$).
BMD: bone mineral density; RBPCR: retinol binding protein creatinine ratio

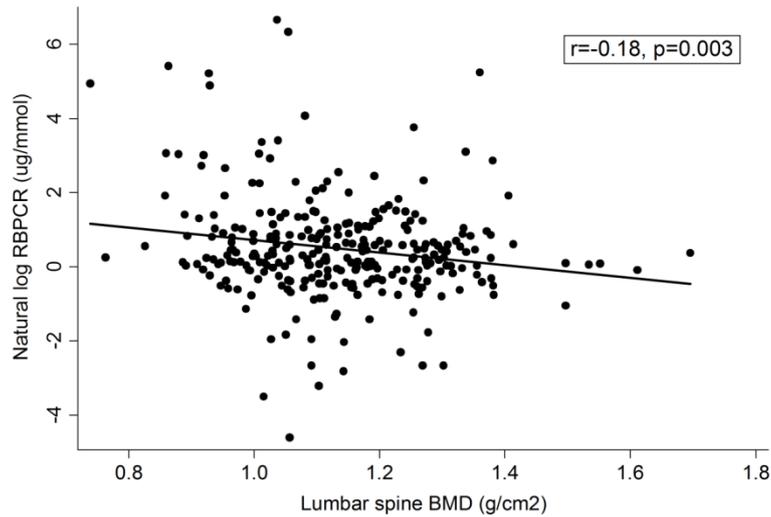


Figure 7.6.6.2 Relationship between lumbar spine BMD and FePO₄ in patients on TDF

Plot of lumbar spine BMD (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=0.02$, $p=0.74$).
BMD: bone mineral density; FePO₄: fractional excretion of phosphate

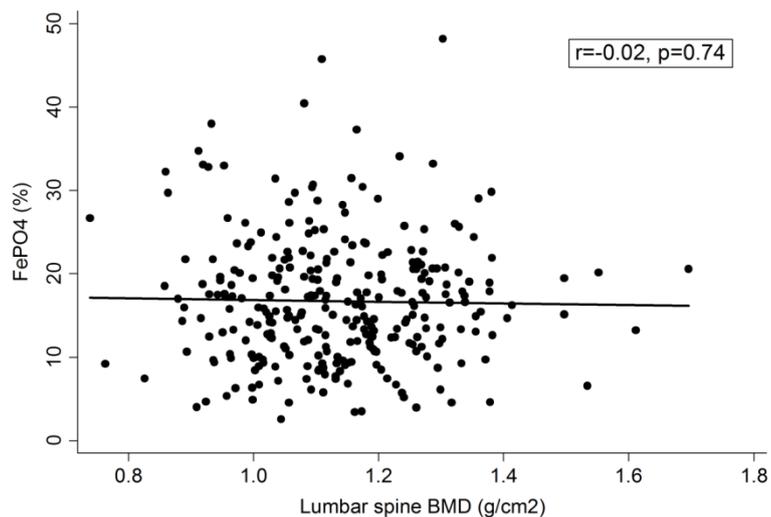


Table 7.6.6.1 Factors associated with lumbar spine BMD when RBPCR was measured as a continuous variable in patients on TDF

There was a significant association between higher RBPCR and lower BMD at the lumbar spine. Being a current smoker or an ex-smoker was associated with a lower BMD whilst normal or high BMI was associated with a higher BMD.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	0.01 (-0.01, 0.03)	0.35		
Ethnicity				
White	0.00	-		
Other	-0.01 (-0.08, 0.06)	0.74		
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.04 (-0.08, 0.004)	0.07	-0.06 (-0.10, -0.01)	0.01
Current smoker	-0.09 (-0.13, -0.05)	<0.0001	-0.07 (-0.11, -0.03)	<0.0001
BMI				
<25	0.00	-	0.00	-
25-30	0.08 (0.05, 0.12)	<0.0001	0.08 (0.04, 0.11)	<0.0001
>30	0.11 (0.06, 0.16)	<0.0001	0.10 (0.04, 0.16)	0.001
Diabetes				
No	0.00	-		
Yes	-0.03 (-0.12, 0.05)	0.43		
Hypertension				
No	0.00	-		
Yes	0.003 (-0.07, 0.08)	0.93		
eGFR**, per 10 ml/min decrease	-0.01 (-0.02, 0.003)	0.17		
Duration of HIV infection, years, per year	-0.002 (-0.004, 0.001)	0.12		
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.07, 0.02)	0.25		
Symptomatic AIDS	-0.03 (-0.07, 0.01)	0.11		
CD4 count, per 50 cells/ μ l				
Nadir	0.004 (-0.003, 0.01)	0.23		
Current	0.002 (-0.001, 0.006)	0.21		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	-0.003 (-0.08, 0.07)	0.94		
HBV co-infection				
No	0.00	-		
Yes	-0.03 (-0.11, 0.05)	0.43		
HCV co-infection				
No	0.00	-		
Yes	-0.02 (-0.07, 0.02)	0.30		
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.03 (-0.07, 0.001)	0.06	-0.03 (-0.06, 0.01)	0.11
Log RBPCR, μ g/mmol	-0.02 (-0.03, -0.01)	0.002	-0.02 (-0.03, -0.003)	0.02

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for smoking status, BMI, current boosted PI use and RBPCR measured as a continuous variable

**eGFR defined using CKD-Epi formula

Table 7.6.6.2 Factors associated with lumbar spine BMD when RBPCR was measured as a categorical variable in patients on TDF

When RBPCR was included in the multivariable model as a categorical variable, severe RTD (RBPCR >14.65 µg/mmol) was associated with a lower BMD at the lumbar spine. Factors that remained associated with a lower BMD were current and ex-smoking and a higher BMD were normal and high BMI.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β* (95% CI)	P-value
Age, years, per 10 years	0.01 (-0.01, 0.03)	0.35		
Ethnicity				
White	0.00	-		
Other	-0.01 (-0.08, 0.06)	0.74		
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.04 (-0.08, 0.004)	0.07	-0.05 (-0.09, -0.01)	0.02
Current smoker	-0.09 (-0.13, -0.05)	<0.0001	-0.07 (-0.11, -0.03)	<0.0001
BMI				
<25	0.00	-	0.00	-
25-30	0.08 (0.05, 0.12)	<0.0001	0.08 (0.05, 0.11)	<0.0001
>30	0.11 (0.06, 0.16)	<0.0001	0.10 (0.05, 0.16)	<0.0001
Diabetes				
No	0.00	-		
Yes	-0.03 (-0.12, 0.05)	0.43		
Hypertension				
No	0.00	-		
Yes	0.003 (-0.07, 0.08)	0.93		
eGFR**, per 10 ml/min decrease	-0.01 (-0.02, 0.003)	0.17		
Duration of HIV infection, years, per year	-0.002 (-0.004, 0.001)	0.12		
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.07, 0.02)	0.25		
Symptomatic AIDS	-0.03 (-0.07, 0.01)	0.11		
CD4 count, per 50 cells/µl				
Nadir	0.004 (-0.003, 0.01)	0.23		
Current	0.002 (-0.001, 0.006)	0.21		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	-0.003 (-0.08, 0.07)	0.94		
HBV co-infection				
No	0.00	-		
Yes	-0.03 (-0.11, 0.05)	0.43		
HCV co-infection				
No	0.00	-		
Yes	-0.02 (-0.07, 0.02)	0.30		
Current PI/ritonavir				
No	0.00	-	0.00	-
Yes	-0.03 (-0.07, 0.001)	0.06	-0.02 (-0.06, 0.01)	0.13
RBPCR, µg/mmol				
<2.93	0.00	-	0.00	-
2.93-14.65	-0.03 (-0.07, 0.02)	0.24	-0.01 (-0.05, 0.02)	0.45
>14.65	-0.11 (-0.18, -0.04)	0.001	-0.11 (-0.19, -0.03)	0.01

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for smoking status, BMI, current boosted PI use and RBPCR measured as a categorical variable

**eGFR defined using CKD-Epi formula

7.6.6.2 Non-dominant total hip

Figure 7.6.6.3 Relationship between non-dominant total hip BMD and RBPCR in patients on TDF

Plot of non-dominant total hip BMD (x-axis) against natural log RBPCR (y-axis) showing a significant negative correlation ($r=-0.14$, $p=0.02$).

BMD: bone mineral density; RBPCR: retinol binding protein creatinine ratio

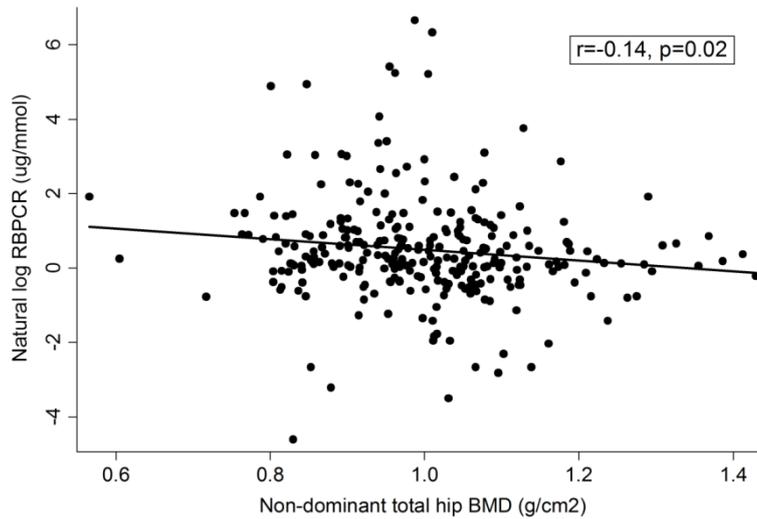


Figure 7.6.6.4 Relationship between non-dominant total hip BMD and FePO₄ in patients on TDF

Plot of non-dominant total hip BMD (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.04$, $p=0.51$).

FePO₄: fractional excretion of phosphate

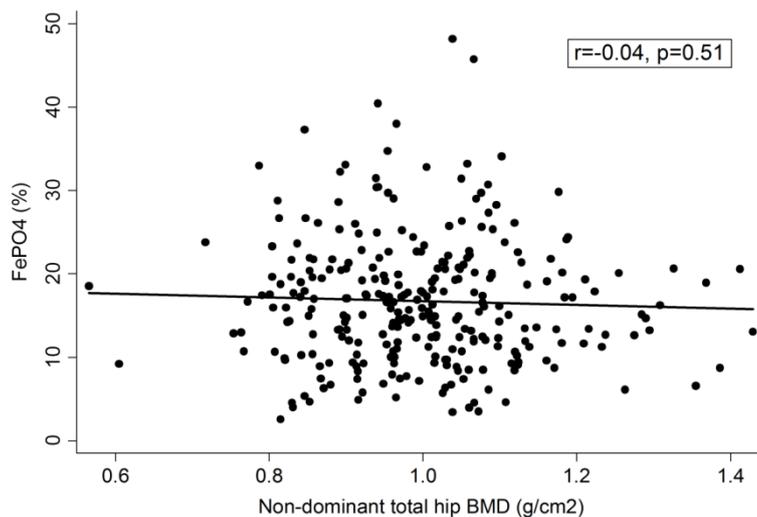


Table 7.6.6.3 Factors associated with non-dominant total hip BMD when RBPCR was measured as a continuous variable in patients on TDF

There was a significant association between higher RBPCR and a lower BMD at the non-dominant total hip. Current smoking was associated with a lower BMD, whilst BMI (normal and high) was associated with a higher BMD.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	-0.004 (-0.02, 0.01)	0.68		
Ethnicity				
White	0.00	-		
Other	0.02 (-0.05, 0.08)	0.58		
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.003 (-0.04, 0.04)	0.88	-0.01 (-0.05, 0.03)	0.55
Current smoker	-0.06 (-0.10, -0.03)	0.001	-0.04 (-0.08, -0.01)	0.01
BMI				
<25	0.00	-	0.00	-
25-30	0.10 (0.07, 0.13)	<0.0001	0.09 (0.06, 0.12)	<0.0001
>30	0.14 (0.10, 0.19)	<0.0001	0.13 (0.09, 0.17)	<0.0001
Diabetes				
No	0.00	-		
Yes	0.02 (-0.06, 0.09)	0.68		
Hypertension				
No	0.00	-		
Yes	0.04 (-0.02, 0.11)	0.21		
eGFR**, per 10 ml/min decrease	-0.005 (-0.01, 0.004)	0.25		
Duration of HIV infection, years, per year	-0.002 (-0.004, 0.0001)	0.07	-0.001 (-0.003, 0.001)	0.20
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.06, 0.01)	0.23		
Symptomatic AIDS	-0.03 (-0.07, 0.01)	0.12		
CD4 count, per 50 cells/ μ l				
Nadir	0.005 (-0.001, 0.01)	0.12		
Current	0.002 (-0.001, 0.005)	0.19		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.03 (-0.04, 0.10)	0.37		
HBV co-infection				
No	0.00	-		
Yes	-0.01 (-0.08, 0.06)	0.85		
HCV co-infection				
No	0.00	-		
Yes	0.002 (-0.04, 0.04)	0.93		
Current PI/ritonavir				
No	0.00	-		
Yes	-0.02 (-0.05, 0.01)	0.20		
Log RBPCR, μ g/mmol	-0.01 (-0.02, -0.002)	0.02	-0.01 (-0.02, 0.0003)	0.04

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for smoking status, BMI, current boosted PI use and RBPCR measured as a continuous variable
**eGFR defined using CKD-Epi formula

Table 7.6.6.4 Factors associated with non-dominant total hip BMD when RBPCR was measured as a categorical variable in patients on TDF

Severe RTD (RBPCR >14.65 µg/mmol) was associated with a lower BMD at the non-dominant total hip in multivariable analysis. Current smoking was associated with a lower BMD and normal or high BMI was associated with a higher BMD.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β* (95% CI)	P-value
Age, years, per 10 years	-0.004 (-0.02, 0.01)	0.68		
Ethnicity				
White	0.00	-		
Other	0.02 (-0.05, 0.08)	0.58		
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	0.003 (-0.04, 0.04)	0.88	-0.01 (-0.05, 0.03)	0.61
Current smoker	-0.06 (-0.10, -0.03)	0.001	-0.04 (-0.08, -0.01)	0.01
BMI				
<25	0.00	-	0.00	-
25-30	0.10 (0.07, 0.13)	<0.0001	0.09 (0.06, 0.12)	<0.0001
>30	0.14 (0.10, 0.19)	<0.0001	0.13 (0.09, 0.17)	<0.0001
Diabetes				
No	0.00	-		
Yes	0.02 (-0.06, 0.09)	0.68		
Hypertension				
No	0.00	-		
Yes	0.04 (-0.02, 0.11)	0.21		
eGFR**, per 10 ml/min decrease	-0.005 (-0.01, 0.004)	0.25		
Duration of HIV infection, years, per year	-0.002 (-0.004, 0.0001)	0.07	-0.001 (-0.003, 0.001)	0.17
HIV clinical stage				
Asymptomatic	0.00	-		
Symptomatic non-AIDS	-0.02 (-0.06, 0.01)	0.23		
Symptomatic AIDS	-0.03 (-0.07, 0.01)	0.12		
CD4 count, per 50 cells/µl				
Nadir	0.005 (-0.001, 0.01)	0.12		
Current	0.002 (-0.001, 0.005)	0.19		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.03 (-0.04, 0.10)	0.37		
HBV co-infection				
No	0.00	-		
Yes	-0.01 (-0.08, 0.06)	0.85		
HCV co-infection				
No	0.00	-		
Yes	0.002 (-0.04, 0.04)	0.93		
Current PI/ritonavir				
No	0.00	-		
Yes	-0.02 (-0.05, 0.01)	0.20		
RBPCR, µg/mmol				
<2.93	0.00	-	0.00	-
2.93-14.65	-0.04 (-0.08, 0.001)	0.06	-0.03 (-0.07, 0.01)	0.13
>14.65	-0.04 (-0.11, 0.01)	0.13	-0.05 (-0.10, 0.0001)	0.05

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for smoking status, BMI, current boosted PI use and RBPCR measured as a categorical variable

**eGFR defined using CKD-Epi formula

7.6.6.3 Non-dominant femoral neck

Figure 7.6.6.5 Relationship between non-dominant femoral neck BMD and RBPCR in patients on TDF

Plot of non-dominant femoral neck BMD (x-axis) against natural log RBPCR (y-axis) showing a significant negative correlation ($r=-0.13$, $p=0.02$).

BMD: bone mineral density; RBPCR: retinol binding protein creatinine ratio

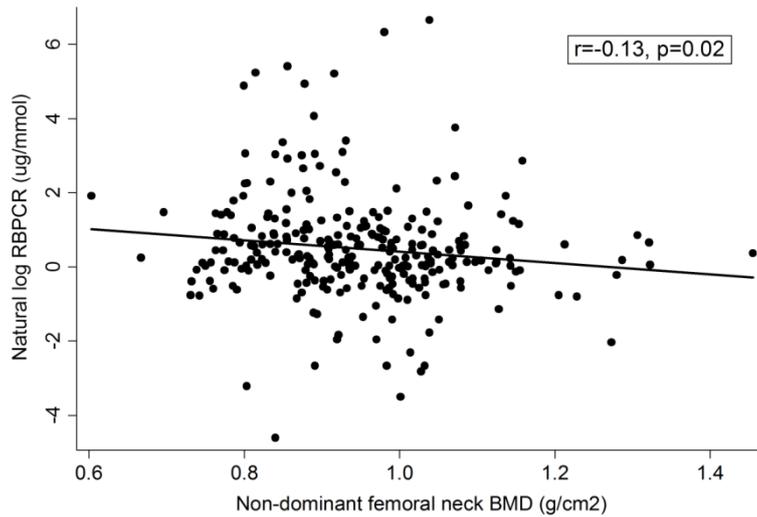


Figure 7.6.6.6 Relationship between non-dominant femoral neck BMD and FePO₄ in patients on TDF

Plot of non-dominant femoral neck BMD (x-axis) against FePO₄ (y-axis) showing no significant correlation ($r=-0.05$, $p=0.41$).

BMD: bone mineral density; FePO₄: fractional excretion of phosphate

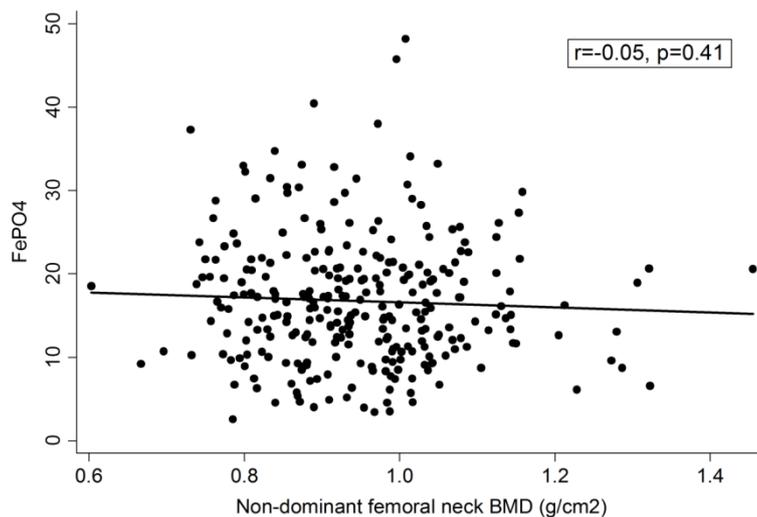


Table 7.6.6.5 Factors associated with non-dominant femoral neck BMD when RBPCR was measured as a continuous variable in patients on TDF

Although there was a significant association between BMD at the non-dominant femoral neck and RBPCR measured continuously on unilateral analyses, this association became borderline in multivariable analyses. Current smoking was associated with a lower BMD whilst BMI (normal and high) was associated with a higher BMD.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β^* (95% CI)	P-value
Age, years, per 10 years	-0.01 (-0.03, 0.003)	0.12		
Ethnicity				
White	0.00	-		
Other	0.03 (-0.03, 0.09)	0.33		
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.001 (-0.04, 0.04)	0.97	-0.01 (-0.05, 0.03)	0.63
Current smoker	-0.05 (-0.09, -0.02)	0.004	-0.03 (-0.07, -0.0003)	0.048
BMI				
<25	0.00	-	0.00	-
25-30	0.07 (0.04, 0.10)	<0.0001	0.06 (0.03, 0.09)	<0.0001
>30	0.11 (0.07, 0.16)	<0.0001	0.10 (0.06, 0.15)	<0.0001
Diabetes				
No	0.00	-		
Yes	-0.02 (-0.09, 0.05)	0.58		
Hypertension				
No	0.00	-		
Yes	0.01 (-0.05, 0.07)	0.78		
eGFR**, per 10 ml/min decrease	-0.003 (-0.01, 0.01)	0.57		
Duration of HIV infection, years, per year	-0.002 (-0.004, -0.0002)	0.04	-0.001 (-0.003, 0.001)	0.21
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.03 (-0.07, 0.005)	0.09	-0.02 (-0.06, 0.01)	0.18
Symptomatic AIDS	-0.02 (-0.06, 0.01)	0.21	-0.01 (-0.04, 0.03)	0.68
CD4 count, per 50 cells/ μ l				
Nadir	0.003 (-0.002, 0.009)	0.24		
Current	0.0008 (-0.002, 0.004)	0.58		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.02 (-0.04, 0.09)	0.48		
HBV co-infection				
No	0.00	-		
Yes	0.01 (-0.06, 0.07)	0.78		
HCV co-infection				
No	0.00	-		
Yes	0.001 (-0.04, 0.04)	0.97		
Current PI/ritonavir				
No	0.00	-		
Yes	-0.01 (-0.04, 0.02)	0.57		
Log RBPCR, μ g/mmol	-0.01 (-0.02, -0.001)	0.03	-0.01 (-0.02, 0.001)	0.08

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for smoking status, BMI, duration and clinical stage of HIV infection and RBPCR measured as a continuous variable

**eGFR defined using CKD-Epi formula

Table 7.6.6.6 Factors associated with non-dominant femoral neck BMD when RBPCR was measured as a categorical variable in patients on TDF

When RBPCR was measured as a categorical variable, there was a borderline association between severe RTD (RBPCR >14.65 µg/mmol) and non-dominant femoral neck BMD. The only factor associated with a higher BMD was BMI (normal and high), although being a current smoker showed a borderline association with a lower BMD.

	Univariable estimates		Multivariable estimates	
	β (95% CI)	P-value	Adjusted β* (95% CI)	P-value
Age, years, per 10 years	-0.01 (-0.03, 0.003)	0.12		
Ethnicity				
White	0.00	-		
Other	0.03 (-0.03, 0.09)	0.33		
Smoking				
Never	0.00	-	0.00	-
Ex-smoker	-0.001 (-0.04, 0.04)	0.97	-0.01 (-0.04, 0.03)	0.69
Current smoker	-0.05 (-0.09, -0.02)	0.004	-0.03 (-0.06, 0.001)	0.06
BMI				
<25	0.00	-	0.00	-
25-30	0.07 (0.04, 0.10)	<0.0001	0.06 (0.03, 0.09)	<0.0001
>30	0.11 (0.07, 0.16)	<0.0001	0.11 (0.06, 0.15)	<0.0001
Diabetes				
No	0.00	-		
Yes	-0.02 (-0.09, 0.05)	0.58		
Hypertension				
No	0.00	-		
Yes	0.01 (-0.05, 0.07)	0.78		
eGFR**, per 10 ml/min decrease	-0.003 (-0.01, 0.01)	0.57		
Duration of HIV infection, years, per year	-0.002 (-0.004, -0.0002)	0.04	-0.001 (-0.003, 0.001)	0.17
HIV clinical stage				
Asymptomatic	0.00	-	0.00	-
Symptomatic non-AIDS	-0.03 (-0.07, 0.005)	0.09	-0.02 (-0.06, 0.01)	0.16
Symptomatic AIDS	-0.02 (-0.06, 0.01)	0.21	-0.06 (-0.04, 0.03)	0.74
CD4 count, per 50 cells/µl				
Nadir	0.003 (-0.002, 0.009)	0.24		
Current	0.0008 (-0.002, 0.004)	0.58		
HIV viral load <40, copies/mL				
Yes	0.00	-		
No	0.02 (-0.04, 0.09)	0.48		
HBV co-infection				
No	0.00	-		
Yes	0.01 (-0.06, 0.07)	0.78		
HCV co-infection				
No	0.00	-		
Yes	0.001 (-0.04, 0.04)	0.97		
Current PI/ritonavir				
No	0.00	-		
Yes	-0.01 (-0.04, 0.02)	0.57		
RBPCR, µg/mmol				
<2.93	0.00	-	0.00	-
2.93-14.65	-0.04 (-0.08, -0.001)	0.05	-0.03 (-0.07, 0.01)	0.11
>14.65	-0.05 (-0.11, 0.01)	0.11	-0.05 (-0.10, 0.01)	0.08

95% CI: 95% confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; HBV: hepatitis B; HCV: hepatitis C; PI: protease inhibitor; RBPCR: retinol binding protein creatinine ratio; TDF: tenofovir

*Adjusted for smoking status, BMI, duration of HIV infection, HIV clinical stage and RBPCR measured as a categorical variable

**eGFR defined using CKD-Epi formula

Chapter 8: Discussion

8.1 Introduction

My main aim in this thesis was to conduct a cross-sectional study with changes over time to investigate BMD and RTD in a relatively homogenous group of white, ART-experienced HIV-positive men in the UK, who were mostly MSM and mainly on TDF. My aims included evaluating the prevalence of reduced BMD in this cohort, the risk factors for reduced BMD, the change in BMD over 12 months and the factors associated with this, calculating fracture risk using FRAX[®] scores, assessing FRAX[®] and pDXA as screening tools, and evaluating RTD, including using APR to differentiate RTD from other proteinuria and the relationship between RTD and bone.

To address my aims, I designed and initiated a large prospective cohort study of HIV-positive men attending the HIV outpatient clinic at BSUH. I used the data from my cohort to investigate the following:

1. The prevalence and risk factors associated with reduced BMD at baseline (Chapter 3), as well as the change in BMD over 12 months and the factors associated with loss of BMD (Chapter 4).
2. The utility of the FRAX[®] score and pDXA as screening tools (Chapter 5).
3. The utility of APR in differentiating RTD from other proteinuria (Chapter 6), as well as the relationship between RTD and bone (Chapter 7).

In 2017, even with advances in testing, treatment and prevention, HIV remains a major health concern worldwide. There is still no cure. The latest figures show that 36.7 million HIV-positive individuals were alive in 2015 [510]. In the UK, the overall prevalence rate in 2015 was 2.26 people per 1,000 population [511]. The number of new diagnoses has continued to decline since a peak in 2005. In 2015, 6,095 people (4,551 men and 1,537 women) were newly diagnosed in the UK [511]. Although the number of HIV-positive MSM has declined slightly since 2014, the rate remains high, with 55% of all diagnoses occurring in MSM [511].

Interestingly, the proportion of people >50 years old who were newly diagnosed with HIV infection in the UK has increased from approximately 9% in 2006 to 17% in 2015 [511]. As survival rates remain high, the number of older people accessing care has also increased, with 34% of all HIV-positive patients aged >50 years old attending for HIV care [511]. This reduction in morbidity and mortality is attributed to ART [14].

However, improved survival has a major impact on long-term complications (e.g. reduced BMD, renal disease), most of which are associated with ageing [20]. Although ART has changed the natural history of HIV infection, it has also been associated with numerous side effects, including bone and renal complications [20]. The inflammatory state associated with ageing, sometimes termed 'inflammaging' [512], as well as inflammation due to chronic HIV infection, has led to a premature ageing phenotype, making HIV-positive patients susceptible to many chronic conditions, including bone and renal disease.

In this final chapter, I summarise how the findings of my study have contributed to existing knowledge in this field. The summary then guides an exploration of possible future research.

8.2 Main findings

My main findings have been discussed in their respective chapters and are summarised below.

8.2.1 Prevalence of reduced BMD

I recruited 422 HIV-positive men into my study. The prevalence of reduced BMD was relatively small at each site. This is probably a reflection of the young age of the cohort, good immune function and well-controlled HIV infection. Many also had a long duration of exposure to TDF, and as most BMD loss associated with TDF occurs in the first 24 to 48 months after initiation [29,251], there may have been stabilisation of BMD in men in my cohort. The prevalence of reduced BMD (both osteopenia and osteoporosis) in my study was lower than that reported in both the pilot study [160] and in a meta-analysis of 11 studies [136]. Interestingly, the prevalence of osteoporosis and osteopenia calculated using the T-score in all participants in my cohort was similar to those quoted in the Probono-1 study [306], which was also a UK cohort, with men of a similar age to those recruited into my study, as well as a similar number of patients on TDF.

Recent studies comparing HIV-positive subjects to HIV-negative controls show variability in prevalence of reduced BMD. Some have reported a higher prevalence of reduced BMD compared to HIV-negative patients [306,323,513]. This included a study from the UK [306] and one from Ireland [323], as well as one from the Netherlands

which mainly investigated MSM [513]. In contrast, one study showed no difference in BMD or 25(OH)D levels in HIV-positive South African women compared to HIV-negative women [514]. However, one study showed that the prevalence of osteoporosis varied considerably depending on the definition used [408]. Interestingly, a study investigating changes in bone microstructure showed that premenopausal HIV-positive women had a lower trabecular density at the tibia compared to HIV-negative women, and that HIV status was the only variable that differed in the two groups [515].

In the Probono-1 study, the prevalence of osteopenia and osteoporosis in HIV-negative male controls was 39% and 14%, respectively [306]. They reported no difference in the rates between HIV-positive and HIV-negative men. As this study is UK-based, its study population is the closest to mine. As there are no cohort data on the prevalence of reduced BMD in males in the UK, the rates in the Probono-1 study are useful as they provide some data on reduced BMD in the general population. However, their HIV-negative male cohort was small with only 44 patients [306]. Therefore, larger cohort data from the UK general population would be useful to ensure a good comparison could be made with prevalence rates in HIV-positive men.

8.2.2 Factors associated with reduced BMD

The factors associated with BMD in my cohort were the 'traditional' risk factors, such as older age, white ethnicity, smoking and use of steroids [61]. These findings suggest that traditional factors may be of more importance than HIV-related factors in reducing BMD in men with well-controlled HIV infection. Although there was an association between longer duration of exposure to a boosted PI and lower BMD at the lumbar spine, there were no other associations with lower BMD and HIV- or ART-related factors. Interestingly, there was no association between TDF use and lower BMD, which has previously been implicated in the loss of BMD [516]. However, as most studies have shown that the effect of TDF on loss of BMD is related to initiation of TDF [252], the lack of an association in my cohort may be because the patients were TDF-experienced.

Although I found no association with any specific HIV-related factors and BMD, the published evidence suggests that HIV-positive patients are at greater risk of lower BMD than that conferred by traditional risk factors alone. The pathogenesis of reduced BMD is multifactorial, and includes risk from co-morbidities, co-infections, behavioural risk factors, the persistent immune dysfunction associated with chronic HIV infection and ART [161]. Interestingly, in a study which measured bone material strength using

microindentation, HIV-positive patients had significantly lower bone strength compared to HIV-negative controls [517]. However, there was no difference in BMD between the two groups, suggesting that HIV infection may cause bone damage independent of BMD. Additionally, as HIV-positive patients continue to age, they are at risk of 'inflammaging' [512], as well as being at risk from other factors related to both older age and HIV infection (e.g. falls, frailty) [518].

8.2.3 ART and reduced BMD

Since the start of my thesis, a number of newer antiretroviral drugs have become available. These include the integrase inhibitors dolutegravir and elvitegravir, rilpivirine (NNRTI) and TAF. In addition to ritonavir, there is now another booster called cobicistat that can be used when initiating ART.

Although ART has transformed the natural course of HIV infection, it has also been associated with bone and renal complications [20]. Initiation of ART has been associated with a loss of BMD between 2% and 6%, but also induces a catabolic state in which patients are susceptible to developing fragility fractures [519]. However, with improvements in the side effect profiles of antiretroviral drugs, and evidence from the START study, HIV-positive patients are now advised to commence ART at higher CD4 counts [520]. It appears that the importance of early initiation of ART is not just related to immunologic benefits, but is also involved in reducing metabolic complications associated with ART (e.g. BMD loss) [362].

ART, including individual drugs (e.g.TDF), have been discussed in detail throughout this thesis. Here I will concentrate on the more recent developments relating to ART and bone and renal disease. I will also discuss some of the individual drugs in more detail (e.g.TAF).

8.2.3.1 TDF and TAF

There are numerous studies showing that TDF has an effect on reducing BMD compared to other NRTIs (Chapters 1,3 and 4). Although the exact mechanism has not been elucidated, it has been postulated that the process occurs indirectly either via RTD or the PTH axis as TDF has been linked to secondary hyperparathyroidism [516]. Further work from the STEAL study has shown that TDF exposure was associated with reduced indices of bone strength as measured by hip structural analysis [521]. In this study, none of the parameters improved with discontinuation of TDF [521]. In a recent

systemic review of TDF/emtricitabine/EFV, the renal safety profile was better than when TDF was prescribed with either a PI or cobicistat [522].

As the majority of patients in my cohort were on TDF, I was able to investigate the possible association of TDF and reduced BMD, although I did not find such an association. This may be because most studies have shown that the effect of TDF on BMD occurs in the first 24 to 48 months of commencing TDF [516], and as my cohort was ART-experienced, many of the participants had been on TDF for a number of years. This helps strengthen the argument that the association of TDF and low BMD occurs in the first couple of years.

Interestingly, TDF has a negative effect on BMD even in HIV-negative individuals, and this has been demonstrated in PrEP studies [338,363]. In both of these studies, there was a small but significant decrease in BMD in the TDF/emtricitabine arm [338,363]. In the iPrEx study, reduction in BMD occurred by week 24, and then stabilised [338], which is in keeping with studies in HIV-positive populations.

TAF is an alanine ester prodrug of tenofovir (different to TDF) [42]. It is a more targeted drug with higher viral activity but less systemic exposure than TDF, and therefore, has more favourable bone and renal outcomes. In ART-naïve patients, TAF-containing regimens have been non-inferior in virologic outcomes compared to TDF-containing ones and have so far shown minimal adverse effects with regards to both reduced BMD and RTD [523-525]. Two of these studies have combined TAF or TDF with emtricitabine/elvitegravir/cobicistat and have shown smaller BMD reductions in the TAF arm [523-524]. Similar results were reported in the third study which compared TDF to TAF in patients on emtricitabine/darunavir/cobicistat [525].

There are also data from studies where ART-experienced patients have been switched from TDF to TAF [526-528]. They have all shown an improvement in BMD in patients switched to TAF [526-528]. This was also the case in patients with renal impairment [527].

As more co-formulations with TAF are coming on to the market, and TAF is being used more routinely, the adverse effects of TDF may not be such a problem in the future. TDF remains one of the first-line antiretroviral drugs, and therefore, using the alternative formulation of TAF in patients at risk of low BMD or RTD could be very useful. NHS England has recently issued guidance on which patients to start on TAF and which patients to switch to TAF, including the rationale and the benefits of such decisions [529]. However, the use of TAF needs to be further investigated for any long-

term adverse bone and renal effects, as well as fracture risk, evidence of which are currently lacking.

8.2.3.2 Other antiretroviral drugs

In my study, longer cumulative exposure to a boosted PI was significantly associated with a lower BMD at the lumbar spine. Early studies showed contradictory evidence relating to the effect of boosted PIs on BMD [142,144,151,199,233,296,300]. In a meta-analysis, the overall pooled data showed that exposure to boosted PIs was associated with reduced BMD compared to never having being exposed [136]. In longitudinal studies following ART-naïve patients, some studies showed an association between loss of BMD and PIs [252,301,302], whilst others did not [215].

Recent studies have shown that PIs contribute to more bone loss than other classes of ART. In the ACTG substudy 5260s, there was loss of BMD in all arms, but a greater loss in those containing boosted PIs (atazanavir/ritonavir or darunavir/ritonavir) compared to the arm containing the integrase inhibitor raltegravir [332]. However, the magnitude of the effect appears to vary depending on the PI, with a greater loss of BMD being seen in patients on lopinavir/ritonavir compared to those on atazanavir/ritonavir [530]. In contrast, a study in middle-aged female illicit drug users showed that PI use for 3 years or more was associated with an increase in BMD at the lumbar spine compared to HIV-negative women [531].

Other studies have shown that eliminating NRTIs was more beneficial than discontinuing PIs in relation to BMD [334,350,532]. In the study by Hamzah *et al*, switching to darunavir/ritonavir monotherapy led to an improvement in serum vitamin D levels, BTMs and BMD [350]. Although NRTI-sparing regimens may be better from a bone perspective, they can cause more virologic failures [334]. Additionally, in a study in which patients had failed first-line therapy, second-line therapy with lopinavir/ritonavir and replacement of TDF with raltegravir reduced loss of BMD [364]. This study further suggests that there is an interaction between TDF and boosted PIs that cause reduction in BMD.

Although the exact mechanisms by which boosted PIs affect BMD are not known, it is thought that they have an effect on bone cells [533]. An *in vitro* study has shown that some PIs can induce premature ageing of human bone marrow mesenchymal cells, which affect their potential to differentiate into osteoblasts [534]. Furthermore, it has been postulated that PIs impair vitamin D metabolism [533]. PIs have been found to impair conversion of 25(OH)D to 1,25(OH)₂D [535], although the clinical significance of this is yet not known.

Studies have shown a lesser effect on BMD of integrase inhibitors (mainly raltegravir) compared to other classes of ART [332,345]. In the study by Brown *et al*, ART-naïve patients randomised to TDF/emtricitabine with atazanavir/ritonavir, darunavir/ritonavir or raltegravir showed that the smallest reduction in BMD at 96 weeks was seen in the integrase inhibitor arm, with similar reduction in both boosted PI arms [332]. Martin *et al* reported that an NRTI-sparing regimen containing a boosted PI and raltegravir led to less bone loss at 48 weeks than a regimen containing NRTIs and a boosted PI in ART-experienced patients failing first-line therapy [345]. Similar results were reported by the study group at 96 weeks [364]. In a study comparing TDF/emtricitabine with either elvitegravir/cobicistat or atazanavir/ritonavir, a similar loss of BMD at week 96 occurred in both arms [536]. It will be interesting to see more data on the effect of integrase inhibitors on BMD, particularly with some of the newer drugs in this class, such as dolutegravir and elvitegravir, especially as these drugs are being more widely prescribed and are now recommended as preferred agents in the latest ART prescribing guidelines [26].

8.2.4 Change in BMD over time and factors associated with loss of BMD

There were 338 men who returned for a second visit at 12 months. The change in absolute BMD at 12 months was small, probably reflecting the short length of follow-up. In order to account for precision errors, I used a greater than SDD decrease in BMD to assess the difference in BMD from baseline to 12 months which has been used by a French group [352]. This group then investigated changes in SDD with different densitometers, and found that the SDD was greater for scans performed using two different devices compared to two scans obtained with the same device [353].

Unlike at baseline, no traditional factors were associated with a greater than SDD reduction in BMD. The only factor associated with a greater than SDD reduction in BMD was a detectable HIV viral load. This suggests that uncontrolled HIV infection may have an effect on BMD reduction. This is in keeping with published data, where it has been postulated that the inflammatory nature of HIV infection may lead to a catabolic state where the rate of bone resorption is greater than that of bone formation [519]. Several HIV-related factors were found to be associated with lower odds of a greater than SDD reduction in BMD, including some relating to ART exposure. These suggest that patients on ART have returned to health and by maintaining good immunologic and virologic control, ART may actually be important in reducing BMD loss, especially in ART-experienced patients.

8.2.5 Fracture assessment, fracture risk and screening tools

In my cohort, there was a low rate of risk factors for osteoporotic fracture, family history of osteoporotic fractures, falls and reduced mobility. These findings were not unexpected as the men in my cohort were relatively young and healthy with well-controlled HIV infection.

I assessed the utility of pDXA and FRAX[®] as screening tools. There was a low probability of either a major osteoporotic fracture or a hip fracture using FRAX[®]. Unfortunately, FRAX[®] alone was not sufficiently sensitive enough in my cohort. Interestingly, pDXA was slightly more sensitive than FRAX[®] alone in my cohort, although the addition of pDXA resulted in only a small increase in specificity and the combined sensitivity of both tests was not much better than using FRAX[®] alone. Finally, I was not able to calculate incident fracture rates, in particular, the 10-year fracture prediction rates for a major osteoporotic or hip fracture, nor was I able to validate the FRAX[®] tool. Further work needs to be done in order to validate the FRAX[®] score in HIV-positive populations. Although the FRAX[®] tool has been used in HIV-positive patients, it has not been validated in this population. A recent review has suggested that using current FRAX[®] application guidelines may not be accurate [405], although there is currently no suitable alternative.

8.2.6 Methods to diagnose RTD

There are a number of tests that can be used to determine RTD [473]. These include measuring PCR and ACR to calculate APR [172], or to use novel LMWP biomarkers, such as RBP [170,171]. Although the latter have been shown to be highly sensitive and specific for RTD, they can be expensive and are not routinely available. In contrast, PCR and ACR are routinely available and are cheap, and could be measured in all patients. I have previously shown the utility of APR in distinguishing RTD from GP in a retrospective study from the same HIV outpatient clinic [472]. I had planned to do a similar analysis on patients with proteinuria in my cohort. However, only 28 had proteinuria (PCR ≥ 30 mg/mmol) and APR measurements, which limited my ability to perform these analyses. However, other groups have confirmed the utility of APR in diagnosing RTD in HIV-positive patients, including one that used the same cut-offs as me [450] and another which compared the results to biopsy-proven diagnoses [481].

The low prevalence of proteinuria is likely a reflection of the fact that although the participants had longstanding HIV infection, the majority were on ART and had good

virologic control and immune function. However, another avenue may have been to include all those with low-level proteinuria. Gravemann *et al* studied a cohort that was similar to mine and found that low-level proteinuria was associated with older age, diabetes and exposure to an NRTI independent of TDF [482].

HIV-positive patients are at risk of developing RTD, and patients on TDF and/or a boosted PI may be more susceptible [475]. With the advent of newer more renal-friendly ART (e.g. TAF) [524,527], it will be interesting to follow the course of RTD and to see whether prevalence rates lower further.

8.2.7 RTD and bone

I investigated RTD measured both by RBPCR and phosphate wasting. In my cohort, 20.7% of HIV-positive men had RBPCR-defined RTD and 5.4% had severe RTD (RBPCR >5 times ULN). When assessing phosphate-defined RTD using FePO_4 >20% in those with hypophosphataemia, 12.7% were affected.

Factors associated with RBPCR-defined RTD included a lower eGFR and hypertension (in mild to moderate RTD), both of which are traditionally known to be associated with renal disease [449]. The only HIV factor associated with RBPCR was current TDF use, and this was only associated with severe RTD. Although studies have reported an association between TDF and RTD, most have shown that concurrent prescribing of TDF and a boosted PI led to a greater risk of developing a severe form of RTD called Fanconi syndrome [35,271,502,503]. However, I found no association between RTD and exposure to a boosted PI, but this may be because only one-quarter of the cohort were on both.

With regards to RTD and bone turnover, there was no association with either resorption or formation. This is in keeping with a previous study of 11 cases of RTD which also found no association with BTMs [199]. Although there was no correlation with FePO_4 , there was a negative correlation between RBPCR and BMD at all three sites. However, in multivariable analyses, the only association that remained was a borderline association between severe RTD (measured categorically) and BMD at the lumbar spine.

The presence of RTD in up to one-fifth (when measured using RBPCR) of a relatively young group of men with well-established HIV infection is of concern. These results are in contrast to a study by Calmy *et al* where there was no association between RTD and

BMD [199]. However, although I had a much larger number of RTD cases in my cohort, the scatter plots of the correlations between RBPCR and BMD demonstrated a wide degree of scatter with low correlation coefficients. My results suggest that although mild RTD does not seem to affect BMD, patients with severe RTD may be a sub-group that is at greater risk of BMD changes.

In a sensitivity analysis of patients on TDF, the results were similar to those seen with the entire cohort. However, there was a significant negative association between RBPCR-defined RTD and BMD at the lumbar spine (RBPCR measured as either a continuous or categorical variable) and the non-dominant total hip (RBPCR measured as a continuous variable only), with a borderline association seen with BMD at the non-dominant femoral neck (RBPCR measured as either a continuous or a categorical variable). Although the clinical significance of these findings for patients with RTD and the impact on their bone health is yet to be fully elucidated, as TDF has been linked to a reduction in BMD, quantification of RBPCR may help identify patients who could benefit from TDF discontinuation to preserve BMD and reduce fracture risk.

8.3 Implications of results and contribution to knowledge

This study adds to the data already published in the field of BMD and RTD in HIV-positive patients. Although the prevalence of reduced BMD was small at each site, the majority of patients from my cohort had good immunologic and virologic control. They had also been exposed to TDF long-term. These results suggest that BMD may have stabilised in the patients in my cohort, which is in keeping with other studies.

My results further confirm that the aetiology of reduced BMD is multifactorial and that HIV-positive patients are at risk of the traditional risk factors found in the general population. Although HIV-positive patients have additional risks associated with HIV infection and exposure to ART, management of traditional risk factors may be useful in reducing the prevalence of reduced BMD in this patient population.

The loss of absolute BMD over 12 months was small. This may be because the length of follow-up was too short. However, it may also reflect the fact that my cohort comprised a group of HIV-positive men with longstanding HIV infection who were well-established on ART. Follow-up of these patients over a longer period will help differentiate between the two potential reasons.

Although some guidelines recommend calculating FRAX[®] scores, it is important to remember that this tool has not been validated in HIV-positive patients, and therefore, may not be accurate. Validation of the FRAX[®] tool over 10 years to calculate incident fragility fracture rates would be most useful in gaining a better understanding of the utility of FRAX[®] tool in HIV populations. I also evaluated the use of pDXA as a screening tool, and in my cohort, this was slightly more sensitive than FRAX[®] alone.

HIV-positive patients are at risk of developing RTD, and patients on TDF and/or a boosted PI may be more susceptible. There are different tests that can be used to determine RTD. These include measuring PCR and ACR to calculate APR, or the use of novel low molecular weight protein biomarkers, such as RBP. I found an association between severe RTD (RBPCR >14.65 µg/mmol) and lower BMD at the lumbar spine in patients on TDF. Although the clinical significance of these findings are not yet fully understood, calculating APR or measuring LMWPs may be useful in monitoring TDF-induced RTD and the impact of RTD on bone.

8.4 Strengths of my study

8.4.1 Findings are generalisable to other settings

One of the main strengths of my study was the homogeneity of the study population. The majority of participants were white MSM who were ART-experienced, which enabled me to gain an accurate description of reduced BMD in this group of patients. Additionally, as most men were on TDF, I was able to study TDF in relation to BMD and RTD, both of which are reported complications of the drug [516].

At the time of recruitment into my study, there were no published data from such a group of patients in the UK. Although there are many cohorts worldwide (mainly from the USA), my cohort is one of a handful which have investigated BMD in British and Irish HIV-positive patients. Other studies include a cohort from South London which compared HIV-positive men (comprising 60% of the cohort) and women with age-matched controls [306]. The men in this study were of a similar age to those in my study, but only 48% of the entire study population was white (the percentage of white men was not stated) and the duration of HIV infection was shorter. Interestingly, the number of patients on ART and TDF were similar to those in my study. The HIV UPBEAT study from Ireland compared HIV-positive men and women to HIV-negative patients [323]. However, their demographics were different to those of my cohort, with a higher number of African participants and a lower number of MSM, although they had

a similar number on ART. Finally, the ASSERT study was a multicentre European RCT including patients from the UK which compared HIV-positive ART-naïve patients started on TDF/emtricitabine with abacavir/lamivudine [251,537]. In the 96-week analysis, 81% of study subjects were male and 78% were white [537].

However, the results from my study are not generalisable to all HIV-positive patients, including women and those from a non-white ethnicity.

8.4.2 Selection bias was minimised

Although the study participants were all attendees at the HIV outpatient clinic at BSUH, they were chosen in two ways. All 168 men who had participated in the pilot study were invited to join my study. Additionally, there were 1329 HIV-positive men who were listed as being current attendees of the clinic in January 2010. From this group, 600 men were randomly chosen to be eligible to join my study, which helped to reduce selection bias.

In order to assess ascertainment bias, patient characteristics from the pilot study were compared to those randomly selected to see if the two populations were different. Pilot study and randomly-selected patients showed no difference in demographic factors (including age, ethnicity and BMI) or HIV-related (including HIV transmission risk, duration of HIV infection, clinical stage of HIV infection, immune and virological status, ART use) parameters, so ascertainment bias was minimised. To assess attrition bias, the baseline demographic and HIV-related characteristics of men who returned for a second visit at 12 months and those that did not were compared. The only significant difference between the men who returned for a second visit and those that did not was that those who returned were older on average by four years. Overall, the two populations were similar, which enabled me to analyse both recruitment groups as one entity.

8.4.3 Good precision, accuracy and sensitivity of DXA scanning

Although measuring BMD with DXA is considered the gold standard, this technique can have limitations. The areas of importance relating to bone densitometry are precision, accuracy and sensitivity. Precision measures the reproducibility of DXA and varies depending on the site being scanned [94,95]. Precision can be improved by minimising inter-operator variability, which can be optimised by using a small number of

radiographers. In my study, all the scans were conducted by four radiographers who were trained in using the densitometer, which helped minimise inter-operator variability, and thereby increased precision.

Accuracy is the term used to determine how close the BMD measured by DXA is to the actual calcium content of the bone [96]. It is affected by under- or overweight patients. Fortunately, the mean BMI was 25.2 (SD 4.1) kg/m² and the percentages of patients with low (<18.5 kg/m²) or high BMI (>30 kg/m²) were 2.6% and 12.1%, respectively.

Sensitivity refers to the ability of the DXA measurement to distinguish between patients with and without fractures, as well as the ability to measure small changes over time and/or with treatment [102]. As changes in BMD over time are relatively small, sensitivity is improved if there is an adequate time interval (e.g. 18 to 24 months) between measurements [102]. The follow-up visit was at 12 months, and this length of time may have been too short for good sensitivity. Therefore, a longer interval before the follow-up visit (e.g. 2 years, 5 years) may have been better, and this limitation is further discussed in Section 8.5.2.

One of the issues with studies involving BMD measurement is the densitometer used. This is because once BMD is measured, the bone densitometer interprets the result using normal reference databases. These databases were mainly derived from a white, female, post-menopausal American population [56]. In an effort to standardise results, the NHANES reference database was selected as the reference database for interpreting hip data [103]. Apart from the majority of study participants being white, it is unknown how relevant a database based on a white, female, post-menopausal American population is to a male UK HIV-positive study population.

Additionally, different densitometers use different programs and algorithms to interpret BMD data. There is no universally accepted cross-calibration procedure or standard. When comparisons are made between DXA results obtained from different machines, it is better to compare age-stratified standardised mean BMD [109]. However, as only one densitometer was used, this was not needed in my study, which enabled easier comparison of results between patients and also between baseline and the 1-year follow-up visits in each patient.

8.4.4 Fasted blood and urine sampling taken at the same time each day

All samples were taken at the same time in the morning (between 8.30am and 10am) and after a minimum 8 hour fast to ensure diurnal variation and calcium and vitamin D in food did not affect results, respectively. This was particularly important for BTMs, which are not only affected by time of day and fasting status, but also by season, smoking, exercise and alcohol consumption [123,131,132].

A range of markers were analysed, including markers of both bone resorption (CTX) and formation (P1NP). Although there are numerous BTMs that can be measured, many other studies have also measured these two markers, which enabled comparisons to be made between studies.

8.5 Limitations of my study

8.5.1 High rate of loss to follow-up

Out of 422 patients recruited into the study, only 338 returned for a follow-up visit at 12 months, leading to a 19.9% dropout rate. The power calculation done prior to starting the study recommended 400 patients to return for follow-up at 24 months, with an estimated dropout rate of 20% (Chapter 2). At 12 months, this dropout rate had already been reached, which would have been expected to be lower to account for more participants not returning at 24 months. So, although over 400 patients were recruited, there was a high rate of loss to follow-up at 12 months, which may affect the validity of my results. Therefore, in order to maintain statistical power, I should have recruited more than 400 patients at baseline. Finally, when comparing those who returned to those that did not, those who returned were significantly older, but there was no difference in other demographic characteristics. It was actually better to have an older population return as loss of BMD increases with age, as do risk factors for reduced BMD.

8.5.2 Short follow-up period

In general, as men do not have an equivalent phase to the accelerated bone loss experienced by women after the menopause, they experience a gradual loss in bone volume of 0.5% to 1% per year [70,71]. Even if bone loss is accelerated in HIV-positive men, and allowing for small errors in precision between scans, a follow-up period of 12 months is relatively short to be able to detect significant changes in BMD. This issue

could be improved if the patients from this study are further scanned, possibly at 10 years, which would also enable the FRAX[®] score to be validated in this group of men.

8.5.3 Lack of a control group for comparison

Although I attempted to recruit HIV-negative men from the sexual health clinic, the numbers recruited were too small to analyse. The lack of an HIV-negative control group meant that the effects of the virus alone could not be accounted for and may be partly responsible for the absence of an association to HIV-related factors. Additionally, a control group would have enabled the prevalence of reduced BMD in HIV-positive men in the UK to be compared to the prevalence in HIV-negative men with similar demographics, of which data are lacking.

8.5.4 Limitations of self-reported details

Some of the questions in the self-reported questionnaire were not as detailed as they could have been (e.g. length and strength of steroid use). Additionally, ever and current use of medications was combined, but the effect of some medications reduce with stopping, some to the point that they no longer have an effect. Unfortunately, the details from the questionnaire were unable to take this into account.

A major drawback of the questionnaire was that previous fracture history was poorly captured. Firstly, recall bias was an issue as many participants could not recall the details of fractures sustained in childhood or many years previously. Secondly, the questionnaire did not capture details relating to mode of injury, which would have helped to determine whether fractures were fragility fractures or not.

Finally, I was unable to verify whether self-reported details in the questionnaire were correct, in particular with questions relating to past fracture history, past medication and family history.

8.5.5 Effect of newer classes of ART on BMD not investigated

Although I investigated the association between ART, and specific classes of ART, this thesis mainly concentrates on changes relating to TDF, boosted PIs and NNRTIs, which reflects the regimens these men were on when the study was commenced. Over

the years, data have emerged relating to the effect of integrase inhibitors, in particular, raltegravir [332,345,364]. Although there were patients on raltegravir in this cohort, the numbers were too small to perform any specific analyses. There are also newer agents being used now, including TAF, which has a better bone and renal profile, which was not available when my study was started, so none of the participants are on this. However, it will be interesting to see what effect TAF has on this group of ART-experienced men from a bone and renal perspective.

8.6 Future research

8.6.1 Longitudinal data

Although there are a number of longitudinal cohorts investigating low BMD worldwide in HIV-positive patients, this is one of a handful of cohorts from the UK. Longer follow-up of this cohort would be useful as this was a relatively homogenous cohort comprising of mainly white, young (mean age 47 years) HIV-positive MSM, who had a long duration of HIV infection (median 9.6 years) and were ART-experienced (90.3% on ART at baseline) with good immunological (median CD4 547 cells/ μ L at recruitment) and virological control (86.5% with HIV RNA viral load <40 copies/mL).

BMD changes occur at a relatively slow rate in men, at approximately 0.5% to 1.0% per year [70,71]. Even if this is accelerated in HIV-positive men, longer follow-up would enable true differences to be investigated. The lack of a high percentage of men exhibiting a greater than SDD decrease in BMD at 12 months is probably reflective of the short time period rather than a reduction in BMD not occurring. Therefore, long-term follow-up of this cohort (e.g. over 10 years) would enable this to be further investigated.

8.6.2 Standardisation of methods used to define osteoporosis and reduced BMD in HIV-positive men

The T-scores used by the WHO to diagnose osteoporosis and osteopenia were calculated using data from white females aged 20 to 29 years old from the NHANES III database [103]. Additionally, the normal reference databases used by DXA scanners to interpret BMD results were mainly derived from data from white, post-menopausal American women [56]. This means that T-score data are most accurate for diagnosing reduced BMD in post-menopausal women [59].

Studies in the general population have shown that some patients with reduced BMD have not been correctly identified [95]. Therefore, BMD results in men, ethnic minorities (e.g. black, Asian) and pre-menopausal women need to be interpreted with caution. The rate of reduction of BMD in men is different to that seen in women and occurs at a later age in the general population as men do not experience a period equivalent to the menopause [70,71]. Although reduced BMD does seem to occur at a younger age than in the general population in HIV-positive men, using T-scores in young, HIV-positive men may not be the most suitable way of diagnosing osteoporosis. The Z-score may be better in this context, which is recommended in pre-menopausal women and men <50 years old [60]. However, many studies report T-score data (even in men <50 years old).

One avenue for future research is to derive a reference range specific to BMD in HIV-positive patients, which takes into account that HIV-positive patients may have a skeletal biological age that is higher than their real age. This database could then be used to compare BMD in other HIV-positive patients.

8.6.3 Further evaluation of FRAX® in HIV-positive patients

Although many management guidelines in HIV-positive patients recommend calculating FRAX® scores [402,403], this tool has not been validated in HIV-positive patients and is only recommended to be used in those over the age of 40 years. As the FRAX® tool assesses the 10-year probability of a major osteoporotic or hip fracture, a longer period of follow-up would enable the 10-year incidence of fragility fractures in my cohort to be assessed. Due to the length of my study, I was unable to do this, which could be done in the future. Additionally, it would be extremely beneficial to ensure FRAX® is validated in HIV-positive patients, both men and women, although that is beyond the scope of my cohort alone.

A study by Yin *et al* investigated the use of a 'modified' FRAX® score where the fields relating to secondary risk factors and parental hip fracture were left blank as the data in their cohort were incomplete [410]. They found that the modified FRAX® score underestimated the risk compared to the observed fracture rate in HIV-positive patients, but that the modified FRAX® score using HIV infection as a secondary risk factor improved performance. The EACS guidelines have for a number of years recommended using HIV as a secondary risk factor [403]. Although the guidelines are based on expert opinion rather than evidence, it appears that the study by Yin *et al*

helps strengthen these recommendations. It would be useful to further evaluate the performance of FRAX[®] in HIV-positive patients using all the parameters in the FRAX[®] tool, so that the results are comparable to those in the general population.

Although in the majority of cases, low BMD leads to fragility fractures, fragility fractures can occur even in the presence of normal BMD [61,388]. FRAX[®] looks specifically at the 10-year risk of developing both a major osteoporotic fracture (at the spine, hip and wrist) and a hip fracture and can be used with or without BMD data [63,64]. Although DXA remains the gold standard investigation for diagnosing low BMD, it cannot, however, diagnose those who will go on to develop a fragility fracture. As minimising fragility fractures is the clinical indication for diagnosing, and hence, preventing low BMD, FRAX[®], which is non-invasive and does not expose patients to radiation, may become a first-line diagnostic tool in the future. Therefore, validating FRAX[®] in different patient populations, including HIV-positive patients, would be an important area of research for the future. I have already calculated the FRAX[®] scores in this cohort using all the parameters in the tool. The participants in my study could be followed-up over 10 years to enable the actual fracture rate to be calculated, which could then be compared with the predicted fracture risk rate estimated using FRAX[®].

One avenue for future research may be investigating FRAX[®] scores in those who have been on TDF long-term when compared with those on ART but unexposed to TDF, which would enable further evaluation of TDF's role in fracture risk. Furthermore, if there were 10-year follow-up data, the FRAX[®] scores could be compared with incident fractures, which would help ascertain whether TDF has an effect on fracture risk in addition to its association with reducing BMD.

Another area of research could involve comparing FRAX[®] scores in HIV and HCV mono-infected patients to those that are HIV/HCV co-infected. HCV has been shown to be associated with significant fracture risk [432,433]. As the rate of HCV co-infection in my cohort was 14.0%, comparison of FRAX[®] scores in HIV/HCV co-infected men to those with HCV mono-infection and HIV mono-infection from within my cohort may be an interesting study for the future.

8.6.4 Comparison of BMD in HIV-positive patients with HIV-negative patients

The iPrEx study interestingly found that 10% of HIV-negative MSM on PrEP with TDF had reduced BMD at baseline [294]. In this study, reduced BMD was associated with recreational drug use, in particular, amphetamine use. In another study, BMD of MSM

with PHI was compared with those with chronic HIV infection and with HIV-negative MSM [538]. This study found that HIV infection was not associated with BMD, suggesting that reduced BMD seen in HIV-positive MSM may have occurred prior to HIV acquisition.

To accurately investigate whether HIV infection has an effect on BMD, my cohort of patients should be compared to age- and ethnicity-matched HIV-negative controls. I attempted to recruit HIV-negative controls during the course of the study, but as the numbers were too small, no statistical analyses were attempted and the findings are not reported in this thesis. Further work could involve specifically recruiting age-, ethnicity- and sexual orientation-matched HIV-negative controls and assessing the differences in BMD, factors associated with reduced BMD and fracture rates in these two groups.

8.6.5 Comparison of BMD in HIV-positive patients with seroconverters

It has been postulated that the inflammatory state induced by HIV infection is partly responsible for directly causing reduced BMD by increasing the rate of bone resorption and reducing the ability to form new bone [22]. During PHI, the inflammatory state of HIV infection is at its greatest [10]. A study of 33 men with PHI found that reduced BMD was highly prevalent in this group, with 15/33 men (45%) having osteopenia and 2/33 (6%) osteoporosis [293]. In this study, reduced BMD was associated with increased age, lower BMI and thyroid stimulating hormone (TSH) levels, and higher levels of HIV viremia. Therefore, comparing BMD in seroconverters to those with established HIV infection may be useful in helping to identify whether the HIV infection *per se* is a contributing factor.

As there are high numbers of seroconverters diagnosed in and attending the HIV outpatient clinic, it would be useful to pursue this area of research to further add to the few studies investigating BMD in seroconverters. I attempted to recruit age and ethnicity-matched seroconverters during the course of the study, but as the numbers were too small, no statistical analyses were attempted and the findings are not reported in this thesis.

8.6.6 Association of ART and reduced BMD

As the majority of patients in this cohort were on TDF, I was able to investigate the association of reduced BMD with TDF. However, it would be useful to investigate other antiretroviral drugs, including newer drugs (e.g. integrase inhibitors) and new formulations (e.g. TAF).

8.6.7 Body composition

Although I have concentrated on BMD in this thesis, studies have shown that HIV infection affects body composition, especially in those diagnosed with the metabolic syndrome [539]. Most of these studies were done in the earlier ART era when drugs causing lipodystrophy and lipoatrophy (e.g. zidovudine, didanosine, PIs) were either being used more commonly or as first-line drugs [540]. The side effect profiles of the newer drugs are better than these earlier drugs. More recent studies have shown a neutral effect of atazanavir, darunavir and raltegravir on lipoatrophy and conflicting results relating to lipohypertrophy [540]. However, there are little data relating to some of the most recent antiretroviral drugs in use (e.g. rilpivirine, dolutegravir). HIV-positive patients are at risk of lipoatrophy and lipohypertrophy associated with HIV infection and ART [539], but also of fat redistribution associated with ageing [541]. Therefore, it would be useful to investigate whether the drugs used more commonly now still have an effect on body composition, especially as muscle and fat mass contribute to the strength or weakness of bone. I have collected data on body composition in my cohort using DXA measurements, although these data have not been analysed for this thesis.

Leptin and adiponectin are hormones secreted by adipose cells that may have an impact on BMD [542]. I have stored plasma and serum samples that could be used to test for adiponectin and leptin. Together with DXA data, these could be used to provide further information regarding changes in body composition in this cohort of HIV-positive men who are ART-experienced and ageing, and therefore, at risk of metabolic syndrome, lipodystrophy and abdominal obesity.

8.6.8 Metabolomics

Metabolomics is the analysis of low molecular weight compounds (<1000 Daltons) in a biological fluid or tissue, including blood and urine. The collective term used for all metabolites in the urine is the urinary metabolome. Urine is thought to contain

thousands of metabolites, including peptides, amino acids, nucleotides, lipids, carbohydrates and inorganic compounds, as well as numerous dietary compounds, pharmaceuticals and environmental contaminants [543,544]. Although targeted metabolomics has been used for a number of years in a clinical setting (e.g. measuring glucose in diabetes or creatinine for kidney injuries), non-targeted metabolomics is a method used to detect as many of the urinary metabolites as possible [545].

One area of interest is the effect of pharmaceutical intervention on the metabolome in order to identify markers of toxicity arising from prolonged exposure to ART medication. It is currently not possible to predict which patients are more likely to develop particular non-AIDS co-morbidities after starting ART. Therefore, biomarkers that could predict and guide ART in HIV-positive patients would be really helpful, especially as this would be a non-invasive screening method. To date, although a few studies have distinguished HIV-negative, HIV-positive and ART-experienced patient profiles from one another, these were mainly qualitative studies and they have not attributed particular metabolic changes to specific drug regimens [546]. Few metabolomic studies have investigated the effects of ART intervention. Our group has published the first study analysing urine as a biofluid for these types of analyses in HIV-positive patients and has shown that certain antiretroviral drugs can disrupt the urinary metabolite profile in HIV-positive patients [547]. As the majority of patients in my cohort were on TDF, metabolomic analysis of urine samples could be used to investigate the effect of TDF on bone and kidney metabolomes. Urine samples were collected for this purpose during my study, and there are also stored blood samples (plasma and serum), which could be used in the future for further research investigating the effect of ART on the metabolome.

8.7 Summary

As HIV-positive patients continue to live longer, long-term complications of ageing, such as bone and renal disease, are becoming more common. Although ART has transformed the natural history of HIV infection, it can cause side effects, including reduced BMD and RTD. In this cohort of mainly white, ART-experienced (mainly exposed to TDF) HIV-positive MSM in the UK, the prevalence of both reduced BMD and RTD was low. The factors associated with reduced BMD were mainly 'traditional' factors and probably reflects a 'return to health' with ART in these men. There was not much change in BMD over 12 months, which is probably reflective of the short follow-up period. Using FRAX[®] and pDXA may be useful as screening tools, but further work

is needed before any firm conclusions can be made in this cohort. Although one-fifth had RBPCR-defined RTD, the clinical significance of these findings and the impact on bone health is yet to be fully elucidated. It will be interesting to see how the earlier introduction of ART, the use of more bone- and renal-friendly ART and an ageing population have an impact on long-term bone and renal disease in HIV-positive patients.

Chapter 9: References

1. Centers for Disease Control (CDC). Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. *MMWR Morb Mortal Wkly Rep.* 1981 Jul 3;30(25):305-308.
2. Wohl DA, McComsey G, Tebas P, Brown TT, Glesby MJ, Reeds D, Shikuma C, Mulligan K, Dube M, Wininger D, Huang J, Revuelta M, Currier J, Swindells S, Fichtenbaum C, Basar M, Tungsiripat M, Meyer W, Weihe J, Wanke C. Current concepts in the diagnosis and management of metabolic complications of HIV infection and its therapy. *Clin Infect Dis.* 2006 Sep 1;43(5):645-653.
3. Greene WC. A history of AIDS: looking back to see ahead. *Eur J Immunol.* 2007 Nov;37 Suppl 1:S94-S102.
4. de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. *Trends Microbiol.* 2008 Dec;16(12):588-595.
5. Wang WK, Chen MY, Chuang CY, Jeang KT, Huang LM. Molecular biology of human immunodeficiency virus type 1. *J Microbiol Immunol Infect.* 2000 Sep;33(3):131-140.
6. Sierra S, Kupfer B, Kaiser R. Basics of the virology of HIV-1 and its replication. *J Clin Virol.* 2005 Dec;34(4):233-244.
7. Fanales-Belasio E, Raimondo M, Suligo B, Buttò S. HIV virology and pathogenetic mechanisms of infection: a brief overview. *Ann Ist Super Sanita.* 2010;46(1):5-14.
8. Munier ML, Kelleher AD. Acutely dysregulated, chronically disabled by the enemy within: T-cell responses to HIV-1 infection. *Immunol Cell Biol.* 2007 Jan;85(1):6-15.
9. Gomez C, Hope TJ. The ins and outs of HIV replication. *Cell Microbiol.* 2005 May;7(5):621-626.
10. Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med.* 1993 Feb 4;328(5):327-335.
11. Grund B, Peng G, Gibert CL, Hoy JF, Isaksson RL, Shlay JC, Martinez E, Reiss P, Visnegarwala F, Carr AD; INSIGHT SMART Body Composition Substudy Group. Continuous antiretroviral therapy decreases bone mineral density. *AIDS.* 2009 Jul 31;23(12):1519-1529.
12. World Health Organization. HIV/AIDS Data and Statistics 2011. Last accessed 24/04/2011.
13. Health Protection Agency. HIV in the UK: 2010 Report. 2010.
14. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med.* 1998 Mar 26;338(13):853-860.
15. Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet.* 2008 Jul 26;372(9635):293-299.
16. Lohse N, Hansen AB, Gerstoft J, Obel N. Improved survival in HIV-infected persons: consequences and perspectives. *J Antimicrob Chemother.* 2007 Sep;60(3):461-463.
17. van Sighem AI, Gras LA, Reiss P, Brinkman K, de Wolf F; ATHENA national observational cohort study. Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals. *AIDS.* 2010 Jun 19;24(10):1527-1535.
18. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, d'Arminio Monforte A, Yust I, Bruun JN, Phillips AN, Lundgren JD. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. *Lancet.* 1998 Nov 28;352(9142):1725-1730.

19. Marin B, Thiébaud R, Bucher HC, Rondeau V, Costagliola D, Dorrucchi M, Hamouda O, Prins M, Walker S, Porter K, Sabin C, Chêne G. Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy. *AIDS*. 2009 Aug 24;23(13):1743-1753.
20. Deeks SG, Phillips AN. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. *BMJ*. 2009 Jan 26;338:a3172.
21. Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N Engl J Med*. 2005 Jan 6;352(1):48-62.
22. Borderi M, Gibellini D, Vescini F, De Crignis E, Cimatti L, Biagetti C, Tampellini L, Re MC. Metabolic bone disease in HIV infection. *AIDS*. 2009 Jul 17;23(11):1297-1310.
23. Amorosa V, Tebas P. Bone disease and HIV infection. *Clin Infect Dis*. 2006 Jan 1;42(1):108-114.
24. Compston J. HIV infection and bone disease. *J Intern Med*. 2016 Oct;280(4):350-358.
25. Gazzard BG, Anderson J, Babiker A, Boffito M, Brook G, Brough G, Churchill D, Cromarty B, Das S, Fisher M, Freedman A, Geretti AM, Johnson M, Khoo S, Leen C, Nair D, Peters B, Phillips A, Pillay D, Pozniak A, Walsh J, Wilkins E, Williams I, Williams M, Youle M; BHIVA Treatment Guidelines Writing Group. British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. *HIV Med*. 2008 Oct;9(8):563-608.
26. Churchill D, Waters L, Ahmed N, Angus B, Boffito M, Bower M, Dunn D, Edwards S, Emerson C, Fidler S, Fisher M, Horne R, Khoo S, Leen C, Mackie N, Marshall N, Monteiro F, Nelson M, Orkin C, Palfreeman A, Pett S, Phillips A, Post F, Pozniak A, Reeves I, Sabin C, Trevelion R, Walsh J, Wilkins E, Williams I, Winston A. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015. *HIV Med*. 2016 Aug;17 Suppl 4:s2-s104.
27. Durand-Gasselín L, Van Rompay KK, Vela JE, Henne IN, Lee WA, Rhodes GR, Ray AS. Nucleotide analogue prodrug tenofovir disoproxil enhances lymphoid cell loading following oral administration in monkeys. *Mol Pharm*. 2009 Jul-Aug;6(4):1145-1151.
28. Ray AS, Cihlar T, Robinson KL, Tong L, Vela JE, Fuller MD, Wieman LM, Eisenberg EJ, Rhodes GR. Mechanism of active renal tubular efflux of tenofovir. *Antimicrob Agents Chemother*. 2006 Oct;50(10):3297-3304.
29. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, Coakley DF, Lu B, Toole JJ, Cheng AK; 903 Study Group. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients: a 3-year randomized trial. *JAMA*. 2004 Jul 14;292(2):191-201.
30. Pozniak AL, Gallant JE, DeJesus E, Arribas JR, Gazzard B, Campo RE, Chen SS, McColl D, Enejosa J, Toole JJ, Cheng AK. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naïve patients: virologic, immunologic, and morphologic changes--a 96-week analysis. *J Acquir Immune Defic Syndr*. 2006 Dec 15;43(5):535-540.
31. Mocroft A, Kirk O, Gatell J, Reiss P, Gargalianos P, Zilmer K, Beniowski M, Viard JP, Staszewski S, Lundgren JD. Chronic renal failure among HIV-1-infected patients. *AIDS*. 2007 May 31;21(9):1119-1127.
32. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, Lazzarin A, Schewe K, Lange J, Wyatt C, Curtis S, Chen SS, Smith S, Bischofberger N, Rooney JF. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *AIDS*. 2007 Jun 19;21(10):1273-1281.
33. Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D, Edwards SG, Johnson MA, Connolly JO. Tenofovir-associated renal and bone toxicity. *HIV Med*. 2009 Sep;10(8):482-487.
34. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougnot B, Girard PM, Ronco P, Rossert J. Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am J Kidney Dis*. 2002 Dec;40(6):1331-1333.
35. Earle KE, Seneviratne T, Shaker J, Shoback D. Fanconi's syndrome in HIV+ adults: report of three cases and literature review. *J Bone Miner Res*. 2004 May;19(5):714-721.

36. Peyrière H, Reynes J, Rouanet I, Daniel N, de Boever CM, Mauboussin JM, Leray H, Moachon L, Vincent D, Salmon-Céron D. Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr*. 2004 Mar 1;35(3):269-273.
37. Parsonage MJ, Wilkins EG, Snowden N, Issa BG, Savage MW. The development of hypophosphataemic osteomalacia with myopathy in two patients with HIV infection receiving tenofovir therapy. *HIV Med*. 2005 Sep;6(5):341-346.
38. Gupta SK. Tenofovir-associated Fanconi syndrome: review of the FDA adverse event reporting system. *AIDS Patient Care STDS*. 2008 Feb;22(2):99-103.
39. Grigsby IF, Pham L, Gopalakrishnan R, Mansky LM, Mansky KC. Downregulation of *Gnas*, *Got2* and *Snord32a* following tenofovir exposure of primary osteoclasts. *Biochem Biophys Res Commun*. 2010 Jan 15;391(3):1324-1329.
40. Grigsby IF, Pham L, Mansky LM, Gopalakrishnan R, Carlson AE, Mansky KC. Tenofovir treatment of primary osteoblasts alters gene expression profiles: implications for bone mineral density loss. *Biochem Biophys Res Commun*. 2010 Mar 26;394(1):48-53.
41. Childs KE, Fishman SL, Constable C, Gutierrez JA, Wyatt CM, Dieterich DT, Mullen MP, Branch AD. Short communication: Inadequate vitamin D exacerbates parathyroid hormone elevations in tenofovir users. *AIDS Res Hum Retroviruses*. 2010 Aug;26(8):855-859.
42. Ray AS, Fordyce MW, Hitchcock MJ. Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. *Antiviral Res*. 2016 Jan;125:63-70.
43. Seeman E, Delmas PD. Bone quality--the material and structural basis of bone strength and fragility. *N Engl J Med*. 2006 May 25;354(21):2250-2261.
44. Eastell R, Mosekilde L, Hodgson SF, Riggs BL. Proportion of human vertebral body bone that is cancellous. *J Bone Miner Res*. 1990 Dec;5(12):1237-1241.
45. Kuiper JW, Van Kuijk C, Grashuis JL. Distribution of trabecular and cortical bone related to geometry. A quantitative computed tomography study of the femoral neck. *Invest Radiol*. 1997 Feb;32(2):83-89.
46. Becker C. Pathophysiology and clinical manifestations of osteoporosis. *Clin Cornerstone*. 2006;8(1):19-27.
47. Quinn JM, Saleh H. Modulation of osteoclast function in bone by the immune system. *Mol Cell Endocrinol*. 2009 Oct 30;310(1-2):40-51.
48. Bossard MJ, Tomaszek TA, Thompson SK, Amegadzie BY, Hanning CR, Jones C, Kurdyla JT, McNulty DE, Drake FH, Gowen M, Levy MA. Proteolytic activity of human osteoclast cathepsin K. Expression, purification, activation, and substrate identification. *J Biol Chem*. 1996 May 24;271(21):12517-12524.
49. Halleen JM, Tiitinen SL, Ylipahkala H, Fagerlund KM, Väänänen HK. Tartrate-resistant acid phosphatase 5b (TRACP 5b) as a marker of bone resorption. *Clin Lab*. 2006;52(9-10):499-509.
50. Caplan AI. Mesenchymal stem cells. *J Orthop Res*. 1991 Sep;9(5):641-650.
51. Martin TJ, Seeman E. Bone remodelling: its local regulation and the emergence of bone fragility. *Best Pract Res Clin Endocrinol Metab*. 2008 Oct;22(5):701-722.
52. Matsuo K, Irie N. Osteoclast-osteoblast communication. *Arch Biochem Biophys*. 2008 May 15;473(2):201-209.
53. Raisz LG. Local and systemic factors in the pathogenesis of osteoporosis. *N Engl J Med*. 1988 Mar 31;318(13):818-828.
54. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone Miner Res*. 2001 Sep;16(9):1575-1582.

55. Caetano-Lopes J, Canhão H, Fonseca JE. Osteoimmunology--the hidden immune regulation of bone. *Autoimmun Rev.* 2009 Jan;8(3):250-255.
56. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med.* 1993 Jun;94(6):646-650.
57. World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser.* 1994;843:1-129.
58. Sambrook P, Cooper C. Osteoporosis. *Lancet.* 2006 Jun 17;367(9527):2010-2018.
59. Lewiecki EM, Gordon CM, Baim S, Leonard MB, Bishop NJ, Bianchi ML, Kalkwarf HJ, Langman CB, Plotkin H, Rauch F, Zemel BS, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Silverman S. International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. *Bone.* 2008 Dec;43(6):1115-1121.
60. National Institute of Health Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA.* 2001 Feb 14;285(6):785-795.
61. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. *Lancet.* 2002 Jun 1;359(9321):1929-1936.
62. Kanis JA, McCloskey EV, Johansson H, Oden A. Approaches to the targeting of treatment for osteoporosis. *Nat Rev Rheumatol.* 2009 Aug;5(8):425-431.
63. Kanis JA, Oden A, Johansson H, Borgström F, Ström O, McCloskey E. FRAX and its applications to clinical practice. *Bone.* 2009 May;44(5):734-743.
64. Kanis JA, McCloskey EV, Johansson H, Oden A, Ström O, Borgström F. Development and use of FRAX in osteoporosis. *Osteoporos Int.* 2010 Jun;21 Suppl 2:S407-S413.
65. Looker AC, Melton LJ 3rd, Harris TB, Borrud LG, Shepherd JA. Prevalence and trends in low femur bone density among older US adults: NHANES 2005-2006 compared with NHANES III. *J Bone Miner Res.* 2010 Jan;25(1):64-71.
66. Madeo B, Zirilli L, Caffagni G, Diazzi C, Sanguanini A, Pignatti E, Carani C, Rochira V. The osteoporotic male: overlooked and undermanaged? *Clin Interv Aging.* 2007;2(3):305-312.
67. Amin S, Felson DT. Osteoporosis in men. *Rheum Dis Clin North Am.* 2001 Feb;27(1):19-47.
68. Seeman E. Clinical review 137: Sexual dimorphism in skeletal size, density, and strength. *J Clin Endocrinol Metab.* 2001 Oct;86(10):4576-4584.
69. Khosla S, Riggs BL. Pathophysiology of age-related bone loss and osteoporosis. *Endocrinol Metab Clin North Am.* 2005 Dec;34(4):1015-1030.
70. Orwoll ES, Klein RF. Osteoporosis in men. *Endocr Rev.* 1995 Feb;16(1):87-116.
71. Seeman E. Pathogenesis of bone fragility in women and men. *Lancet.* 2002 May 25;359(9320):1841-1850.
72. Khosla S, Riggs BL, Atkinson EJ, Oberg AL, McDaniel LJ, Holets M, Peterson JM, Melton LJ 3rd. Effects of sex and age on bone microstructure at the ultradistal radius: a population-based noninvasive in vivo assessment. *J Bone Miner Res.* 2006 Jan;21(1):124-131.
73. Scane AC, Francis RM. Risk factors for osteoporosis in men. *Clin Endocrinol (Oxf).* 1993 Jan;38(1):15-16.
74. Kanis JA, Johnell O, Oden A, De Laet C, Mellstrom D. Diagnosis of osteoporosis and fracture threshold in men. *Calcif Tissue Int.* 2001 Oct;69(4):218-221.
75. Ebeling PR. Clinical practice. Osteoporosis in men. *N Engl J Med.* 2008 Apr 3;358(14):1474-1482.

76. Vanderschueren D, Boonen S, Bouillon R. Osteoporosis and osteoporotic fractures in men: a clinical perspective. *Baillieres Best Pract Res Clin Endocrinol Metab.* 2000 Jun;14(2):299-315.
77. Schousboe JT, Taylor BC, Fink HA, Kane RL, Cummings SR, Orwoll ES, Melton LJ 3rd, Bauer DC, Ensrud KE. Cost-effectiveness of bone densitometry followed by treatment of osteoporosis in older men. *JAMA.* 2007 Aug 8;298(6):629-637.
78. Donaldson LJ, Cook A, Thomson RG. Incidence of fractures in a geographically defined population. *J Epidemiol Community Health.* 1990 Sep;44(3):241-245.
79. Holroyd C, Cooper C, Dennison E. Epidemiology of osteoporosis. *Best Pract Res Clin Endocrinol Metab.* 2008 Oct;22(5):671-685.
80. Gullberg B, Johnell O, Kanis JA. World-wide projections for hip fracture. *Osteoporos Int.* 1997;7(5):407-413.
81. Jiang HX, Majumdar SR, Dick DA, Moreau M, Raso J, Otto DD, Johnston DW. Development and initial validation of a risk score for predicting in-hospital and 1-year mortality in patients with hip fractures. *J Bone Miner Res.* 2005 Mar;20(3):494-500.
82. Schwartz AV, Kelsey JL, Maggi S, Tuttleman M, Ho SC, Jónsson PV, Poór G, Sisson de Castro JA, Xu L, Matkin CC, Nelson LM, Heyse SP. International variation in the incidence of hip fractures: cross-national project on osteoporosis for the World Health Organization Program for Research on Aging. *Osteoporos Int.* 1999;9(3):242-253.
83. Seeman E, Bianchi G, Khosla S, Kanis JA, Orwoll E. Bone fragility in men--where are we? *Osteoporos Int.* 2006;17(11):1577-1583.
84. Szulc P, Munoz F, Duboeuf F, Marchand F, Delmas PD. Bone mineral density predicts osteoporotic fractures in elderly men: the MINOS study. *Osteoporos Int.* 2005 Oct;16(10):1184-1192.
85. Nguyen TV, Eisman JA, Kelly PJ, Sambrook PN. Risk factors for osteoporotic fractures in elderly men. *Am J Epidemiol.* 1996 Aug 1;144(3):255-263.
86. Dennison E, Eastell R, Fall CH, Kellingray S, Wood PJ, Cooper C. Determinants of bone loss in elderly men and women: a prospective population-based study. *Osteoporos Int.* 1999;10(5):384-391.
87. Tinetti ME. Clinical practice. Preventing falls in elderly persons. *N Engl J Med.* 2003 Jan 2;348(1):42-49.
88. Kukuljan S, Nowson CA, Bass SL, Sanders K, Nicholson GC, Seibel MJ, Salmon J, Daly RM. Effects of a multi-component exercise program and calcium-vitamin-D3-fortified milk on bone mineral density in older men: a randomised controlled trial. *Osteoporos Int.* 2009 Jul;20(7):1241-1251.
89. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet.* 2007 Aug 25;370(9588):657-666.
90. Blake GM, Fogelman I. The role of DXA bone density scans in the diagnosis and treatment of osteoporosis. *Postgrad Med J.* 2007 Aug;83(982):509-517.
91. Fogelman I, Blake GM. Bone densitometry: an update. *Lancet.* 2005 Dec 17;366(9503):2068-2070.
92. Cummings SR, Bates D, Black DM. Clinical use of bone densitometry: scientific review. *JAMA.* 2002 Oct 16;288(15):1889-1897.
93. Grampp S, Genant HK, Mathur A, Lang P, Jergas M, Takada M, Glüer CC, Lu Y, Chavez M. Comparisons of noninvasive bone mineral measurements in assessing age-related loss, fracture discrimination, and diagnostic classification. *J Bone Miner Res.* 1997 May;12(5):697-711.
94. Glüer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK. Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. *Osteoporos Int.* 1995;5(4):262-270.

95. Kanis JA, Glüer CC. An update on the diagnosis and assessment of osteoporosis with densitometry. Committee of Scientific Advisors, International Osteoporosis Foundation. *Osteoporos Int.* 2000;11(3):192-202.
96. Blake GM, Fogelman I. How important are BMD accuracy errors for the clinical interpretation of DXA scans? *J Bone Miner Res.* 2008 Apr;23(4):457-462.
97. Tothill P, Pye DW. Errors due to non-uniform distribution of fat in dual X-ray absorptiometry of the lumbar spine. *Br J Radiol.* 1992 Sep;65(777):807-813.
98. Svendsen OL, Hassager C, Skødt V, Christiansen C. Impact of soft tissue on in vivo accuracy of bone mineral measurements in the spine, hip, and forearm: a human cadaver study. *J Bone Miner Res.* 1995 Jun;10(6):868-873.
99. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper DA. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS.* 1998 May 7;12(7):F51-F58.
100. Kendler DL, Borges JL, Fielding RA, Itabashi A, Krueger D, Mulligan K, Camargos BM, Sabowitz B, Wu CH, Yu EW, Shepherd J. The Official Positions of the International Society for Clinical Densitometry: Indications of Use and Reporting of DXA for Body Composition. *J Clin Densitom.* 2013 Oct-Dec;16(4):496-507.
101. Bickel M, Eisen J, Stephan C, Crespi CM, Lutz T, Klauke S, Vogl TJ, Jacobi V, Yang OO, Staszewski S, Zangos S. A standardized, comprehensive magnetic resonance imaging protocol for rapid and precise quantification of HIV-1-associated lipodystrophy. *HIV Med.* 2007 Oct;8(7):413-419.
102. Glüer CC. Monitoring skeletal changes by radiological techniques. *J Bone Miner Res.* 1999 Nov;14(11):1952-1962.
103. Looker AC, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP, Johnston CC Jr, Lindsay R. Updated data on proximal femur bone mineral levels of US adults. *Osteoporos Int.* 1998;8(5):468-489.
104. Compston J. Guidelines for the management of osteoporosis: the present and the future. *Osteoporos Int.* 2005 Oct;16(10):1173-1176.
105. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ.* 1996 May 18;312(7041):1254-1259.
106. Compston J. Monitoring osteoporosis treatment. *Best Pract Res Clin Rheumatol.* 2009 Dec;23(6):781-788.
107. Kalender WA. Effective dose values in bone mineral measurements by photon absorptiometry and computed tomography. *Osteoporos Int.* 1992 Mar;2(2):82-87.
108. Blake GM, Naeem M, Boutros M. Comparison of effective dose to children and adults from dual X-ray absorptiometry examinations. *Bone.* 2006 Jun;38(6):935-942.
109. Genant HK, Grampp S, Glüer CC, Faulkner KG, Jergas M, Engelke K, Hagiwara S, Van Kuijk C. Universal standardization for dual x-ray absorptiometry: patient and phantom cross-calibration results. *J Bone Miner Res.* 1994 Oct;9(10):1503-1514.
110. Kalender WA, Felsenberg D, Genant HK, Fischer M, Dequeker J, Reeve J. The European Spine Phantom--a tool for standardization and quality control in spinal bone mineral measurements by DXA and QCT. *Eur J Radiol.* 1995 Jul;20(2):83-92.
111. Augat P, Fuerst T, Genant HK. Quantitative bone mineral assessment at the forearm: a review. *Osteoporos Int.* 1998;8(4):299-310.
112. Miller PD, Siris ES, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Chen YT, Berger ML, Santora AC, Sherwood LM. Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: evidence from the National Osteoporosis Risk Assessment. *J Bone Miner Res.* 2002 Dec;17(12):2222-2230.

113. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. *Lancet*. 1993 Jan 9;341(8837):72-75.
114. Patel R, Blake GM, Fogelman I. An evaluation of the United Kingdom National Osteoporosis Society position statement on the use of peripheral dual-energy X-ray absorptiometry. *Osteoporos Int*. 2004 Jun;15(6):497-504.
115. Harrison EJ, Adams JE. Application of a triage approach to peripheral bone densitometry reduces the requirement for central DXA but is not cost effective. *Calcif Tissue Int*. 2006 Oct;79(4):199-206.
116. Abbott TA 3rd, Mucha L, Manfredonia D, Schwartz EN, Berger ML. Efficient patient identification strategies for women with osteoporosis. *J Clin Densitom*. 1999 Fall;2(3):223-230.
117. Lawrenson R, Nicholls P, Rivers-Latham R, Brown T, Barnardo J, Gray R. PIXI bone density screening for osteoporosis in postmenopausal women. *Maturitas*. 2006 Feb 20;53(3):245-251.
118. Pérez-Castrillón JL, Martín-Escudero JC, del Pino-Montes J, Blanco FS, Martín FJ, Paredes MG, Fernández FP, Arés TA. Prevalence of osteoporosis using DXA bone mineral density measurements at the calcaneus: cut-off points of diagnosis and exclusion of osteoporosis. *J Clin Densitom*. 2005 Winter;8(4):404-408.
119. Picard D, Brown JP, Rosenthal L, Couturier M, Lévesque J, Dumont M, Ste-Marie LG, Tenenhouse A, Dodin S. Ability of peripheral DXA measurement to diagnose osteoporosis as assessed by central DXA measurement. *J Clin Densitom*. 2004 Spring;7(1):111-118.
120. Calmy A, Chevalley T, Delhumeau C, Toutous-Trellu L, Spycher-Elbes R, Ratib O, Zawadzinski S, Rizzoli R. Long-term HIV infection and antiretroviral therapy are associated with bone microstructure alterations in premenopausal women. *Osteoporos Int*. 2013 Jun;24(6):1843-1852.
121. Meier C, Nguyen TV, Center JR, Seibel MJ, Eisman JA. Bone resorption and osteoporotic fractures in elderly men: the dubbo osteoporosis epidemiology study. *J Bone Miner Res*. 2005 Apr;20(4):579-587.
122. Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, Kanis JA. International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. *Clin Chem Lab Med*. 2011 Aug;49(8):1271-1274.
123. Singer FR, Eyre DR. Using biochemical markers of bone turnover in clinical practice. *Cleve Clin J Med*. 2008 Oct;75(10):739-750.
124. Meier C, Seibel MJ, Kraenzlin ME. Use of bone turnover markers in the real world: are we there yet? *J Bone Miner Res*. 2009 Mar;24(3):386-388.
125. Lewiecki EM. Benefits and limitations of bone mineral density and bone turnover markers to monitor patients treated for osteoporosis. *Curr Osteoporos Rep*. 2010 Mar;8(1):15-22.
126. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Bréart G, Meunier PJ, Delmas PD. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. *J Bone Miner Res*. 1996 Oct;11(10):1531-1538.
127. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. *J Bone Miner Res*. 2000 Aug;15(8):1526-1536.
128. Johnell O, Odén A, De Laet C, Garnero P, Delmas PD, Kanis JA. Biochemical indices of bone turnover and the assessment of fracture probability. *Osteoporos Int*. 2002 Jul;13(7):523-526.
129. Stepan JJ. Prediction of bone loss in postmenopausal women. *Osteoporos Int*. 2000;11 Suppl 6:S45-S54.
130. Rogers A, Hannon RA, Eastell R. Biochemical markers as predictors of rates of bone loss after menopause. *J Bone Miner Res*. 2000 Jul;15(7):1398-1404.
131. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. *Bone*. 2002 Jul;31(1):57-61.

132. Henriksen DB, Alexandersen P, Bjarnason NH, Vilsbøll T, Hartmann B, Henriksen EE, Byrjalsen I, Krarup T, Holst JJ, Christiansen C. Role of gastrointestinal hormones in postprandial reduction of bone resorption. *J Bone Miner Res*. 2003 Dec;18(12):2180-2189.
133. Fatayerji D, Eastell R. Age-related changes in bone turnover in men. *J Bone Miner Res*. 1999 Jul;14(7):1203-1210.
134. Szulc P, Delmas PD. Biochemical markers of bone turnover in men. *Calcif Tissue Int*. 2001 Oct;69(4):229-234.
135. Szulc P, Montella A, Delmas PD. High bone turnover is associated with accelerated bone loss but not with increased fracture risk in men aged 50 and over: the prospective MINOS study. *Ann Rheum Dis*. 2008 Sep;67(9):1249-1255.
136. Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS*. 2006 Nov 14;20(17):2165-2174.
137. O'Brien KO, Razavi M, Henderson RA, Caballero B, Ellis KJ. Bone mineral content in girls perinatally infected with HIV. *Am J Clin Nutr*. 2001 Apr;73(4):821-826.
138. Arpadi SM, Horlick M, Thornton J, Cuff PA, Wang J, Kotler DP. Bone mineral content is lower in prepubertal HIV-infected children. *J Acquir Immune Defic Syndr*. 2002 Apr 15;29(5):450-454.
139. Jacobson DL, Spiegelman D, Duggan C, Weinberg GA, Bechard L, Furuta L, Nicchitta J, Gorbach SL, Miller TL. Predictors of bone mineral density in human immunodeficiency virus-1 infected children. *J Pediatr Gastroenterol Nutr*. 2005 Sep;41(3):339-346.
140. Jacobson DL, Lindsey JC, Gordon CM, Moyer J, Hardin DS, Mulligan K, Aldrovandi GM; Pediatric AIDS Clinical Trials Group P1045 team. Total body and spinal bone mineral density across Tanner stage in perinatally HIV-infected and uninfected children and youth in PACTG 1045. *AIDS*. 2010 Mar 13;24(5):687-696.
141. Mallon PW. HIV and bone mineral density. *Curr Opin Infect Dis*. 2010 Feb;23(1):1-8.
142. Tebas P, Powderly WG, Claxton S, Marin D, Tantisiriwat W, Teitelbaum SL, Yarasheski KE. Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. *AIDS*. 2000 Mar 10;14(4):F63-F67.
143. Huang JS, Wilkie SJ, Sullivan MP, Grinspoon S. Reduced bone density in androgen-deficient women with acquired immune deficiency syndrome wasting. *J Clin Endocrinol Metab*. 2001 Aug;86(8):3533-3539.
144. Knobel H, Guelar A, Vallecillo G, Nogués X, Díez A. Osteopenia in HIV-infected patients: is it the disease or is it the treatment? *AIDS*. 2001 Apr 13;15(6):807-808.
145. Loiseau-Pérès S, Delaunay C, Poupon S, Lespessailles E, Ballouche N, Arsac P, Benhamou CL. Osteopenia in patients infected by the human immunodeficiency virus. A case control study. *Joint Bone Spine*. 2002 Oct;69(5):482-485.
146. Bruera D, Luna N, David DO, Bergoglio LM, Zamudio J. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS*. 2003 Sep 5;17(13):1917-1923.
147. Teichmann J, Stephan E, Lange U, Discher T, Friese G, Lohmeyer J, Stracke H, Bretzel RG. Osteopenia in HIV-infected women prior to highly active antiretroviral therapy. *J Infect*. 2003 May;46(4):221-227.
148. Amiel C, Ostertag A, Slama L, Baudoin C, N'Guyen T, Lajeunie E, Neit-Ngeilh L, Rozenbaum W, De Vernejoul MC. BMD is reduced in HIV-infected men irrespective of treatment. *J Bone Miner Res*. 2004 Mar;19(3):402-409.
149. Brown TT, Ruppe MD, Kassner R, Kumar P, Kehoe T, Dobs AS, Timpone J. Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. *J Clin Endocrinol Metab*. 2004 Mar;89(3):1200-1206.
150. Dolan SE, Huang JS, Killilea KM, Sullivan MP, Aliabadi N, Grinspoon S. Reduced bone density in HIV-infected women. *AIDS*. 2004 Feb 20;18(3):475-483.

151. Madeddu G, Spanu A, Solinas P, Calia GM, Lovigu C, Chessa F, Mannazzu M, Falchi A, Mura MS, Madeddu G. Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. *Q J Nucl Med Mol Imaging*. 2004 Mar;48(1):39-48.
152. Yin M, Dobkin J, Brudney K, Becker C, Zadel JL, Manandhar M, Adesso V, Shane E. Bone mass and mineral metabolism in HIV+ postmenopausal women. *Osteoporos Int*. 2005 Nov;16(11):1345-1352.
153. Arnsten JH, Freeman R, Howard AA, Floris-Moore M, Santoro N, Schoenbaum EE. HIV infection and bone mineral density in middle-aged women. *Clin Infect Dis*. 2006 Apr 1;42(7):1014-1020.
154. Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Bone mineral density is not reduced in HIV-infected Caucasian men treated with highly active antiretroviral therapy. *Clin Endocrinol (Oxf)*. 2006 Aug;65(2):191-197.
155. Anastos K, Lu D, Shi O, Mulligan K, Tien PC, Freeman R, Cohen MH, Justman J, Hessol NA. The association of bone mineral density with HIV infection and antiretroviral treatment in women. *Antivir Ther*. 2007;12(7):1049-1058.
156. Arnsten JH, Freeman R, Howard AA, Floris-Moore M, Lo Y, Klein RS. Decreased bone mineral density and increased fracture risk in aging men with or at risk for HIV infection. *AIDS*. 2007 Mar 12;21(5):617-623.
157. Jones S, Restrepo D, Kasowitz A, Korenstein D, Wallenstein S, Schneider A, Keller MJ. Risk factors for decreased bone density and effects of HIV on bone in the elderly. *Osteoporos Int*. 2008 Jul;19(7):913-918.
158. Yin MT, McMahon DJ, Ferris DC, Zhang CA, Shu A, Staron R, Colon I, Laurence J, Dobkin JF, Hammer SM, Shane E. Low bone mass and high bone turnover in postmenopausal human immunodeficiency virus-infected women. *J Clin Endocrinol Metab*. 2010 Feb;95(2):620-629.
159. Yin MT, Lu D, Cremers S, Tien PC, Cohen MH, Shi Q, Shane E, Golub ET, Anastos K. Short-term bone loss in HIV-infected premenopausal women. *J Acquir Immune Defic Syndr*. 2010 Feb;53(2):202-208.
160. Short CE, Shaw SG, Fisher MJ, Walker-Bone K, Gilleece YC. Prevalence of and risk factors for osteoporosis and fracture among a male HIV-infected population in the UK. *Int J STD AIDS*. 2014 Feb;25(2):113-121.
161. Glesby MJ. Bone disorders in human immunodeficiency virus infection. *Clin Infect Dis*. 2003;37 Suppl 2:S91-S95.
162. Shiau S, Broun EC, Arpadi SM, Yin MT. Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. *AIDS*. 2013 Jul 31;27(12):1949-1957.
163. Post FA, Holt SG. Recent developments in HIV and the kidney. *Curr Opin Infect Dis*. 2009; 22:43-48.
164. Berliner AR, Fine DM, Lucas GM, Rahman MH, Racusen LC, Scheel PJ, Atta MG. Observations on a cohort of HIV-infected patients undergoing native renal biopsy. *Am J Nephrol*. 2008;28(3):478-486.
165. Wyatt CM, Morgello S, Katz-Malamed R, Wei C, Klotman ME, Klotman PE, D'Agati VD. The spectrum of kidney disease in patients with AIDS in the era of antiretroviral therapy. *Kidney Int*. 2009 Feb;75(4):428-434.
166. Campbell LJ, Ibrahim F, Fisher M, Holt SG, Hendry BM, Post FA. Spectrum of chronic kidney disease in HIV-infected patients. *HIV Med*. 2009 Jul;10(6):329-336.
167. Szczech LA, Gupta SK, Habash R, Guasch A, Kalayjian R, Appel R, Fields TA, Svetkey LP, Flanagan KH, Klotman PE, Winston JA. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney Int*. 2004 Sep;66(3):1145-1152.
168. Haas M, Kaul S, Eustace JA. HIV-associated immune complex glomerulonephritis with "lupus-like" features: a clinicopathologic study of 14 cases. *Kidney Int*. 2005 Apr;67(4):1381-1390.
169. Nielsen R, Christensen EI. Proteinuria and events beyond the slit. *Pediatr Nephrol*. 2010; 25: 813-822.

170. Bernard AM, Moreau D, Lauwerys R. Comparison of retinol-binding protein and beta 2-microglobulin determination in urine for the early detection of tubular proteinuria. *Clin Chim Acta*. 1982 Nov 24;126(1):1-7.
171. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet*. 2005 Apr 2-8;365(9466):1231-1238.
172. Smith ER, Cai MMX, McMahon LP, Wright DA, Holt SG. The value of simultaneous measurements of urinary albumin and total protein in proteinuric patients. *Nephrol Dial Transplant*. 2012; 27:1534–1541.
173. Mazzaferro S, Pasquali M, Pirrò G, Rotondi S, Tartaglione L. The bone and the kidney. *Arch Biochem Biophys*. 2010 Nov 1;503(1):95-102.
174. Bernard AM, Vyskocil AA, Mahieu P, Lauwerys RR. Assessment of urinary retinol-binding protein as an index of proximal tubular injury. *Clin Chem*. 1987 Jun;33(6):775-779.
175. Office of National Statistics Census 2001.
176. Office of National Statistics Census 2011.
177. Public Health England England HIV Prevalence by Local Authority of Residence 2013.
178. Brighton and Sussex University Hospitals website. www.bsuh.nhs.uk, last accessed 01/04/2017.
179. Fisher M Personal Communication 2010.
180. Ben Sedrine W, Broers P, Devogelaer JP, Depresseux G, Kaufman JM, Goemaere S, Reginster JY. Interest of a prescreening questionnaire to reduce the cost of bone densitometry. *Osteoporos Int*. 2002 May;13(5):434-442.
181. Ministry of Agriculture, Fisheries and Food (GB). Food portion sizes. London, UK: HMSO; 1993.
182. Ministry of Agriculture, Fisheries and Food (GB). McCance and Widdowson's The Composition of Foods. 5th ed. London, UK: HMSO; 1991.
183. Food Standards Agency. Dietary Reference Values of Food Energy and Nutrients for the United Kingdom 1991.
184. Maynard MJ, Blane D. Dietary assessment in early old age: experience from the Boyd Orr cohort. *Eur J Clin Nutr*. 2009 Feb;63 Suppl 1:S58-S63.
185. Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, Oakes S, Khaw KT, Wareham N, Day NE. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr*. 2001 Jun;4(3):847-858.
186. Emmett P, Rogers I, Symes C; ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Food and nutrient intakes of a population sample of 3-year-old children in the south west of England in 1996. *Public Health Nutr*. 2002 Feb;5(1):55-64.
187. Rogers I, Emmett P. Diet during pregnancy in a population of pregnant women in South West England. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *Eur J Clin Nutr*. 1998 Apr;52(4):246-250.
188. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancina G, Mengden T, Myers M, Padfield P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, Verdecchia P; European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens*. 2003 May;21(5):821-848.
189. International Conference on Harmonisation (ICH). ICH Harmonised Tripartite Guideline for Good Clinical Practice. 1996.
190. The World Medical Association. Declaration Of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. October 2008.

191. Department of Health, The Caldicott Committee. Report on the review of patient-identifiable information. 1 December 1997.
192. Jacobson DL, Spiegelman D, Knox TK, Wilson IB. Evolution and predictors of change in total bone mineral density over time in HIV-infected men and women in the nutrition for healthy living study. *J Acquir Immune Defic Syndr*. 2008 Nov 1;49(3):298-308.
193. Mondy K, Yarasheski K, Powderly WG, Whyte M, Claxton S, DeMarco D, Hoffmann M, Tebas P. Longitudinal evolution of bone mineral density and bone markers in human immunodeficiency virus-infected individuals. *Clin Infect Dis*. 2003 Feb 15;36(4):482-490.
194. Dolan SE, Kanter JR, Grinspoon S. Longitudinal analysis of bone density in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab*. 2006 Aug;91(8):2938-2945.
195. Pedrazzoni M, Vescovi PP, Maninetti L, Michelini M, Zaniboni G, Pioli G, Costi D, Alfano FS, Passeri M. Effects of chronic heroin abuse on bone and mineral metabolism. *Acta Endocrinol (Copenh)*. 1993 Jul;129(1):42-45.
196. Lo Re V 3rd, Guaraldi G, Leonard MB, Localio AR, Lin J, Orlando G, Zirilli L, Rochira V, Kostman JR, Tebas P. Viral hepatitis is associated with reduced bone mineral density in HIV-infected women but not men. *AIDS*. 2009 Oct 23;23(16):2191-2198.
197. Maas JJ, Dukers N, Krol A, van Ameijden EJ, van Leeuwen R, Roos MT, de Wolf F, Coutinho RA, Keet IP. Body mass index course in asymptomatic HIV-infected homosexual men and the predictive value of a decrease of body mass index for progression to AIDS. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998 Nov 1;19(3):254-259.
198. Bolland MJ, Grey AB, Gamble GD, Reid IR. Low body weight mediates the relationship between HIV infection and low bone mineral density: a meta-analysis. *J Clin Endocrinol Metab*. 2007 Dec;92(12):4522-4528.
199. Calmy A, Fux CA, Norris R, Vallier N, Delhumeau C, Samaras K, Hesse K, Hirschel B, Cooper DA, Carr A. Low bone mineral density, renal dysfunction, and fracture risk in HIV infection: a cross-sectional study. *J Infect Dis*. 2009 Dec 1;200(11):1746-1754.
200. Childs K., Welz T., Samarawickrama A., Post F.A. Effects of vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. *AIDS*. Jan 2012;26(3):253-262.
201. Sharma A, Flom PL, Weedon J, Klein RS. Prospective study of bone mineral density changes in aging men with or at risk for HIV infection. *AIDS*. 2010 Sep 24;24(15):2337-2345.
202. Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev*. 2002 Jun;23(3):279-302.
203. Lunt M, Felsenberg D, Adams J, Benevolenskaya L, Cannata J, Dequeker J, Dodenhof C, Falch JA, Johnell O, Khaw KT, Masaryk P, Pols H, Poor G, Reid D, Scheidt-Nave C, Weber K, Silman AJ, Reeve J. Population-based geographic variations in DXA bone density in Europe: the EVOS Study. *European Vertebral Osteoporosis*. *Osteoporos Int*. 1997;7(3):175-189.
204. Cauley JA, Fullman RL, Stone KL, Zmuda JM, Bauer DC, Barrett-Connor E, Ensrud K, Lau EM, Orwoll ES; Mr. OS Research Group. Factors associated with the lumbar spine and proximal femur bone mineral density in older men. *Osteoporos Int*. 2005 Dec;16(12):1525-1537.
205. Bendavid EJ, Shan J, Barrett-Connor E. Factors associated with bone mineral density in middle-aged men. *J Bone Miner Res*. 1996 Aug;11(8):1185-1190.
206. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PW, Kiel DP. Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. *J Bone Miner Res*. 2000 Apr;15(4):710-720.
207. Szulc P, Marchand F, Duboeuf F, Delmas PD. Cross-sectional assessment of age-related bone loss in men: the MINOS study. *Bone*. 2000 Feb;26(2):123-129.
208. Carr A, Miller J, Eisman JA, Cooper DA. Osteopenia in HIV-infected men: association with asymptomatic lactic acidemia and lower weight pre-antiretroviral therapy. *AIDS*. 2001 Apr 13;15(6):703-709.

209. Fausto A, Bongiovanni M, Cicconi P, Menicagli L, Ligabò EV, Melzi S, Bini T, Sardanelli F, Cornalba G, Monforte Ad. Potential predictive factors of osteoporosis in HIV-positive subjects. *Bone*. 2006 Jun;38(6):893-897.
210. Cazanave C, Dupon M, Lavignolle-Aurillac V, Barthe N, Lawson-Ayayi S, Mehsen N, Mercié P, Morlat P, Thiébaud R, Dabis F; Groupe d'Epidémiologie Clinique du SIDA en Aquitaine. Reduced bone mineral density in HIV-positive patients: prevalence and associated factors. *AIDS*. 2008 Jan 30;22(3):395-402.
211. Paul TV, Asha HS, Thomas N, Seshadri MS, Rupali P, Abraham OC, Pulimood SA, Jose A. Hypovitaminosis D and bone mineral density in human immunodeficiency virus-infected men from India, with or without antiretroviral therapy. *Endocr Pract*. 2010 Jul-Aug;16(4):547-553.
212. Bell NH, Shary J, Stevens J, Garza M, Gordon L, Edwards J. Demonstration that bone mass is greater in black than in white children. *J Bone Miner Res*. 1991 Jul;6(7):719-723.
213. Kleerekoper M, Nelson DA, Peterson EL, Flynn MJ, Pawluszka AS, Jacobsen G, Wilson P. Reference data for bone mass, calciotropic hormones, and biochemical markers of bone remodeling in older (55-75) postmenopausal white and black women. *J Bone Miner Res*. 1994 Aug;9(8):1267-1276.
214. Finkelstein JS, Lee ML, Sowers M, Ettinger B, Neer RM, Kelsey JL, Cauley JA, Huang MH, Greendale GA. Ethnic variation in bone density in premenopausal and early perimenopausal women: effects of anthropometric and lifestyle factors. *J Clin Endocrinol Metab*. 2002 Jul;87(7):3057-3067.
215. Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, da Silva BA. Loss of bone mineral density after antiretroviral therapy initiation, independent of antiretroviral regimen. *J Acquir Immune Defic Syndr*. 2009 Aug 15;51(5):554-561.
216. Fuster M, Estrada V, Fernandez-Pinilla MC, Fuentes-Ferrer ME, Tellez MJ, Vergas J, Serrano-Villar S, Fernandez-Cruz A. Smoking cessation in HIV patients: rate of success and associated factors. *HIV Med*. 2009 Nov;10(10):614-619.
217. Levine AM, Seaberg EC, Hessol NA, Preston-Martin S, Silver S, Cohen MH, Anastos K, Minkoff H, Orenstein J, Dominguez G, Watts DH. HIV as a risk factor for lung cancer in women: data from the Women's Interagency HIV Study. *J Clin Oncol*. 2010 Mar 20;28(9):1514-1519.
218. Sherwood JE, Mesner OC, Weintrob AC, Hadigan CM, Wilkins KJ, Crum-Cianflone NF, Aronson NE. Vitamin D deficiency and its association with low bone mineral density, HIV-related factors, hospitalization, and death in a predominantly black HIV-infected cohort. *Clin Infect Dis*. 2012 Dec;55(12):1727-1736.
219. Mdodo R, Frazier EL, Dube SR, Mattson CL, Sutton MY, Brooks JT, Skarbinski J. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. *Ann Intern Med*. 2015 Mar 3;162(5):335-344.
220. Hopper JL, Seeman E. The bone density of female twins discordant for tobacco use. *N Engl J Med*. 1994 Feb 10;330(6):387-392.
221. Papaioannou A, Kennedy CC, Cranney A, Hawker G, Brown JP, Kaiser SM, Leslie WD, O'Brien CJ, Sawka AM, Khan A, Siminoski K, Tarulli G, Webster D, McGowan J, Adachi JD. Risk factors for low BMD in healthy men age 50 years or older: a systematic review. *Osteoporos Int*. 2009 Apr;20(4):507-518.
222. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, Fujiwara S, Kroger H, McCloskey EV, Mellstrom D, Melton LJ, Pols H, Reeve J, Silman A, Tenenhouse A. Smoking and fracture risk: a meta-analysis. *Osteoporos Int*. 2005 Feb;16(2):155-162.
223. Laitinen K, Välimäki M. Alcohol and bone. *Calcif Tissue Int*. 1991;49 Suppl:S70-S73.
224. Kanis JA, Johansson H, Johnell O, Oden A, De Laet C, Eisman JA, Pols H, Tenenhouse A. Alcohol intake as a risk factor for fracture. *Osteoporos Int*. 2005 Jul;16(7):737-742.
225. Malmivaara A, Heliövaara M, Knekt P, Reunanen A, Aromaa A. Risk factors for injurious falls leading to hospitalization or death in a cohort of 19,500 adults. *Am J Epidemiol*. 1993 Sep 15;138(6):384-394.
226. Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *J Bone Miner Res*. 2004 Jun;19(6):1006-1012.

227. Snow-Harter C, Whalen R, Myburgh K, Arnaud S, Marcus R. Bone mineral density, muscle strength, and recreational exercise in men. *J Bone Miner Res.* 1992 Nov;7(11):1291-1296.
228. Kelley GA, Kelley KS, Tran ZV. Exercise and bone mineral density in men: a meta-analysis. *J Appl Physiol.* 1985. 2000 May;88(5):1730-1736.
229. Bonaiuti D, Shea B, Iovine R, Negrini S, Robinson V, Kemper HC, Wells G, Tugwell P, Cranney A. Exercise for preventing and treating osteoporosis in postmenopausal women. *Cochrane Database Syst Rev.* 2002;(3):CD000333.
230. Fairfield WP, Finklestein JS, Klibanski A, Grinspoon SK. Osteopenia in eugonadal men with acquired immune deficiency syndrome wasting syndrome. *J Clin Endocrinol Metab.* 2001; 86(5): 2020-2026.
231. Knoke JD, Barrett-Connor E. Weight loss: a determinant of hip bone loss in older men and women. The Rancho Bernardo Study. *Am J Epidemiol.* 2003 Dec 15;158(12):1132-1138.
232. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey EV, Mellstrom D, Melton LJ 3rd, Meunier PJ, Pols HA, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporos Int.* 2005 Nov;16(11):1330-1338.
233. Nolan D, Upton R, McKinnon E, John M, James I, Adler B, Roff G, Vasikaran S, Mallal S. Stable or increasing bone mineral density in HIV-infected patients treated with nelfinavir or indinavir. *AIDS.* 2001 Jul 6;15(10):1275-1280.
234. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007 Jul 19;357(3):266-281.
235. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011 Jul;96(7):1911-1930.
236. Turner AG, Anderson PH, Morris HA. Vitamin D and bone health. *Scand J Clin Lab Invest Suppl.* 2012;243:65-72.
237. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* 2004 Dec;80(6 Suppl):1689S-1696S.
238. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. *Am J Med.* 2004 May 1;116(9):634-639.
239. Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, Nickelsen T. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. *J Clin Endocrinol Metab.* 2001 Mar;86(3):1212-1221.
240. Ensrud KE, Taylor BC, Paudel ML, Cauley JA, Cawthon PM, Cummings SR, Fink HA, Barrett-Connor E, Zmuda JM, Shikany JM, Orwoll ES; Osteoporotic Fractures in Men Study Group. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. *J Clin Endocrinol Metab.* 2009 Aug;94(8):2773-2780.
241. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. *JAMA.* 2005 Nov 9;294(18):2336-2341.
242. Curtis JR, Smith B, Weaver M, Landers K, Lopez-Ben R, Raper JL, Saag M, Venkataraman R, Saag KG. Ethnic variations in the prevalence of metabolic bone disease among HIV-positive patients with lipodystrophy. *AIDS Res Hum Retroviruses.* 2006 Feb;22(2):125-131.
243. García Aparicio AM, Muñoz Fernández S, González J, Arribas JR, Peña JM, Vázquez JJ, Martínez ME, Coya J, Martín Mola E. Abnormalities in the bone mineral metabolism in HIV-infected patients. *Clin Rheumatol.* 2006 Jul;25(4):537-539.
244. Bang UC, Shakar SA, Hitz MF, Jespersen MS, Andersen O, Nielsen SD, Jensen JE. Deficiency of 25-hydroxyvitamin D in male HIV-positive patients: a descriptive cross-sectional study. *Scand J Infect Dis.* 2010 Apr;42(4):306-310.

245. Ramayo E, González-Moreno MP, Macías J, Cruz-Ruiz M, Mira JA, Villar-Rueda AM, García-García JA, Gómez-Mateos JM, Lozano F, Pineda JA. Relationship between osteopenia, free testosterone, and vitamin D metabolite levels in HIV-infected patients with and without highly active antiretroviral therapy. *AIDS Res Hum Retroviruses*. 2005 Nov;21(11):915-921.
246. Seminari E, Castagna A, Soldarini A, Galli L, Fusetti G, Dorigatti F, Hasson H, Danise A, Guffanti M, Lazzarin A, Rubinacci A. Osteoprotegerin and bone turnover markers in heavily pretreated HIV-infected patients. *HIV Med*. 2005 May;6(3):145-150.
247. Tomazic J, Ul K, Volcansk G, Gorenek S, Pfeifer M, Karner P, Prezelj J, Vidmar G, Vidmar L. Prevalence and risk factors for osteopenia/osteoporosis in an HIV-infected male population. *Wien Klin Wochenschr*. 2007;119(21-22):639-646.
248. Horizon AA, Joseph RJ, Liao Q, Ross ST, Pakes GE. Characteristics of foot fractures in HIV-infected patients previously treated with tenofovir versus non-tenofovir-containing highly active antiretroviral therapy. *HIV AIDS (Auckl)*. 2011;3:53-59.
249. Van Vonderen MG, Lips P, van Agtmael MA, Hassink EA, Brinkman K, Geerlings SE, Sutinen J, Ristola M, Danner SA, Reiss P. First line zidovudine/lamivudine/lopinavir/ritonavir leads to greater bone loss compared to nevirapine/lopinavir/ritonavir. *AIDS*. 2009 Jul 17;23(11):1367-1376.
250. Martin A, Bloch M, Amin J, Baker D, Cooper DA, Emery S, Carr A; STEAL Study Group. Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-lamivudine: a randomized, 96-week trial. *Clin Infect Dis* 2009; 49:1591–1601.
251. Stellbrink HJ, Orkin C, Arribas JR, Compston J, Gerstoft J, Van Wijngaerden E, Lazzarin A, Rizzardini G, Sprenger HG, Lambert J, Sture G, Leather D, Hughes S, Zucchi P, Pearce H; ASSERT Study Group. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-positive adults: 48-week results from the ASSERT study. *Clin Infect Dis*. 2010 Oct 15;51(8):963-972.
252. McComsey GA, Kitch D, Daar ES, Tierney C, Jahed NC, Tebas P, Myers L, Melbourne K, Ha B, Sax PE. Bone mineral density and fractures in antiretroviral-naïve persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with EFV or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. *J Infect Dis*. 2011 Jun 15;203(12):1791-1801.
253. Lodder MC, de Jong Z, Kostense PJ, Molenaar ET, Staal K, Voskuyl AE, Hazes JM, Dijkmans BA, Lems WF. Bone mineral density in patients with rheumatoid arthritis: relation between disease severity and low bone mineral density. *Ann Rheum Dis*. 2004 Dec;63(12):1576-1580.
254. Cooper MS. Thyroid gland: Variation in 'normal' thyroid function--effect on bone health? *Nat Rev Endocrinol*. 2010 Nov;6(11):599-600.
255. Ninkovic M, Love SA, Tom B, Alexander GJ, Compston JE. High prevalence of osteoporosis in patients with chronic liver disease prior to liver transplantation. *Calcif Tissue Int*. 2001 Dec;69(6):321-326.
256. Turner J, Bansi L, Gilson R, Gazzard B, Walsh J, Pillay D, Orkin C, Phillips A, Easterbrook P, Johnson M, Porter K, Schwenk A, Hill T, Leen C, Anderson J, Fisher M, Sabin C; UK Collaborative HIV Cohort (UK CHIC) Study. The prevalence of hepatitis C virus (HCV) infection in HIV-positive individuals in the UK - trends in HCV testing and the impact of HCV on HIV treatment outcomes. *J Viral Hepat*. 2010 Aug;17(8):569-577.
257. Price H, Bansi L, Sabin CA, Bhagani S, Burroughs A, Chadwick D, Dunn D, Fisher M, Main J, Nelson M, Pillay D, Rodger A, Taylor C, Gilson R; UK Collaborative HIV Cohort Hepatitis Group, Steering Committee. Hepatitis B virus infection in HIV-positive individuals in the UK collaborative HIV cohort (UK CHIC) study. *PLoS One*. 2012;7(11):e49314.
258. Orwoll ES. Androgens: basic biology and clinical implication. *Calcif Tissue Int*. 2001 Oct;69(4):185-188.
259. Khosla S, Melton LJ 3rd, Riggs BL. Estrogen and the male skeleton. *J Clin Endocrinol Metab*. 2002 Apr;87(4):1443-1450.
260. Fink HA, Ewing SK, Ensrud KE, Barrett-Connor E, Taylor BC, Cauley JA, Orwoll ES. Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. *J Clin Endocrinol Metab*. 2006 Oct;91(10):3908-3915.

261. Kalyani RR, Gavini S, Dobs AS. Male hypogonadism in systemic disease. *Endocrinol Metab Clin North Am.* 2007 Jun;36(2):333-348.
262. Bouillon R, Bex M, Van Herck E, Laureys J, Doms L, Lesaffre E, Ravussin E. Influence of age, sex, and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. *J Clin Endocrinol Metab.* 1995 Apr;80(4):1194-1202.
263. Inzerillo AM, Epstein S. Osteoporosis and diabetes mellitus. *Rev Endocr Metab Disord.* 2004 Aug;5(3):261-268.
264. Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res.* 2007 Sep;22(9):1317-1328.
265. Young B, Dao CN, Buchacz K, Baker R, Brooks JT; HIV Outpatient Study (HOPS) Investigators. Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000-2006. *Clin Infect Dis.* 2011 Apr 15;52(8):1061-1068.
266. Bedimo R, Maalouf NM, Zhang S, Drechsler H, Tebas P. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. *AIDS.* 2012 Apr 24;26(7):825-831.
267. Cunningham J, Sprague SM, Cannata-Andia J, Coco M, Cohen-Solal M, Fitzpatrick L, Goltzmann D, Lafage-Proust MH, Leonard M, Ott S, Rodriguez M, Stehman-Breen C, Stern P, Weisinger J; Osteoporosis Work Group. Osteoporosis in chronic kidney disease. *Am J Kidney Dis.* 2004 Mar;43(3):566-571.
268. Pelletier S, Chapurlat R. Optimizing bone health in chronic kidney disease. *Maturitas.* 2010 Apr;65(4):325-333.
269. Miller PD. Diagnosis and treatment of osteoporosis in chronic renal disease. *Semin Nephrol.* 2009 Mar;29(2):144-155.
270. Campbell LJ, Dew T, Salota R, Cheserem E, Hamzah L, Ibrahim F, Sarafidis PA, Moniz CF, Hendry BM, Poulton M, Sherwood RA, Post FA. Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients. *BMC Nephrol* 2012, 13:85.
271. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, Rivas P, Albalater M, Blanco F, Moreno V, Vispo E, Soriano V. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS* 2009, 23:689–696.
272. Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. *Osteoporos Int.* 2007 Oct;18(10):1319-1328.
273. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton III LJ, Tenenhouse A, Reeve J, Silman AJ, Pols HA, Eisman JA, McCloskey EV, Mellstrom D. A meta-analysis of prior corticosteroid use and fracture risk. *J Bone Miner Res.* 2004 Jun;19(6):893-899.
274. Nelson M, Dockrell D, Edwards S; BHIVA Guidelines Subcommittee, Angus B, Barton S, Beeching N, Bergin C, Boffito M, Breen R, Cartledge J, Clarke S, Fisher M, Freedman A, Gazzard B, Grant A, Greig J, Jones R, Khoo S, Leen C, Lipman M, Manji H, Miller R, Mitchell S, Ong E, Pozniak A, Schmid M, Shiew M, Singer M, Wilkins E, Williams I, Wood C, Weston R. British HIV Association and British Infection Association guidelines for the treatment of opportunistic infection in HIV-seropositive individuals 2011. *HIV Med.* 2011 Sep;12 Suppl 2:1-140.
275. Kinjo M, Setoguchi S, Schneeweiss S, Solomon DH. Bone mineral density in subjects using central nervous system-active medications. *Am J Med.* 2005 Dec; 118(12):1414.
276. Kim TW, Alford DP, Malabanan A, Holick MF, Samet JH. Low bone density in patients receiving methadone maintenance treatment. *Drug Alcohol Depend.* 2006 Dec 1; 85(3):258-262.
277. Reece AS. Chronic toxicology of cannabis. *Clin Toxicol (Phila).* 2009 Jul;47(6):517-524.
278. Alsina M, Guise TA, Roodman GD. Cytokine regulation of bone cell differentiation. *Vitam Horm.* 1996;52:63-98.
279. Wang L, Mondal D, La Russa VF, Agrawal KC. Suppression of clonogenic potential of human bone marrow mesenchymal stem cells by HIV type 1: putative role of HIV type 1 tat protein and inflammatory cytokines. *AIDS Res Hum Retroviruses.* 2002 Sep 1;18(13):917-931.

280. Cotter EJ, Mallon PW, Doran PP. Is PPAR γ a prospective player in HIV-1-associated bone disease? *PPAR Res.* 2009;2009:421376.
281. Beaupere C, Garcia M, Larghero J, Fève B, Capeau J, Lagathu C. The HIV proteins Tat and Nef promote human bone marrow mesenchymal stem cell senescence and alter osteoblastic differentiation. *Aging Cell.* 2015 Aug;14(4):534-546.
282. Cotter EJ, Malizia AP, Chew N, Powderly WG, Doran PP. HIV proteins regulate bone marker secretion and transcription factor activity in cultured human osteoblasts with consequent potential implications for osteoblast function and development. *AIDS Res Hum Retroviruses.* 2007 Dec;23(12):1521-1530.
283. Gibellini D, De Crignis E, Ponti C, Cimatti L, Borderi M, Tschon M, Giardino R, Re MC. HIV-1 triggers apoptosis in primary osteoblasts and HOBIT cells through TNF α activation. *J Med Virol.* 2008 Sep;80(9):1507-1514.
284. Cotter EJ, Ip HS, Powderly WG, Doran PP. Mechanism of HIV protein induced modulation of mesenchymal stem cell osteogenic differentiation. *BMC Musculoskelet Disord.* 2008 Mar 13;9:33.
285. Gruber MF, Weih KA, Boone EJ, Smith PD, Clouse KA. Endogenous macrophage CSF production is associated with viral replication in HIV-1-infected human monocyte-derived macrophages. *J Immunol.* 1995 May 15;154(10):5528-5535.
286. Fakruddin JM, Laurence J. HIV envelope gp120-mediated regulation of osteoclastogenesis via receptor activator of nuclear factor kappa B ligand (RANKL) secretion and its modulation by certain HIV protease inhibitors through interferon-gamma/RANKL cross-talk. *J Biol Chem.* 2003 Nov 28;278(48):48251-48258.
287. Fakruddin JM, Laurence J. HIV-1 Vpr enhances production of receptor of activated NF-kappaB ligand (RANKL) via potentiation of glucocorticoid receptor activity. *Arch Virol.* 2005 Jan;150(1):67-78.
288. Chew N, Tan E, Li L, Lim R. HIV-1 tat and rev upregulates osteoclast bone resorption. *J Int AIDS Soc.* 2014 Nov 2;17(4 Suppl 3):19724.
289. Titanji K, Vunnavu A, Sheth AN, Delille C, Lennox JL, Sanford SE, Foster A, Knezevic A, Easley KA, Weitzmann MN, Ofotokun I. Dysregulated B cell expression of RANKL and OPG correlates with loss of bone mineral density in HIV infection. *PLoS Pathog.* 2014 Nov 13;10(10):e1004497.
290. Hileman CO, Labbato DE, Storer NJ, Tangpricha V, McComsey GA. Is bone loss linked to chronic inflammation in antiretroviral-naïve HIV-infected adults? A 48-week matched cohort study. *AIDS.* 2014 Jul 31;28(12):1759-1767.
291. Kelley CF, Barbour JD, Hecht FM. The relation between symptoms, viral load, and viral load set point in primary HIV infection. *J Acquir Immune Defic Syndr.* 2007 Aug 1;45(4):445-448.
292. Aukrust P, Haug CJ, Ueland T, Lien E, Müller F, Espevik T, Bollerslev J, Frøland SS. Decreased bone formative and enhanced resorptive markers in human immunodeficiency virus infection: indication of normalization of the bone-remodeling process during highly active antiretroviral therapy. *J Clin Endocrinol Metab.* 1999 Jan;84(1):145-150.
293. Grijzen ML, Vrouwenraets SM, Steingrover R, Lips P, Reiss P, Wit FW, Prins JM. High prevalence of reduced bone mineral density in primary HIV-1-infected men. *AIDS.* 2010 Sep 10;24(14):2233-2238.
294. Liu AY, Vittinghoff E, Sellmeyer DE, Irvin R, Mulligan K, Mayer K, Thompson M, Grant R, Pathak S, O'Hara B, Gvetadze R, Chillag K, Grohskopf L, Buchbinder SP. Bone mineral density in HIV-negative men participating in a tenofovir pre-exposure prophylaxis randomized clinical trial in San Francisco. *PLoS One.* 2011;6(8):e23688.
295. Dave JA, Cohen K, Micklesfield LK, Maartens G, Levitt NS. Antiretroviral therapy, especially EFV, is associated with low bone mineral density in HIV-infected South Africans. *PLoS One.* 2015 Dec 3;10(12):e0144286.
296. Fernández-Rivera J, García R, Lozano F, Macías J, García-García JA, Mira JA, Corzo JE, Gómez-Mateos J, Rueda A, Sánchez-Burson J, Pineda JA. Relationship between low bone mineral density and highly active antiretroviral therapy including protease inhibitors in HIV-infected patients. *HIV Clin Trials.* 2003 Sep-Oct;4(5):337-346.

297. Vescini F, Borderi M, Buffa A, Sinicropi G, Tampellini L, Chiodo F, Caudarella R. Bone mass in HIV-infected patients: focus on the role of therapy and sex. *J Acquir Immune Defic Syndr*. 2003 Jul 1;33(3):405-407.
298. Landonio S, Quirino T, Bonfanti P, Gabris A, Boccassini L, Gulisano C, Vulpio L, Ricci E, Carrabba M, Vigevani GM. Osteopenia and osteoporosis in HIV+ patients, untreated or receiving HAART. *Biomed Pharmacother*. 2004 Nov;58(9):505-508.
299. Bongiovanni M, Fausto A, Cicconi P, Aliprandi A, Cornalba G, Bini T, Sardanelli F, D'Arminio Monforte A. Non-nucleoside-reverse-transcriptase-inhibitor-based HAART and osteoporosis in HIV-infected subjects. *J Antimicrob Chemother*. 2006 Aug;58(2):485-486.
300. Moore AL, Vashisht A, Sabin CA, Mocroft A, Madge S, Phillips AN, Studd JW, Johnson MA. Reduced bone mineral density in HIV-positive individuals. *AIDS*. 2001 Sep 7;15(13):1731-1733.
301. Rivas P, Górgolas M, García-Delgado R, Díaz-Curiel M, Goyenechea A, Fernández-Guerrero ML. Evolution of bone mineral density in AIDS patients on treatment with zidovudine/lamivudine plus abacavir or lopinavir/ritonavir. *HIV Med*. 2008 Feb;9(2):89-95.
302. Duvivier C, Kolta S, Assoumou L, Ghosn J, Rozenberg S, Murphy RL, Katlama C, Costagliola D; ANRS 121 Hippocampe study group. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naïve patients. *AIDS*. 2009 Apr 27;23(7):817-824.
303. Bonjoch A, Figueras M, Estany C, Perez-Alvarez N, Rosales J, del Rio L, di Gregorio S, Puig J, Gómez G, Clotet B, Negredo E; Osteoporosis Study Group. High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study. *AIDS*. 2010 Nov 27;24(18):2827-2833.
304. Yin MT, Zhang CA, McMahon DJ, Ferris DC, Irani D, Colon I, Cremers S, Shane E. Higher rates of bone loss in postmenopausal HIV-infected women: a longitudinal study. *J Clin Endocrinol Metab*. 2012 Feb;97(2):554-562.
305. National Osteoporosis Society: Exercise and Osteoporosis. Last reviewed November 2014 NOS/00108.
306. Peters BS, Perry M, Wierzbicki AS, Wolber LE, Blake GM, Patel N, Hoile R, Duncan A, Kulasegaram R, Williams FM. A cross-sectional randomised study of fracture risk in people with HIV infection in the probono 1 study. *PLoS One*. 2013 Oct 29;8(10):e78048.
307. Liu G, Peacock M, Eilam O, Dorulla G, Braunstein E, Johnston CC. Effect of osteoarthritis in the lumbar spine and hip on bone mineral density and diagnosis of osteoporosis in elderly men and women. *Osteoporos Int*. 1997;7(6):564-569.
308. Chapurlat RD, Delmas PD. Bone microdamage: a clinical perspective. *Osteoporos Int* 2009 Aug;20(8):1299-1308.
309. Liberman UA, Weiss SR, Bröll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. *N Engl J Med*. 1995 Nov 30;333(22):1437-1443.
310. Casado JL, Bañon S, Andrés R, Perez-Elías MJ, Moreno A, Moreno S. Prevalence of causes of secondary osteoporosis and contribution to lower bone mineral density in HIV-infected patients. *Osteoporos Int*. 2014 Mar;25(3):1071-1079.
311. Reisner SL, Mimiaga MJ, Bland S, Skeer M, Cranston K, Isenberg D, Driscoll M, Mayer KH. Problematic alcohol use and HIV risk among Black men who have sex with men in Massachusetts. *AIDS Care*. 2010 May;22(5):577-587.
312. Wosje KS, Kalkwarf HJ. Bone density in relation to alcohol intake among men and women in the United States. *Osteoporos Int*. 2007 Mar;18(3):391-400.
313. Berg KM, Kunins HV, Jackson JL, Nahvi S, Chaudhry A, Harris KA Jr, Malik R, Arnsten JH. Association between alcohol consumption and both osteoporotic fracture and bone density. *Am J Med*. 2008 May;121(5):406-418.

314. Kim EY, Kwon do H, Lee BD, Kim YT, Ahn YB, Yoon KY, Sa SJ, Cho W, Cho SN. Frequency of osteoporosis in 46 men with methamphetamine abuse hospitalized in a National Hospital. *Forensic Sci Int*. 2009 Jul 1;188(1-3):75-80.
315. Daskalopoulou M, Rodger A, Phillips AN, Sherr L, Speakman A, Collins S, Elford J, Johnson MA, Gilson R, Fisher M, Wilkins E, Anderson J, McDonnell J, Edwards S, Perry N, O'Connell R, Lascar M, Jones M, Johnson AM, Hart G, Miners A, Geretti AM, Burman WJ, Lampe FC. Recreational drug use, polydrug use, and sexual behaviour in HIV-diagnosed men who have sex with men in the UK: results from the cross-sectional ASTRA study. *Lancet HIV*. 2014 Oct;1(1):e22-e31.
316. Santos WR, Santos WR, Paes PP, Ferreira-Silva IA, Santos AP, Vercese N, Machado DR, de Paula FJ, Donadi EA, Navarro AM, Fernandes AP. Impact of Strength Training on Bone Mineral Density in Patients Infected With HIV Exhibiting Lipodystrophy. *J Strength Cond Res*. 2015 Dec;29(12):3466-3471.
317. Frost HM. Defining osteopenias and osteoporoses: another view (with insights from a new paradigm). *Bone*. 1997 May;20(5):385-391.
318. Turner CH, Robling AG. Mechanisms by which exercise improves bone strength. *J Bone Miner Metab*. 2005;23 Suppl:16-22.
319. Whedon GD. Disuse osteoporosis: physiological aspects. *Calcif Tissue Int*. 1984;36 Suppl 1:S146-S150.
320. Shea B, Wells G, Cranney A, Zytaruk N, Robinson V, Griffith L, Ortiz Z, Peterson J, Adachi J, Tugwell P, Guyatt G; Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocr Rev*. 2002 Aug;23(4):552-559.
321. Haskelberg H, Hoy JF, Amin J, Ebeling PR, Emery S, Carr A, STEAL Study Group. Changes in bone turnover and bone loss in HIV-positive patients changing treatment to tenofovir-emtricitabine or abacavir-lamivudine. *PLoS One*. 2012;7(6):e38377.
322. Assoumou L, Katlama C, Viard JP, Bentata M, Simon A, Roux C, Kolta S, Costagliola D, Rozenberg S; ANRS Osteovir study group. Changes in bone mineral density over a 2-year period in HIV-1-infected men under combined antiretroviral therapy with osteopenia. *AIDS*. 2013 Sep 24;27(15):2425-2430.
323. Cotter AG, Sabin CA, Simelane S, Macken A, Kavanagh E, Brady JJ, McCarthy G, Compston J, Mallon PW; HIV UPBEAT Study Group. Relative contribution of HIV infection, demographics and body mass index to bone mineral density. *AIDS*. 2014 Sep 10;28(14):2051-2060.
324. Mallon PW. Aging with HIV: osteoporosis and fractures. *Curr Opin HIV AIDS*. 2014 Jul;9(4):428-435.
325. Mussolino ME, Looker AC, Orwoll ES. Jogging and bone mineral density in men: results from NHANES III. *Am J Public Health*. 2001 Jul;91(7):1056-1059.
326. Smith BA, Neidig JL, Nickel JT, Mitchell GL, Para MF, Fass RJ. Aerobic exercise: effects on parameters related to fatigue, dyspnea, weight and body composition in HIV-positive adults. *AIDS*. 2001 Apr 13;15(6):693-701.
327. Carr A, Grund B, Neuhaus J, Schwartz A, Bernardino JI, White D, Badel-Faesens S, Avihingsanon A, Ensrud K, Hoy J; International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) START Study Group. Prevalence of and risk factors for low bone mineral density in untreated HIV infection: a substudy of the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. *HIV Med*. 2015 Apr;16 Suppl 1:137-146.
328. Mallon PW, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS*. 2003 May 2;17(7):971-979.
329. Cassetti I, Madruga JV, Suleiman JM, Etzel A, Zhong L, Cheng AK, Enejosa J; Study 903E Team. The safety and efficacy of tenofovir DF in combination with lamivudine and EFV through 6 years in antiretroviral-naïve HIV-1-infected patients. *HIV Clin Trials*. 2007 May-Jun;8(3):164-172.
330. Huang JS, Hughes MD, Riddler SA, Haubrich RH; Aids Clinical Trials Group A5142 Study Team. Bone mineral density effects of randomized regimen and nucleoside reverse transcriptase inhibitor selection from ACTG A5142. *HIV Clin Trials*. 2013 Sep-Oct;14(5):224-234.

331. Bernardino JI, Mocroft A, Mallon PW, Wallet C, Gerstoft J, Russell C, Reiss P, Katlama C, De Wit S, Richert L, Babiker A, Buño A, Castagna A, Girard PM, Chene G, Raffi F, Arribas JR; NEAT001/ANRS143 Study Group. Bone mineral density and inflammatory and bone biomarkers after darunavir-ritonavir combined with either raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults with HIV-1: a substudy of the NEAT001/ANRS143 randomised trial. *Lancet HIV*. 2015 Nov;2(11):e464-e473.
332. Brown TT, Moser C, Currier JS, Ribaldo HJ, Rothenberg J, Kelesidis T, Yang O, Dubé MP, Murphy RL, Stein JH, McComsey GA. Changes in Bone Mineral Density After Initiation of Antiretroviral Treatment With Tenofovir Disoproxil Fumarate/Emtricitabine Plus Atazanavir/Ritonavir, Darunavir/Ritonavir, or Raltegravir. *J Infect Dis*. 2015 Oct 15;212(8):1241-1249.
333. Reynes J, Trinh R, Pulido F, Soto-Malave R, Gathe J, Qaqish R, Tian M, Fredrick L, Podsadecki T, Norton M, Nilius A. Lopinavir/ritonavir combined with raltegravir or tenofovir/emtricitabine in antiretroviral-naive subjects: 96-week results of the PROGRESS study. *AIDS Res Hum Retroviruses*. 2013 Feb;29(2):256-265.
334. Bedimo RJ, Drechsler H, Jain M, Cutrell J, Zhang S, Li X, Farukhi I, Castanon R, Tebas P, Maalouf NM. The RADAR study: week 48 safety and efficacy of RALtegravir combined with boosted DARunavir compared to tenofovir/emtricitabine combined with boosted darunavir in antiretroviral-naive patients. Impact on bone health. *PLoS One*. 2014 Aug 29;9(8):e106221.
335. Taiwo BO, Chan ES, Fichtenbaum CJ, Ribaldo H, Tsibris A, Klingman KL, Eron JJ, Berzins B, Robertson K, Landay A, Ofotokun I, Brown T AIDS Clinical Trials Group A5303 Study Team. Less Bone Loss With Maraviroc- Versus Tenofovir-Containing Antiretroviral Therapy in the AIDS Clinical Trials Group A5303 Study. *Clin Infect Dis*. 2015 Oct 1;61(7):1179-1188.
336. Young L, Wohl DA, Hyslop WB, Lee YZ, Napravnik S, Wilkin A. Effects of raltegravir combined with tenofovir/emtricitabine on body shape, bone density, and lipids in African-Americans initiating HIV therapy. *HIV Clin Trials*. 2015 Oct;16(5):163-169.
337. Bolland MJ, Wang TK, Grey A, Gamble GD, Reid IR. Stable bone density in HAART-treated individuals with HIV: a meta-analysis. *J Clin Endocrinol Metab*. 2011 Sep;96(9):2721-2731.
338. Mulligan K, Glidden DV, Anderson PL, Liu A, McMahan V, Gonzales P, Ramirez-Cardich ME, Namwongprom S, Chodacki P, de Mendonca LM, Wang F, Lama JR, Chariyalertsak S, Guanira JV, Buchbinder S, Bekker LG, Schechter M, Veloso VG, Grant RM; Preexposure Prophylaxis Initiative Study Team. Effects of Emtricitabine/Tenofovir on Bone Mineral Density in HIV-Negative Persons in a Randomized, Double-Blind, Placebo-Controlled Trial. *Clin Infect Dis*. 2015 Aug 15;61(4):572-580.
339. Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Bone mineral density remains stable in HAART-treated HIV-infected men over 2 years. *Clin Endocrinol (Oxf)*. 2007 Aug;67(2):270-275.
340. Bolland MJ, Grey A, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Gamble GD, Reid IR. Stable bone mineral density over 6 years in HIV-infected men treated with highly active antiretroviral therapy (HAART). *Clin Endocrinol (Oxf)*. 2012 May;76(5):643-648.
341. Dubé MP, Qian D, Edmondson-Melançon H, Sattler FR, Goodwin D, Martinez C, Williams V, Johnson D, Buchanan TA. Prospective, intensive study of metabolic changes associated with 48 weeks of aprenavir-based antiretroviral therapy. *Clin Infect Dis*. 2002 Aug 15;35(4):475-481.
342. McComsey GA, Lo Re V 3rd, O'Riordan M, Walker UA, Lebrecht D, Baron E, Mounzer K, Frank I. Effect of reducing the dose of stavudine on body composition, bone density, and markers of mitochondrial toxicity in HIV-infected subjects: a randomized, controlled study. *Clin Infect Dis*. 2008 Apr 15;46(8):1290-1296.
343. Curran A, Martinez E, Podzamczer D, Lonca M, Barragan P, Crespo M, Falco V, Vidal-Sicart S, Imaz A, Martinez M, Gatell JM, Ribera E. Changes in body composition and mitochondrial DNA in HIV-1-infected patients switching to fixed-dose abacavir/lamivudine or tenofovir/emtricitabine: a substudy of the BICOMBO trial. *Antivir Ther*. 2012;17(4):711-718.
344. Cotter AG, Vrouenraets SM, Brady JJ, Wit FW, Fux CA, Furrer H, Brinkman K, Sabin CA, Reiss P, Mallon PW; PREPARE (Preventing Progression of Adipose Tissue Redistribution) Investigators. Impact of switching from zidovudine to tenofovir disoproxil fumarate on bone mineral density and markers of bone metabolism in virologically suppressed HIV-1 infected patients; a substudy of the PREPARE study. *J Clin Endocrinol Metab*. 2013 Apr;98(4):1659-1666.

345. Martin A, Moore C, Mallon PW, Hoy J, Emery S, Belloso W, Phanuphak P, Ferret S, Cooper DA, Boyd MA; Second Line study team. Bone mineral density in HIV participants randomized to raltegravir and lopinavir/ritonavir compared with standard second line therapy. *AIDS*. 2013 Sep 24;27(15):2403-2411.
346. Bianco C, Rossetti B, Gagliardini R, Lamonica S, Fanti L, Lombardi F, Cauda R, Di Giambenedetto S, De Luca A. Bone mineral density improvement after 48 weeks of switch to maraviroc+darunavir/ritonavir 300/800/100 mg QD, preliminary results of GUSTA study. *J Int AIDS Soc*. 2014 Nov 2;17(4 Suppl 3):19816.
347. Bloch M, Tong WW, Hoy J, Baker D, Lee FJ, Richardson R, Carr A; TROP (Switch from Tenofovir to Raltegravir for Low Bone Density) study team. Switch from tenofovir to raltegravir increases low bone mineral density and decreases markers of bone turnover over 48 weeks. *HIV Med*. 2014 Jul;15(6):373-380.
348. Negredo E, Domingo P, Pérez-Álvarez N, Gutiérrez M, Mateo G, Puig J, Escrig R, Echeverría P, Bonjoch A, Clotet B. Improvement in bone mineral density after switching from tenofovir to abacavir in HIV-1-infected patients with low bone mineral density: two-centre randomized pilot study (OsteoTDF study). *J Antimicrob Chemother*. 2014 Dec;69(12):3368-3371.
349. Calza L, Magistrelli E, Colangeli V, Borderi M, Conti M, Mancini R, Viale P. Improvement in renal function and bone mineral density after a switch from tenofovir/emtricitabine plus ritonavir-boosted protease inhibitor to raltegravir plus nevirapine: a pilot study. *Antivir Ther*. 2016;21(3):217-224.
350. Hamzah L, Tiraboschi JM, Iveson H, Toby M, Mant C, Cason J, Burling K, Wandolo E, Jendrulek I, Taylor C, Ibrahim F, Kulasegaram R, Teague A, Post FA, Fox J. Effects on vitamin D, bone and the kidney of switching from fixed-dose tenofovir disoproxil fumarate/emtricitabine/EFV to darunavir/ritonavir monotherapy: a randomized, controlled trial (MIDAS). *Antivir Ther*. 2016;21(4):287-296.
351. Shepherd JA, Fan B, Lu Y, Lewiecki EM, Miller P, Genant HK. Comparison of BMD precision for Prodigy and Delphi spine and femur scans. *Osteoporos Int*. 2006;17(9):1303-1308.
352. Ravaud P, Reny JL, Giraudeau B, Porcher R, Dougados M, Roux C. Individual smallest detectable difference in bone mineral density measurements. *J Bone Miner Res*. 1999 Aug;14(8):1449-1456.
353. Kolta S, Ravaud P, Fechtenbaum J, Dougados M, Roux C. Follow-up of individual patients on two DXA scanners of the same manufacturer. *Osteoporos Int*. 2000;11(8):709-713.
354. Walters J, Koo WW, Bush A, Hammami M. Effect of hand dominance on bone mass measurement in sedentary individuals. *J Clin Densitom*. 1998 Winter;1(4):359-367.
355. Rao AD, Reddy S, Rao DS. Is there a difference between right and left femoral bone density? *J Clin Densitom*. 2000 Spring;3(1):57-61.
356. Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Melton LJ 3rd. Differential changes in bone mineral density of the appendicular and axial skeleton with aging: relationship to spinal osteoporosis. *J Clin Invest*. 1981 Feb;67(2):328-335.
357. Mazess RB, Barden HS, Drinka PJ, Bauwens SF, Orwoll ES, Bell NH. Influence of age and body weight on spine and femur bone mineral density in U.S. white men. *J Bone Miner Res*. 1990 Jun;5(6):645-652.
358. Hannan MT, Felson DT, Anderson JJ. Bone mineral density in elderly men and women: results from the Framingham osteoporosis study. *J Bone Miner Res*. 1992 May;7(5):547-553.
359. Orwoll ES, Oviatt SK, McClung MR, Deftos LJ, Sexton G. The rate of bone mineral loss in normal men and the effects of calcium and cholecalciferol supplementation. *Ann Intern Med*. 1990 Jan 1;112(1):29-34.
360. Davis JW, Ross PD, Vogel JM, Wasnich RD. Age-related changes in bone mass among Japanese-American men. *Bone Miner*. 1991 Dec;15(3):227-236.
361. Slemenda CW, Christian JC, Reed T, Reister TK, Williams CJ, Johnston CC Jr. Long-term bone loss in men: effects of genetic and environmental factors. *Ann Intern Med*. 1992 Aug 15;117(4):286-291.

362. Grant PM, Kitch D, McComsey GA, Dube MP, Haubrich R, Huang J, Riddler S, Tebas P, Zolopa AR, Collier AC, Brown TT. Low baseline CD4+ count is associated with greater bone mineral density loss after antiretroviral therapy initiation. *Clin Infect Dis*. 2013 Nov;57(10):1483-1488.
363. Kasonde M, Niska RW, Rose C, Henderson FL, Segolodi TM, Turner K, Smith DK, Thigpen MC, Paxton LA. Bone mineral density changes among HIV-uninfected young adults in a randomised trial of pre-exposure prophylaxis with tenofovir-emtricitabine or placebo in Botswana. *PLoS One*. 2014 Mar 13;9(3):e90111.
364. Haskelberg H, Mallon PW, Hoy J, Amin J, Moore C, Phanuphak P, Ferret S, Belloso WH, Boyd MA, Cooper DA, Emery S. Bone mineral density over 96 weeks in adults failing first-line therapy randomized to raltegravir/lopinavir/ritonavir compared with standard second-line therapy. *J Acquir Immune Defic Syndr*. 2014 Oct 1;67(2):161-168.
365. Orwoll ES, Bevan L, Phipps KR. Determinants of bone mineral density in older men. *Osteoporos Int*. 2000;11(10):815-821.
366. Kung AW, Ho AY, Ross PD, Reginster JY. Development of a clinical assessment tool in identifying Asian men with low bone mineral density and comparison of its usefulness to quantitative bone ultrasound. *Osteoporos Int*. 2005 Jul;16(7):849-855.
367. Lau EM, Leung PC, Kwok T, Woo J, Lynn H, Orwoll E, Cummings S, Cauley J. The determinants of bone mineral density in Chinese men--results from Mr. Os (Hong Kong), the first cohort study on osteoporosis in Asian men. *Osteoporos Int*. 2006 Feb;17(2):297-303.
368. Naves M, Díaz-López JB, Gómez C, Rodríguez-Rebollar A, Serrano-Arias M, Cannata-Andía JB. Prevalence of osteoporosis in men and determinants of changes in bone mass in a non-selected Spanish population. *Osteoporos Int*. 2005 Jun;16(6):603-609.
369. Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, Eisman J, Fujiwara S, Garnero P, Kroger H, McCloskey EV, Mellstrom D, Melton LJ, Pols H, Reeve J, Silman A, Tenenhouse A. A meta-analysis of previous fracture and subsequent fracture risk. *Bone*. 2004 Aug;35(2):375-382.
370. Yin MT, Shi Q, Hoover DR, Anastos K, Sharma A, Young M, Levine A, Cohen MH, Shane E, Golub ET, Tien PC. Fracture incidence in HIV-infected women: results from the Women's Interagency HIV Study. *AIDS*. 2010 Nov 13;24(17):2679-2686.
371. Sharma A, Shi Q, Hoover DR, Anastos K, Tien PC, Young MA, Cohen MH, Golub ET, Gustafson D, Yin MT. Increased fracture incidence in middle-aged HIV-infected and HIV-uninfected women: updated results from the Women's Interagency HIV Study. *J Acquir Immune Defic Syndr*. 2015 Sep 1;70(1):54-61.
372. Kanis JA, Oden A, Johnell O, Jonsson B, de Laet C, Dawson A. The burden of osteoporotic fractures: a method for setting intervention thresholds. *Osteoporos Int*. 2001;12(5):417-427.
373. Looker AC, Beck TJ. Maternal history of osteoporosis and femur geometry. *Calcif Tissue Int*. 2004 Oct;75(4):277-285.
374. Lunt M, Masaryk P, Scheidt-Nave C, Nijs J, Poor G, Pols H, Falch JA, Hammermeister G, Reid DM, Benevolenskaya L, Weber K, Cannata J, O'Neill TW, Felsenberg D, Silman AJ, Reeve J. The effects of lifestyle, dietary dairy intake and diabetes on bone density and vertebral deformity prevalence: the EVOS study. *Osteoporos Int*. 2001;12(8):688-698.
375. Kanis JA, Johansson H, Oden A, Johnell O, De Laet C, Eisman JA, McCloskey EV, Mellstrom D, Melton LJ 3rd, Pols HA, Reeve J, Silman AJ, Tenenhouse A. A family history of fracture and fracture risk: a meta-analysis. *Bone*. 2004 Nov;35(5):1029-1037.
376. Campbell AJ, Reinken J, Allan BC, Martinez GS. Falls in old age: a study of frequency and related clinical factors. *Age Ageing*. 1981 Nov;10(4):264-270.
377. Tinetti ME, Speechley M, Ginter SF. Risk factors for falls among elderly persons living in the community. *N Engl J Med*. 1988 Dec 29;319(26):1701-1707.
378. Cummings SR, Nevitt MC. Non-skeletal determinants of fractures: the potential importance of the mechanics of falls. Study of Osteoporotic Fractures Research Group. *Osteoporos Int*. 1994;4 Suppl 1:67-70.

379. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med*. 1995 Mar 23;332(12):767-773.
380. Melton LJ 3rd, Crowson CS, O'Fallon WM. Fracture incidence in Olmsted County, Minnesota: comparison of urban with rural rates and changes in urban rates over time. *Osteoporos Int*. 1999;9(1):29-37.
381. Nevitt MC, Cummings SR. Type of fall and risk of hip and wrist fractures: the study of osteoporotic fractures. The Study of Osteoporotic Fractures Research Group. *J Am Geriatr Soc*. 1993 Nov;41(11):1226-1234.
382. Graafmans WC, Ooms ME, Hofstee HM, Bezemer PD, Bouter LM, Lips P. Falls in the elderly: a prospective study of risk factors and risk profiles. *Am J Epidemiol*. 1996 Jun 1;143(11):1129-1136.
383. Vellas BJ, Wayne SJ, Romero LJ, Baumgartner RN, Garry PJ. Fear of falling and restriction of mobility in elderly fallers. *Age Ageing*. 1997 May;26(3):189-193.
384. Tinetti ME, Inouye SK, Gill TM, Doucette JT. Shared risk factors for falls, incontinence, and functional dependence. Unifying the approach to geriatric syndromes. *JAMA*. 1995 May 3;273(17):1348-1353.
385. Mowé M, Haug E, Bøhmer T. Low serum calcidiol concentration in older adults with reduced muscular function. *J Am Geriatr Soc*. 1999 Feb;47(2):220-226.
386. Deandrea S, Lucenteforte E, Bravi F, Foschi R, La Vecchia C, Negri E. Risk factors for falls in community-dwelling older people: a systematic review and meta-analysis. *Epidemiology*. 2010 Sep;21(5):658-668.
387. Arden NK, Nevitt MC, Lane NE, Gore LR, Hochberg MC, Scott JC, Pressman AR, Cummings SR. Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fractures. Study of Osteoporotic Fractures Research Group. *Arthritis Rheum*. 1999 Jul;42(7):1378-1385.
388. Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, Oden A, Zethraeus N, Pfleger B, Khaltav N. Assessment of fracture risk. *Osteoporos Int*. 2005 Jun;16(6):581-589.
389. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. *Top HIV Med*. 2009 Sep-Oct;17(4):118-123.
390. Effros RB, Fletcher CV, Gebo K, Halter JB, Hazzard WR, Horne FM, Huebner RE, Janoff EN, Justice AC, Kuritzkes D, Nayfield SG, Plaeger SF, Schmader KE, Ashworth JR, Campanelli C, Clayton CP, Rada B, Woolard NF, High KP. Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin Infect Dis*. 2008 Aug 15;47(4):542-553.
391. Erlandson KM, Allshouse AA, Jankowski CM, Duong S, MaWhinney S, Kohrt WM, Campbell TB. Risk factors for falls in HIV-infected persons. *J Acquir Immune Defic Syndr*. 2012 Dec 1;61(4):484-489.
392. Ruiz MA, Reske T, Cefalu C, Estrada J. Falls in HIV-infected patients: a geriatric syndrome in a susceptible population. *J Int Assoc Provid AIDS Care*. 2013 Jul-Aug;12(4):266-269.
393. Miller PD, Siris ES, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Chen YT, Berger ML, Santora AC, Sherwood LM. Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: evidence from the National Osteoporosis Risk Assessment. *J Bone Miner Res*. 2002 Dec;17(12):2222-2230.
394. Cuddihy MT, Gabriel SE, Crowson CS, O'Fallon WM, Melton LJ 3rd. Forearm fractures as predictors of subsequent osteoporotic fractures. *Osteoporos Int*. 1999;9(6):469-475.
395. Short CE, Shaw SG, Fisher MJ, Gilleece YC, Walker-Bone K. Comparison of peripheral forearm DXA and clinical risk factor screening using FRAX® to assess the risk of HIV-associated low bone mass: a cross-sectional study. *Arch Osteoporos*. 2014;9:181.
396. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos Int*. 2008 Apr;19(4):385-397.
397. Compston J, Cooper A, Cooper C, Francis R, Kanis JA, Marsh D, McCloskey EV, Reid DM, Selby P, Wilkins M; National Osteoporosis Guideline Group (NOGG). Guidelines for the diagnosis and management

of osteoporosis in postmenopausal women and men from the age of 50 years in the UK. *Maturitas*. 2009 Feb 20;62(2):105-108.

398. Cummings SR, Nevitt MC, Kidd S. Forgetting falls. The limited accuracy of recall of falls in the elderly. *J Am Geriatr Soc*. 1988 Jul;36(7):613-616.

399. Sandhu SK, Nguyen ND, Center JR, Pocock NA, Eisman JA, Nguyen TV. Prognosis of fracture: evaluation of predictive accuracy of the FRAX algorithm and Garvan nomogram. *Osteoporos Int*. 2010 May;21(5):863-871.

400. van den Bergh JP, van Geel TA, Lems WF, Geusens PP. Assessment of individual fracture risk: FRAX and beyond. *Curr Osteoporos Rep*. 2010 Sep;8(3):131-137.

401. National Institute for Health and Clinical Excellence. Osteoporosis: assessing the risk of fragility fracture. August 2012.

402. Angus B, Brook G, Awosusi F, Barker G, Boffito M, Das S, Dorrell L, Dixon-Williams E, Hall C, Howe B, Kalwij S, Matin N, Nastouli E, Post F, Tenant-Flowers M, Smit E, Wheals D. BHIVA guidelines for the routine investigation and monitoring of adult HIV-1-positive individuals 2016. Available at: <http://www.bhiva.org/monitoring-guidelines.aspx>. Last accessed on 30/01/2017.

403. European AIDS Clinical Society Treatment Guidelines. Version 8.1. 2016. Available at: <http://www.eacsociety.org/guidelines/eacs-guidelines/eacsguidelines.html>. Last accessed on 30/01/2017.

404. Peters B, Post F, Wierzbicki AS, Phillips A, Power L, Das S, Johnson M, Moyle G, Hughes L, Wilkins E, McCloskey E, Compston J, Di Angelantonio E. Screening for chronic comorbid diseases in people with HIV: the need for a strategic approach. *HIV Med*. 2013 Jan;14 Suppl 1:1-11.

405. Yin MT, Falutz J. How to predict the risk of fracture in HIV? *Curr Opin HIV AIDS*. 2016 May;11(3):261-267.

406. Gazzola L, Comi L, Savoldi A, Tagliabue L, Del Sole A, Pietrogrande L, Bini T, d'Arminio Monforte A, Marchetti G. Use of the FRAX equation as first-line screening of bone metabolism alteration in the HIV-infected population. *J Infect Dis*. 2010 Jul 15;202(2):330-331.

407. Pepe J, Isidori AM, Falciano M, Iaiani G, Salotti A, Diacinti D, Del Fiacco R, Sbardella E, Cipriani C, Piemonte S, Romagnoli E, Lenzi A, Minisola S. The combination of FRAX and Ageing Male Symptoms scale better identifies treated HIV males at risk for major fracture. *Clin Endocrinol (Oxf)*. 2012 Nov;77(5):672-678.

408. Mary-Krause M, Viard JP, Ename-Mkoumazok B, Bentata M, Valantin MA, Missy P, Darasteanu I, Roux C, Kolta S, Costagliola D, Rozenberg S. Prevalence of low bone mineral density in men and women infected with human immunodeficiency virus 1 and a proposal for screening strategy. *J Clin Densitom*. 2012 Oct-Dec;15(4):422-433.

409. Mazzotta E, Ursini T, Agostinone A, Di Nicola AD, Polilli E, Sozio F, Vadini F, Pieri A, Trave F, De Francesco V, Capasso L, Borderi M, Manzoli L, Viale P, Parruti G. Prevalence and predictors of low bone mineral density and fragility fractures among HIV-infected patients at one Italian center after universal DXA screening: sensitivity and specificity of current guidelines on bone mineral density management. *AIDS Patient Care STDS*. 2015 Apr;29(4):169-180.

410. Yin MT, Shiao S, Rimland D, Gibert CL, Bedimo RJ, Rodriguez-Barradas MC, Harwood K, Aschheim J, Justice AC, Womack JA. Fracture prediction with modified-FRAX in older HIV-infected and uninfected men. *J Acquir Immune Defic Syndr*. 2016 Aug 15;72(5):513-520.

411. Güerri-Fernández R, Villar-García J, Díez-Pérez A, Prieto-Alhambra D. HIV infection, bone metabolism, and fractures. *Arq Bras Endocrinol Metabol*. 2014 Jul;58(5):478-483.

412. Prior J, Burdge D, Maan E, Milner R, Hankins C, Klein M, Walmsley S. Fragility fractures and bone mineral density in HIV positive women: a case-control population-based study. *Osteoporos Int*. 2007 Oct;18(10):1345-1353.

413. Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. *J Clin Endocrinol Metab*. 2008 Sep;93(9):3499-3504.

414. Womack JA, Goulet JL, Gibert C, Brandt C, Chang CC, Gulanski B, Fraenkel L, Mattocks K, Rimland D, Rodriguez-Barradas MC, Tate J, Yin MT, Justice AC; Veterans Aging Cohort Study Project Team. Increased risk of fragility fractures among HIV infected compared to uninfected male veterans. *PLoS One*. 2011 Feb 16;6(2):e17217.
415. Hansen AB, Gerstoft J, Kronborg G, Larsen CS, Pedersen C, Pedersen G, Obel N. Incidence of low and high-energy fractures in persons with and without HIV infection: a Danish population-based cohort study. *AIDS*. 2012 Jan 28;26(3):285-293.
416. Torti C, Mazziotti G, Soldini PA, Focà E, Maroldi R, Gotti D, Carosi G, Giustina A. High prevalence of radiological vertebral fractures in HIV-infected males. *Endocrine*. 2012 Jun;41(3):512-517.
417. Güerri-Fernandez R, Vestergaard P, Carbonell C, Knobel H, Avilés FF, Castro AS, Nogués X, Prieto-Alhambra D, Diez-Perez A. HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. *J Bone Miner Res*. 2013 Jun;28(6):1259-1263.
418. Prieto-Alhambra D, Güerri-Fernández R, De Vries F, Lalmohamed A, Bazelier M, Starup-Linde J, Diez-Perez A, Cooper C, Vestergaard P. HIV infection and its association with an excess risk of clinical fractures: a nationwide case-control study. *J Acquir Immune Defic Syndr*. 2014 May 1;66(1):90-95.
419. Byrne DD, Newcomb CW, Carbonari DM, Nezamzadeh MS, Leidl KB, Herlim M, Yang YX, Hennessy S, Kostman JR, Leonard MB, Localio AR, Lo Re V 3rd. Increased risk of hip fracture associated with dually treated HIV/hepatitis B virus coinfection. *J Viral Hepat*. 2015 Nov;22(11):936-947.
420. Collin F, Duval X, Le Moing V, Piroth L, Al Kaied F, Massip P, Villes V, Chêne G, Raffi F; ANRS CO8 APROCO-COPILOTE study group. Ten-year incidence and risk factors of bone fractures in a cohort of treated HIV1-infected adults. *AIDS*. 2009 May 15;23(8):1021-1024.
421. Hasse B, Ledergerber B, Furrer H, Battegay M, Hirschel B, Cavassini M, Bertisch B, Bernasconi E, Weber R; Swiss HIV Cohort Study. Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin Infect Dis*. 2011 Dec;53(11):1130-1139.
422. Yong MK, Elliott JH, Woolley IJ, Hoy JF. Low CD4 count is associated with an increased risk of fragility fracture in HIV-infected patients. *J Acquir Immune Defic Syndr*. 2011 Jul 1;57(3):205-210.
423. Womack JA, Goulet JL, Gibert C, Brandt CA, Skanderson M, Gulanski B, Rimland D, Rodriguez-Barradas MC, Tate J, Yin MT, Justice AC; Veterans Aging Cohort Study Project Team.. Physiologic frailty and fragility fracture in HIV-infected male veterans. *Clin Infect Dis*. 2013 May;56(10):1498-1504.
424. Battalora L, Buchacz K, Armon C, Overton ET, Hammer J, Patel P, Chmiel JS, Wood K, Bush TJ, Spear JR, Brooks JT, Young B; HIV Outpatient Study (HOPS) and SUN Study Investigators. Low bone mineral density and risk of incident fracture in HIV-infected adults. *Antivir Ther*. 2016;21(1):45-54.
425. Yin MT, Kendall MA, Wu X, Tassiopoulos K, Hochberg M, Huang JS, Glesby MJ, Bolivar H, McComsey GA. Fractures after antiretroviral initiation. *AIDS*. 2012 Nov 13;26(17):2175-2184.
426. Borderi M, Calza L, Colangeli V, Vanino E, Viale P, Gibellini D, Re MC. Prevalence of sub-clinical vertebral fractures in HIV-infected patients. *New Microbiol*. 2014 Jan;37(1):25-32.
427. Porcelli T, Gotti D, Cristiano A, Maffezzoni F, Mazziotti G, Focà E, Castelli F, Giustina A, Quiros-Roldan E. Role of bone mineral density in predicting morphometric vertebral fractures in patients with HIV infection. *Osteoporos Int*. 2014 Sep;25(9):2263-2269.
428. Gazzola L, Savoldi A, Bai F, Magenta A, Dziubak M, Pietrogrande L, Tagliabue L, Del Sole A, Bini T, Marchetti G, d'Arminio Monforte A. Assessment of radiological vertebral fractures in HIV-infected patients: clinical implications and predictive factors. *HIV Med*. 2015 Oct;16(9):563-571.
429. Stephens KI, Rubinsztain L, Payan J, Rentsch C, Rimland D, Tangpricha V. Dual-energy x-ray absorptiometry and calculated FRAX risk scores may underestimate osteoporotic fracture risk in vitamin d-deficient veterans with HIV infection. *Endocr Pract*. 2016 Apr;22(4):440-446.
430. Maalouf NM, Zhang S, Drechsler H, Brown GR, Tebas P, Bedimo R. Hepatitis C co-infection and severity of liver disease as risk factors for osteoporotic fractures among HIV-infected patients. *J Bone Miner Res*. 2013 Dec;28(12):2577-2583.

431. Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A; National Osteoporosis Guideline Group. Case finding for the management of osteoporosis with FRAX--assessment and intervention thresholds for the UK. *Osteoporos Int*. 2008 Oct;19(10):1395-1408.
432. Lo Re V 3rd, Volk J, Newcomb CW, Yang YX, Freeman CP, Hennessy S, Kostman JR, Tebas P, Leonard MB, Localio AR. Risk of hip fracture associated with hepatitis C virus infection and hepatitis C/human immunodeficiency virus coinfection. *Hepatology*. 2012 Nov;56(5):1688-1698.
433. Dong HV, Cortés YI, Shiao S, Yin MT. Osteoporosis and fractures in HIV/hepatitis C virus coinfection: a systematic review and meta-analysis. *AIDS*. 2014 Sep 10;28(14):2119-2131.
434. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*. 2002 Feb;39(2 Suppl 1):S1-S266.
435. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2005 Jun;67(6):2089-2100.
436. Ibrahim F, Hamzah L, Jones R, Nitsch D, Sabin C, Post FA; UK Collaborative HIV Cohort (CHIC)/CKD Study Group. Baseline kidney function as predictor of mortality and kidney disease progression in HIV-positive patients. *Am J Kidney Dis*. 2012 Oct;60(4):539-547.
437. Asboe D, Aitken C, Boffito M, Booth C, Cane P, Fakoya A, Geretti AM, Kelleher P, Mackie N, Muir D, Murphy G, Orkin C, Post F, Rooney G, Sabin C, Sherr L, Smit E, Tong W, Ustianowski A, Valappil M, Walsh J, Williams M, Yirell D; BHIVA Guidelines Subcommittee. British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals 2011. *HIV Med*. 2012 Jan;13(1):1-44.
438. Kabanda A, Vandercam B, Bernard A, Lauwerys R, van Ypersele de Strihou C. Low molecular weight proteinuria in human immunodeficiency virus-infected patients. *Am J Kidney Dis*. 1996 Jun;27(6):803-808.
439. Szczech LA. Renal diseases associated with human immunodeficiency virus infection: epidemiology, clinical course, and management. *Clin Infect Dis*. 2001 Jul 1;33(1):115-119.
440. Hamzah L, Booth JW, Jose S, McAdoo SP, Kumar EA, O'Donnell P, Hilton R, Sabin C, Williams DI, Jones R, Post FA; HIVCKD Study. Renal tubular disease in the era of combination antiretroviral therapy. *AIDS*. 2015 Sep 10;29(14):1831-1836.
441. Diana NE, Naicker S. Update on current management of chronic kidney disease in patients with HIV infection. *Int J Nephrol Renovasc Dis*. 2016 Sep 16;9:223-234.
442. Campos P, Ortiz A, Soto K. HIV and kidney diseases: 35 years of history and consequences. *Clin Kidney J*. 2016 Dec;9(6):772-781.
443. Gupta SK, Mamlin BW, Johnson CS, Dollins MD, Topf JM, Dubé MP. Prevalence of proteinuria and the development of chronic kidney disease in HIV-infected patients. *Clin Nephrol*. 2004 Jan;61(1):1-6.
444. Szczech LA, Gange SJ, van der Horst C, Bartlett JA, Young M, Cohen MH, Anastos K, Klassen PS, Svetkey LP. Predictors of proteinuria and renal failure among women with HIV infection. *Kidney Int*. 2002 Jan;61(1):195-202.
445. Kimmel PL, Umana WO, Bosch JP. Abnormal urinary protein excretion in HIV-infected patients. *Clin Nephrol*. 1993 Jan;39(1):17-21.
446. Szczech LA, Grunfeld C, Scherzer R, Canchola JA, van der Horst C, Sidney S, Wohl D, Shlipak MG. Microalbuminuria in HIV infection. *AIDS*. 2007 May 11;21(8):1003-1009.
447. Lucas GM, Mehta SH, Atta MG, Kirk GD, Galai N, Vlahov D, Moore RD. End-stage renal disease and chronic kidney disease in a cohort of African-American HIV-infected and at-risk HIV-seronegative participants followed between 1988 and 2004. *AIDS*. 2007 Nov 30;21(18):2435-2443.
448. Ryom L, Kirk O, Lundgren JD, Reiss P, Pedersen C, De Wit S, Buzunova S, Gasiorowski J, Gatell JM, Mocroft A; EuroSIDA in EuroCoord. Advanced chronic kidney disease, end-stage renal disease and renal death among HIV-positive individuals in Europe. *HIV Med*. 2013 Sep;14(8):503-508.

449. Achhra AC, Mocroft A, Ross MJ, Ryom L, Lucas GM, Furrer H, Neuhaus J, Somboonwit C, Kelly M, Gatell JM, Wyatt CM; International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) START Study Group. Kidney disease in antiretroviral-naïve HIV-positive adults with high CD4 counts: prevalence and predictors of kidney disease at enrolment in the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. *HIV Med.* 2015 Apr;16 Suppl 1:55-63.
450. Reynes J, Cournil A, Peyriere H, Psomas C, Guiller E, Chatron M, Cristol JP, Badiou S. Tubular and glomerular proteinuria in HIV-infected adults with estimated glomerular filtration rate ≥ 60 ml/min per 1.73m^2 . *AIDS.* 2013 May 15;27(8):1295-1302.
451. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of renal disease among people living with HIV: a systematic review and meta-analysis. *BMC Public Health.* 2012 Mar 23;12:234.
452. Jotwani V, Li Y, Grunfeld C, Choi AI, Shlipak MG. Risk factors for ESRD in HIV-infected individuals: traditional and HIV-related factors. *Am J Kidney Dis.* 2012 May;59(5):628-635.
453. Ryom L, Mocroft A, Kirk O, Worm SW, Kamara DA, Reiss P, Ross M, Fux CA, Morlat P, Moranne O, Smith C, Lundgren JD; D:A:D Study Group. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis.* 2013 May 1;207(9):1359-1369.
454. Santiago P, Grinsztejn B, Friedman RK, Cunha CB, Coelho LE, Luz PM, de Oliveira AV, Moreira RI, Cardoso SW, Veloso VG, Suassuna JH. Screening for decreased glomerular filtration rate and associated risk factors in a cohort of HIV-infected patients in a middle-income country. *PLoS One.* 2014 Apr 3;9(4):e93748.
455. Ryom L, Mocroft A, Kirk O, Ross M, Reiss P, Fux CA, Morlat P, Moranne O, Smith C, El-Sadr W, Law M, Lundgren JD. Predictors of advanced chronic kidney disease and end-stage renal disease in HIV-positive persons. *AIDS.* 2014 Jan 14;28(2):187-199.
456. El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, Cohen CJ, Cohn D, Cooper D, Darbyshire J, Emery S, Fätkenheuer G, Gazzard B, Grund B, Hoy J, Klingman K, Losso M, Markowitz N, Neuhaus J, Phillips A, Rappoport C, Strategies for Management of Antiretroviral Therapy (SMART) Study Group. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med.* 2006 Nov 30;355(22):2283-2296.
457. Kalayjian RC, Lau B, Mechekano RN, Crane HM, Rodriguez B, Salata RA, Krishnasami Z, Willig JH, Martin JN, Moore RD, Eron JJ, Kitahata MM. Risk factors for chronic kidney disease in a large cohort of HIV-1 infected individuals initiating antiretroviral therapy in routine care. *AIDS.* 2012 Sep 24;26(15):1907-1915.
458. Lucas GM, Eustace JA, Sozio S, Mentari EK, Appiah KA, Moore RD. Highly active antiretroviral therapy and the incidence of HIV-1-associated nephropathy: a 12-year cohort study. *AIDS* 2004; 18:541–546.
459. Shah S, Weber-Shrikant E, Panesar M. Discontinuation of antiretroviral therapy causing progression to end-stage renal disease in an HIV patient diagnosed with immune complex 'lupus-like' glomerulonephritis. *Clin Kidney J.* 2012 Jun;5(3):276-278.
460. Jao J, Wyatt CM. Antiretroviral medications: adverse effects on the kidney. *Adv Chronic Kidney Dis.* 2010 Jan;17(1):72-82.
461. Kalyesubula R, Perazella MA. Nephrotoxicity of HAART. *AIDS Res Treat.* 2011;2011:562790.
462. Wikman P, Safont P, Del Palacio M, Moreno A, Moreno S, Casado JL. The significance of antiretroviral-associated acute kidney injury in a cohort of ambulatory human immunodeficiency virus-infected patients. *Nephrol Dial Transplant.* 2013 Aug;28(8):2073-2081.
463. Flandre P, Pugliese P, Cuzin L, Bagnis CI, Tack I, Cabié A, Poizot-Martin I, Katlama C, Brunet-François C, Yazdanpanah Y, Dellamonica P; New AIDS Data group. Risk factors of chronic kidney disease in HIV-infected patients. *Clin J Am Soc Nephrol.* 2011 Jul;6(7):1700-1707.
464. Achhra AC, Nugent M, Mocroft A, Ryom L, Wyatt CM. Chronic Kidney Disease and Antiretroviral Therapy in HIV-Positive Individuals: Recent Developments. *Curr HIV/AIDS Rep.* 2016 Jun;13(3):149-157.

465. Côté HC, Magil AB, Harris M, Scarth BJ, Gadawski I, Wang N, Yu E, Yip B, Zalunardo N, Werb R, Hogg R, Harrigan PR, Montaner JS. Exploring mitochondrial nephrotoxicity as a potential mechanism of kidney dysfunction among HIV-infected patients on highly active antiretroviral therapy. *Antivir Ther.* 2006;11(1):79-86.
466. Kiser JJ, Carten ML, Aquilante CL, Anderson PL, Wolfe P, King TM, Delahunty T, Bushman LR, Fletcher CV. The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients. *Clin Pharmacol Ther.* 2008 Feb;83(2):265-272.
467. Rodríguez-Nóvoa S, Labarga P, Soriano V, Egan D, Albalater M, Morello J, Cuenca L, González-Pardo G, Khoo S, Back D, Owen A. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis.* 2009 Jun 1;48(11):e108-e116.
468. Herlitz LC, Mohan S, Stokes MB, Radhakrishnan J, D'Agati VD, Markowitz GS. Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. *Kidney Int.* 2010; 78:1171–1177.
469. Peterson PA, Evrin PE, Berggard I. Differentiation of glomerular, tubular, and normal proteinuria: determinations of urinary excretion of beta-2-macroglobulin, albumin, and total protein. *J Clin Invest.* 1969; 48: 1189–1198.
470. Shihabi ZK, Konen JC, O'Connor ML. Albuminuria vs urinary total protein for detecting chronic renal disorders *Clin Chem.* 1991; 37:621-624.
471. Parikh CR, Lu JC, Coca SG, Devarajan P. Tubular proteinuria in acute kidney injury: a critical evaluation of current status and future promise. *Ann Clin Biochem.* 2010 Jul;47(Pt 4):301-312.
472. Samarawickrama A, Cai M, Smith ER, Nambiar K, Sabin C, Fisher M, Gilleece Y, Holt SG. Simultaneous measurement of urinary albumin and total protein may facilitate decision-making in HIV-infected patients with proteinuria. *HIV Med.* 2012; 13; 526–532.
473. Post FA, Wyatt CM, Mocroft A. Biomarkers of impaired renal function. *Curr Opin HIV AIDS.* 2010 Nov;5(6):524-530.
474. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. *Am J Kidney Dis.* 2011; 57(5):773-780.
475. Dauchy FA, Lawson-Ayayi S, de la Faille R, Bonnet F, Rigotherier C, Mehse N, iremont-Salame G, Cazanave C, Greib C, dabis F, Dupon M. Increased risk of abnormal proximal renal tubular function with HIV infection and antiretroviral therapy. *Kidney Int.* 2011; 80:302–309.
476. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, Gatell J, Phillips AN, Ledergerber B, Lundgren JD. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. *AIDS.* 2010; 24: 1667–1678.
477. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med.* 2003; 139: 137–147.
478. Fux CA, Simcock M, Wolbers M, Bucher HC, Hirschel B, Opravil M, Vernazza P, Cavassini M, Bernasconi E, Elzi L, Furrer H. Swiss HIV Cohort Study. Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. *Antivir Ther.* 2007; 12: 1165–1173.
479. Goicoechea M, Liu S, Best B, Sun S, Jain S, Kemper C, Witt M, Diamond C, Haubrich R, Louie S. Greater tenofovir-associated renal function decline with protease inhibitor-based versus nonnucleoside reverse-transcriptase inhibitor-based therapy. *J Infect Dis.* 2008; 197: 102–108.
480. Gallant JE, Moore RD. Renal function with use of a tenofovir-containing initial antiretroviral regimen. *AIDS.* 2009; 23: 1971–1975.
481. Sise ME, Hirsch JS, Canetta PA, Herlitz L, Mohan S. Nonalbumin proteinuria predominates in biopsy-proven tenofovir nephrotoxicity. *AIDS.* 2015; 29:941–946.
482. Gravemann S, Brinkkoetter PT, Vehreschild JJ, Franke B, Ehren K, Bünemann E, Orbach H, Weiß V, Hellmich M, Benzinger T, Fätkenheuer G. Low-grade proteinuria is highly prevalent in HIV-positive patients on antiretroviral treatment. *AIDS.* 2014 Jul 31;28(12):1783-1789.

483. Jose S, Hamzah L, Campbell LJ, Hill T, Fisher M, Leen C, Gilson R, Walsh J, Nelson M, Hay P, Johnson M, Chadwick D, Nitsch D, Jones R, Sabin CA, Post FA; UK Collaborative HIV Cohort Study Steering Committee. Incomplete reversibility of estimated glomerular filtration rate decline following tenofovir disoproxil fumarate exposure. *J Infect Dis.* 2014 Aug 1;210(3):363-373.
484. Gupta SK, Parker RA, Robbins GK, Dubé MP. The effects of highly active antiretroviral therapy on albuminuria in HIV-infected persons: results from a randomized trial. *Nephrol Dial Transplant.* 2005 Oct;20(10):2237-2242.
485. Hall AM, Edwards SG, Lapsley M, Connolly JO, Chetty K, O'Farrell S, Unwin RJ, Williams IG. Subclinical tubular injury in HIV-infected individuals on antiretroviral therapy: a cross-sectional analysis. *Am J Kidney Dis.* 2009 Dec;54(6):1034-1042.
486. Izzedine H, Hulot JS, Villard E, Goyenvalle C, Dominguez S, Ghosn J, Valantin MA, Lechat P, Deray AG. Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. *J Infect Dis.* 2006 Dec 1;194(11):1481-1491.
487. Pushpakom SP, Liptrott NJ, Rodríguez-Nóvoa S, Labarga P, Soriano V, Albalater M, Hopper-Borge E, Bonora S, Di Perri G, Back DJ, Khoo S, Pirmohamed M, Owen A. Genetic variants of ABCC10, a novel tenofovir transporter, are associated with kidney tubular dysfunction. *J Infect Dis.* 2011 Jul 1;204(1):145-153.
488. Gallant JE, Parish MA, Keruly JC, Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. *Clin Infect Dis.* 2005 Apr 15;40(8):1194-1198.
489. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L, Chang J, Blank J, Quesenberry C Jr, Klein D. Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. *J Acquir Immune Defic Syndr.* 2010 Jan;53(1):62-69.
490. De Beaudrap P, Diallo MB, Landman R, Guèye NF, Ndiaye I, Diouf A, Kane CT, Etard JF, Girard PM, Sow PS, Delaporte E; ANRS 1215 Study Group. Changes in the renal function after tenofovir-containing antiretroviral therapy initiation in a Senegalese cohort (ANRS 1215). *AIDS Res Hum Retroviruses.* 2010 Nov;26(11):1221-1227.
491. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczek D, Fisher M, Norden AG, Cavassini M, Rieger A, Khuong-Josses MA, Branco T, Pearce HC, Givens N, Vavro C, Lim ML. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. *J Acquir Immune Defic Syndr* 2010;55:49-57.
492. Vrouwenraets SM, Fux CA, Wit FW, Garcia EF, Furrer H, Brinkman K, Hoek FJ, Abeling NG, Krediet RT, Reiss P; Prepare Study Group. Persistent decline in estimated but not measured glomerular filtration rate on tenofovir may reflect tubular rather than glomerular toxicity. *AIDS.* 2011 Nov 13;25(17):2149-2155.
493. Izzedine H, Isnard-Bagnis C, Hulot JS, Vittecoq D, Cheng A, Jais CK, Launay-Vacher V, Deray G. Renal safety of tenofovir in HIV treatment-experienced patients. *AIDS.* 2004 Apr 30;18(7):1074-1076.
494. Madeddu G, Bonfanti P, De Socio GV, Carradori S, Grosso C, Marconi P, Penco G, Rosella E, Miccolis S, Melzi S, Mura MS, Landonio S, Ricci E, Quirino T; CISA Group. Tenofovir renal safety in HIV-infected patients: results from the SCOLTA Project. *Biomed Pharmacother.* 2008 Jan;62(1):6-11.
495. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl.* 2009 Aug;(113):S1-130.
496. Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G; Kidney Disease: Improving Global Outcomes (KDIGO). Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int.* 2006 Jun;69(11):1945-1953.
497. Fux CA, Hasse B, Opravil M, Cavassini M, Calmy A, Gurtner-de la Fuente V, Schmid P, Stoeckle M, Flepp M, Furrer H, Swiss HIV Cohort Study. Bone turnover, in particular osteoclast activity, is increased in patients with confirmed proximal renal tubulopathy within the Swiss HIV Cohort Study. Conference on Retroviruses and Opportunistic Infections, San Francisco, USA, 2010, abstract #748.

498. Burling KA, Cutillas PR, Church D, Lapsley M, Norden AG. Analysis of molecular forms of urine retinol-binding protein in Fanconi syndrome and design of an accurate immunoassay. *Clin Chim Acta* 2012;413:483-489.
499. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604–612.
500. Stevens LA, Schmid CH, Greene T, Zhang YL, Beck GJ, Froissart M, Hamm LL, Lewis JB, Mauer M, Navis GJ, Steffes MW, Eggers PW, Coresh J, Levey AS. Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m². *Am J Kidney Dis* 2010; 56:486–495.
501. Ibrahim F, Hamzah L, Jones R, Nitsch D, Sabin C, Post FA; UK CHIC/CKD* Study Group. Comparison of CKD-EPI and MDRD to estimate baseline renal function in HIV-positive patients. *Nephrol Dial Transplant*. 2012 Jun;27(6):2291-2297.
502. Gupta SK, Anderson AM, Ebrahimi R, Fralich T, Graham H, Scharen-Guivel V, Flaherty JF, Fortin C, Kalayjian RC, Rachlis A, Wyatt CM. Fanconi syndrome accompanied by renal function decline with tenofovir disoproxil fumarate: a prospective, case-control study of predictors and resolution in HIV-infected patients. *PLoS One* 2014;9:e92717.
503. Mateo L, Holgado S, Mariñoso ML, Pérez-Andrés R, Bonjoch A, Romeu J, Olivé A. Hypophosphatemic osteomalacia induced by tenofovir in HIV-infected patients. *Clin Rheumatol*. 2016 May;35(5):1271-1279.
504. Bonnick SL. *Bone Densitometry in Clinical Practice Application and Interpretation; Third Edition Bone Densitometry in Clinical Practice* 2010.
505. Nishijima T, Shimbo T, Komatsu H, Takano M, Tanuma J, Tsukada K, Teruya K, Gatanaga H, Kikuchi Y, Oka S. Urinary beta-2 microglobulin and alpha-1 microglobulin are useful screening markers for tenofovir-induced kidney tubulopathy in patients with HIV-1 infection: a diagnostic accuracy study. *J Infect Chemother*. 2013 Oct;19(5):850-857.
506. Rasmussen TA, Jensen D, Tolstrup M, Nielsen US, Erlandsen EJ, Birn H, Østergaard L, Langdahl BL, Laursen AL. Comparison of bone and renal effects in HIV-infected adults switching to abacavir or tenofovir based therapy in a randomized trial. *PLoS One*. 2012;7(3):e32445.
507. Masiá M, Padilla S, Robledano C, López N, Ramos JM, Gutiérrez F. Early changes in parathyroid hormone concentrations in HIV-infected patients initiating antiretroviral therapy with tenofovir. *AIDS Res Hum Retroviruses*. 2012 Mar;28(3):242-246.
508. Gökçe C, Gökçe O, Baydıncı C, İlhan N, Alaşehirli E, Ozkücüük F, Taşçı M, Atılkeler MK, Celebi H, Arslan N. Use of random urine samples to estimate total urinary calcium and phosphate excretion. *Arch Intern Med* 1991;151:1587-1588.
509. Robinson-Cohen C, Ix JH, Smits G, Persky M, Chertow GM, Block GA, Kestenbaum BR. Estimation of 24-hour urine phosphate excretion from spot urine collection: development of a predictive equation. *J Ren Nutr*. 2014 May;24(3):194-199.
510. World Health Organization. Progress report 2016: Prevent HIV, test and treat all. Last accessed 01/05/2017.
511. Public Health England. HIV diagnoses, late diagnoses and numbers accessing treatment and care: 2016 report. October 2016.
512. Lencel P, Magne D. Inflammaging: the driving force in osteoporosis? *Med Hypotheses*. 2011 Mar;76(3):317-321.
513. Kooij KW, Wit FW, Bisschop PH, Schouten J, Stolte IG, Prins M, van der Valk M, Prins JM, van Eck-Smit BL, Lips P, Reiss P; AGEHIV Cohort Study group. Low bone mineral density in patients with well-suppressed HIV infection: association with body weight, smoking, and prior advanced HIV disease. *J Infect Dis*. 2015 Feb 15;211(4):539-548.

514. Hamill MM, Ward KA, Pettifor JM, Norris SA, Prentice A. Bone mass, body composition and vitamin D status of ARV-naïve, urban, black South African women with HIV infection, stratified by CD₄ count. *Osteoporos Int*. 2013 Nov;24(11):2855-2861.
515. Calmy A, Chevalley T, Delhumeau C, Toutous-Trellu L, Spycher-Elbes R, Ratib O, Zawadzinski S, Rizzoli R. Long-term HIV infection and antiretroviral therapy are associated with bone microstructure alterations in premenopausal women. *Osteoporos Int*. 2013 Jun;24(6):1843-1852.
516. Grant PM, Cotter AG. Tenofovir and bone health. *Curr Opin HIV AIDS*. 2016 May;11(3):326-332.
517. Güerri-Fernández R, Molina D, Villar-García J, Prieto-Alhambra D, Mellibovsky L, Nogués X, González-Mena A, Guelar A, Trenchs-Rodríguez M, Herrera-Fernández S, Horcajada JP, Díez-Pérez A, Knobel H. Brief Report: HIV Infection Is Associated With Worse Bone Material Properties, Independently of Bone Mineral Density. *J Acquir Immune Defic Syndr*. 2016 Jul 1;72(3):314-318.
518. Erlandson KM, Guaraldi G, Falutz J. More than osteoporosis: age-specific issues in bone health. *Curr Opin HIV AIDS*. 2016 May;11(3):343-350.
519. Battalora LA, Young B, Overton ET. Bones, Fractures, Antiretroviral Therapy and HIV. *Curr Infect Dis Rep*. 2014 Feb;16(2):393.
520. INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, Sharma S, Avihingsanon A, Cooper DA, Fätkenheuer G, Llibre JM, Molina JM, Munderi P, Schechter M, Wood R, Klingman KL, Collins S, Lane HC, Phillips AN, Neaton JD. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med*. 2015 Aug 27;373(9):795-807.
521. Haselberg H, Pocock N, Amin J, Ebeling PR, Emery S, Carr A; STEAL study investigators, Allworth A. Hip structural parameters over 96 weeks in HIV-infected adults switching treatment to tenofovir-emtricitabine or abacavir-lamivudine. *PLoS One*. 2014 Apr 10;9(4):e94858.
522. Bedimo R, Rosenblatt L, Myers J. Systematic review of renal and bone safety of the antiretroviral regimen efavirenz, emtricitabine, and tenofovir disoproxil fumarate in patients with HIV infection. *HIV Clin Trials*. 2016 Nov;17(6):246-266.
523. Sax PE, Zolopa A, Brar I, Elion R, Ortiz R, Post F, Wang H, Callebaut C, Martin H, Fordyce MW, McCallister S. Tenofovir alafenamide vs. tenofovir disoproxil fumarate in single tablet regimens for initial HIV-1 therapy: a randomized phase 2 study. *J Acquir Immune Defic Syndr*. 2014 Sep 1;67(1):52-58.
524. Sax PE, Wohl D, Yin MT, Post F, DeJesus E, Saag M, Pozniak A, Thompson M, Podzamczar D, Molina JM, Oka S, Koenig E, Trottier B, Andrade-Villanueva J, Crofoot G, Custodio JM, Plummer A, Zhong L, Cao H, Martin H, Callebaut C, Cheng AK, Fordyce MW, McCallister S; GS-US-292-0104/0111 Study Team. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, non-inferiority trials. *Lancet*. 2015 Jun 27;385(9987):2606-2615.
525. Mills A, Crofoot G Jr, McDonald C, Shalit P, Flamm JA, Gathe J Jr, Scribner A, Shambraw D, Saag M, Cao H, Martin H, Das M, Thomas A, Liu HC, Yan M, Callebaut C, Custodio J, Cheng A, McCallister S. Tenofovir Alafenamide Versus Tenofovir Disoproxil Fumarate in the First Protease Inhibitor-Based Single-Tablet Regimen for Initial HIV-1 Therapy: A Randomized Phase 2 Study. *J Acquir Immune Defic Syndr*. 2015 Aug 1;69(4):439-445.
526. Mills A, Arribas JR, Andrade-Villanueva J, DiPerri G, Van Lunzen J, Koenig E, Elion R, Cavassini M, Madruga JV, Brunetta J, Shambraw D, DeJesus E, Orkin C, Wohl DA, Brar I, Stephens JL, Girard PM, Huhn G, Plummer A, Liu YP, Cheng AK, McCallister S; GS-US-292-0109 team. Switching from tenofovir disoproxil fumarate to tenofovir alafenamide in antiretroviral regimens for virologically suppressed adults with HIV-1 infection: a randomised, active-controlled, multicentre, open-label, phase 3, non-inferiority study. *Lancet Infect Dis*. 2016 Jan;16(1):43-52.
527. Pozniak A, Arribas JR, Gathe J, Gupta SK, Post FA, Bloch M, Avihingsanon A, Crofoot G, Benson P, Lichtenstein K, Ramgopal M, Chetchotisakd P, Custodio JM, Abram ME, Wei X, Cheng A, McCallister S, SenGupta D, Fordyce MW; GS-US-292-0112 Study Team. Switching to Tenofovir Alafenamide, Coformulated With Elvitegravir, Cobicistat, and Emtricitabine, in HIV-Infected Patients With Renal Impairment: 48-Week Results From a Single-Arm, Multicenter, Open-Label Phase 3 Study. *J Acquir Immune Defic Syndr*. 2016 Apr 15;71(5):530-537.

528. Post FA, Tebas P, Clarke A, Cotte L, Short WR, Abram ME, Jiang S, Cheng A, Das M, Fordyce MW. Brief Report: Switching to Tenofovir Alafenamide, Coformulated With Elvitegravir, Cobicistat, and Emtricitabine, in HIV-Infected Adults With Renal Impairment: 96-Week Results From a Single-Arm, Multicenter, Open-Label Phase 3 Study. *J Acquir Immune Defic Syndr*. 2017 Feb 1;74(2):180-184.
529. National Health Service England Specialised Services Clinical Reference Group for HIV. Clinical Commissioning Policy: Tenofovir Alafenamide for treatment of HIV 1 in adults and adolescents. February 2017.
530. Moyle GJ, Hardy H, Farajallah A, McGrath SJ, Kaplita S, Ward D. Changes in bone mineral density after 96 weeks of treatment with atazanavir/ritonavir or lopinavir/ritonavir plus tenofovir DF/emtricitabine in treatment-naïve patients with HIV-1 infection: the CASTLE body composition substudy. *J Acquir Immune Defic Syndr*. 2015 Jan 1;68(1):40-45.
531. Sharma A, Cohen HW, Freeman R, Santoro N, Schoenbaum EE. Prospective evaluation of bone mineral density among middle-aged HIV-infected and uninfected women: Association between methadone use and bone loss. *Maturitas*. 2011 Nov;70(3):295-301.
532. Guaraldi G, Zona S, Cossarizza A, Vernacotola L, Carli F, Lattanzi A, Nardini G, Orlando G, Garlassi E, Termini R, Garau M. Switching to darunavir/ritonavir monotherapy vs. triple-therapy on body fat redistribution and bone mass in HIV-infected adults: the Monarch randomized controlled trial. *Int J STD AIDS*. 2014 Mar;25(3):207-212.
533. Moran CA, Weitzmann MN, Ofotokun I. The protease inhibitors and HIV-associated bone loss. *Curr Opin HIV AIDS*. 2016 May;11(3):333-342.
534. Hernandez-Vallejo SJ, Beaupere C, Larghero J, Capeau J, Lagathu C. HIV protease inhibitors induce senescence and alter osteoblastic potential of human bone marrow mesenchymal stem cells: beneficial effect of pravastatin. *Aging Cell*. 2013 Dec;12(6):955-965.
535. Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS. HIV protease inhibitor impair vitamin D bioactivation 1,25-dihydroxyvitamin D. *AIDS*. 2003;17(4):513-520.
536. Rockstroh JK, DeJesus E, Henry K, Molina JM, Gathe J, Ramanathan S, Wei X, Plummer A, Abram M, Cheng AK, Fordyce MW, Szwarcberg J; GS-236-0103 Study Team. A randomized, double-blind comparison of coformulated elvitegravir/cobicistat/emtricitabine/tenofovir DF vs ritonavir-boosted atazanavir plus coformulated emtricitabine and tenofovir DF for initial treatment of HIV-1 infection: analysis of week 96 results. *J Acquir Immune Defic Syndr*. 2013 Apr 15;62(5):483-486.
537. Moyle GJ, Stellbrink HJ, Compston J, Orkin C, Arribas JR, Domingo P, Granier C, Pearce H, Sedani S, Gartland M; ASSERT Team. 96-Week results of abacavir/lamivudine versus tenofovir/emtricitabine, plus efavirenz, in antiretroviral-naïve, HIV-1-infected adults: ASSERT study. *Antivir Ther*. 2013;18(7):905-913.
538. Grijzen ML, Vrouwenraets SM, Wit FW, Stolte IG, Prins M, Lips P, Reiss P, Prins JM. Low bone mineral density, regardless of HIV status, in men who have sex with men. *J Infect Dis*. 2013 Feb 1;207(3):386-391.
539. Calvo M, Martinez E. Update on metabolic issues in HIV patients. *Curr Opin HIV AIDS*. 2014 Jul;9(4):332-339.
540. Guaraldi G, Stentarelli C, Zona S, Santoro A. HIV-associated lipodystrophy: impact of antiretroviral therapy. *Drugs*. 2013 Sep;73(13):1431-1450.
541. Shanmugasundaram M, Rough SJ, Alpert JS. Dyslipidemia in the elderly: should it be treated. *Clin Cardiol*. 2010;33:1-4.
542. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, Kanaya AM, Harris TB, Cauley JA; Health ABC Study. The effects of adiponectin and leptin on changes in bone mineral density. *Osteoporos Int*. 2012 Jun;23(6):1699-1710.
543. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J, Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS. The human urine metabolome. *PLoS One*. 2013 Sep 4;8(9):e73076.
544. Chetwynd AJ, Abdul-Sada A, Hill EM. Solid-phase extraction and nanoflow liquid chromatography-nano electrospray ionization mass spectrometry for improved global urine metabolomics. *Anal Chem*. 2015 Jan 20;87(2):1158-1165.

545. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. *Mass Spectrom Rev.* 2007 Jan-Feb;26(1):51-78.
546. Sitole LJ, Williams AA, Meyer D. Metabonomic analysis of HIV-infected biofluids. *Mol Biosyst.* 2013 Jan 27;9(1):18-28.
547. Chetwynd AJ, Samarawickrama A, Vera JH, Bremner SA, Abdul-Sada A, Gillece Y, Holt SG, Hill EM. Nanoflow-Nanospray Mass Spectrometry Metabolomics Reveals Disruption of the Urinary Metabolite Profiles of HIV-Positive Patients on Combination Antiretroviral Therapy. *J Acquir Immune Defic Syndr.* 2017 Feb 1;74(2):e45-e53.

Chapter 10: Appendices

10.1 Publications

10.2 Conference oral and poster presentations

10.2.1 Oral presentations

10.2.2 Poster presentation and themed discussion

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10.6 Protocol for optimisation check of BTM assays

10.1 Publications

Below is a list of publications relating to both work included in this thesis and allied work conducted during the course of my study.

Post FA, McCloskey EV, Compston JE, Bowman CA, Hay PE, Johnson MA, Mallon PWG, Peters BS, **Samarawickrama A**, Tudor-Williams G. Prevention of bone loss and management of fracture risk in HIV-infected individuals: case studies and recommendations for different patient subgroups. *Fut Virol* 2011 June;6(6):769-782.

Childs K, Welz T, **Samarawickrama A**, Post FA. Effects of vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. *AIDS* 2012 Jan 28;26(3):253-262.

Samarawickrama A, Cai M, Smith E, Nambiar K, Sabin C, Fisher M, Gilleece Y, Holt S. Simultaneous measurement of urinary albumin and total protein may facilitate decision-making in HIV-infected patients with proteinuria. *HIV Med* 2012 Oct;13(9):526-532.

Hamzah L, **Samarawickrama A**, Campbell L, Pope M, Burling K, Fisher M, Gilleece Y, Walker-Bone K, Post F. Effects of renal tubular dysfunction on bone in tenofovir-exposed HIV-positive patients. *AIDS* 2015 Sep 10;29(14):1785-1792 (joint 1st author).

Chetwynd AJ, **Samarawickrama A**, Vera JH, Bremner SA, Abdul-Sada A, Gilleece Y, Holt SG, Hill EM. Nanoflow-nanospray mass spectrometry metabolomics reveals disruption of the urinary metabolite profiles of HIV-positive patients on combination antiretroviral therapy. *J Acquir Immune Defic Syndr* 2017 Feb 1;74(2):e45-e53.

10.2 Conference oral and poster presentations

Below is a list of oral and poster presentations relating to work included in this thesis, as well as allied work conducted during the course of my study.

10.2.1 Oral presentations

Samarawickrama A, Holt S, Nambiar K, Fisher M, Gilleece Y. The value of urine protein/creatinine and albumin/creatinine ratios in assessing renal disease in HIV infection. *HIV Med* 2010;11(10-11):1464-2662.

Malik R, **Samarawickrama A**, Walker-Bone K. Fracture epidemiology in HIV-infected adults in the UK. *Osteoporos Int* 2010 Nov;21(S452):937-941.

Hamzah L, **Samarawickrama A**, Campbell L, Pope M, Burling K, Norden A, Fisher M, Gilleece Y, Walker-Bone K, Post F. Effects of renal tubular dysfunction on bone in HIV positive patients. *HIV Med* 2013 Apr;14(5):1464-2662.

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. No association between vitamin D deficiency and parathyroid hormone, bone density and bone turnover in a large cohort of HIV-infected men on tenofovir. *HIV Med* 2015;16 (Suppl. 2):1–11.

10.2.2 Poster presentation and themed discussion

Samarawickrama A, Nambiar K, Gilleece Y, Fisher M, Holt S. The value of urine protein/creatinine and albumin/creatinine ratios in assessing renal disease in HIV infection – Poster 737 and themed discussion, 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, USA, February 2010.

10.2.3 Poster presentations

Walker-Bone K, Malik R, Fisher M, Gilleece Y, **Samarawickrama A**. Fracture epidemiology among a cohort of HIV-infected adults in the UK: an epidemiological study. *Arth Rheum* 2010;62(966):0004-3591.

Surah S, **Samarawickrama A**, Campbell L, Kulasegeram R, Post F, Fisher M, Peters B, Fox J. Prevalence and factors associated with severe vitamin D deficiency in HIV/hepatitis C co-infected patients. *J Int AIDS Soc* 2010 Nov;13:1758-2652.

Samarawickrama A, Malik R, Fisher M, Gilleece Y, Walker-Bone K. Rates of bone fractures in a cohort of HIV-infected adults in the UK. *J Int AIDS Soc* 2010 Nov;13:1758-2652.

Shaw S, White S, Gilleece Y, Fisher M, **Samarawickrama A**, Walker-Bone K. Risk factors for low bone mass among a cohort of male HIV-infected adults. *Osteoporos Int* 2010 Nov;21(S495-S496):937-941.

Samarawickrama A, Nambiar K, Sabin C, Smith E, McMahon L, Fisher M, Gilleece Y, Holt S. The value of using both urine protein/creatinine and albumin/creatinine ratios in assessing renal disease in HIV infection. *Nephrol* 2011 Sep; 16(59):1320-5358.

Samarawickrama A, Iwuji C, Fisher M, Gilleece Y, Walker-Bone K. Diagnosis of osteoporosis: comparison of the effect of using T-scores derived from Caucasian male populations with those derived from HIV-infected males. *HIV Med* 2011 Apr;12(Suppl 1):87-91.

Surah S, **Samarawickrama A**, Hill A, Sawyer W, Peters B, Kulasegaram R, Fisher M, Fox J. Factors associated with vitamin D deficiency in HIV/Hepatitis C co-infected patients and relationship between vitamin D levels and Hepatitis C treatment outcomes. *HIV Med* 2012 Apr;13(79):1464-2662.

Mahendran P, **Samarawickrama A**, Churchill D, Walker-Bone K.E. Risk factors for hyperuricaemia among a large cohort of HIV-infected men. *Rheum* 2013 Apr;52(i67);1462-0324.

Hamzah L, **Samarawickrama A**, Campbell L, Pope M, Burling K, Norden A, Fisher M, Gilleece Y, Walker-Bone K, Post F. Effects of renal tubular dysfunction on bone in HIV positive patients – Poster MOPE076, 7th International AIDS Society (IAS) Conference, Kuala Lumpur, Malaysia, July 2013.

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. Minimal change in bone density and no association with HIV factors over 12 months in HIV-infected men – Poster 777, 21st Conference on Retroviruses and Opportunistic Infections, Boston, USA, March 2014.

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. Minimal change in bone density over 12 months in cART-experienced HIV-infected men – Poster 163, HIV Med 2014;15(3):1–16.

Samarawickrama A, Jose S, Sabin C, Walker-Bone K, Fisher M, Gilleece Y. No association between vitamin D deficiency and parathyroid hormone, bone density and bone turnover in a large cohort of HIV-infected men on tenofovir – Poster 36, J Int AIDS Soc 2014 Nov 2;17(4 Suppl 3):19568.

Hamzah L, **Samarawickrama A**, Walker-Bone K, Gilleece Y, Fisher M, Post F. Relationship between phosphate reabsorption, age, tenofovir and bone mineral density – Poster 768, 22nd Conference on Retroviruses and Opportunistic Infections, Seattle, USA, February 2015.

Payne L, Hayes M, **Samarawickrama A**, Gilleece Y, Fisher M, Walker-Bone K. Reduced bone mineral density in HIV infection: HIV, ART or lifestyle – Poster 95, HIV Med 2015; 16 (Suppl. 2), 12–77.

Hamzah L, **Samarawickrama A**, Walker-Bone K, Gilleece Y, Fisher M, Post F. Relationship between phosphate reabsorption, age, tenofovir and bone mineral density – Poster 74, HIV Med 2015; 16 (Suppl. 2), 12–77.

Chetwynd AJ, **Samarawickrama A**, Abdul-Sada A, Vera JH, Holt SG, Fisher M, Gilleece Y, Hill EM. Differences in urine metabolomes with HIV infection and antiretroviral drug exposure – Poster 692, 23rd Conference on Retroviruses and Opportunistic Infections, Boston, USA, February 2016.

Chetwynd AJ, **Samarawickrama A**, Abdul-Sada A, Vera JH, Holt SG, Fisher M, Gilleece Y, Hill EM. Differences in urine metabolomes with HIV infection and antiretroviral drug exposure – Poster 70, HIV Med 2016; 17 (Suppl. 1), 14–71.

10.3 Ethics documents

10.3.1 Ethics approval

10.3.2 Letter of invitation for participants of the pilot study

10.3.3 Participant information sheet

10.3.4 Email invitation for patients attending the email clinic

10.3.5 Written consent form

10.3.1 Ethics approval



Health Research Authority

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07 October 2009
Letter reissued 20 May 2013

Dr Amanda Samarawickrama
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Dear Dr Samarawickrama

Study Title: Bone mineral status in HIV infection: screening, prevalence, risk factors and prognosis
REC reference number: 09/H1107/101
Protocol number: 1.0

The Research Ethics Committee reviewed the above application at the meeting held on 01 October 2009. Thank you for attending to discuss the study along with your supervisor, Dr Karen-Walker-Bone.

Ethical opinion

Members were of the view that this was a well thought out research project. Members noted that the study was being done as part of an educational qualification for a PhD and that this was a follow-on, after a pilot project.

Members were satisfied that the risks from radiation had been adequately addressed in the information sheet.

Members asked what fall-back mechanisms were in place should an abnormality be picked up during any of the tests for both groups of participants. At the meeting, you explained that if an abnormality was detected in the HIV group of participants, the care physician of the participant would be notified and in case of the non-HIV group of participants, their GP would be informed. Members were satisfied with this response.

Members asked what guidelines would be followed if osteoporosis was detected in any of the younger participants as the current guidelines were of the older age group rather than the younger age group. You explained that most patients in the younger age group in the past had not needed any treatment and in some cases patients were prescribed calcium and vitamin D supplements. You also told the committee that you would be using the study to define guidelines for management of patients with osteoporosis in the younger age

group. Members accepted this as a reasonable explanation.

Members wanted to know if participants diagnosed with osteoporosis from the non-HIV group would be referred to their GP. Members did not think that this was appropriate, as the GPs may not know how to manage this group of participants. At the meeting, your supervisor, Dr Karen Walker-Bone agreed that these participants could be referred to her and at the same time the GP would be informed. Members agreed that this was a better solution.

Members felt that participants needed to be re-consented, as there was a year's time gap between the various tests. At the meeting, your supervisor explained that the participant's attendance could be considered as implied consent (as the participant would not keep the appointment if they wanted to withdraw from the study). Members accepted this explanation as a reasonable compromise.

At the meeting members wanted to know why you would be storing some of the blood samples. You explained that as the bone marker tests were expensive, it was possible that you would run out of funding to do these tests. You added that additional funding had been applied for and upon receipt of the additional funding, the bone marker tests would be carried out on the stored samples. At the meeting, you also told the committee that you would also be storing blood samples for future projects and that ethics approval will be obtained at that stage for the project. Members accepted your explanation.

Members agreed that it was acceptable to inform the GP only if an abnormality was detected.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

Please submit final versions of the information sheets and consent form to the committee for information only along with a covering letter. While sending the documents to the

committee please make sure that you enclose a copy of the relevant document showing the tracked changes and a copy of the same document in its final format

Amendments requested:

1 It was noted that if participants withdrew from the study, their data collected before their withdrawal would still be used. At the meeting, it was noted that you wanted to retain this data because recruitment was difficult and in addition, this data would prove to be useful. But members were of the view that the data should be removed if the participant withdrew and requested removal of their data. You agreed that the data would be removed and not used, if the participant specifically asked for their data to be removed. Please confirm in writing.

2 It needs to be made clear in the participant information sheet why and for what purpose the blood will be stored.

3. Rename the "patient information sheet" to read "participant information sheet".

4. Members felt that the font size of the information sheet needed to be increased. Members were of the view that it may be preferable to use the information sheet in the regular portrait format rather than in a leaflet format. Please amend appropriately and resubmit.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering Letter		10 September 2009
REC application		10 September 2009
Protocol	1.0	24 July 2009
Investigator CV		17 August 2009
CV of supervisor, Dr Karen-Walker-Bone		25 August 2009
Validated questionnaire	1.0 (Appendix 1)	11 September 2009
Participant Information Sheet	1.0 (appendix 3)	24 July 2009
Participant Consent Form	1.0 (appendix 4)	24 July 2009
Letter from Dr Samarawickrama about peer review		03 September 2009
Letter from statistician		03 September 2009
Study design	1.0 (appendix 2)	24 July 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Conflict declared by Dr Stuart White and Mr Bill Kent, but as both members did not have any personal or professional interest in the study it was agreed that they could take part in the review of the study. There were no declarations of conflicts of interests from any of the other members present.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1107/101	Please quote this number on all correspondence
--------------	--

With the Committee's best wishes for the success of this project

Yours sincerely



Dr Paul Seddon
Chair

Email: nischinth.cherodian@bhcpct.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

"After ethical review – guidance for researchers"

Copy to: *Scott Harfield
R&D Manager
Brighton and Sussex University Hospitals
Eastern Road
Brighton, BN2 5BE
(Sponsor and R&D office at lead site)*

Brighton East Research Ethics Committee

Attendance at Committee meeting on 01 October 2009

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Mrs Rosemary Bolinger	Charity Worker	No	
Ms Sue Eckstein	Lecturer in Clinical and Biomedical Ethics	Yes	
Prof Angie Hart	Professor of Child, Family & Community Health	No	
Dr Rachael James	Consultant Cardiologist	No	
Dr Puneet Kakar	Specialist Registrar in Medicine & Geriatrics	Yes	
Mr Bill Kent	Retired Solicitor	Yes	*conflict declared
Mr Maurice Marchant	Lay member/Statistician	Yes	
Ms Nicola Mason	Specialist Midwife - Practice Development	Yes	
Dr Martin Parry	Consultant Paediatric Anaesthetist	No	
Mr Ian Parsons	Supervisor - Sterile Services (training)	Yes	
Dr Paul Seddon	Consultant Paediatrician	No	
Mr Christopher Snowling	Retired Solicitor	Yes	
Ms Kathy Stott	Pharmacist	Yes	
Dr Simon Walton	Consultant Anaesthetist	Yes	In the chair
Dr Stuart White	Consultant Anaesthetist	Yes	*conflict declared
Ms Vanessa Wright	Nurse Consultant	No	
Ms Debra Young	Head of Midwifery	No	

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Mrs Nischinth Cherodian	Administrator

*Conflict declared by Dr Stuart White and Mr Bill Kent, but as both members did not have any personal or professional interest in the study, it was agreed that they could take part in the review of the study.

10.3.2 Letter of invitation for participants of the pilot study



Brighton and Sussex 
University Hospitals

NHS Trust

Clinical Director: Dr Steve Holt
Director of Research: Professor Kevin Davies
Manager: Mr Scott Harfield
Lead Research Nurse: Sr Victoria Sellick
Senior Research Nurse: Wendy Harman
Research Methodologist: David Crook PhD

Clinical Investigation & Research Unit (CIRU)
Level 5, The Royal Sussex County Hospital
Eastern Road, Brighton, BN2 5BE
Tel: 01273 696955 ext 3522/3528
Fax: 01273 664855
www.bsuh.nhs.uk/research/

Pilot Study Participants' Letter of Invitation

Date

Dear Mr _____

Invitation to participate in the Bone Study

As a patient who took part in the pilot Bone Study investigating bone thinning (osteoporosis) in men, we are writing to invite you to take part in the main study which is now recruiting. It is similar to the pilot study, in that it involves having a whole body DXA scan, as well as a peripheral DXA scan. We are interested in looking at risk factors that may specifically affect men. The results of the study may affect how osteoporosis is managed in the future, so you may gain a direct benefit from taking part.

This study will take place over 3 years, and will involve one additional visit each year for 3 years (i.e. 3 additional visits in total). At each visit, you will be asked to complete a questionnaire (similar to the one you completed for the pilot study) and be examined. We will also ask if we can take blood and urine tests, and the DXA scans. The good news is that the whole body DXA scan will take place at the same place and time as the peripheral DXA scan, so there is no need for an additional visit.

We will contact you in the next week to see if you want to take part in the study. If you decide you do not want to take part, your care at the Lawson Unit will not be affected. If you are keen to take part, or want to find out more about the study, please contact me on 01273 696955 x 3902 or by email on amanda.samarawickrama@bsuh.nhs.uk.

Thank you very much.

Yours sincerely

Dr Amanda Samarawickrama
Clinical Research Fellow and Specialist Registrar
Clinical Investigation and Research Unit (CIRU)
Brighton and Sussex University Hospitals
Tel: 01273 696955 x 3902

Version 1.0

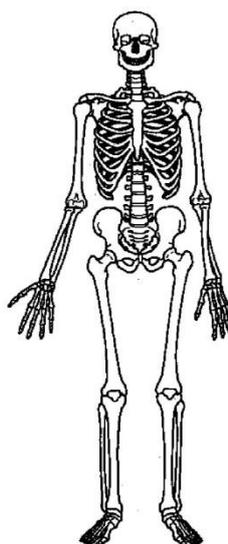
18/12/09

10.3.3 Participant information sheet



Clinical Investigation and Research Unit
Royal Sussex County Hospital
Eastern Road, Brighton, BN2 5BE
Phone: 01273 696955 ext 3522
Fax: 01273 664855
www.bsms.ac.uk/research/ciru

Screening for Bone Health in Men Participant Information Sheet



We would like to invite you to take part in a screening project for low bone mineral density (osteoporosis) run by the Lawson Unit, the Claude Nicol Clinic and the Clinical Investigation Research Unit (CIRU). Before you decide we need you to understand why the research is being done and what it would involve. Talk to others about the study if you wish.

What is osteoporosis?

Osteoporosis means porous bones. It is the so-called 'silent disease' which causes bones to become thin and fracture more easily later in life. In men, osteoporosis remains under diagnosed, underreported, and inadequately researched.

Risk factors for osteoporosis in men

- Prolonged exposure to certain medications, such as steroids used to treat asthma or arthritis, anticonvulsants, certain cancer treatments and aluminium-containing antacids
- Chronic diseases that affects the kidneys, lungs, stomach and intestines, and alters hormone levels
- Undiagnosed low levels of the sex hormone testosterone
- Lifestyle habits:
 - Heavy cigarette smoking
 - Excessive alcohol use
 - Low calcium intake
 - Inadequate physical exercise (which can cause increased body fat)
- Age: bone loss increases with age
- Family history
- Race: of all men, white men appear to be at greatest risk for osteoporosis. However, men from all ethnic groups can develop osteoporosis.

Osteoporosis and HIV infection

People with HIV infection are at greater risk of osteoporosis compared to the general population. This is believed to be the result of the virus itself and through associations with low body weight, low testosterone levels, smoking and alcohol intake. There may also be a role played by antiretroviral medication.

How is osteoporosis diagnosed?

The diagnosis of osteoporosis in men is often overlooked. A physician should firstly assess an individual's risk of osteoporosis by asking relevant questions about risk factors and conducting a complete physical exam, including height, weight, x-rays, and blood and urine tests.

The 'gold standard' test is called a Bone Mineral Densitometry or DXA scan, a special type of very low dose x-rays that can diagnose osteoporosis, and also measure body fat and muscle mass.

Treatment and prevention

Medical research on osteoporosis in men has been inadequate especially in association with HIV infection. However, experts agree that the following steps can preserve bone health and reduce fracture risk in men:

- Recognise and treat any underlying medical conditions that affect bone health
- Change unhealthy habits, such as smoking, excessive alcohol intake, and inactivity
- Ensure adequate calcium and vitamin D intake.

Can medications slow or stop bone loss in men?

A class of drugs called bisphosphonates can be used to stop or slow bone loss. Alendronate (brand name Fosamax[®]), risedronate (brand name Actonel[®]) and ibandronate (brand name Bonviva[®]) are common examples. Another option is for men at risk of osteoporosis to take calcium and vitamin D supplements. Testosterone supplements may be helpful if low testosterone is diagnosed.

What is the purpose of the study?

We want to find out if there is a relationship between HIV infection and osteoporosis. We want to follow-up men for 2 years to see how many have osteoporosis, and how quickly bone loss occurs. We want to identify those HIV positive men who are at risk of osteoporosis and those who may benefit from treatment to reduce fracture risk.

We are also interested in comparing results from a portable DXA scan of the wrist to the gold standard whole body DXA scan. If the results match sufficiently, the plan is to offer the portable screening test to more HIV positive men as part of routine care.

To answer these questions, we are interested in different groups of men:

- Men with new HIV infection
- Men who are known to be HIV positive but have not yet started antiretroviral medication
- Men who are known to be HIV positive and have started antiretroviral medication.

As we are especially interested in whether the HIV infection itself is linked to osteoporosis, we want to compare the results of men with new HIV infection with HIV negative men. This will form a small subgroup of the study.

Why have I been invited?

You are being invited to take part in this study because you are male, HIV positive or negative and over 18 years of age. We are offering the screening test to 500 men to get meaningful results, and you are one of these.

What is expected from participants?

If you agree to participate in this study, we would ask you to attend for 3 sets of appointments over 2 years, each set 1 year apart. If you are HIV positive and you start antiretroviral treatment during the study and your last study appointment was more than 6 months previously, we would ask you to attend for 1 extra set of appointments, so you would have 4 sets of appointments in total.

For each set of appointments, we would ask you to attend a research appointment and a 'gold standard' whole body DXA scan appointment in CIRU.

During the research appointment we would take your consent (first appointment only), measure your height, weight and blood pressure, and perform a 5 minute DXA scan of your wrist. If you agree, we would take samples of fasted blood and urine for conditions linked to osteoporosis. We will check routine blood tests (e.g. kidney markers, glucose, lipids, calcium, phosphate, thyroid function, testosterone and vitamin D) as well as special tests (e.g. inflammatory and bone markers). We will check routine and special urine tests for signs of kidney damage. In HIV positive men, we will also check HIV markers (e.g. CD4 count and viral load). We will give you a questionnaire regarding risk factors for osteoporosis which takes 20 minutes to complete. You will be asked to complete this whilst you wait for your whole body DXA scan.

The whole body DXA scan appointment will take 20 minutes. During this we would take measurements of your hip, spine and forearm, as well as a total body scan to measure body fat and muscle mass.

Some of the blood and urine samples you have given will not be tested immediately, but stored and tested at a later date. Your samples may be stored after the study is completed and used for further research into osteoporosis. This is because newer and better tests for diagnosing osteoporosis may become available in the future. We will not do any testing after the study has ended without your consent.

What will happen with my results?

The results from the study will be analysed to calculate how many men have low bone mass and to assess what risk factors and conditions are most likely to cause osteoporosis. They may also help us to understand why osteoporosis is more common in those with HIV infection. The portable DXA scan results will be compared with the results of the 'gold standard' whole body DXA scan to check how good the wrist scan is at identifying osteoporosis.

The data may be used for presentations or publication. However you will not be identifiable from this. Your clinic doctor will inform you if this data is published.

Are there any benefits?

If we find that you have low bone mass you will be referred for specialist care as you may benefit from regular monitoring with DXA scans or specific treatment as mentioned above. However if we find you have normal bone mass we can reassure you. If you are HIV positive, your results will be copied into your clinic notes and may guide your future HIV treatment.

Are there any risks of the bone scans?

The DXA scans in this study are performed for research purposes and would not normally be done as routine practice. However, DXA scans are done daily throughout the UK for assessing bones. Although involving x-rays, the total radiation dose you will receive every year from participating in this project is approximately 3 days of natural background radiation for this part of the country. These doses are trivial and you can expect no consequence from them.

Will my taking part be confidential?

Yes, all information pertaining to you will be coded under your clinic number and date of birth, and will only be accessed by the doctors, nurses and administrative staff involved in the study. The data may need to be accessed by the sponsor of the project (Brighton and Sussex University Hospitals) and regulatory authorities. The data will be stored in a confidential manner in CIRU.

Will you inform my GP?

We will not inform your GP that you have taken part in the study unless you ask us to do so. But we may inform your GP, with your permission, if you have abnormal results.

How do I take part?

Your HIV clinic doctor or the doctor or nurse looking after you in the sexual health clinic will ask if you want to take part in the study. If you agree to take part, you will be given this leaflet to read and be asked to make an appointment in CIRU at a time that suits you. You will then attend CIRU for all your appointments, blood and urine tests and DXA scans.

What happens if I do not want to take part?

Please be assured that if you decide you do not want to take part it will not affect the care at the Lawson Unit or the Claude Nicol Clinic in any way. You are free to withdraw at any time, but your data to that point may be used, unless you ask us not to.

What if there is a problem?

Complaints: If you have concerns regarding this study please contact Dr Amanda Samarawickrama at CIRU, who will endeavour to answer your questions. If you remain unhappy and wish to formally complain, you can do so through the NHS complaints procedure.

Harm: In the unlikely event that something goes wrong and you are harmed during the research and this is due to someone's negligence, then you may have grounds for legal compensation against Brighton and Sussex University Hospitals NHS Trust.

Thank you for taking the time to read this leaflet. If you have any questions please do not hesitate to contact CIRU on 01273 696955 x 3522/3528 or Dr Amanda Samarawickrama on 01273 696955 x 3902. Independent advice is also available from Brighton and Sussex Patient Advice and Liaison Service via RSCH switch board 01273 696955 Ex 4588.

For further information about Osteoporosis and HIV please refer to AIDSmap or the National Osteoporosis Society www.nos.org.uk

10.3.4 Email invitation for patients attending the email clinic

Email Clinic Patients' Template Email Invitation

Dear Mr _____

The Lawson Unit is conducting a large study investigating bone thinning (osteoporosis) in HIV-infected men. We are interested in looking at risk factors that may specifically affect HIV-infected men. The results of the study may affect how osteoporosis is managed in the future, so you may gain a direct benefit from taking part.

When you signed on to the Connect Clinic, you agreed to be contacted if there were any suitable studies or clinical trials taking part in the Lawson Unit. You have been selected as being eligible to take part in the above study.

The study involves one additional visit each year for 3 years (i.e. 3 additional visits in total). At each visit, you will be asked to complete a questionnaire and be examined. We will also ask if we can take blood and urine tests, and offer special scans to look for osteoporosis.

Please reply to this email, even if you do not want to take part. If we have not heard from you in the next week, we will contact you to see if you want to take part in the study. If you decide you do not want to take part, your care at the Lawson Unit will not be affected.

If you are keen to take part, or want to find out more about the study, please contact me on 01273 696955 x 3902 or by email on amanda.samarawickrama@bsuh.nhs.uk.

Thank you very much.

Yours sincerely

Dr Amanda Samarawickrama
Clinical Research Fellow and Specialist Registrar
Clinical Investigation and Research Unit (CIRU)
Brighton and Sussex University Hospitals
Tel: 01273 696955 x 3902

10.3.5 Written consent form



Clinical Investigation and Research Unit
Royal Sussex County Hospital
Eastern Road, Brighton, BN2 5BE
Phone: 01273 696955 ext 3522
Fax: 01273 664855
www.bsms.ac.uk/research/ciru

Bone mineral status in HIV infection: screening, prevalence, risk factors and prognosis

Study Participant Consent Form

Name of Researcher: Dr Amanda Samarawickrama

Clinic Number: _____

Date of Birth: _____

Please
initial box

I confirm that I have read and understand the information sheet dated 18th December 2009 (Version 2.0) for the above study. I have had the opportunity to consider the information, ask questions, and have these answered satisfactorily.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

I understand that relevant sections of my clinic notes and data collected during the study may be looked at by individuals from regulatory authorities or from Brighton and Sussex University Hospitals NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

I agree to my blood and urine tests being stored for testing at a later date if necessary.

I agree to being referred to my GP or clinic doctor in the event of an abnormal investigation being discovered during this study.

I agree to my GP being contacted to obtain further details regarding my medications.

I agree to take part in the above study.

Name of Participant	Signature of Participant	Date
_____	_____	_____

Name of Researcher	Signature of Researcher	Date
_____	_____	_____

Version 2.0

18/12/09

10.4 Questionnaire



Brighton and Sussex 
University Hospitals
NHS Trust

Clinical Investigation and Research Unit
Royal Sussex County Hospital
Eastern Road, Brighton, BN2 5BE
Phone: 01273 696955 ext 3522
Fax: 01273 664855
www.bsms.ac.uk/research/ciru

Study No
Lawson Unit No -

Questionnaire: Factors that influence bone strength

This questionnaire is for patients
enrolled in the following study at the
Clinical Investigation and Research Unit
(CIRU)

Bone mineral status in HIV-infected
patients

INSTRUCTIONS FOR THIS QUESTIONNAIRE

The responses you give to questions asked in this survey enable us to assess your risk of osteoporosis (thin bones). Your answers will be used to see if it is possible to predict which HIV positive men attending the Lawson Unit may have started to develop thin bones and would therefore benefit from screening for osteoporosis. If you are HIV negative, your answers will be used to compare with those from HIV positive men.

People who have answered this questionnaire have found it takes about **20** minutes to complete.

Your responses are extremely valuable to us and will be handled in the strictest confidence. They will be recorded under your clinic number and date of birth and will not be identifiable by name.

Most of the questions may be answered by putting a tick in a box under, next to or in a column further along a line containing your answer. For example, if you wished to answer 'Yes' to the example question below, then tick the appropriate box like this:

Is this the first time you have been prescribed this medicine?	Yes	No
	<input checked="" type="checkbox"/>	<input type="checkbox"/>

In your response to some questions, it may be appropriate to tick more than one box. Please make sure you answer ALL the questions.

Should you make a mistake or change your mind after inserting a tick, then please simply blot out the 'wrong' box by putting a line through it and re-enter a tick in the correct box.

Please take time to read and answer each question carefully. If you are unsure about any of the questions or need any help, please ask to speak to the research Doctor or Nurse.

Should you have any other queries, please contact Dr Amanda Samarawickrama on 01273 696955 x 3902, by emailing on amanda.samarawickrama@bsuh.nhs.uk or ask in person when you attend your research appointment.

SECTION 1: Personal details

1 **Date of birth:** __/__/____

SECTION 2: This section is about any health problems or illnesses with which you have been diagnosed.

2 Have you ever been told by a doctor that you have any of the following medical conditions?

- | | | | | | | | |
|---|---|--|---------------------------------------|---|--|--|---------------------------------------|
| a | Diabetes | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | b | Low testosterone | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| c | Hyperthyroidism
(overactive thyroid) | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | d | Hypothyroidism
(underactive thyroid) | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| e | Hyperparathyroidism | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | f | Hypoparathyroidism | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| g | Kidney disease | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | h | Liver disease | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| i | Inflammatory bowel
disease | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | j | Coeliac disease | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| k | Depression | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | l | Anorexia nervosa | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| m | Rheumatoid arthritis | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | n | Problems with fat or lipid
levels in the blood (e.g.
high cholesterol) | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |

If you ticked 'Yes' to any of the above boxes or you are unsure if you suffer from any of the conditions listed above, please give details below.

Details:

SECTION 3: This section is about your bone health

3 Have you ever broken a bone (had a bone fracture)? **Yes** **No**

If '**No**', please go to question 10
 If '**Yes**', please answer questions 4 to 9

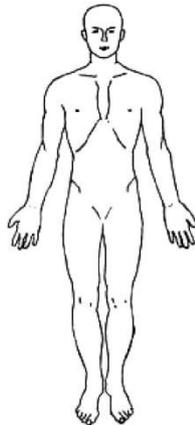
4 Did you receive medical attention for the broken bone? **Yes** **No**

5 Where did you receive this medical attention? **Brighton hospital** **Other hospital**

6 Was an x-ray taken of the broken bone? **Yes** **No**

7 What was the approximate date you broke your bone? (**Please state month/year** _____)

8 Please mark on the drawing below the location of the broken bone(s) and the approximate date of the broken bone at that site?



a
 M M Y Y Y Y

b
 M M Y Y Y Y

c
 M M Y Y Y Y

d
 M M Y Y Y Y

e
 M M Y Y Y Y

9 If you were told which bone you had broken, please give its name (**Please state** _____)

SECTION 4: This section covers medication that you are currently taking or have taken in the past

10 Are you currently taking OR have you ever taken any of the following medication?

- | | | | | | |
|--|--|---------------------------------------|--|--|---------------------------------------|
| a Steroid inhalers | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | b Steroid tablets | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| c Anabolic steroids | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | d Growth hormones | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| e Ketoconazole | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | f Chemotherapy | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| g Antidepressants | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | h Anticonvulsants | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| i Bendrofluazide | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | j Testosterone | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| k Calcium tablets | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | l Vitamin D supplements | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| m Bisphosphonates (e.g. alendronate/Fosamax [®] , risedronate/Actonel [®] , ibandronate/Bonviva [®]) | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | n Antacids containing aluminium (e.g. Maalox [®] , Nucogel [®] , Gastrocote [®]) | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| o Any other medication | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> | | | |

If you ticked 'Yes' to any of the above boxes please give details of the drugs you currently use or have used in the past.

Details:

- 11 I give my permission for the research team to contact my GP to check my medications **Yes** **No**

SECTION 5: This section is about your background, which includes any recreational drugs that you are currently taking or have taken in the past. This information will be treated in strict confidence and will NOT be entered into your clinic notes

- | | | | | | | | |
|----|---|---|---|--|--|--|---------------------------------------|
| 12 | Please indicate if you smoke tobacco | Never smoked
<input type="checkbox"/> | Used to smoke but stopped
<input type="checkbox"/> | Smoke less than 10 cigarettes/day
<input type="checkbox"/> | Smoke 10 to 20 cigarettes/day
<input type="checkbox"/> | Smoke more than 20 cigarettes/day
<input type="checkbox"/> | |
| 13 | Please indicate your average consumption of alcoholic beverages | Never drink
<input type="checkbox"/> | Drink less than one or one glass/day
<input type="checkbox"/> | Drink two glasses/day
<input type="checkbox"/> | Drink three glasses/day
<input type="checkbox"/> | Drink four or more glasses/day
<input type="checkbox"/> | |
| 14 | Have you ever used drugs for recreational purposes? | | | | | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| | <p style="margin-left: 40px;">If 'No', please go to question 18
 If 'Yes', please answer questions 15 to 17</p> | | | | | | |
| 15 | Have you ever regularly injected drugs? | | | | | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| 16 | Have you ever been in a methadone programme? | | | | | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| 17 | Are you currently taking methadone? | | | | | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |

If you ticked 'Yes' to any of the above boxes please give details of the drugs you currently use or have used in the past.

Details:

SECTION 6: This section is about your family background

- 18 Have you or a member of your family ever been directly affected by osteoporosis? **Yes** **No** **Don't Know**
- 19 Did your mother and/or your maternal grandmother ever suffer a hip fracture? **Yes** **No** **Don't Know**

SECTION 7: This section is about your diet

- 20 How often nowadays are you eating the following foods?

	Never or rarely	Once in 2 weeks	1-3 times a week	4-6 times a week	Once a day	More than once a day
a Oat cereals (e.g. porridge, Ready Brek, muesli etc.)	<input type="checkbox"/>					
b Other breakfast cereals (e.g. Rice Krispies, Special K, Weetabix, Shredded Wheat, Cornflakes, Shreddies, Cheerios etc.)	<input type="checkbox"/>					
c Tuna	<input type="checkbox"/>					
d Oily fish (e.g. pilchards, sardines, mackerel, herrings, kippers, trout, salmon etc.)	<input type="checkbox"/>					
e Eggs, quiche/flans, omelettes, etc.	<input type="checkbox"/>					
f Cheese	<input type="checkbox"/>					
g Pizza	<input type="checkbox"/>					
h Cabbage, brussels sprouts, spinach, broccoli and other dark green leafy vegetables	<input type="checkbox"/>					
i Tofu, tofu burgers, tofu sausages	<input type="checkbox"/>					
j Yoghurt, fromage frais, milk puddings (e.g. rice pudding), mousse	<input type="checkbox"/>					
k Soya yoghurts	<input type="checkbox"/>					

		Never or rarely	Once in 2 weeks	1-3 times a week	4-7 times a week	Once a day	More than once a day
l	Ice cream, choc ice etc.	<input type="checkbox"/>					
m	Custard, cream, Elmlea, evaporated milk on puddings, etc.	<input type="checkbox"/>					
n	Cakes or buns (e.g. fruit cake, sponge, teacake, doughnut, scone, custard tart, cream cake etc.)	<input type="checkbox"/>					
o	Full-coated chocolate biscuits (e.g. Kit Kat, Penguin etc.)	<input type="checkbox"/>					
p	Other biscuits (e.g. Rich Tea, shortcake, digestive, chocolate digestive, Nice etc.)	<input type="checkbox"/>					
q	Chocolate (milk or white) or chocolate bars (e.g. Mars, Snickers, etc.)	<input type="checkbox"/>					
r	Meal replacement bars or drinks	<input type="checkbox"/>					

If you eat or drink meal replacement bars or drinks (Question 20r), please give name and brand below.

Details:

21	What kind of milk do you use most often?	Full fat/ whole milk	Semi-skimmed milk	Skimmed milk	Goat/ sheep milk	Soya milk	Other milk
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

If you ticked 'Other milk', please give details below.

Details:

22	Do you have milk with breakfast cereal or in porridge?	Yes usually	Yes sometimes	No	Do not eat breakfast cereal or porridge
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

23 How often do you drink the following drinks?

- | | | Never
or
rarely | Once
in 2
weeks | 1-3
times
a
week | 4-7
times
a
week | Once a
day | More
than
once a
day |
|---|--|--------------------------------|--------------------------------|-------------------------------------|-------------------------------------|--------------------------|---|
| a | Milk on its own | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b | Flavoured milk drinks
(e.g. Horlicks, Ovaltine,
milkshakes) or yoghurt
drinks | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c | Cola drinks, Red Bull,
Lucozade, Dr Pepper | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
- 24 Do you normally choose decaffeinated fizzy drinks (e.g Coca cola, Red Bull, Dr Pepper)?
- | | Yes
usually | Yes
sometimes | No | Do not drink
fizzy drinks |
|--|--------------------------|--------------------------|--------------------------|--------------------------------------|
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
- 25 What type of bread do you eat most often?
- | | White
bread
(including
French
stick/
baguette) | Brown/
granary
bread | Wholemeal
bread | Chappatis
or pitta
bread | Naan
bread | Do not
eat
bread |
|--|---|-------------------------------------|----------------------------|---|--------------------------|---------------------------------|
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
- 26 How many pieces of bread, rolls or chappatis do you eat on a usual day?
- | | None or
less than 1 | 1-2 | 3-4 | 5 or
more |
|--|--------------------------------|--------------------------|--------------------------|--------------------------|
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
- 27 What sort of fat do you use most often?

**On bread or For frying
vegetables**

a	Butter, ghee, lard, solid cooking fat	Yes <input type="checkbox"/>	No <input type="checkbox"/>
b	Spreads and margarines: sunflower spread (e.g. Flora), olive oil spread (e.g. Bertolli), other spreads (e.g. Clover, I Can't Believe it's Not Butter), cholesterol-lowering spreads (e.g. Benecol, Flora Proactive), own-brand spreads	Yes <input type="checkbox"/>	No <input type="checkbox"/>
c	Sunflower oil, corn oil, soya oil, olive oil, other vegetable oil	Yes <input type="checkbox"/>	No <input type="checkbox"/>
d	Other	Yes <input type="checkbox"/>	No <input type="checkbox"/>

If you ticked 'Other' (Question 27d), please give details below.

Details:

- 28 Do you normally choose low-fat versions of spreads/butter on bread? **Always** **Sometimes** **No** **Do not use spreads**
- 29 How many slices of bread (or rolls) spread with fat do you eat each day (including shop bought sandwiches)? **(Please state exact number_____)**
- 30 How many cups of tea do you drink in a day (do NOT include herbal teas)? **(Please state exact number_____)**
- 31 How many cups of tea are decaffeinated? **Do not drink tea** **(Please state exact number_____)**
- 32 How much milk do you have in your tea? **Black** **Dash of milk** **Average amount of milk** **Milky tea** **Do not drink tea**
- 33 How many cups of coffee do you drink in a day? **(Please state exact number_____)**
- 34 How many cups of coffee are decaffeinated? **Do not drink coffee** **(Please state exact number_____)**
- 35 How much milk do you have in your coffee? **Black** **Dash of milk** **Average amount of milk** **Milky tea** **Do not drink coffee**
- 36 Do you take antacids or indigestion remedies regularly? **Yes** **No**

If you ticked 'Yes', please give name and brand below.

Details:

- 37 Do you take any kinds of supplements regularly? Yes No

If you ticked 'Yes', please give name and brand below.

Details:

SECTION 8: This section is about your mobility and any exercise you do

- 38 Do you have any problems walking? **Can walk without any problems** **Can walk but have some difficulties** **Can't walk without a walking aid** **Can't walk without someone else's help** **Can't walk at all**
- 39 Do you ever fall? Yes No
- 40 If you answered 'Yes' to Question 35, how many times have you fallen in the last year? **(Please state exact number _____)**
- 41 The National Osteoporosis Society recommends that you should walk for 30 minutes 3 times a week. Do you do this? **Never** **Some weeks** **Most Weeks** **Every week**
- 42 Do you do any weight-bearing exercise (e.g. jogging, running)? **Never** **Some weeks** **Most Weeks** **Every week**
- 43 Do you do any muscle toning exercise (e.g. pilates, weight training, yoga)? **Never** **Some weeks** **Most Weeks** **Every week**

SECTION 9: In this section you are invited to make any comments that you feel may be relevant to the questions you have just answered

THANK YOU VERY MUCH FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE

10.5 DXA reports

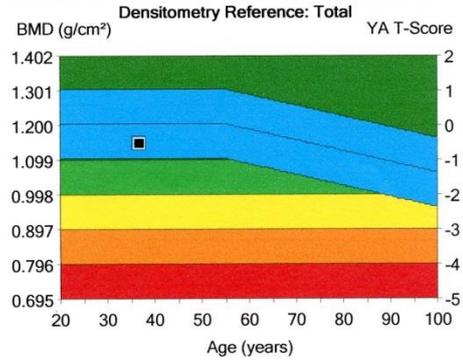
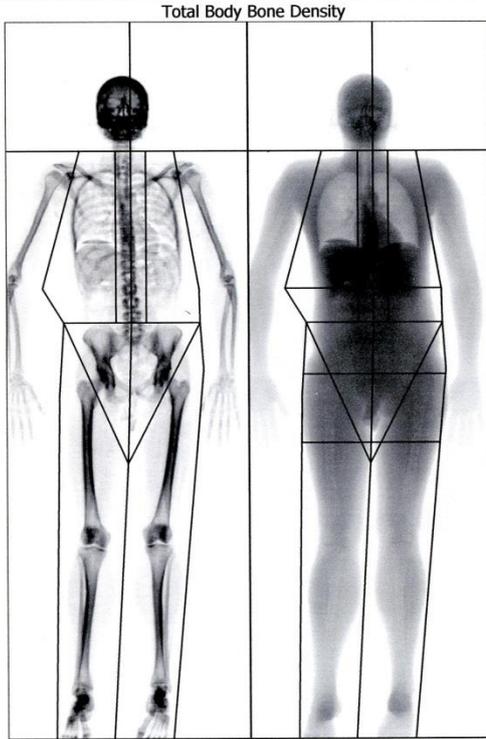
10.5.1 cDXA results printout

10.5.2 pDXA results printout

10.5.1 cDXA results printout

Brighton County Hospital
Research Unit

Patient: ██████████	Facility ID:
Birth Date: ██████████ 36.8 years	Referring Physician: WALKER-BONE KE
Height / Weight: 174.4 cm 78.9 kg	Measured: 20/09/2010 10:14:07 (11.40)
Sex / Ethnic: Male White	Analyzed: 20/09/2010 10:53:03 (11.40)



(e) - Estimated	¹ BMD (g/cm ²)	² Young-Adult T-Score	³ Age-Matched Z-Score
Region			
Head	2.036	-	-
(e) Arms	0.767	-	-
(e) Legs	1.306	-	-
(e) Trunk	0.976	-	-
(e) Ribs	0.881	-	-
(e) Pelvis	1.022	-	-
Spine	1.014	-	-
(e) Total	1.147	-0.5	-0.6

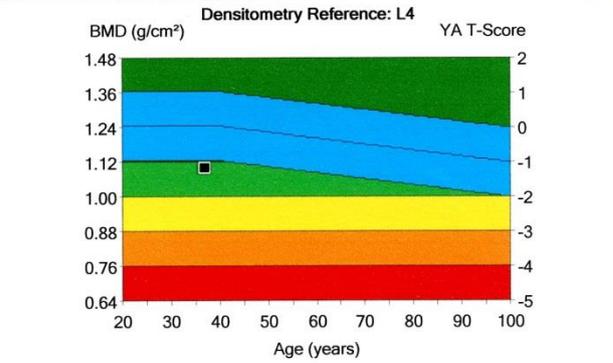
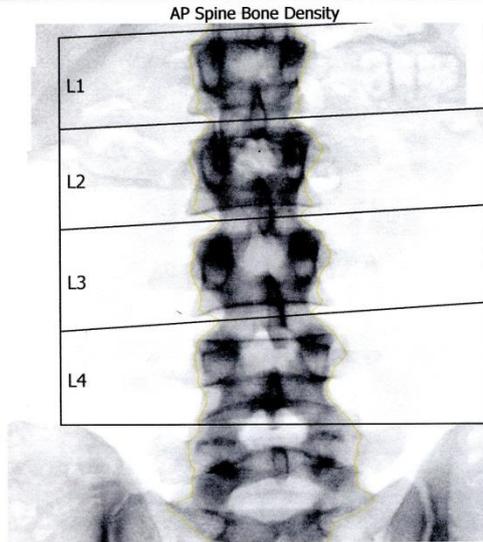
COMMENTS:

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 Printed: 20/09/2010 11:01:17 (11.40)100:0.19:153.85:15.6 0.00:-1.00
 2.40x3.04 11.8:%Fat=30.3%
 0.00:0.00 0.00:0.00
 Filename: 61g19f4aj5.mex
 Scan Mode: Standard 3.0 µGy

1 - Statistically 68% of repeat scans fall within 1SD (± 0.010 g/cm² for Total Body Total)
 2 - Custom UK Total Body Reference Population (v0)
 3 - Matched for Age, Weight (males 25-100 kg), Ethnic

Brighton County Hospital Research Unit

Patient: ██████████ Birth Date: ██████████ 36.8 years Height / Weight: 174.4 cm 78.9 kg Sex / Ethnic: Male White	Facility ID: ██████████ Referring Physician: WALKER-BONE KE Measured: 20/09/2010 10:17:47 (11.40) Analyzed: 20/09/2010 10:53:03 (11.40)
---	--



Region	BMD ¹ (g/cm ²)	Young-Adult ² T-Score	Age-Matched ³ Z-Score
L1	1.159	0.0	0.0
L2	1.235	0.0	-0.1
L3	1.245	0.0	0.0
L4	1.097	-1.2	-1.2
L1-L4	1.180	-0.3	-0.4

COMMENTS:

Image not for diagnosis
 Printed: 20/09/2010 11:01:15 (11.40)100:2.50:50.00:6.0 0.00:8.10 0.30x0.25
 20.9:%Fat=23.4%
 0.00:0.00 0.00:0.00
 Verify bone is centered and there is sufficient tissue next to bone.
 Filename: 61g19f4aj5.mex
 Scan Mode: Standard 146.0 µGy

1 - Statistically 68% of repeat scans fall within 1SD (± 0.030 g/cm² for AP Spine L4)
 2 - Custom UK AP Spine Reference Population (v0)
 3 - Matched for Age, Weight (males 25-100 kg), Ethnic
 11 - World Health Organization - Definition of Osteoporosis and Osteopenia for Caucasian Women:
 Normal = T-Score at or above -1.0 SD; Osteopenia = T-Score between -1.0 and -2.5 SD;
 Osteoporosis = T-Score at or below -2.5 SD; (WHO definitions only apply when a young healthy Caucasian Women reference database is used to determine T-Scores.)

Brighton County Hospital Research Unit

Patient: ██████████	Facility ID: ██████████	
Birth Date: ██████████ 36.8 years	Referring Physician: WALKER-BONE KE	
Height / Weight: 174.4 cm 78.9 kg	Measured: 20/09/2010 10:22:44 (11.40)	
Sex / Ethnic: Male White	Analyzed: 20/09/2010 10:49:38 (11.40)	

DualFemur Bone Density

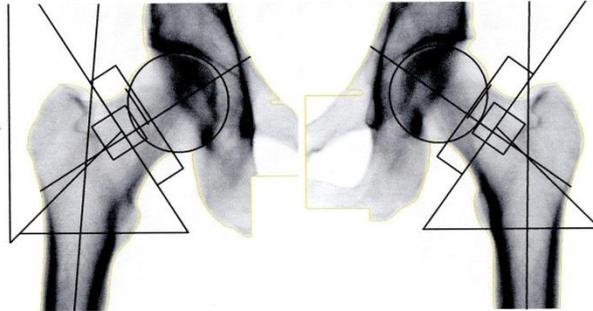
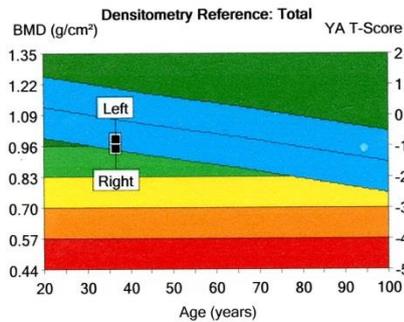


Image not for diagnosis



HAL chart results unavailable

Region	1 BMD (g/cm ²)	2,7 Young-Adult T-Score	3 Age-Matched Z-Score
Neck			
Left	0.939	-1.0	-0.8
Right	0.936	-1.0	-0.8
Mean	0.937	-1.0	-0.8
Difference	0.004	0.0	0.0
Total			
Left	0.990	-0.8	-0.6
Right	0.948	-1.1	-1.0
Mean	0.969	-0.9	-0.8
Difference	0.043	0.3	0.3

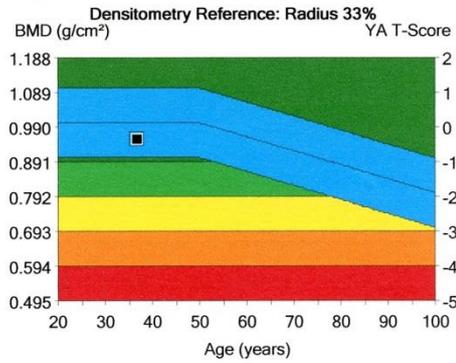
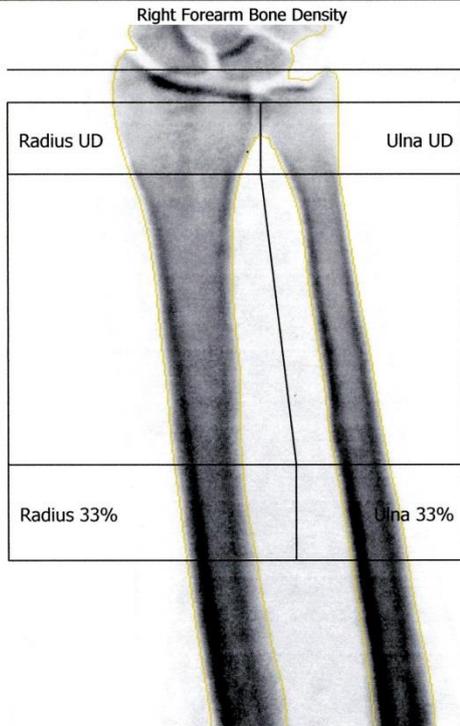
COMMENTS:

- 1 - Statistically 68% of repeat scans fall within 1SD (± 0.010 g/cm² for DualFemur Total Mean)
- 2 - Custom UK Femur Reference Population (v0)
- 3 - Matched for Age, Weight (males 25-100 kg), Ethnic
- 7 - DualFemur Total Mean T-Score difference is 0.3. Asymmetry is None.
- 11 - World Health Organization - Definition of Osteoporosis and Osteopenia for Caucasian Women: Normal = T-Score at or above -1.0 SD; Osteopenia = T-Score between -1.0 and -2.5 SD; Osteoporosis = T-Score at or below -2.5 SD; (WHO definitions only apply when a young healthy Caucasian Women reference database is used to determine T-Scores.)

Printed: 20/09/2010 11:01:20 (11.40); Filename: 61g1914aj5.mex; Right Femur; 16.7:%Fat=21.7%; Neck Angle (deg)= 57; Scan Mode: Standard 146.0 μ Gy; Left Femur; 17.2:%Fat=21.9%; Neck Angle (deg)= 54; Scan Mode: Standard 146.0 μ Gy

Brighton County Hospital Research Unit

Patient: ██████████ Birth Date: ██████████ 36.8 years Height / Weight: 174.4 cm 78.9 kg Sex / Ethnic: Male White	Facility ID: ██████████ Referring Physician: WALKER-BONE KE Measured: 20/09/2010 10:30:15 (11.40) Analyzed: 20/09/2010 10:40:03 (11.40)
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Region	¹ BMD (g/cm ²)	² Young-Adult T-Score	³ Age-Matched Z-Score
Radius UD	0.499	-0.4	-0.4
Ulna UD	0.403	-	-
Radius 33%	0.954	-0.4	-0.4
Ulna 33%	1.017	-	-
Both UD	0.466	-	-
Both 33%	0.982	-	-
Radius Total	0.739	-0.2	-0.2
Ulna Total	0.773	-	-
Both Total	0.752	-	-

COMMENTS:

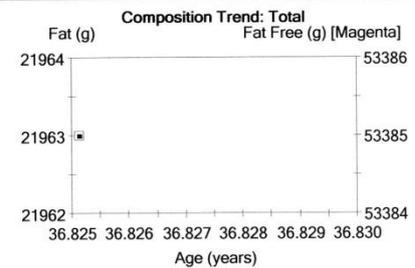
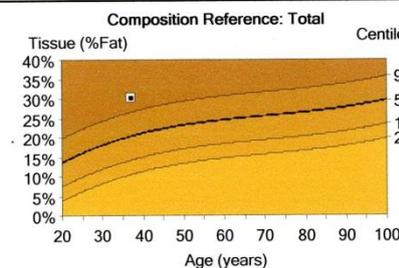
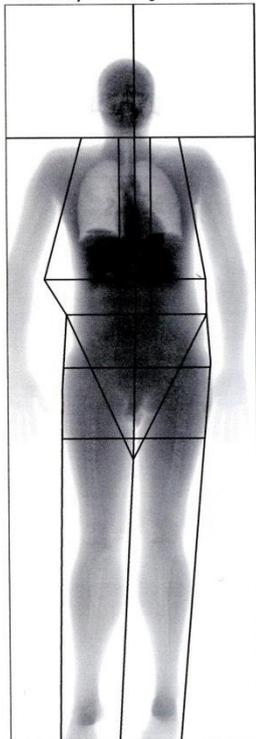
Image not for diagnosis
 Printed: 20/09/2010 11:04:34 (11.40)100:0.19:50.00:6.0 0.00:6.20 0.30x0.25
 6.3:%Fat=24.8%
 0.00:0.00 0.00:0.00
 Forearm Length: 28.0 cm
 Filename: iyg1914aj5.meas
 Scan Mode: Standard;Not seated 10.0 µGy

- 1 - Statistically 68% of repeat scans fall within 1SD (± 0.020 g/cm² for Right Forearm Radius 33%)
- 2 - Custom UK Forearm Reference Population (v0)
- 3 - Matched for Age, Ethnic
- 9 - Lunar calibration in use.
- 11 - World Health Organization - Definition of Osteoporosis and Osteopenia for Caucasian Women:
 Normal = T-Score at or above -1.0 SD; Osteopenia = T-Score between -1.0 and -2.5 SD;
 Osteoporosis = T-Score at or below -2.5 SD; (WHO definitions only apply when a young healthy Caucasian Women reference database is used to determine T-Scores.)

Brighton County Hospital Research Unit

Patient: ██████████	Facility ID: ██████████
Birth Date: ██████████ 36.8 years	Referring Physician: WALKER-BONE KE
Height / Weight: 174.4 cm 78.9 kg	Measured: 20/09/2010 10:14:07 (11.40)
Sex / Ethnic: Male White	Analyzed: 20/09/2010 10:53:03 (11.40)

Total Body Tissue Quantitation



Trend: Total

Measured Date	Age (years)	Tissue ¹ (%Fat)	Centile ^{2,3}	Total Mass (kg)	Region (%Fat)	Tissue ¹ (g)	Fat ¹ (g)	Lean ¹ (g)	BMC (g)	Fat Free (g)
20/09/2010	36.8	30.3	98	75.3	29.1	72,555	21,963	50,593	2,792	53,385

Trend: Fat Distribution

Measured Date	Age (years)	Android (%Fat)	Gynoid (%Fat)	A/G Ratio	Total Body ¹ (%Fat)
20/09/2010	36.8	40.9	35.9	1.14	30.3

COMMENTS:

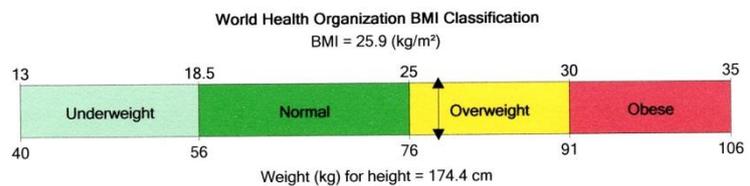


Image not for diagnosis
 Printed: 20/09/2010 11:01:19 (11.40)100:0.19:153.85:15.6 0.00:-1.00 2.40x3.04
 11.8:%Fat=30.3%
 0.00:0.00 0.00:0.00
 Filename: 61g19f4aj5.mex
 Scan Mode: Standard 3.0 µGy

1 - Statistically 68% of repeat scans fall within 1SD (± 0.4 % Fat, ±150 g Tissue Mass, ±280 g Fat Mass, ±310 g Lean Mass for Total Body Total)
 2 - Custom UK Total Body Composition Reference Population (v0)
 3 - Composition Matched for Age

Brighton County Hospital Research Unit

Patient: ██████████	Facility ID:
Birth Date: ██████████ 36.8 years	Referring Physician: WALKER-BONE KE
Height / Weight: 174.4 cm 78.9 kg	Measured: 20/09/2010 10:14:07 (11.40)
Sex / Ethnic: Male White	Analyzed: 20/09/2010 10:53:03 (11.40)

BODY COMPOSITION

(e) - Estimated Region	Tissue ¹ (%Fat)	Region (%Fat)	Tissue ¹ (g)	Fat ¹ (g)	Lean ¹ (g)	BMC (g)	Total Mass (kg)
Left Arm	26.3	25.1	4,442	1,170	3,272	215	4.7
Left Leg	31.7	30.3	12,545	3,980	8,564	581	13.1
Left Trunk	31.4	30.8	17,098	5,375	11,724	333	17.4
Left Total	30.5	29.4	35,447	10,814	24,633	1,283	36.7
(e) Right Arm	26.3	25.1	4,442	1,170	3,272	215	4.7
(e) Right Leg	31.7	30.3	12,545	3,980	8,564	581	13.1
(e) Right Trunk	31.3	30.6	17,317	5,418	11,899	407	17.7
(e) Right Total	30.0	28.9	37,108	11,149	25,959	1,510	38.6
(e) Arms	26.3	25.1	8,883	2,340	6,543	429	9.3
(e) Legs	31.7	30.3	25,090	7,961	17,129	1,161	26.3
(e) Trunk	31.4	30.7	34,415	10,792	23,623	740	35.2
(e) Android	40.9	40.5	5,294	2,164	3,130	51	5.3
(e) Gynoid	35.9	35.1	11,886	4,264	7,622	278	12.2
(e) Total	30.3	29.1	72,555	21,963	50,593	2,792	75.3

FAT MASS RATIOS

Trunk/ Total	Legs/ Total	(Arms+Legs)/ Trunk
0.49	0.36	0.95

1 -Statistically 68% of repeat scans fall within 1SD (± 0.4 % Fat, ±150 g Tissue Mass, ±280 g Fat Mass, ±310 g Lean Mass for Total Body Total)
Filename: 61g19k4aj5.mex

10.5.2 pDXA results printout

CIRU LUNAR PIXI
 BRIGHTON AND SUSSEX UNIVERSITY HOSPITALS
PIXI BONE DENSITY REPORT

Patient :		LEFT FOREARM
Patient ID :	██████████	
Date of Birth :	██████████	
180.70cm 82.80kg	White Male	
Physician :	AS	Acquired : 29/03/2012
Referring Physician :	AS	Analyzed : 29/03/2012 (2.10)
		Printed : 29/03/2012

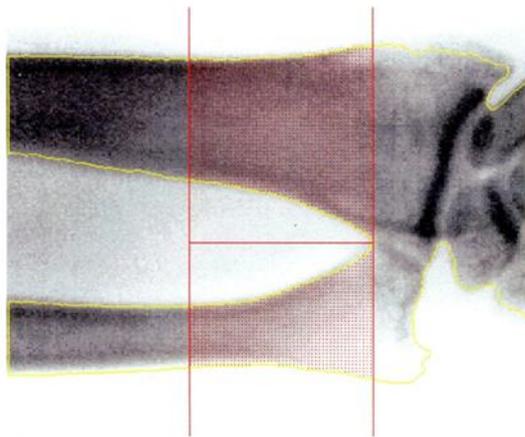
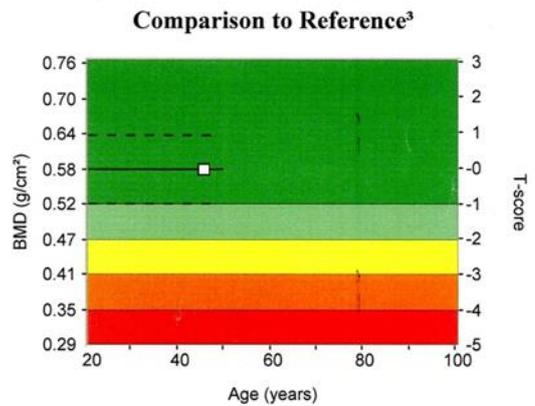


IMAGE NOT FOR DIAGNOSIS



Left Forearm BMD¹	0.584 g/cm²
Percent of Young Adult ²	99.9 %
T-score of Young Adult²	-0.0
Percent of Age-Matched ¹¹	99.9 %
Z-score of Age-Matched¹¹	-0.0

1 - Precision error(1 SD = 0.01 g/cm² or CV = 2%). Statistically 68% of repeat scans will fall within ±1 SD. (see appendix A)
 2 - England Forearm Reference Population, Young Adult Ages 20-50.
 3 - WHO has defined for white women that: >-1.0 SD = normal; -1.0 to -2.5 SD = osteopenia; <-2.5 SD = osteoporosis.
 11 - Matched for Age.

Comments :

Filename : 03-29-12 08'51'26.img

LUNAR® PIXI # 51362

10.6 Protocol for optimisation check of bone marker assays

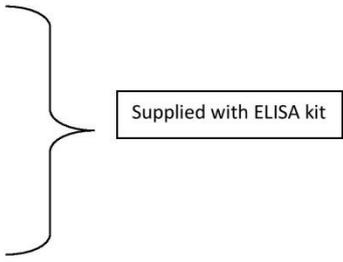
10.6 Protocol for optimisation check of BTM assays

P1NP

Sample collection

- A 6ml serum separator vacuette tube is drawn before any other tube in sequence
- Pre-label the aliquot cryovial with Test, Patient Study Number, Fluid Type, Time Point, M-No, DOB and Date
- Allow the serum to clot, standing upright, for 30 minutes minimum
- Centrifuge the tubes at 3500rpm for 15 minutes
- Carefully remove the lid from the vacuette, breaking the vacuum, but do not disturb the layer division
- Aspirate the serum layer from the tube
- Aliquot 1ml into the appropriate cryovial
- Freeze the cryovial immediately at -20°C and then when storage box is full transfer into -80°C.

Equipment and materials

- Standard
 - Standard dilutant
 - Detection reagent A (Green)
 - Detection reagent B (Red)
 - Assay diluent A
 - Assay diluent B
 - TMB substrate
 - Stop solution
 - Wash buffer
 - Deionised water
 - Calibrated single and multichannel pipettes (1ul-1000ul) with disposable tips
 - Absorbent paper
 - Eppendoff tubes (2ml)
 - Falcon tubes (15ml)
 - Microplate reader with 450±10nm filter
- 
- Supplied with ELISA kit

Reagent preparation

*****Bring all components to room temperature before working*****

*****Serum should be brought to room temperature slowly to prevent random agglutination of samples; first defrosted in the fridge for approximately 30-45 minutes, then on the bench*****

*****Serial dilution in well is not permitted in pre-coated plate*****

*****Reconstituted, standard, detection reagent A and detection reagent B can only be used once*****

*****Standard should be prepared maximum of 15minutes before the assay*****

*****Mix all reagents gently and do not foam to prevent imprecision in pipetting*****

Standard

Reconstitute the standard from the anhydrous form to the working standard with 1.0ml of standard dilutant.

The concentration of the working standard is 4000pg/ml.

First concentration of the standard curve is the reconstituted standard. Then perform a 4x serial dilution in 6 subsequent tubes to cut the dilution by half each time and in the final tube which is blank with 2ml of standard dilutant.

- Pipette 1750ul of standard dilutant into Eppendorff number 1
- Pipette 1000ul of standard dilutant in 6 Eppendorffs labelled with sequential standard dilutions and one blank
- Remove 250ul from the stock standard (4000pg/ml) and place in first Eppendorff and mix, making this 1000pg/ml
- Remove a subsequent 1000ul from the first Eppendorff (1000pg/ml) and place in third tube and mix, making this 500pg/ml
- Repeat the process until the 6th tube, and where after mixing, take off 1000ul and discard, leaving Eppendorff 7 blank with only standard dilutant
- This leaves 7 stock standard tubes with 1000ul volumes at subsequently halving concentrations and one blank (Figure1).

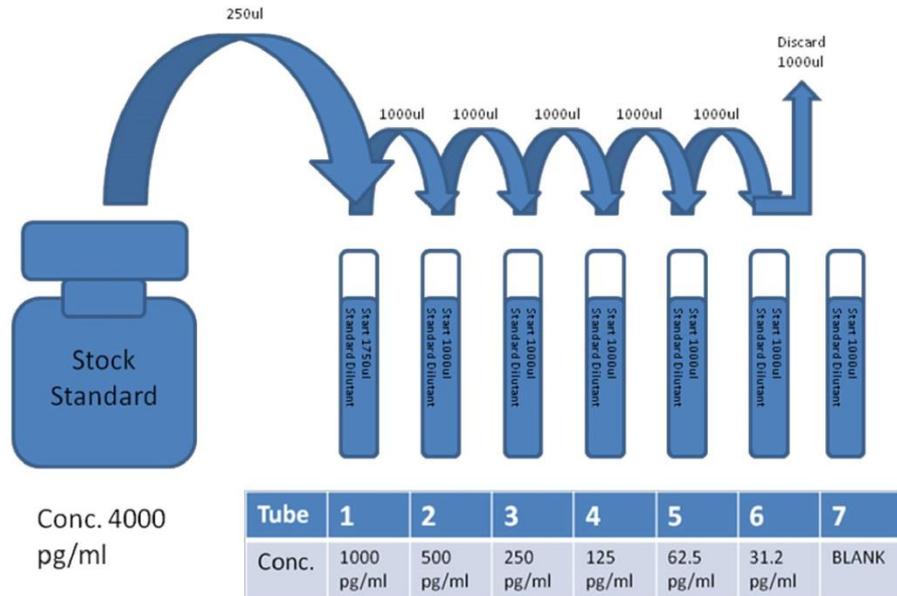


Figure1

Assay dilutant

Assay dilutants A and B in the kit is 2x concentrated and must be diluted first with deionised water before being used to dilute detection reagents A and B.

- Combine 6ml of assay dilutant A with 6ml of deionised water in a 15ml falcon tube
- Repeat for assay dilutant B
- Ensure diluents are labelled appropriately.

Detection reagent

Detection reagents A and B are diluted using the working concentrations (diluted forms) of assay dilutants A and B.

- First micro-centrifuge Eppendorffs containing detection reagents A and B at 500g for 1minute using full brakes
- Pipette 120ul of detection reagent A into the previously prepared 12ml of assay dilutant A in the 15ml falcon tubes
- Repeat this step for assay dilutant B.

Wash solution

Wash solution is 30x concentrated.

- Dilute 20ml of wash solution with 580ml of deionised water.

TMB substrate and stop solution

TMB substrate and stop solution are already at working concentrations. They need to be protected from light until use, when they can be decanted into multichannel pipetting troughs.

Assay procedure (Figure 2)

- Determine wells for the diluted standard, blank and sample. See **ELISA plate organisation**
- Add 100ul of dilution/standard/sample to the appropriate wells
- Cover with the plate sealer and incubate for 2 hours at 37°C
- Aspirate the liquid from each well. **DO NOT WASH**
- Add 100ul of detection reagent A to each well
- Cover with the plate sealer and incubate for 1 hour at 37°C
- Aspirate the liquid from each well, and wash with 350ul of wash solution to each well and leave to sit for 1-2 minutes, then remove from the remaining wells from snapping the plate on absorbent paper, **REPEAT 3 TIMES**
- Add 100ul of detection reagent B to each well
- Cover with the plate sealer and incubate for 30 minutes at 37°C
- Aspirate the liquid from each well, and wash with 350ul of wash solution to each well and leave to sit for 1-2 minutes, then remove from the remaining wells by snapping the plate on absorbent paper, **REPEAT 5 TIMES**
- Add 90ul of substrate solution to each well and the liquid will turn blue
- Cover with the plate sealer and incubate for 25-30 minutes at 37°C depending on development, **DO NOT EXCEED 30 MINUTES**
- Add 50ul of stop solution and the liquid will turn yellow; then mix the solution on the plate shaker at low intensity
- Remove water or finger prints from the bottom of the plate and run on HIDEEX.

**Flow chart : Order of work
for running ELISA P1NP kits**

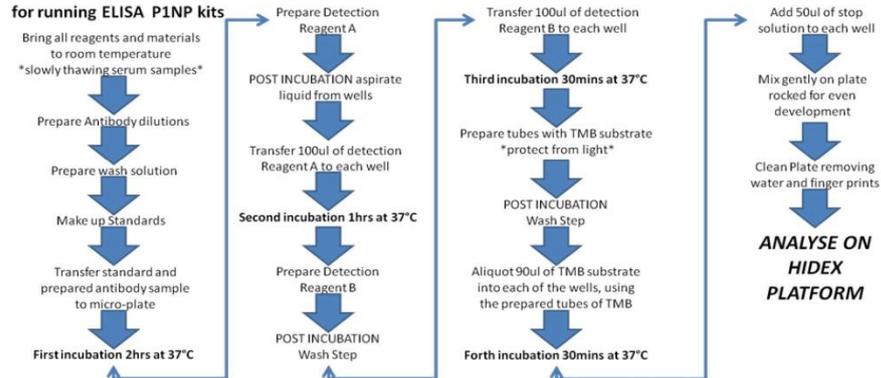


Figure 2

Running on the HIDEX

Sample and run on the HIDEX using MicroWin2000 software to interact with the plate reader. Set up is designated to read absorption at 450nm±10nm and output the optical density readings for each well in triplicate. The file is then output as a text rich file that can be imported into Excel for preliminary data analysis.

ELISA plate organisation – optimisation P1NP (Figure 3)

It is necessary to try to predict the concentration of antibody before running the assay, i.e. to ensure the values are within the range of the standard dilution curve. Optimisation is required to determine optimal sample concentrations.

To optimise the plate, dilution of the antibody is required and a variety of samples from normal healthy donors to experimental samples to determine assay interaction and optimal concentration. All samples are diluted in 0.02mol/l PBS (pH=7.0-7.2) which is made up using anhydrous tablets and deionised water.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 4000pg/ml	Standard 4000pg/ml	Sample (A) undiluted	Sample (A) undiluted	Sample (B) Undiluted	Sample (B) Undiluted	Sample (C) Undiluted	Sample (C) Undiluted	Sample (D) Undiluted	Sample (D) Undiluted	Sample (E) Undiluted	Sample (E) Undiluted
B	Standard 1000pg/ml	Standard 1000pg/ml	Sample (A) 2x dilution	Sample (A) 2x dilution	Sample (B) 2x dilution	Sample (B) 2x dilution	Sample (C) 2x dilution	Sample (C) 2x dilution	Sample (D) 2x dilution	Sample (D) 2x dilution	Sample (E) 2x dilution	Sample (E) 2x dilution
C	Standard 500pg/ml	Standard 500pg/ml	Sample (A) 10x dilution	Sample (A) 10x dilution	Sample (B) 10x dilution	Sample (B) 10x dilution	Sample (C) 10x dilution	Sample (C) 10x dilution	Sample (D) 10x dilution	Sample (D) 10x dilution	Sample (E) 10x dilution	Sample (E) 10x dilution
D	Standard 250pg/ml	Standard 250pg/ml	Sample (A) 100x dilution	Sample (A) 100x dilution	Sample (B) 100x dilution	Sample (B) 100x dilution	Sample (C) 100x dilution	Sample (C) 100x dilution	Sample (D) 100x dilution	Sample (D) 100x dilution	Sample (E) 100x dilution	Sample (E) 100x dilution
E	Standard 125pg/ml	Standard 125pg/ml	Sample (A) 250x dilution	Sample (A) 250x dilution	Sample (B) 250x dilution	Sample (B) 250x dilution	Sample (C) 250x dilution	Sample (C) 250x dilution	Sample (D) 250x dilution	Sample (D) 250x dilution	Sample (E) 250x dilution	Sample (E) 250x dilution
F	Standard 62.5pg/ml	Standard 62.5pg/ml	Sample (A) 500x dilution	Sample (A) 500x dilution	Sample (B) 500x dilution	Sample (B) 500x dilution	Sample (C) 500x dilution	Sample (C) 500x dilution	Sample (D) 500x dilution	Sample (D) 500x dilution	Sample (E) 500x dilution	Sample (E) 500x dilution
G	Standard 31.2pg/ml	Standard 31.2pg/ml	Sample (A) 1000x dilution	Sample (A) 1000x dilution	Sample (B) 1000x dilution	Sample (B) 1000x dilution	Sample (C) 1000x dilution	Sample (C) 1000x dilution	Sample (D) 1000x dilution	Sample (D) 1000x dilution	Sample (E) 1000x dilution	Sample (E) 1000x dilution
H	BLANK standard dilutant	BLANK standard dilutant	BLANK PBS									

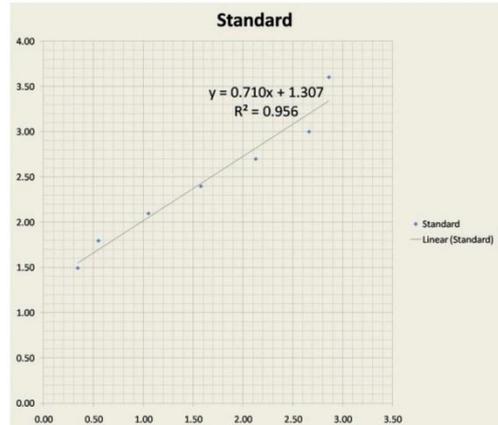
Figure 3

Table 1

Analysis of assay optimisation

Results from the preliminary optimisation assay for P1NP, post averaging from 3 acquisition runs and background correction are shown in Table 1 and Table 2.

Table 1 shows the figure derived from linear regression analysis of the data acquired for the standard curve producing the line equation $y = 0.710x + 1.307$ with $R^2 = 0.956$ as shown in Graph 1. Concentration of P1NP plotted on the y-axis is plotted in natural logarithm, and optical density plotted on the x-axis. As concentration of P1NP is plotted as a log value to the base 10, after conversion with the $y =$ equation, it needs to be raised to the power of 10. Finally before the experimental concentration of the titred optimisation samples based on optical density can be determined, the figure derived needs to be multiplied by its dilution factor.



Graph 1

Linear regression analysis is suggested in the text that accompanies the P1NP ELISA kit. However, polynomial regression analysis was also performed to see the comparison between the R squared values.

Polynomial values are shown in Table2. Figures in the table are derived from the equation $y = 4.714x^5 - 6.838x^4 + 27.06x^3 - 11.40x^2 + 107.1x - 1.495$ with $R^2 = 0.999$ (Graph 2). Linear concentration is shown on the y-axis and linear optical density on the x-axis.

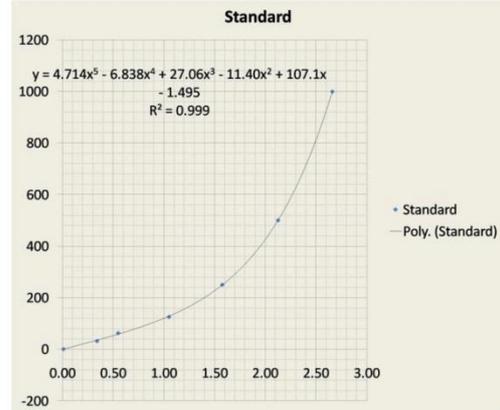
Averaged Values of Three acquisitions to two replicates of optical density extrapolated to concentration of P1NP against a Standard Curve Formula of best fit ($y = 0.710x + 1.307$)

Standard	log concentration of P1NP	Optical Density (D.D)	log curve formula for concentration of P1NP	Concentration of P1NP (log)	Dilution factor	Concentration of P1NP before dilution
A	4000	1.80	2.81984 104.674	1	104.67	2.81984
B	246	0.57	1.71294 51.8816	2	103.76	2.99813
C	213	0.50	1.56961 49.3875	10	91.8075	3.28842
D	138	0.43	1.37272 47.2671	100	72.9671	3.49352
E	105	0.42	1.31856 46.8342	250	52.0355	3.55493
F	62.5	0.40	1.26996 46.4154	500	38.9877	3.61147
G	31.25	0.40	1.26996 46.4154	1000	26.3243	3.61147

Averaged Values of Three Acquisitions to two Duplicates of Optical Density extrapolated to concentration of Pt1W against a Standard Curve Formula of best fit $y = 4.714x^5 - 6.838x^4 + 27.06x^3 - 11.40x^2 + 107.1x - 1.495$

	Standard		PT A 428		PT B 429		PT C 437		PT D 430		PT E D3M											
A	2.86	4000	1.00	119.789393	1	119.8	2.89	1338.86879	1	1338.5	0.57	60.6999588	1	60.5	1.13	142.330392	1	142.3	0.66	71.4430889	1	71.4
B	2.66	1000	0.57	60.972525	2	171.9	2.37	689.424191	2	1378.8	0.56	36.7999077	2	79.6	0.77	85.6189751	2	171.2	0.32	32.7092016	2	65.4
C	2.13	500	3.74	3755.21862	10	3755.1	2.79	1177.583634	10	11725.6	3.57	3107.289546	10	31077.9	3.17	1903.95365	10	19039.4	7.58	907.681295	10	9076.6
D	1.58	250	0.03	1.61971895	100	162.0	0.17	16.74634675	100	1675.7	0.02	0.838187589	100	83.8	0.03	1.44218767	100	144.2	0.03	1.30032647	100	130.0
E	1.05	125	0.02	0.215291925	250	53.9	0.06	5.17838947	250	1294.6	-0.01	-2.26267888	250	-561.3	-0.01	-3.06832796	250	-767.1	-0.07	-6.82103395	250	-2706.3
F	0.55	62.5	0.00	-1.028295361	500	-524.5	0.01	-0.76589353	500	-377.9	-0.05	-6.64702399	500	-3233.5	-0.05	-6.55672689	500	-3278.4	-0.07	-9.20281647	500	-4601.4
G	0.34	31.25	0.00	-1.22868895	1000	-1298.7	-0.03	-4.25394484	1000	-2333.9	-0.03	-4.21929284	1000	-2216.0	0.00	-1.441622847	1000	-1441.5	-0.07	-9.82794709	1000	-12677.6

Table 2



Graph 2

Conclusions

On both the linear and polynomial regression analysis, the R squared values are both above 0.95. Therefore either lines are a suitable fit to be used to derive the calculated concentrations of the titred samples.

The undiluted samples appear to also produce suitable optical density readings via the HIDEK that appear on the standard deviation curve, towards the lower end of the range. To place these values in the centre of the range, lower the standard curve from its starting frame and place it at 1000-15.6pg/ml as a double dilution without the first jump in the scale. This was discussed with the manufactures of the ELIZA kits and they considered this appropriate.

It is preferable to use the undiluted samples as antibody, in order to reduce pipetting and human error.

CTX

Sample collection

- A 6ml serum separator vacuette tube is drawn before any other tube in sequence
- Pre-label the aliquot cryovial with Test, Patient Study Number, Fluid Type, Time Point, M-No, DOB and Date
- Allow the serum to clot, standing upright, for 30 minutes minimum
- Centrifuge the tubes at 3500rpm for 15 minutes
- Carefully remove the lid from the vacuette, breaking the vacuum, but do not disturb the layer division
- Aspirate the serum layer from the tube
- Aliquot 1ml into the appropriate cryovial
- Freeze the cryovial immediately at -20°C and then when storage box is full transfer into -80°C.

Equipment and materials

- Standard
- Standard dilutant
- Detection reagent A (Green)
- Detection reagent B (Red)
- Assay dilutant A
- Assay dilutant B
- TMB substrate
- Stop solution
- Wash buffer
- Deionised water
- Calibrated single and multichannel pipettes (1ul-1000ul) with disposable tips
- Absorbent paper
- Eppendoff tubes (2ml)
- Falcon tubes (15ml)
- Microplate reader with 450±10nm filter

Supplied with ELISA kit

Reagent preparation

*****Bring all components to room temperature before working*****

*****Serum should be brought to room temperature slowly to prevent random agglutination of samples; first defrosted in the fridge for approximately 30-45 minutes, then on the bench*****

*****Serial dilution in well is not permitted in pre-coated plate*****

*****Reconstituted, standard, detection reagent A and detection reagent B can only be used once*****

*****Standard should be prepared maximum of 15minutes before the assay*****

*****Mix all reagents gently and do not foam to prevent imprecision in pipetting*****

Standard

Reconstitute the standard from the anhydrous form to the working standard with 1.0ml of standard dilutant.

The concentration of the working standard is 10000pg/ml.

First concentration of the standard curve is the reconstituted standard. Then perform a 3x serial dilution in 6 subsequent tubes to cut the dilution by one-third each time and in the final tube which is blank with 2ml of standard dilutant.

- Pipette 600ul of standard dilutant into Eppendorffs number 1 to 7
- Remove 300ul from the stock standard (10000pg/ml) and place in first Eppendorff and mix, making this 3333.3pg/ml
- Remove a subsequent 300ul from the first Eppendorff (3333.3pg/ml) and place in third tube and mix, making this 1111.1pg/ml
- Repeat the process until the 6th tube, and where after mixing, take off 300ul and discard, leaving Eppendorff 7 blank with only standard dilutant
- This leaves 7 stock standard tubes with 600ul volumes at subsequently reducing concentrations and one blank (Figure 4).

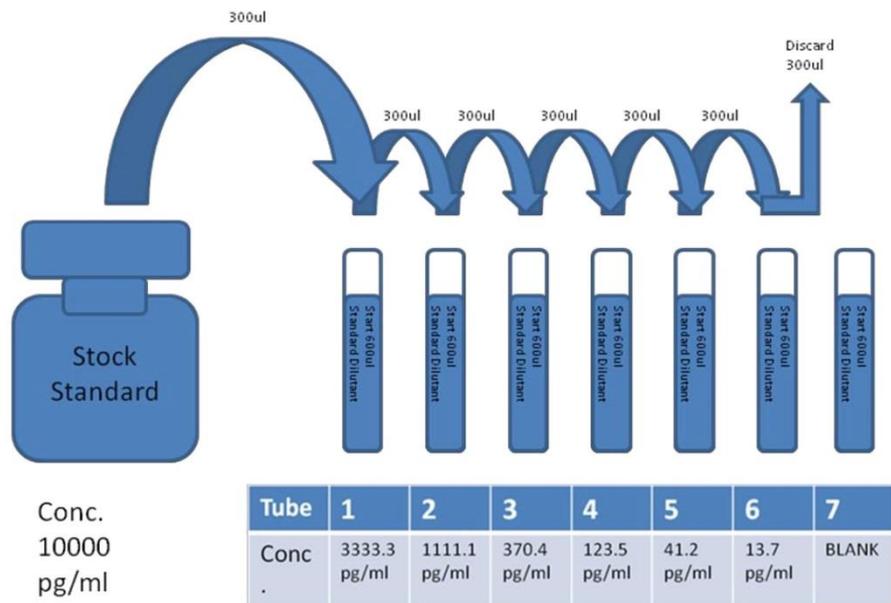


Figure 4

Assay dilutant

Assay dilutant A and B is in the kit 2x concentrated and must be diluted first with deionised water before being used to dilute detection reagents A and B.

- Combine 6ml of assay dilutant A with 6ml of deionised water in a 15ml falcon tube
- Repeat for assay dilutant B
- Ensure diluents are labelled appropriately.

Detection reagent

Detection reagents A and B are diluted using the working concentrations (diluted forms) of assay dilutants A and B.

- First micro-centrifuge Eppendorffs containing detection reagents A and B at 500g for 1 minute using full brakes
- Pipette 120ul of detection reagent A into the previously prepared 12ml of assay dilutant A in the 15ml falcon tubes
- Repeat this step for assay dilutant B.

Wash solution

Wash solution is 30x concentrated.

- Dilute 20ml of wash concentrated with 580ml of deionised water.

TMB substrate and stop solution

TMB substrate and stop solution are already at working concentrations. They need to be protected from light until use, when they can be decanted into multichannel pipetting troughs.

Assay procedure (Figure 5)

- Determine wells for the diluted standard, blank and sample. See **ELISA plate organisation**
- Add 50ul of dilution/standard/sample to the appropriate wells
- Add 50ul of detection reagent A to all of the wells
- Shake the plate gently with the microplate shaker
- Cover with the plate sealer and incubate for 1hour at 37°C
- Aspirate the liquid from each well, and wash with 350ul of wash solution to each well and leave to sit for 1-2 minutes, then remove from the remaining wells by snapping the plate on absorbent paper, **REPEAT 3 TIMES**
- Add 100ul of detection reagent B to each well
- Cover with the plate sealer and incubate for 30 minutes at 37°C
- Aspirate the liquid from each well, and wash with 350ul of wash solution to each well and leave to sit for 1-2 minutes, then remove from the remaining wells by snapping the plate on absorbent paper, **REPEAT 5 TIMES**
- Add 90ul of the substrate solution to each well and the liquid will turn blue
- Cover with the plate sealer and incubate for 15-2 5minutes at 37°C depending on development, **DO NOT EXCEED 30 MINUTES**
- Add 50ul of the stop solution and the liquid will turn yellow; then mix the solution on the plate shaker at low intensity
- Remove water or finger prints from the bottom of the plate and run on HIDEX.

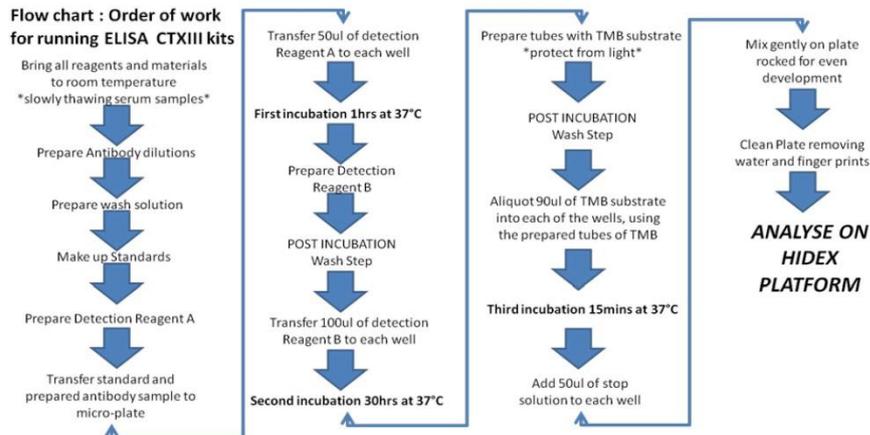


Figure 5

Running on the HIDE X

Sample and run on the Hidex using MicroWin2000 software to interact with the plate reader. Set up is designated to read absorption at 450nm±10nm and output optical density readings for each well in triplicate. The file is then output as a text rich file that can be imported into Excel for preliminary data analysis.

ELISA Plate Organisation – Optimisation CTX(Figure 6)

It is necessary to try to predict the concentration of antibody before running the assay. i.e. to ensure the values are within the range of the standard dilution curve. Optimisation is required to determine optimal sample concentrations.

To optimise the plate, dilution of the antibody is required and a variety of samples from normal healthy donors to experimental samples to determine assay interaction and optimal concentration. All samples are diluted in 0.02mol/l PBS (pH=7.0-7.2) which is made up using anhydrous tablets and deionised water.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 10000pg/ml	Standard 10000pg/ml	Sample (A) undiluted	Sample (A) undiluted	Sample (B) Undiluted	Sample (B) Undiluted	Sample (C) Undiluted	Sample (C) Undiluted	Sample (D) Undiluted	Sample (D) Undiluted	Sample (E) Undiluted	Sample (E) Undiluted
B	Standard 3333.3pg/ml	Standard 3333.3pg/ml	Sample (A) 2x dilution	Sample (A) 2x dilution	Sample (B) 2x dilution	Sample (B) 2x dilution	Sample (C) 2x dilution	Sample (C) 2x dilution	Sample (D) 2x dilution	Sample (D) 2x dilution	Sample (E) 2x dilution	Sample (E) 2x dilution
C	Standard 1111.1pg/ml	Standard 1111.1pg/ml	Sample (A) 10x dilution	Sample (A) 10x dilution	Sample (B) 10x dilution	Sample (B) 10x dilution	Sample (C) 10x dilution	Sample (C) 10x dilution	Sample (D) 10x dilution	Sample (D) 10x dilution	Sample (E) 10x dilution	Sample (E) 10x dilution
D	Standard 370.4pg/ml	Standard 370.4pg/ml	Sample (A) 100x dilution	Sample (A) 100x dilution	Sample (B) 100x dilution	Sample (B) 100x dilution	Sample (C) 100x dilution	Sample (C) 100x dilution	Sample (D) 100x dilution	Sample (D) 100x dilution	Sample (E) 100x dilution	Sample (E) 100x dilution
E	Standard 123.5pg/ml	Standard 123.5pg/ml	Sample (A) 250x dilution	Sample (A) 250x dilution	Sample (B) 250x dilution	Sample (B) 250x dilution	Sample (C) 250x dilution	Sample (C) 250x dilution	Sample (D) 250x dilution	Sample (D) 250x dilution	Sample (E) 250x dilution	Sample (E) 250x dilution
F	Standard 41.2pg/ml	Standard 41.2pg/ml	Sample (A) 500x dilution	Sample (A) 500x dilution	Sample (B) 500x dilution	Sample (B) 500x dilution	Sample (C) 500x dilution	Sample (C) 500x dilution	Sample (D) 500x dilution	Sample (D) 500x dilution	Sample (E) 500x dilution	Sample (E) 500x dilution
G	Standard 13.7pg/ml	Standard 13.7pg/ml	Sample (A) 1000x dilution	Sample (A) 1000x dilution	Sample (B) 1000x dilution	Sample (B) 1000x dilution	Sample (C) 1000x dilution	Sample (C) 1000x dilution	Sample (D) 1000x dilution	Sample (D) 1000x dilution	Sample (E) 1000x dilution	Sample (E) 1000x dilution
H	BLANK standard dilutant	BLANK standard dilutant	BLANK PBS									

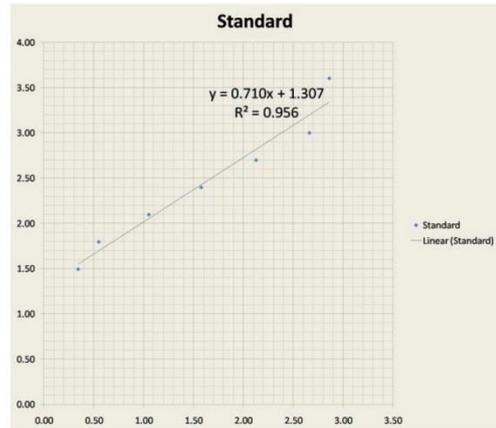
Figure 6

Table 3

Analysis of assay optimisation

Results from the preliminary optimisation assay for CTX-III, post averaging from 3 acquisition runs and background correction are shown in Table 3.

Table 3 shows the figure derived from logarithmic regression analysis of the data acquired for the standard curve producing the line equation $y = -0.631\ln(x) + 2.054$ with $R^2 = 0.989$ as shown in Graph 3. Concentration of CTX is plotted on the y-axis is plotted in natural logarithm, and optical density plotted on the x-axis. As concentration of CTX is plotted as a log value to the base 10, after conversion with the $y =$ equation, it needs to be raised to the power of 10. Finally before the experimental concentration of the titred optimisation samples based on optical density can be determined, the figure derived needs to be multiplied by its dilution factor.



Graph 3

Conclusions

On the logarithmic regression analysis, the R squared value is above 0.95 therefore the lines are a suitable fit to be used to derive the calculated concentrations of the titred samples.

The undiluted samples appear to also produce suitable optical density readings via the HIDEK that appear on the standard deviation curve, towards the centre end of the range.

	Standard	PT a 261		PT b 262		PT c 242		PT d 243		PT e 244												
A	0.05	Optical Density (O.D)	Concentration of CTX (log)	Optical Density (O.D)	Concentration of CTX (log)	Optical Density (O.D)	Concentration of CTX (log)	Optical Density (O.D)	Concentration of CTX (log)	Optical Density (O.D)	Concentration of CTX (log)											
B	0.09	n/a																				
C	0.25	1111.111	0.41	2.6187/409.1448	10	4.0914	0.39	2.6461/442.7294	10	4.427.3	0.49	2.5014/63.71106	10	3173.3	0.37	2.7751/33.953452	10	5958.5	0.37	2.8817/482.8793	10	4828.3
D	0.40	370.3704	1.72	1.7133/851.7080	100	5170.8	1.61	1.7540/836.7939	100	5675.9	1.99	1.6201/41.71737	100	4171.7	1.37	1.8549/71.54794	100	7154.8	1.51	1.7924/62.00083	100	6200.5
E	1.13	123.4568	3.05	1.3509/272.4374	250	5609.4	2.86	1.3012/24.61506	250	6153.9	3.02	1.3586/22.83872	250	5709.6	2.34	1.5194/38.10708	250	8367.6	2.57	1.4593/38.79647	250	7190.1
F	1.73	41.1328	1.89	1.6516/244.8357	500	22417.9	2.44	1.40311/30.99632	500	15498.2	1.78	1.6920/49.21435	500	24607.2	1.89	1.6530/6.44383	500	22497.5	2.18	1.5642/31.966372	500	18331.6
G	1.88	13.27142	2.10	1.5865/29.59488	1000	38594.9	1.73	1.7077/45.10291	1000	51027.9	1.62	1.7469/25.27664	1000	56276.6	1.87	1.6581/45.51395	1000	45514.0	2.00	1.6162/16.432527	1000	41325.3
H	3.20	0	2.10	1.5864/29	Blank	0	1.35	1.8026/83	Blank	0	1.54	1.7837/27	Blank	0	1.78	1.6891/45	Blank	0	1.85	1.6614/58	Blank	0

ELISA plate organisation (Figure 7)

All samples, including standards, to plot the standard curve that concentrations are derived from and blanks to determine background are done in duplicate. It is possible to accommodate 39 patient samples per plate in an undiluted state.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 10ng/ml	Standard 10ng/ml	Patient 1	Patient 1	Patient 8	Patient 8	Patient 16	Patient 16	Patient 24	Patient 24	Patient 32	Patient 32
B	Standard 5ng/ml	Standard 5ng/ml	Patient 2	Patient 2	Patient 9	Patient 9	Patient 17	Patient 17	Patient 25	Patient 25	Patient 33	Patient 33
C	Standard 2.5ng/ml	Standard 2.5ng/ml	Patient 3	Patient 3	Patient 10	Patient 10	Patient 18	Patient 18	Patient 26	Patient 26	Patient 34	Patient 34
D	Standard 1.25ng/ml	Standard 1.25ng/ml	Patient 4	Patient 4	Patient 11	Patient 11	Patient 19	Patient 19	Patient 27	Patient 27	Patient 35	Patient 35
E	Standard 0.625ng/ml	Standard 0.625ng/ml	Patient 5	Patient 5	Patient 12	Patient 12	Patient 20	Patient 20	Patient 28	Patient 28	Patient 36	Patient 36
F	Standard 0.312ng/ml	Standard 0.312ng/ml	Patient 6	Patient 6	Patient 13	Patient 13	Patient 21	Patient 21	Patient 29	Patient 29	Patient 37	Patient 37
G	Standard 0.156ng/ml	Standard 0.156ng/ml	Patient 7	Patient 7	Patient 14	Patient 14	Patient 22	Patient 22	Patient 30	Patient 30	Patient 38	Patient 38
H	BLANK 500ul standard dilutant	BLANK 500ul standard dilutant	BLANK 500ul PBS	BLANK 500ul PBS	Patient 15	Patient 15	Patient 23	Patient 23	Patient 31	Patient 31	Patient 39	Patient 39

Figure 7

References

Ambroszkiewicz J, Klemarczyk W, Gajewska J, Chelchowska M, Laskowska-Klita T. Serum concentration of biochemical bone turnover markers in vegetarian children. *Adv Med Sci.* 2007;52:279-282.

Ravn P, Clemmesen B, Riis BJ, Christiansen C. The effect on bone mass and bone markers of different doses of ibandronate: a new bisphosphonate for prevention and treatment of postmenopausal osteoporosis: a 1-year, randomized, double-blind, placebo-controlled dose-finding study. *Bone.* 1996 Nov;19(5):527-533.

Rosen HN, Moses AC, Garber J, Ilpoutaife ID, Ross DS, Lee SL, Greenspan SL. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. *Calcif Tissue Int.* 2000 Feb;66(2):100-103.