Glucose Metabolism during & following Acute Hypoxia & Exercise in Individuals with Type 2 Diabetes

RICHARD W.A. MACKENZIE

A thesis submitted in full fulfilment of the requirements of the University of Brighton for degree of Doctor of Philosophy

June 2009

University of Brighton

Abstract

The current work is novel in that it investigated *in vivo* analysis of glucose metabolism during and following hypoxic exposure in type 2 diabetics. Using moderate levels of hypoxia (O_2 ~ 14.8%; equivalent ~3100 m), study one found that 60 min of resting hypoxic (Hy Rest) exposure reduced blood glucose concentrations in type 2 diabetics. Insulin sensitivity (S_I^{2*}) was also found to be significantly greater following hypoxic exposure when compared to the normoxic control [mean (SEM); Nor Rest; 1.39 (0.08) and Hy Rest; 2.25 (0.50) $x10^{-4} \cdot min^{-1}$(μU/ml)] (*P* = 0.047). The second study showed that exercise under hypoxic (Hy Ex) conditions acutely reduced arterialised blood glucose concentrations. The total area under the curve for insulin (AUC_{Ins}) was also significantly lower subsequent to an intravenously administered glucose load (IVGTT) in the 4 hr following Hy Ex versus normoxic exercise [Hy Ex; 4334 (617) vs. Nor Ex; 5637 (820) $\mu U \cdot ml^{-1} \cdot min$], respectively (*P* = 0.007). S_I^{2*} was also significantly greater in the Hy Ex trial (*P* = 0.049).

The third study demonstrated that glucose disposal was acutely enhanced in exercise bouts lasting 60 (Hy Ex^{60}; *P* = 0.001) and 40 (Hy Ex^{40}; *P* = 0.005) min (of equal work) in hypoxia. Arterialised blood glucose concentration were unchanged following short-duration exercise in hypoxia (Hy Ex^{20}) (*P* = 0.118). Indices of insulin resistance and insulin sensitivity were also improved in the 48 hr following both Hy Ex^{60} and Hy Ex^{40}, indicating that insulin-dependent mechanisms were up-regulated. The results from study 4 suggest that intermittent exercise in hypoxia (Hy 5:5) may acutely encourage glucose disposal and provide moderate-term improvements in glycaemic control (~24 hr). Indices of insulin resistance and sensitivity were not changed following intermittent exercise in normoxia (Nor 5:5). The rate of glucose disappearance (R_d) was found to be lower 24 hr post continuous moderate intensity exercise in hypoxia (Hy Ex60). Hy Ex60 also demonstrated the greatest improvements in glucose tolerance with plasma insulin (*P* = 0.025), homeostasis model for insulin resistance ($HOMA_{IR}$; *P* = 0.028) and fasting insulin resistance Index (FIRI; *P* = 0.028) all improved in the 48 hr following exercise.

The key conclusions drawn are ***1)*** hypoxia has the ability to increase glucose disposal, both during and following acute exposure and that; ***2)*** hypoxic-induced improvements in glucose tolerance during and in the 4 hr following exposure can be attributed to insulin-independent and -dependent mechanisms, respectively; ***3)*** the effects of exercise on glucose disposal are enhanced by moderate hypoxia; ***4)*** continuous moderate-intensity exercise in normoxia improves S_I^{2*} in type 2 diabetics; ***5)*** intermittent exercise can acutely improve glycaemic control; and finally, ***6)*** the most potent effect on acute and moderate-term glucose control was consistently evident following 60 min of continuous exercise in hypoxia. The finding that hypoxia alters glucose metabolism is in keeping with the original hypothesis and suggests a clinical role for hypoxia in the treatment of type 2 diabetes.

Acknowledgements

There are many individuals who have given their time, energy and uncompromising support to the completion of this thesis. Firstly, a sincere thanks goes to the technical staff at the University of Brighton, namely Ann, David, Ian, Patrick and Ron. You have a great work ethic and it was a pleasure to have you on my side. Your efforts on this as well as my other experimental work over the last 10 years has been outstanding. Special thanks must go to Ann, Brian, Doug, Helmmt, Janet, Jennifer, Ken, Owen, Paul, Peggy and Tina. Your collective contribution was immeasurable and it is by no means a misplacement of the truth to say that this study really couldn't have happened without you. I would also like to express my gratitude to the administrative staff of the University of Brighton. A massive thanks must go to Dr. Clive Castle. You set the standard for me to follow with the work ethic you employed towards the end of your own PhD. Those afternoon spent with a glass of red wine in the bungalow are times to cherish. Although, I mustn't forget that your assistants in proof reading was also greatly appreciated.

I would like to give my sincere thanks to Dr. Gary Brickley. Your commitment was greatly appreciated while your more laidback approach to life was without a doubt useful over the last 4 years. I don't think I have the words to thank Dr. Neil Maxwell enough. I think its best just to say that your unwavering encouragement and confidence have been second to none. I believe I've come a long way since the days of Bishop Carey and a lot of it should be credited to you. It is by no means a small statement when I say that without the knowledge and direction of Dr. Peter Watt, things may have turned out a lot differently and it is your uncompromising experience and knowledge that has allowed me to complete this thesis. Many thanks Peter! To my mum and dad who have always been there for me despite being at the other end of the country. You may be glad to know that I have now completed my studies. And finally Faye. What can I say apart from it was challenging at times but you stuck with me. You are an immense individual and I have a huge amount to thank you for. Your strength of character is unquestionable. Both our lives have been on hold for the last 3 years and the completion of the next sentence will signal the end of that. The rest, as Del Boy would say, "is epidemic"!

[6,6$^2H_2$]glucose	Stable Isotope Tracer
AMPK	Adenosine 5'-monophosphate-activated protein kinase
∝PKC	atypical Protein Kinase C
AMP	Adenosine monophosphate
AS160	Akt substrate of 160 kDa
APE	Atom percent excess
ATP	Adenosine triphosphate
AUC_{Glu}	Area under the curve (glucose)
AUC_{Ins}	Area under the curve (insulin)
BSTFA	Bis(trimethyl)trifluroacetamide
C	Endogenous glucose concentration
cAMPK	Cyclic AMP-dependent kinase
cGMP	Cyclic guanosine 3',5'-monophosphate
Cm	Measured blood glucose concentration
CaMKII	Calmodulin-dependant protein kinase II
CNS	Central nervous system
CV	Coefficient of variation
CVD	Cardiovascular disease
EGP	Endogenous glucose production
EDTA	Ethylenediaminetetraacetic Acid – Anticoagulant
ELISA	Enzyme-Linked Immunosorbent Assay
eNOS	Endothelial Nitric oxide synthases
f	Isotope infusion rate
GTP	Guanosine 5'-triphosphate
GLUT	Glucose transporters
GS	Glycogen snythase
GSK-3	Glycogen synthase kinase 3
Hb	Haemoglobin
HbA_{1c}	Glycated haemoglobin
$HOMA_{β\text{-}Cell}$	Homeostasis model assessment for β-cell function
$HOMA_{IR}$	Homeostasis model assessment for insulin resistance
HR	Heart rate (beats · min^{-1})
Hypoxia	Reduction in O_2 availability
IE	Tracer enrichment
IGT	Impaired glucose tolerance
IL	Interleukin
ISR	Insulin secretion rate
IRS	Insulin receptor substrates
IVGTT	Intravenous Glucose Tolerance Test
[La]	Blood lactate concentration
LKB1	Serine/threonine kinase 11 (sometime referred to as STK11)
LOA	Limits of agreement
LT	Lactate threshold
MCR	Metabolic clearance rate (glucose)
mmol/l	Millimoles per litre
ml	millilitres

FIRI	Fasting insulin resistance index
FFA	Free fatty acid
G-6-P	Glucose-6-phosphate
NEFA	Non-esterified fatty acids
NF-KB	Nuclear factor-kappa B
NO	Nitric Oxide
Normoxia	Ambient oxygen = 20.93%
NRG	Neuregulins
OGTT	Oral Glucose Tolerance Test
P_aO_2	Partial pressure of oxygen in arterial blood (mmHg)
PI-3kinase	Phosphatidylinositol 3-kinase
PKB	Protein Kinase B: also known as Akt or Akt1
POMS	Profile of Mood States Questionnaire
PO_2	Partial pressure of oxygen
PP1	Protein phosphatase-1
QUICKI	Quantitative insulin sensitivity check index
R_a	Rate of appearance (glucose)
R_d	Rate of disappearance (glucose)
RER	Respiratory Exchange Ratio of carbon dioxide output to the oxygen uptake
ROS	Reactive oxygen species
RPE	Rate of perceived exertion
rpm	Revolutions per minute
mmHg	A unit of measurement for pressure, millimetres (mm) of the metal mercury (Hg). At sea level, the earth's atmosphere exerts 760 mmHg of pressure.
mRNA	Messenger RNA
NADPH	Nicotinamide adenine dinucleotide phosphate
SAAMII	Simulation Analysis and Modelling software
S_aO_2	Arterial oxygen saturation
S_G	Glucose effectiveness: assessed using a unlabelled IVGTT
S_G^{2*}	Glucose effectiveness: assessed using a labelled IVGTT
SGLT	Sodium-glucose cotransporters
S_I	Insulin sensitivity : assessed using a unlabelled IVGTT
S_I^{2*}	Insulin sensitivity : assessed using a labelled IVGTT
S_pO_2	Arterial oxygen saturation estimated by pulse oximetery
TEM	Technical error of measurement
TMB	Tetramethylbenzidine
TNF-α	Tumour necrosis factor α
V	Volume of distribution. Assumed equal and constant (145 ml / kg) for glucose
VSMCs	Vascular smooth muscle cells
$\dot{V}O_{2peak}$	Peak oxygen uptake ($ml \cdot kg \cdot min^{-1}$)
$\dot{V}O_{2max}$	Maximal oxygen uptake ($ml \cdot kg \cdot min^{-1}$)

CHAPTER 1

INTRODUCTION

1.0 Introduction

Survival of *Homo sapiens* during evolution was dependent on the acquisition of food, which presumably required regular engagement in physical activity (Chakravarthy et *al.,* 2004). The shift from hunter-gather predominant lifestyles, indicative of the *Homo sapiens* from the Late Paleolithic period (50,000 – 10,000 BC), towards industrialisation has resulted in a fundamental change in the dietary and physical habits of the modern day human. There is overwhelming evidence to support the belief that all environmental factors combined, including physical inactivity (defined as activity equivalent to <30 min of brisk walking per day) and poor dietary habits (defined as high in calorie and fat intake), account for the majority of chronic health conditions (Lichtenstein *et al.,* 2000; Booth *et al.,* 2002). The World Health Organization (WHO) reported that global physical inactivity is estimated to cause, approximately 10–16% of cases of breast, colon and rectal cancers and diabetes mellitus, and about 22% of ischaemic heart disease. Overall, 1.9 million deaths ever year are attributable to physical inactivity (WHO, 2002).

Diabetes *mellitus* is the most prevalent metabolic disorder in modern society and is characterised by habitual hyperglycaemia (elevated blood glucose concentrations). This disease was first documented in 400 BC by the Indian physician, Susruta who referred to it as *Madhumeha* or “honey-urine”. The word diabetes originates from the Greek meaning, ‘to pass through’. An early clinical description of diabetes *mellitus* was reported in the second century AD by Aretaeus of Cappadocia;

"Diabetes is a dreadful affliction, being a melting down of human flesh and limbs into urine. The patient never stops making water and the flow is incessant, like the opening of aqueducts. Life is short, unpleasant and painful, thirst unquenchable and drinking excessive….."

Aretaeus of Cappadocia (2nd century AD).

The sweetness of diabetic urine was rediscovered by the British physician; Thomas Willis in the 1600's who remarked that "although the disease was rare in ancient times, its frequency was increasing". Thomas Willis went further to say that the increase may be linked to changes in dietary intake. The most common form of diabetes in modern society is type 2 diabetes [formerly called non - insulin-dependent diabetes mellitus (NIDDM), or adult-onset diabetes]. A contemporary view of diabetes is one of a multifactorial aetiology, defined by disruptions in lipid, carbohydrate and protein metabolism resulting, in part, to an elevation in circulating blood glucose concentration. It is this consistent elevation in blood glucose levels that causes many of the secondary complications associated with type 2 diabetes. The World Health Organization estimates that the total numbers of deaths caused by diabetes will increase by 50% over the next 10 yr (WHO, 2002).

The financial burden of diabetes reflects the cost of medication, primary and secondary care and educational programmes. A large amount of this expenditure is directly related the treatment of the micro- and macro-vascular complications that progress with the increased severity of diabetes. These complications impose an immense burden on the National Health Service (NHS). A recent report suggests that the NHS spends in the region of £13.7 million a

day on the treatment of diabetes and its related complications (Diabetes UK, 2007). The National Institute of Clinical Excellence (NICE, 2006) places the total cost of diabetes in the UK at ~£7.4 billion. Although, the true medical and social cost of type 2 diabetes is largely unknown. However, the life time cost of treating an individual with diabetic retinopathy in the UK is ~£237,000. Therefore, a reduction of one thousand cases of this complication would potentially save the UK government ~£237 million (Diabetes Report, 2006).

A recent review carried out in the U.S. suggests that the cost of diabetes for 2007 was in excess of $174 billion; $116 billion of this cost was attributed to medical expenditures with the remaining $58 billion associated with a reduction in national productivity [American Diabetes Association (ADA), 2008]. The same study estimated that the greatest cost related to diabetes is hospital inpatient care (50% of total cost). Medication used to treat diabetes accounts for 12% of the total cost (ADA, 2008). The authors also highlighted the substantial cost of this metabolic condition through the reduced productivity of employed diabetic sufferers ($22.6 billion), unemployed sufferers ($0.8 billion), unemployed due to diabetic related disabilities ($7.9 billion) and lost productivity due to early mortality ($26.9 billion) (ADA, 2008).

Regular physical activity and dietary modifications are the first line of defence in the prevention and treatment of diabetes. Exercise can improve glycaemic control both acutely and chronically by increasing blood glucose clearance. The introduction of an exercise programme is known to increase the sensitivity of skeletal muscle to insulin and reverse insulin resistance (Holloszy, 2005), the latter being a major cause of type 2 diabetes. The American Diabetic Association (ADA, 2004) have reported that the benefits of exercise to

type 2 diabetic sufferers is substantial. Although the adherence to this, and other forms of treatment remains low (Vermeire *et al.,* 2006). Furthermore, the most effective type, mode and intensity of exercise, in improving glucose control in type 2 diabetics has proven elusive.

Hypoxia has been shown to stimulate glucose uptake during and following exposure in both animal models (Cartee *et al.,* 1991) and isolated human muscle tissue (Azevedo *et al.,* 1995). However, it remains to be determined whether moderate levels of hypoxia with and without exercise in type 2 diabetic humans can have a similar effect. The aim of this thesis was to assess the effectiveness of varying types of exercise with and without hypoxia on whole body glucose metabolism in individuals with type 2 diabetes.

CHAPTER 2

LITERATURE REVIEW

2.0 Literature Review

2.1 Inactivity and Health

A decrease in general physical activity level is strongly associated with many modern-day chronic diseases (Hamburg *et al.,* 2007). In a comprehensive review Booth *et al.* (2000) defined modern chronic disease as a "disease that is slow in its progression and long in its continuance". This suggests that the cellular defects linked to chronic diseases have been active for many years before clinical features are outwardly recognisable (Figure 2.0) (Booth *et al.,* 2000). The general position of Booth and colleagues (2000; 2002) is that modern life-styles adversely affect health, creating an escalating economic and social burden on society. The same authors go further to purpose that humans have evolved genetically to maintain physically activate life-styles. It is further postulated "that physical inactivity directly contributes to multiple chronic health disorders", including coronary heart disease, obesity, type 2 diabetes, hypertension and some site-specific cancers. A hypothesis has been proposed that environmental factors such as physical inactivity may influence gene expression and consequently enhance genetic susceptibility to chronic diseases (Lichtenstein *et al.,* 2000; Booth *et al.,* 2002). A cancer prevention study conducted in America suggested that 30% of the total number of cancer deaths is associated to dietary habits, physical inactivity and obesity (Harvard Centre For Cancer Prevention, 1996). Elsewhere, Hu *et al.* (2001) and Stampfer *et al.* (2000) estimated that 91% of type 2 diabetic and 82% of coronary heart disease cases respectively, can be attributed to body mass index (BMI) >25, poor dietary habits and sustained levels of physical inactivity. In the UK, physical inactivity is considered a growing problem, with only ~40% of males and ~28% of females meeting the recommendations levels (National Health Service Report, 2008). Hahn *et al.* (1990) reported that physical inactivity in

the USA is the 3rd major cause of death and contributes to obesity, which is regarded as the 2nd leading cause of fatality in western societies.

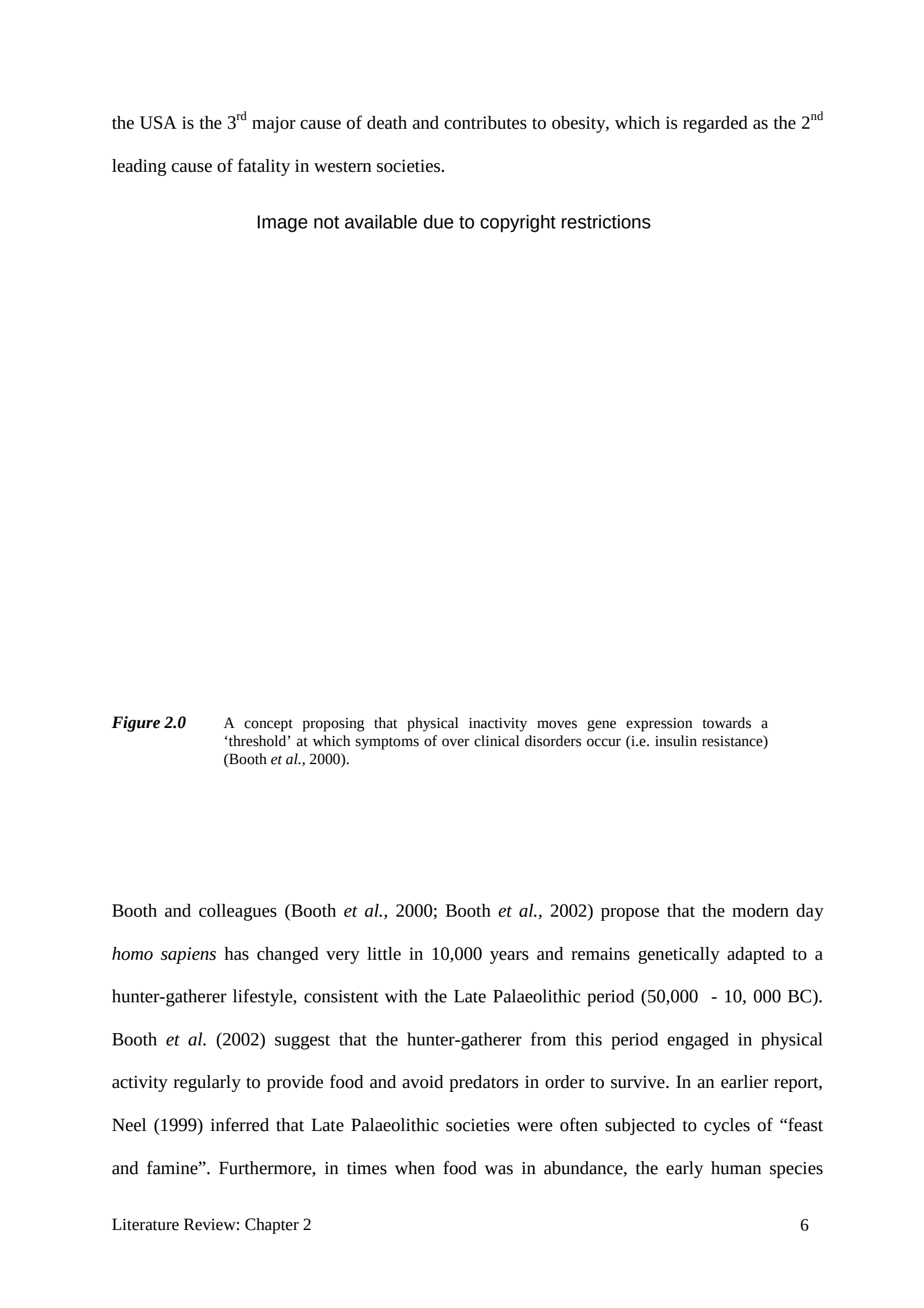

Figure 2.0 A concept proposing that physical inactivity moves gene expression towards a 'threshold' at which symptoms of over clinical disorders occur (i.e. insulin resistance) (Booth *et al.*, 2000).

Booth and colleagues (Booth *et al.*, 2000; Booth *et al.*, 2002) propose that the modern day *homo sapiens* has changed very little in 10,000 years and remains genetically adapted to a hunter-gatherer lifestyle, consistent with the Late Palaeolithic period (50,000 - 10, 000 BC). Booth *et al.* (2002) suggest that the hunter-gatherer from this period engaged in physical activity regularly to provide food and avoid predators in order to survive. In an earlier report, Neel (1999) inferred that Late Palaeolithic societies were often subjected to cycles of "feast and famine". Furthermore, in times when food was in abundance, the early human species

would store energy in the form of lipids, in anticipation of famine. Therefore, the modern human seems genetically equipped to rapidly store excess food within adipose tissue for later use during periods of starvation (Joffe & Zimmet, 1998). This concept can be supported by the 'thrifty' gene hypothesis proposed by (Neel *et al.*, 1998). According to Neel *et al.* (1962; 1998) 'thrifty' genes were incorporated into the genome of *homo sapiens* due to a selective advantage during early evolution. These authors propose that 'thrifty' genes would activate the efficient storage of energy in times of food abundance in comparison to individuals without such genes, giving them an evolutionary advantage (Neel *et al.*, 1962; 1998).

Adapting to lower rates of energy metabolism, as seen during periods of inactivity, has been described as a survival mechanism (Shetty 1999). The ability of metabolic pathways in skeletal muscle to remain flexible, shows an adaptive advantage during episodes of restricted foods and physical inactivity. It is proposed that the Late Palaeolithic phenotype could leave modern day *homo sapiens* predisposed, and therefore contribute to, insulin resistance in skeletal muscle (Wendorf & Goldfine, 1991). These authors hypothesised that reduced insulin action in muscle tissue would slow and / or prevent hypoglycaemia known to occur during periods of low energy intake (Wendorf & Goldfine, 1991). Within this hypothesis it was suggested that during periods of low energy availability fuel would be prevented from entering nonessential (i.e. inactive muscle) tissue and delivered to vital organs, maintaining basal physiological functions. The phenotype inherited by the modern day *homo sapiens* provides them with a collection of highly fluid metabolic processes that evolved to support physical activity (Booth *et al.*, 2002).

Epidemiological evidence supports a link between physical inactivity and the chronic (metabolic) disease, type 2 diabetes. The prevalence of type 2 diabetes is currently 6-10% in adults from western cultural societies and only 0-2% in populations that remain in a hunter-gatherer type civilization (Eaton *et al.,* 1988). The research discussed above seems to be supported by controlled experimental work. A recent publication demonstrated that television viewing has a strong relationship with measures of glucose tolerance in Australian men and woman (Dunstan *et al.,* 2007). This paper reported that those individuals watching the most amount of television exhibited the greatest impairments in glucose tolerance (Dunstan *et al.,* 2007). Using age-adjusted regression models, these authors reported that a 1 hr / day increase in television viewing augmented fasting plasma glucose concentrations by 0.02 mmol/l in females ($P = 0.04$) and 0.04 mmol/l in males ($P = 0.001$).

2.2 Diabetes Mellitus

In modern society diabetes mellitus is the most common metabolic and endocrine disorder, characterised as a multifactorial disease including; defects in insulin secretion, insulin action (insulin sensitivity) (Stumvoll *et al.,* 2005), glucose effectiveness (Del Prato *et al.,* 1993) and endogenous glucose production (Mayer *et al.,* 1998). The combination of these defects, if untreated can result in habitual hyperglycaemia (elevated blood glucose concentrations > 7 mmol/l or 126 mg/dL) (Giugliano *et al.,* 1997). There are numerous pathogenic processes implicated in the maturity of diabetes. These range from autoimmune destruction of pancreas β-cells, resulting insulin secretion deficiency and an associated decrease in insulin action (insulin resistance). Increased insulin resistance is the consequence of inadequate insulin release and / or reduced responsiveness of peripheral tissue to insulin. It is common for both

decreased insulin action and impaired insulin secretion to coexist in the same individual, although it is often indistinguishable which defect, if either, is the principal cause of the hyperglycaemia. Essentially, the inhibition of insulin secretion and / or insulin action results in decreased blood insulin concentrations, elevations in blood glucose and a reduction in glucose uptake (Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002).

Diabetes mellitus is estimated to affect more than 170 million individuals worldwide and has been described as a global epidemic (Stumvoll *et al*., 2005). Estimates for the year 2010 predict a further growth in the world's population of approximately 50% with the greatest increases predicted in developing nations such Africa, Asia, and South America (Zimmet *et al.*, 2001; Stumvoll *et al.*, 2005). This could raise the total number of individuals affected by diabetes to 366 million (Wild *et al*., 2004). Elsewhere, current estimations place the number of individuals diagnosed with diabetes at 194 million with a predicted growth to 333 million by the year 2025 (Table 2.0; International Diabetes Federation, 2005). In the UK, there are approximately 2.35 million people living with diabetes, 90% of whom have type 2 or late-onset diabetes (Section 2.2.2). This is predicted to increase to 2.5 million by the year 2010 resulting in an estimated 2.3 million individuals in the UK diagnosed with this form of diabetes (Department of Health, UK, 2000). King *et al.* (1998) suggests that the number of individuals with diabetes in the UK will grow by 274,000 by the year 2025.

Table 2.0 Number of Individuals with Diabetes by region in 2003 and the projected estimates for 2025

	2003			2025		
	Population (20-79) (million)	**Individuals with diabetes (million)**	**Prevalence (%)**	**Population (20-79) (million)**	**No. of people with diabetes (million)**	**Prevalence (%)**
Africa	295	7.1	2.4	541	15.0	2.8
Eastern Mediterranean & Middle East	276	19.2	7.0	494	39.4	8.0
Europe	621	48.4	7.8	646	58.6	9.1
North America	290	23.0	7.9	374	36.2	9.7
South & Central American	252	14.2	5.6	364	26.2	7.2
South-East Asian	705	39.3	5.6	1,081	81.6	7.5
Western Pacific	1,384	43.0	3.1	1,751	75.8	4.3
Total	**3,823**	**194**	**5.1**	**5,251**	**333**	**6.3**

Values are numbers (million) of individuals between the ages of 20 - 79 yrs. Source: International diabetes Federation (http://eatlas.idf.org).

Worryingly, the occurrence of diabetes in obese white adolescents has reached 4%, with a further 25% having impaired glucose tolerance (Sinha *et al.,* 2002). Sinha *et al.* (2002), Rosenbloom *et al.* (1999) and Dabelea *et al.* (1999) have all described childhood obesity as an epidemic in the United States which is linked to an increasing frequency of type 2 diabetes in adolescent populations. The results from Rosenbloom *et al.* (1999) work do not support the view of some that a family history in type 2 diabetes is a risk factor for glucose intolerance (Ehrman *et al.,* 2005; Pierce *et al.,* 1995). Rather that the increased prevalence of diabetes in adolescence probably reflects a contribution of environmental factors, including dietary habits and inactivity (Rosenbloom *et al.,* 1999). However, Pierce *et al.* (1995) proposed that a family history presents a 2.4 fold increase risk for type 2 diabetes.

2.2.1 Type 1 Diabetes

Insulin-dependent diabetes, type 1 diabetes or immune-mediated diabetes is predominantly the consequence of autoimmune destruction of the β-cells (insulin secreting cells) within the pancreas (Atkinson *et al*., 1994). The exact aetiology of type 1 diabetes is both complex and poorly understood (Williams & Pickup, 1999). Although, it is most likely that environmental, geographical and ethnic factors trigger the onset of diabetes in populations with an inherited predisposition (Dahlquist, 1998). Type 1 diabetes is associated with sudden onset of symptoms, normally developing during childhood and results in little or no insulin being secreted. The subsequent hyperglycaemia may be treated with injections of insulin or more recently via continuous subcutaneous insulin infusion pumps. This form of diabetes increases the risk of cardiovascular disease >10 times when compared to healthy populations (Retnakaran & Zinman, 2008) and is a leading cause of mortality in coronary artery disease (Krolewski *et al.,* 1987).

2.2.2 Type 2 diabetes

Type 2 diabetes has been described as adult-onset diabetes due to it association with increasing age. The basic defects of this metabolic condition are insulin resistance, hyperinsulinaemia (elevated blood insulin), in the early stages of the disease, followed by insulin deficiency in the later stages, decreased glucose effectiveness (Bouche *et al.,* 2004) and elevated EGP (Abuissa *et al*., 2005). A description of the multifactorial dysfunction that contributes to type 2 diabetes follows in sections 2.5 (Figure 2.1). The specific aetiologies that relate to type 2 diabetes are not fully understood, although autoimmune destruction of β-cells does not occur. Obesity, to some degree, causes insulin resistance with many diagnosed with this condition having a body mass index (BMI) >35 kg/m^2. However, it would appear that total fat mass and adipose

distribution, rather than BMI, are more strongly associated with insulin resistance and type 2 diabetes (Azuma *et al.,* 2007). The amount of visceral adipose tissue has been consistently shown to correlate with insulin resistance (Ross *et al.,* 2002) and type 2 diabetes (Gastaldelli *et al.,* 2002; Azuma *et al.,* 2007). Other risk factors for this metabolic condition include; age, physical inactivity, race / geographical location and a genetic predisposition (Stumvoll *et al.,* 2005). It is common for many individuals with type 2 diabetes to remain asymptomatic, which often results in the disorder going undiagnosed. Furthermore, in some cases, individuals with type 2 diabetes have no outwardly showing symptoms at the time of diagnosis. Evans *et al.,* (2008) have recently stated that early diagnosis (i.e. before symptoms are recognised) is beneficial in the treatment of type 2 diabetes and can decrease the occurrence of related complications. Type 2 diabetic symptoms may include polydipsia (increased thirst), polyuria (increased urination), increased appetite, fatigue, blurred vision and unexplained weight loss.

2.2.3 Diagnostic Criteria of Diabetes Mellitus

The diagnostic criteria for diabetes mellitus has recently been modified from those previously recommended by the National Diabetes Data Group (NDDG, 1979) and the World Health Organisation (WHO, 1985), which suggests three methods of diagnosis [Table 2.1; adapted from Report of the Expert Committee on the Classification and Diagnosis of Diabetes (2002) and Stumvoll *et al*., (2005)]. Each method of diagnosis must be confirmed, on a subsequent day using a different measuring criteria outlined in Table 2.1. However, the gold standard for the diagnosis of diabetes is the Oral Glucose Tolerance Test (OGTT; section 2.3.2).

An international Expert Committee (Report of the Expert Committee on the Classification and Diagnosis of Diabetes, 2002) was established in 1995 to review scientific data published since the last classification of diabetes in 1979 by the National Diabetes Data Group (NDDG, 1979). These groups acknowledged two major forms of diabetes and termed them insulin-dependent diabetes mellitus (IDDM, type 1 diabetes) and non-insulin-dependent diabetes mellitus (NIDDM; type 2 diabetes or adult-onset diabetes).

Table 2.1 **Diagnostic Criteria for Diabetes Mellitus and Impaired Glucose Tolerance**

Diabetes Mellitus	o Fasting ≥ 7.0 mmol/l o Glucose concentration ≥ 11.1 two hr post 75g OGTT ≥ 11.1 mmol/l o Symptoms of diabetes + casual glucose concentrations 11.1 mmol/l
Impaired Glucose Tolerance	o Fasting <7.0 and two hr post OGTT ≥ 7.8 and <11.1 mmol/l
Impaired fasting glucose	o Fasting ≥ 6.1 and <7.0 and 2 hr post OGTT <7.8 mmol/l

Fasting*; Defined as no caloric intake for at least 8 hr;* ***Casual****; Random blood glucose test without regard to time of day or feeding****; OGTT****; 75g Oral Glucose Tolerance Test. Values represent Glucose concentrations in venous plasma (mmol/l). Adapted from* Report of the Expert Committee on the Classification and Diagnosis of Diabetes (2002) and Stumvoll *et al*. (2005).

2.3 Assessment of Glucose Tolerance & Insulin Sensitivity

2.3.1 Glycated Haemoglobin (HbA_{1c})

Incidence reflecting glycaemia control can be measured using a range of methods and techniques. The use of glycated haemoglobin (HbA_{1c} or A1c), sampled from venous or finger tip blood is widely used in clinical and research settings. During the normal life cycle of red blood cells, glucose binds with haemoglobin to form HbA_{1c}. The rate of this binding is directly proportional to the concentration of blood glucose. An increase in HbA_{1c} therefore, reflects the level of hyperglycaemia that a red blood cell has been exposed to during its life (Table 2.2). Assay measurement of HbA_{1c} offers a means of assessing glucose control over the preceding 12 wks (Nathan *et al.*, 1984). The United Kingdom Prospective Diabetes Study (Stratton *et al.*, 2000) highlighted a direct association between HbA_{1c} levels and risks of diabetic related complications. The same paper also demonstrated that a 1% fall in HbA_{1c} reduces the occurrence of these complications by 21% (Stratton *et al.*, 2000). Elsewhere, Davidson *et al.* (2001) presented data reporting that if HbA_{1c} is kept $\leq$ 7% (non diabetic values ~5%), development of secondary complications are close to zero and that HbA_{1c} offers a good reflection of glycaemic control and secondary complications over the Oral Glucose Tolerance Test (OGTT).

Table 2.2 Approximate Relationship between Blood Glucose Concentrations and HbA_{1c}

Glyclated Haemoglobin (HbA_{1c}%)	**Mean Blood Glucose Concentrations (mmol/l)**
4	3.3
5	5.0
6	6.7
7	8.3
8	10.0
9	11.7
10	13.3
11	15.0
12	16.7
13	18.3
14	20.0

Adapted from Nathan *et al.* (1984)

2.3.2 Oral Glucose Tolerance Test (OGTT)

The OGTT is routinely used for the diagnosis of diabetes and glucose intolerance (Report of the Expert Committee on the Classification and Diagnosis of Diabetes, 2002). The administration of the OGTT, with the measurement of plasma insulin, allows for the assessment β-cell secretory capacity and peripheral insulin sensitivity (Stumvoll *et al.*, 2000). The protocol for this test involves the ingestion of a solution containing the equivalent of 75-g anhydrous glucose (WHO, 1999) and is therefore, not relative to body mass. Blood samples are then drawn over a 2-4 hr period to allow for the determination of blood glucose and plasma insulin. Stumvoll *et al.* (2000) concluded that the OGTT provides estimations of insulin sensitivity (insulin sensitivity index; $r = 0.79$, $P < 0.0005$) and β-cell function (first-phase

insulin release; r = 0.78, $P < 0.0005$) with reasonable accuracy when comparisons were made with the gold standard assessment of glucose tolerance (euglycaemic-hyperinsulinaemic clamp technique).

2.3.3 Euglycaemic – Hyperinsulinaemic Clamp Technique

The euglycaemic-hyperinsulinaemic clamp is described as a gold standard *in vivo* assessment of insulin sensitivity and β-cell function (DeFronzo *et al.,* 1979; Uwaifo *et al.,* 2002). Quantification of β-cell sensitivity to glucose and peripheral insulin sensitivity can be derived with the intravenous administration of insulin and glucose (Trout *et al.,* 2007). The purpose of insulin infusion is to elevate plasma insulin concentrations to a plateau approximately 100 μU/ml above basal levels. Blood glucose concentrations are then frequently determined, with the administration of exogenous glucose to maintain euglycaemia (5 mmol/l; Soop *et al.,* 2002). The clamp test was first developed by Andres *et al.* in 1965, due to limitations in the OGTT (Trout *et al.,* 2007). However, this procedure is impractical in a clinical diagnosis setting and perhaps undesirable in large experimental investigations as it requires intravenous insulin infusion, frequent blood sampling and continuous adjustment of glucose infusions (Konstantinos *et al.,* 2005).

2.3.4 Intravenous Glucose Tolerance Test (IVGTT)

The intravenous glucose tolerance test (IVGTT) provides estimates of the metabolic indices; glucose effectiveness (S_G), insulin sensitivity (S_I) and endogenous glucose production (EGP). The use of this procedure is frequently favoured over the more invasive and labour intensive euglycaemic–hyperinsulinaemic clamp technique (Vicini & Cobelli, 2001). The IVGTT, analysed as a one-compartment minimal model, requires the rapid intravenous injection of an exogenous glucose bolus with frequent sampling of venous blood in the subsequent ~ 4 hr. Although, this method's precision has been called into question as it provides unreliable measures of endogenous glucose production and is unable to separate measures of glucose effectiveness and plasma clearance rates (Vicini *et al.,* 1997). In an attempt to improve this method, the modified insulin IVGTT has been proposed where by exogenous insulin is administered at time points 20-25min. Although offering improvements in precision, this method has draw backs in that it becomes substantially more complex and can result in hypoglycaemia. Furthermore the introduction of exogenous insulin clearly makes the determination of β-cell function difficult (Vicini & Cobelli, 2001).

In 1993 Caumo *et al.* (1993) proposed that a two-compartment minimal model could solve a problem of the one-compartment IVGTT (i.e. the non-physiological pattern of EGP) and offer plausible profiles of EGP during an intravenous (labelled) glucose load. By rapidly injecting the stable isotope [6,6$^2H_2$]glucose (~10% of total glucose dose) within an unlabelled glucose (0.25 - 0.33 g / kg of body mass) bolus, Vicini *et al.* (1997) were able to demonstrated that the labelled IVGTT allowed for powerful assessment of glucose metabolism in the form of the two-compartment measurement of glucose effectiveness (S_G^{2*}), insulin sensitivity (S_I^{2*}) and EGP in various populations.

2.4 Diabetic Related Complications

The secondary complications associated with type 2 diabetes are the consequence of habitual hyperglycaemia, and can be categorised into either micro- or macro-vascular. Evidence suggests that the severity of micro-vascular complication in type 2 diabetes is linked with both the duration and extent of hyperglycaemia [UK Prospective Diabetes Study (UKPDS 33), 1999]. The same study provided comparable evidence showing a clear link between improvements in glycaemic control and reduction in diabetic related tissue damage (UKPDS 33).

The progression of the numerous long-term complications of diabetes mellitus seems to correlate with the severity and duration of hyperglycaemia. It is known that postprandial glucose levels > 11 mmol (200 mg / dL) are more frequently associated with renal, retinal, and neurologic complications which may transpire 5 to 10 yr after the onset of diabetes (Clark *et al.*, 1995; Gugliucci, 2000). Glucose is known to damage tissue by acute reversible changes in metabolism as well as cumulative effects that include irreversible alterations in stable macromolecules (i.e. forming of advanced glycation end-products). The link between hyperglycaemia and diabetic related complications has been comprehensively reviewed (Gugliucci, 2000). The cause of diabetic complications can be attributed to the aldose reductase reaction (Gabbay, 1973), oxidative stress (Soulis-Liparota *et al.*, 1995), advanced glycation end-product (Mailard) and modified protein kinase C activity (PKC; Gugliucci, 2000). Elevated glucose levels are thought to have an additional effect by causing localised tissue (pseudo) hypoxia, modified regulation of lipoprotein metabolism (Giugliano *et al.*, 1995) and altered cytokine activity (Lopes-Virella & Virella, 1996).

2.5 Type 2 Diabetes & Metabolic Dysfunction(s)

2.5.1 Insulin resistance

Insulin resistance may be defined as impairments in the biological response to endogenous insulin, is a key component of type 2 diabetes. Insulin resistance is categorised by a decrease in insulin-dependent glucose transport in insulin responsive tissue and an inability of insulin to suppress EGP (Dinneen *et al.,* 1992). An understanding of the cellular mechanisms implicated in insulin resistance is essential in developing therapeutic interventions in type 2 diabetes. Exposing skeletal muscle to elevated glucose concentration (4 hr 12 mmol/l) *in vitro* has been shown to inhibit insulin action and decrease glucose transport activity when compared to a euglycaemic control condition (Richter *et al.,* 1988). Kraegen *et al.* (2006) recently demonstrated that the onset of insulin resistance induced by excessive glucose availability reduced adenosine 5'-monophosphate-activated protein kinase (AMPK) phosphorylation and activity in skeletal muscle tissue by 50%. These authors maintained hyperglycaemia (17–18 mmol/l) by intravenous glucose infusion in rats for 3 or 5 hr; before euglycaemia was restored. A subsequent 2 hr hyperinsulinaemic clamp (following euglycaemia) showed that prior hyperglycaemia induced insulin resistance in both skeletal muscle and liver tissue ($P < 0.01$) (Kraegen *et al.,* 2006). This data proposes that hyperglycaemia decreases insulin-dependent glucose transport and may result in insulin resistance (Richter *et al.,* 1988).

The pathophysiology of insulin resistance is complex and is thought to involve a series of mechanisms which inhibit the insulin signalling cascade. Hormones, cytokines and non-esterified fatty acids (NEFA) released by adipocytes have been linked to reduced insulin action (Stumvoll *et al.,* 2005). Accumulation of triglycerides within subcutaneous adipose tissue has

been reported to trigger cell specific insulin resistance with a coupling effect on reduced insulin mediated suppression of lipolysis (Boden, 1997) (Figure 2.1).

In healthy subjects, insulin binds with insulin specific plasma membrane receptors (insulin receptor substrates; IRS) which when phosporlated, control the activity of intracellular tyrosine kinase involved in the insulin signalling cascade (White, 2002) (Figure 2.9). The movement of insulin-stimulated glucose transporters from intracellular sites to the plasma membrane include IRS-1 (muscle tissue) (White, 2002), phosphatidylinositol 3-kinase (PI-3kinase) (Frosig *et al.*, 2007), atypical PKC, PKB (Farese *et al.*, 2005), AMPK (Fisher *et al.*, 2002), Akt substrate of 160 kDa (AS160) (Howlett *et al.*, 2007). Insulin resistance seems to occur through a number of mechanisms that decrease IRS ability to activate subsequent protein kinase involved in downstream insulin signalling (White, 2002) including *1)* dephosphorlyation of protein side chains on the IRS protein, directly inhibiting IRS-1 activation (Zick, 2001); *2)* the degradation of IRS and loss of IRS from the cell surface, via direct action of local cytokines (Rui *et al.*, 2002) which also interfere with insulin stimulated glucose transporter (GLUT) activity; *3)* steady rate lipid-infusion (5 hr) has been reported to increase muscle triglyceride and long-chain acyl CoA content (Chalkey *et al.*, 1998) which is linked with decreasing IRS-1 and PI-3kinase activity (Tremblay *et al.*, 2001). Indeed, rodents feed with high fats diets exhibited impaired PI-3kinase function and downregulated Akt/protein kinase B activity (-40%, $P < 0.01$) with a subsequent decrease in GLUT translocation (Tremblay *et al.*, 2001); *4)* the proinflammatory cytokine tumour necrosis factor α (TNF-α) has been directly shown to stimulate adipocyte specific lipolysis, further increasing NEFA availability. This increase in NEFA encourages serine/threonine phosphorylation of IRS-1 and -2, adversely affecting insulin signalling (Griffin *et al.*, 1999).

Figure 2.1 Metabolic defects contributing to hyperglycaemia in type 2 diabetes (Stumvoll *et al.,* 2005).

2.5.2 β-Cell Dysfunction

Numerous abnormalities in insulin secretion exist in individuals with type 2 diabetes. The importance of insulin in the progression of impaired glucose tolerance (IGT) to overt type 2 diabetes is highlighted in Pima Indian populations who exhibit insulin resistance with a compensatory increase in insulin release. Although clinically insulin resistant, this population has the ability to respond to hyperglycaemia via increased β-cell insulin release which seems to slow or prevent the development of overt diabetes (Weyer *et al.,* 1999). In normally functioning β-cells, glucose is rapidly taken up by a pancreatic specific isoform of GLUT (GLUT-2). Phosphorylation of glucose then follows via the coenzyme glucokinase, converting glucose into glucose-6-phosphate (G-6-P) before further degradation results in the formation of

pyruvate (Stumvoll *et al.,* 2005) (Figure 2.2). Pyruvate then passes into the mitochondria where it is oxidised to produce adenosine triphosphate (ATP). ATP function, within β-cells provides much of the energy needed for both insulin release and cell membrane depolarisation. The increased ATP levels result in the closure of K_{ATP} channels, leading to membrane depolarisation. The consequence of such is the opening of voltage controlled Ca^{2+} channels, an increase in Ca^{2+} influx and release from the endoplasmic reticulum. The increase in intracellular Ca^{2+} concentration causes the release of previously synthesised insulin from secretory vesicles (Gabbay *et al.,* 1975). It is proposed that the coenzyme acts as a glucose sensor, causing increased release of insulin during hyperglycaemic episodes (Stumvoll *et al.,* 2005).

β-cell damage caused by glucose toxicity results in the deterioration of insulin release and is a prominent defect in type 2 diabetes (Wallace *et al.,* 2002). Excessive glucose metabolism has been reported to result in a large production of reactive oxygen species (ROS) within pancreatic β-cells and has been associated with causing cellular damage in the same cell type. The enhanced ROS are known to promote incorrect regulation of nuclear factor-kappa B (NF-κB), resulting in β-cell apoptosis (cell death) (Stumvoll *et al.,* 2005). Furthermore, high levels of circulating glucose have been shown to decrease GLUT-2 activity, which would presumably inhibit glucokinase's ability to sense glucose levels and up regulate insulin release. High fatty acid levels have also been implicated in reduced insulin secretory capacity through the build up of long-chain coenzyme A, a product of preferential glucose oxidation and elevated NEFA (Robertson *et al.,* 2004). It is suggested that the increased presence of long-chain coenzyme A diminishes insulin secretion through the opening of β-cell potassium channels (Robertson *et al.,* 2004).

Figure 2.2 Glucose induced insulin release in a normal functioning β-cell. GLUT-2; glucose transporter-2. G-6-P; glucose-6-phosphate.

2.5.3 *Endogenous Glucose Production (EPG)*

In postprandial states hepatic glucose uptake allows for the conversation of glucose to glycogen (glycogen synthesis) and triglycerides (Viollet *et al.,* 2006). These energy storing processes are reversible and play a key role in substrate availability via glycogenolysis, gluconeogenesis and lipolysis. Excessive EPG is thought to be a major contributor to the progression of elevated blood glucose concentrations in type 2 diabetes (Meyer *et al.,* 1998). In severe cases of diabetes (basal glucose levels > 7.8 mmol/l) it is generally proposed that EGP is the predominant factor (independent of feeding) responsible for hyperglycaemia (Fery, 1994), primarily due to hepatic insulin resistance (Figure 2.1) (Staehr *et al.,* 2002).

Furthermore, insulin treatment has been shown to reduce basal EGP rates by 25-50% in type 2 diabetic humans (Garvey *et al.,* 1985). Figure 2.3 shows the dose response of insulin on EGP in healthy subjects (Staehr *et al.,* 2002). Insulin is able to suppress EGP by as much as 60-70%, when hepatic insulin sensitivity is considered normal (Hother-Nielsen *et al.,* 1996). However, in type 2 diabetics, elevated EGP in the presence of elevated circulating plasma insulin suggests hepatic insulin resistance (Staehr *et al.,* 2002). Basal EGP has been shown to be 10-30% higher in hyperglycaemic glucose intolerant subjects (8-9 mmol/l) (Staehr *et al.,* 2002). In addition, Meyer *et al.* (1998) has shown that renal and hepatic glucose production are not only elevated in type 2 diabetics but are also comparable [renal; 2.2 (0.3) µmol / kg / min^{-1}, hepatic; 2.6 (0.7) µmol / kg / min^{-1}].

EGP is thought to be activated by hepatic specific AMPK which responds to various physiological stimuli including exercise and nutrient availability (Hardie, 2004), adiponection (Yamauchi *et al.,* 2002) and pharmacological agents including metformin (Zhou *et al.,* 2001; Viollet *et al.,* 2006). Hepatic specific AMPKα2 null mice exhibit both high glucose concentrations and increased EGP, suggesting that this AMKPα2 isoform is partly responsible for the control of EGP and maintenance of fasting blood glucose concentrations within the normal physiological range (Viollet *et al.,* 2006).

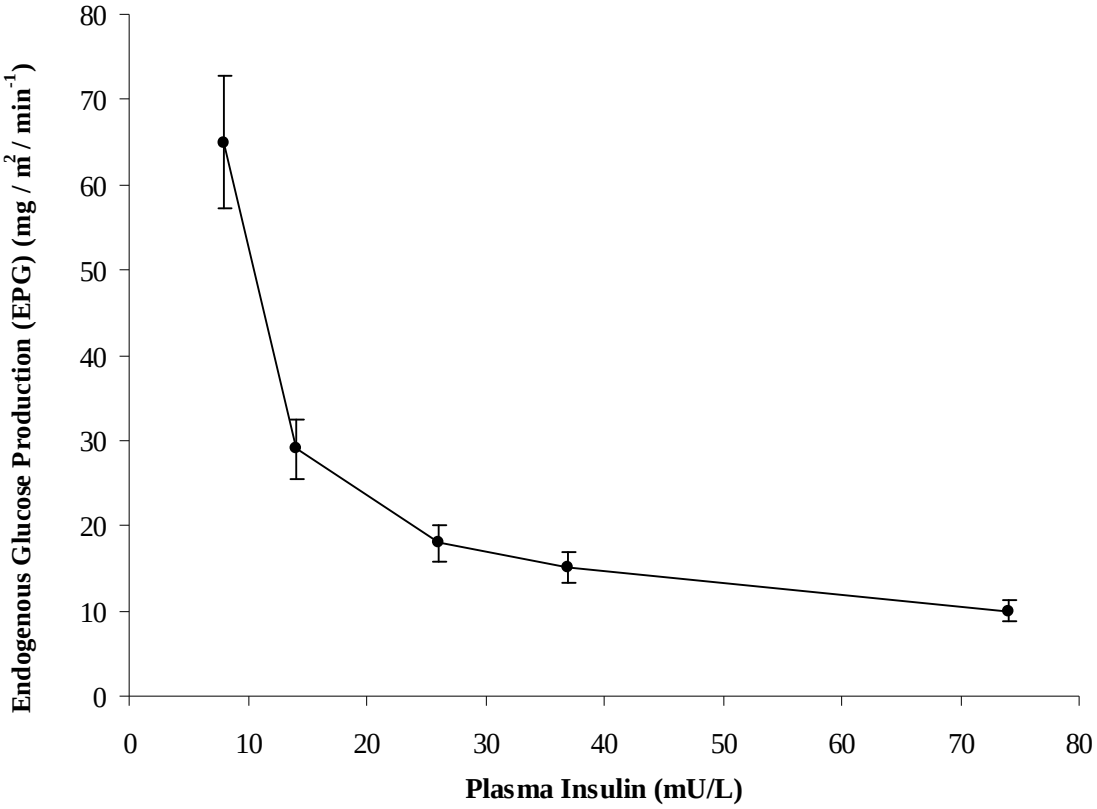

Figure 2.3 Relationship between plasma insulin levels and endogenous glucose production (EGP) (Staehr *et al.,* 2002).

2.5.4 Reduced Glucose Effectiveness

Cellular glucose uptake occurs via facilitated transport proteins which are stimulated *in vivo* by increased levels of circulating glucose and insulin. Blood glucose concentrations influence glucose uptake by a mass action effect that operates independent to insulin (Del Prato *et al.,* 1997). Insulin resistance is a key contributory factor of hyperglycaemia in type 2 diabetics (Schernthaner & Schernthaner, 2005; Henriksen *et al.,* 2006), although little is known about the ability of glucose to stimulate its own transport via GLUT-1 and -4 activities in skeletal muscle (Del Prato *et al.,* 1997). Glucose transport, via an insulin-independent mechanism, can

account for as much as 50% of the decline in blood glucose during periods when blood glucose is elevated (Bouche *et al.,* 2004). Glucose effectiveness (S_G), which is the ability of hyperglycaemia to promote glucose disposal at basal blood insulin concentrations, is a component of importance equal to or greater than insulin itself when determining glucose tolerance (Higaki *et al.,* 1996). Furthermore, glucose has been suggested to play a central role in promoting glucose resistance by decreasing proteins involved (AMPK) in its own metabolism and therefore down regulating glucose clearance (Itani *et al.,* 2003).

Glucose effectiveness (S_G), measured using an intravenous glucose tolerance test (IVGTT), has been shown to be impaired in type 2 diabetic individuals (Del Prato *et al.,* 1997). In maintaining insulin at basal levels Del Prato *et al.* (1997) were able to show that increasing blood glucose concentrations to three different levels (+2.8, +5.6 and +11.2 mmol/l above baseline) caused a progressive and significant increase in glucose uptake in healthy control subjects [13.1 (0.6), 15.7 (0.7) and 26.3 (1.1) µmol / kg / min^{-1}, respectively). Although the same pattern occurred in type 2 diabetic patients, the increase in glucose disposal was reduced [10.5 (0.2), 12.1 (1.0) and 17.5 (1.1) µmol / kg / min^{-1}, respectively) ($P < 0.05$). Using tracer infusion techniques Basu *et al.* (1997) demonstrated that plasma concentrations of [6-^{3}H]glucose were higher in diabetic subjects than in the nondiabetic controls ($P < 0.05$). This was due to both a reduced ability of glucose to stimulate its own disappearance via mass action and to a greater inhibitory effect of glucose on its own clearance (Arnfred *et al.,* 1988). Del Prato *et al.* (1997) concluded that glucose resistance in type 2 diabetic subjects could be attributed to a decrease in the ability of hyperglycaemia to promote its own transport in muscle tissue via an abnormality in glucose transport activity, associated with glucose toxicity.

2.6 Glucose Delivery, Transport & Utilisation

There are three steps that may be rate limiting for glucose uptake by muscle tissue: glucose delivery to the active muscle cell, the quantity of glucose transporter in the myocyte membrane (permeability to glucose) and glucose flux through intracellular metabolic pathways (Rose & Richter, 2005) (Figure 2.4). Once diffused from the capillary towards the muscle cell, glucose is then transported across the surface membrane by facilitative transporter proteins (glucose transporter; GLUT). Once in the muscle cell, glucose undergoes irreversible phosphorylation, making it available for oxidation or energy storage in the form of glycogen.

Figure 2.4 The rate-limiting steps in glucose uptake by skeletal muscle (Rose & Richter, 2005).

There are three major pathways for the cellular fate of glucose: *1)* oxidation to (in the absence of O_2), which may undergo further oxidation in the citric acid cycle; *2)* storage as the polysaccharide glycogen, which can be broken down and utilised during metabolic stress at a later stage; and *3)* conversion to other sugars and intermediates essential for biosynthetic and / or metabolic pathways, including the production of glycerol 3-phosphate used in triglyceride

and phospholipid synthesis, a key pathway of glucose in adipose, liver and muscle tissue (Bouche *et al.,* 2004). Glucose is both an efficient and unique source of fuel as it produces more ATP per molecule over other sources (lipids and proteins) and can produce ATP even in the absence of O_2 (Rose & Richter, 2005).

2.6.1 Glucose Delivery - Nitric Oxide Induced Blood Flow

The gas nitric oxide (NO) has been widely implicated in the regulation of muscle blood flow and glucose transport activity (Stamler & Meissner, 2001). During exercise, NO is synthesised by endothelial cells using L-arginine and NO synthase, after which NO enters adjacent vascular smooth muscle cells and activates guanylyl cyclise (Gill *et al.,* 2007). This enzyme (guanylyl cyclise) catalyses the dephosphorylation of guanosine 5'-triphosphate (GTP), forming cyclic guanosine 3',5'-monophosphate (cGMP) which stimulates the vasodilation of smooth muscles (Roberts *et al.,* 1999). This vasodilatory effect relieves the shear stress caused by exercise and promotes blood-oxygen delivery to skeletal muscle (Maiorana *et al.,* 2003). Electrically induced muscle contractions have also been shown to increase eNOS activity and NO release (Balon and Nadler, 1994), suggesting that exercise can promote both vascular (endothelial) and muscular release of eNOS with others showing that exercise upregulates eNOS gene expression (Yang *et al.,* 2002).

Obesity, insulin resistance and type 2 diabetes are characterised by reduced NO bioactivity and impaired NO vascular function which may inhibit muscle blood flow during exercise (Balon and Nadler, 1994; Maiorana *et al.,* 2003; Kearney *et al.,* 2007). Using a stable isotope (^{15}N Arginine) approach, Avogaro *et al.* (2003) have clearly shown that type 2 diabetics have impaired NO production, providing a link between endothelial dysfunction and atherosclerosis within the same population. A reduced NO production, with an associated decrease in muscle blood flow and glucose uptake, may account for the decreased $\dot{V}O_{2max}$ (Baldi *et al.,* 2003) and exercise capacity (Fang *et al.,* 2005) known to be present in type 2 diabetics. Although reduced mitochondria size (skeletal muscle) in type 2 diabetic (Kelley *et al.,* 2002) may also contribute to a reduced exercise capacity.

2.6.2 Cellular Glucose Transport

The transport of glucose into the cell is mediated by two distinct families of hexose transporter proteins located on the cell membrane: facilitative glucose carriers [glucose transporter (GLUTs)] and sodium-glucose cotransporters (SGLTs) (Bell *et al.,* 1993). A complete description of their structures and functions falls outside the capacity of this thesis. For detailed reviews see (Bell *et al.,* 1993). Sodium-glucose cotransporters (SGLTs) contain 14 transmembrane α-helices and are principally expressed in the intestine and kidney. There are two major isoforms SGLTs that have been characterised in detail (SGLT-1 and SGLT-2) although more may exist (Wright, 2001). The SGLT-1 isoform is primarily responsible for glucose and galactose uptake within the small intestine and has also been implicated in nephron mediated reabsorption of glucose from urine. SGLT-2 is principally found in the kidney and is also implicated in early glucose reabsorption within the proximal tubule (Bouche *et al.,* 2004).

Twelve facilitative glucose transporters (GLUTs) have been classified and can be further characterised into 3 classes. Class I consists of GLUTs with high-affinity binding proteins GLUT-1, GLUT-3 and GLUT-4 and the lower-affinity protein GLUT-2. The second class (II) of GLUTs include GLUT-5, GLUT-7, GLUT-9 and GLUT-11. These transporters have a very low affinity for glucose while favouring the transport of fructose. The third class (III) of GLUT have been described as novel as less is known about this class of transporters and include GLUT-6, GLUT-8, GLUT-10 and GLUT-12. Class I GLUTs will be discussed in more detail as these proteins are principally expressed in the tissue of interest (skeletal muscle). Glucose transporter-1 and -4 are the primary GLUTs found in skeletal muscle. Although found in low levels, GLUT-1 is primarily responsible for cellular glucose uptake under basal conditions (Barnes *et al.,* 2002) and is continually located in the cell membrane.

GLUT-4 mediates insulin-dependent and independent glucose transport through its translocation from intracellular sites to the cell membrane (Figure 2.9). Both insulin and muscular contractions (exercise) are known to stimulate GLUT-4 movement to the cell membrane (Richter *et al.,* 2001). Although insulin and exercise are thought to activate GLUT-4 translocation from different intracellular storage sites (Ploug *et al.,* 1998). GLUT-4 has been shown to have an important function in glucose transport and glucose tolerance (O'Gorman *et al.,* 2006). Insulin stimulated glucose transport, is known to be decreased in GLUT-4 null rodents (Katz *et al.,* 1995) with decreased insulin action also being linked with reduced GLUT-4 trafficking in type 2 diabetic humans (Karlsson *et al.,* 2005). Augmented glucose uptake in contracting skeletal muscle has been shown to be the product of increased GLUT-4 membrane content, both acutely (during exercise) and chronically (post exercise) in type 2 diabetic patients (O'Gorman *et al.,* 2006).

2.6.3 Glucose Phosphorylation and Glucose-6-phosphate Hydrolysis – Implications in Type 2 Diabetes

Upon entering the cell, glucose is rapidly phosphorylated to glucose 6-phosphate (G-6-P) by the enzyme hexokinase, subsequently trapping the glucose molecule within the cell and committing it to metabolic processes that include glycolysis and glycogen synthesis (Figure 2.5). The hexokinase II isoform, found predominantly in skeletal muscle is known to be up regulated by exercise (Koval *et al.*, 1998) and insulin (Vogt *et al.*, 2000), with loss of activity found in insulin resistant skeletal muscle (Katzen *et al.*, 1970). Furthermore, there is evidence to suggest that both glucose phosphorylation and hexokinase II expression are reduced in obese and type 2 diabetic individuals (Pendergrass *et al.*, 1998) and that exercise can increase hexokinase activity in healthy control, but not type 2 diabetic subjects (Cusi *et al.*, 2001).

Figure 2.5 The fate of glucose with its key pathways: glucose membrane transport and phosphorylation, glycolysis, glycogen synthesis, pentose phosphate pathway and hexosamine biosynthesis (adapted from Bouche *et al.*, 2004).

2.6.4 Glucose Utilisation

In skeletal muscle, glucose can be used to generate ATP via both aerobic and anaerobic glycolysis. The metabolic process of glycolysis provides energy via ATP resynthesis in all cell types and involves the breakdown of glucose in an 11 stage enzyme reaction within the cytosol (Figure 2.6). Pyruvate, the end-product of glycolysis, can then be converted to lactate via lactate dehydrogenase. Under aerobic conditions, glucose produces a net of 36 ATP molecules per molecule in skeletal muscle. Following the breakdown of glucose into 2 molecules of pyruvate, the latter can then cross the mitochondrial membrane where it is

rapidly converted to acetyl-CoA (coenzyme A) using the pyruvate dehydrogenase complex. Acetyl-CoA is then able to enter the Krebs cycle (citric acid cycle / tricarboxylic acid cycle) where electrons are made available to the electron transport chain via the coenzymes FAD and NAD^+.

Glucose 6-phosphatase is the enzyme responsible for the hydrolysis of G-6-P into phosphate and glucose. This reaction is the final stage in gluconeogenesis and glycogenolysis and plays a fundamental role in the regulation of blood glucose. Glucose is then free to leave the cell via glucose transporter proteins. Glucose 6-phosphatase, predominately expressed in hepatocytes, is responsive to both hormonal and substrate input and is known to be downregulated in the presence of elevated insulin and glucose concentrations (Bouche *et al.,* 2004). Furthermore, overexpression of this enzyme has been closely associated with insulin resistance in animal models (Lange *et al.,* 1994) with both increased G-6-P activity and increased EGP being implicated in type 2 diabetic humans (Clore *et al.,* 2000).

2.6.5 Glycogen Synthesis - Implications of Type 2 Diabetes

Skeletal muscle is the major site for insulin stimulated glucose disposal. Much of the glucose entering skeletal muscle is stored as glycogen. Therefore, an understanding of this process and how it is altered under different metabolic conditions is vital in understanding how interventions may benefit type 2 diabetics. The linkage of glucose into its polysaccharide form (glycogen) is known as glycogen synthesis.

Upon entering the cell, glucose is rapidly phosphorylated by hexokinase forming G-6-P, subsequently trapping the glucose molecule within the cell and committing it to metabolic processes. The next process involves the conversion of G-6-P to Glucose-1-phosphate using the enzyme phosphoglucomutase. Glucose-1-phosphate is then available to form uridine diphosphoglucose (UDP-glucose) via UDP-glucose pyrophorphorylase. The formation of UDP-glucose represents the preliminary steps in glycogen synthesis, with the latter stage being mediated by the glycosyltransferase protein glycogenin (Bouche *et al.,* 2004). Glycogenin acts as a primer, to which additional glucose monomers are attached by catalyzing additional glucose from UDP-glucose. Once eight further glucose residues have been added, glycogen synthase (GS) acts to extend the glucose polymer, forming a covalently linked oligosaccharide. The final step in glycogen synthesis requires the enzyme glycogen branching transferase, which attaches glycosyl chains in α-1,6-glycosidic bonds to chains of glycogen (Bouche *et al.,* 2004).

Insulin is known to stimulate PKB/Akt in a PI-3kinase dependent manner. In turn PKB/Akt phosphorylates glycogen synthase kinase 3 (GSK-3) on Ser^{21} and Ser^{9} in GSK-3α and β, respectively, leading to their deactivation (King *et al.,* 2006). GSK-3 is known to phosphorylate and so inhibit glycogen synthase activity. Indeed, glycogen synthesis increases, as measured by GS activity, when human hepatic cells are treated with GSK-3 inhibitors in vitro (Coghlan *et al.,* 2000). The inhibition of GSK-3 results in the dephosphorylation and activation of GS, and the stimulation of glycogen synthesis (Yeaman *et al.,* 2001). Insulin also results in the activation of protein phosphatase 1 (PP1), which removes a phosphate group and so activates GS while inactivating glycogen phosphatase (Groop & Orho-Melander, 2008).

In addition, GS can also be regulated in an allosteric manner by G-6-P. That is, the substrate G-6-P is able to activate glycogen formation by binding at a non-active protein site on GS (Roach, 1990). Following glycogen depletion (induced by contractile activity) glucose readily activates glycogen synthesis and GS activity independent of GSK-3 and PP1 activity (Halse *et al.,* 2001). These authors showed that *in vitro* starvation and re administration with glucose reduced and increased glycogen synthesis, respectively in human gastrocnemius muscle cells when using an insulin inhibitor, which presumably did not result in GSK-3 phosphorylation (Halse *et al.,* 2001). These data suggesting that glycogen synthesis can be regulated via an insulin-dependent pathway. It is proposed that GS is a substrate for AMPK and that AMPK activation may be involved in glycogen synthesis. AMPK has been shown to phosphorylate glycogen synthase at Ser^{7}, resulting in a decrease in activity at low levels of G-6-P (Carling & Hardie, 1989). Evidence for the role of AMPK in glycogen synthesis is also based on the finding that mutation of swine PRKAG3 gene, which encodes the regulatory γ subunit of muscle specific AMPK, is associated with higher muscle glycogen content in animal models (Milan *et al.,* 2000). Furthermore, long-term treatment with AICAR *in vivo* increases muscle glycogen content in insulin resistant obese Zucker (fa/fa) rodents (Buhl *et al.,* 2002). Although, this notion remains controversial and that the association of AMPK regulation with glycogen content may merely reflect a correlation between the two (McBride *et al.,* 2009).

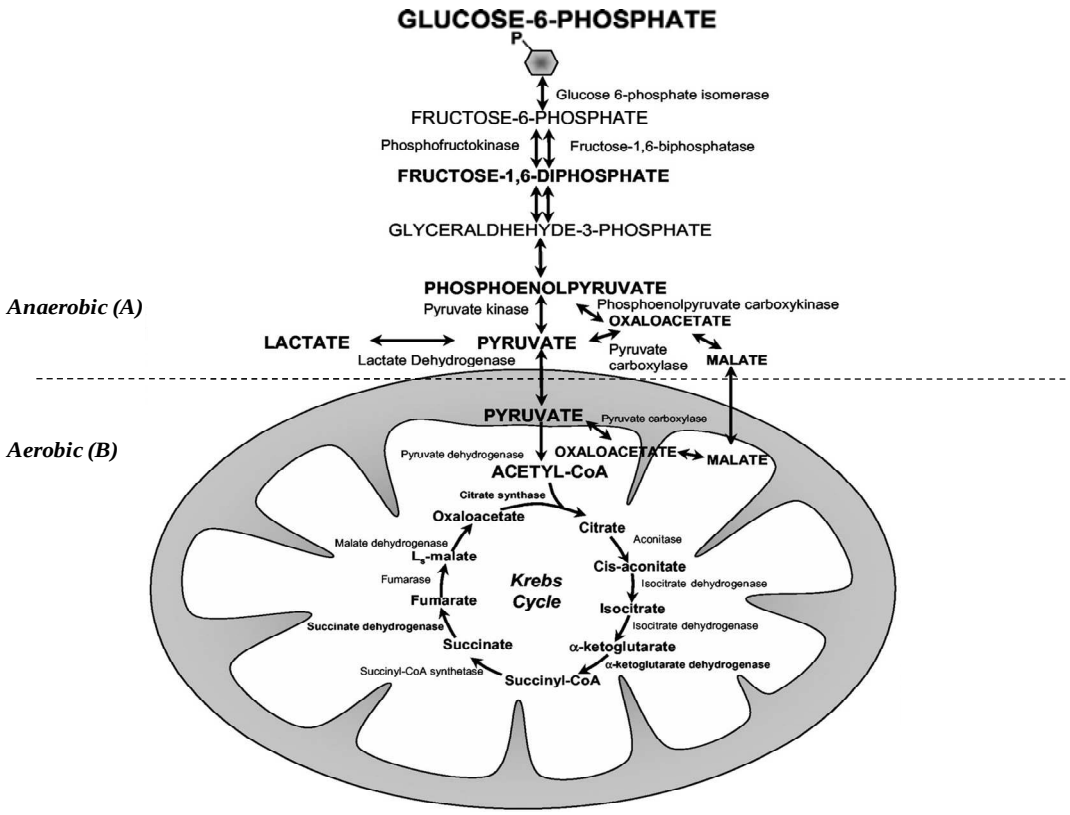

Figure 2.6 Anaerobic and Aerobic Glycolysis (Adapted from Bouche *et al.*, 2004). Shows the fate of glucose after its conversion to glucose-6-phosphate.

2.6.6 Pentose Phosphate Pathway - Implications for Type 2 Diabetes

The pentose phosphate pathway, sometimes referred to as the phosphogluconate or hexose monophosphate pathway, is predominantly active in adipose tissue, hepatocytes, and lactating mammary glands (Houston, 2001). It occurs in the cytosol, producing NADPH and synthesizing pentose (5-carbon sugars). The amount of glucose catabolized through this pathway is tissue dependent. Although it is known to be more active in diabetics due to an increased oxidative stress (Wu & Ren, 2006). The generation of NADPH during the pentose phosphate pathway is used to reduce the coenzyme glutathione, which converts reactive H_2O_2 into H_2O. Without this reaction H_2O_2 would be freely available to form hydroxyl free radicals (Houston, 2001).

2.6.7 Hexosamine Pathway – Implications for Type 2 Diabetes

The hexosamine pathway is a cellular energy sensing pathway that adapts the expression of many nuclear-encoded mitochondrial genes involved in oxidative metabolism, in both skeletal muscle and adipose tissue (Obici *et al.,* 2002). Excluding the first step in the hexosamine pathway, all reactions are reversible and do not require ATP. The preliminary step in the hexosamine biosynthesis pathway requires the conversation of fructose-6-phosphate to glucosamine-6-phosphate using the enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). *N*-Acetyl glucosamine-6-phosphate is then readily adapted to produce *N*-acetyl glucosamine-1,6-phosphate and then to UDP-*N*-Acetyl glucosamine. UDP-*N*-Acetyl glucosamine is then made available as a substrate for O-linked glycosylation, using O-Aceylglucosamine transferase (Kaneto *et al.,* 2001; Bouche *et al.,* 2004). The latter part of this process results in cytoplasmic and nuclear proteins being glycosylated at the hydroxyl group on serine / threonine residues (Kaneto *et al.,* 2001).

Over activity of the hexosamine pathway is a common feature involved in insulin resistance and type 2 diabetes (Buse *et al.,* 1997). It is suggested that when glucose flux is excessive, the hexosamine pathway responds by down-regulating glucose transport activity by inhibiting both insulin action (sensitivity) and S_G, as assessed using an IVGTT (Monauni *et al.,* 2000). Monauni *et al.* (2000) also contributed β-cell impairment with increased activity of hexosamine biosynthesis (Monauni *et al.,* 2000). Furthermore, increased glucose availability to the hexosamine pathway has been shown to down-regulate GLUT-4 translocation (Baron *et al.,* 1995), glycogen synthesis and PI-3kinase activity (Hawkins *et al.,* 1999), highlighting its role in reduced insulin resistance and glycogen formation.

In addition to impaired insulin action and β-cell dysfunction, the hexosamine pathway also seems to be implicated in the development of diabetic related complications. The end products of the hexosamine pathway (hexosamines) have been linked with causing endothelial dysfunction in bovine aortic cells, which was attributed to a decrease in endothelial nitric oxide (eNOS) activity (Du *et al.,* 2001). It is proposed that increased activity of the hexosamine pathway decreases PKB induced Ser^{1177} phosphorylation of eNOS (Du *et al.,* 2001). Elsewhere, Federici *et al.* (2002) have shown that atherosclerotic plaques were greater, as was glycosylation in diabetic patients, and that increased activity in hexosamine biosynthesis contributes to the development of diabetic macrovascular complications through a decrease in eNOS activity (Federici *et al.,* 2002).

2.7 Type 2 Diabetes & Exercise

Exercise can stimulate whole body oxygen consumption by as much as 20-fold (ADA, 2002). The energy provided to the working muscle during exercise is supplied by muscle glycogen, triglycerides, free fatty acids (FFAs) derived from the breakdown of adipose tissue and glucose released through glycogenolysis (EGP). The supply of glucose for exercising muscle is controlled largely by hormonal input. The inhibition of circulating insulin and increase in glucagon release stimulates EGP, providing fuel metabolism during muscular contractions (ADA, 2002). The rate of glucose removal from the blood is increased during moderate intensity exercise in type 2 diabetic individuals (Colberg *et al.,* 2008). As a product of the increased cellular requirements for glucose, exercise has been used as an intervention in the treatment and prevention of type 2 diabetes.

2.7.1 Fuel Metabolism during Exercise

Research conducted using radiolabelled glucose (Reichard *et al.,* 1961) and direct measurement of arteriovenous glucose differences across exercising muscle groups (Sanders *et al.,* 1964) has shown that muscle glucose uptake is increased during exercise (Hargreaves, 1995). Muscle glycogen and circulating blood glucose are key substrates in the resynthesis of ATP in contracting skeletal muscle (Hargreaves and Spriet, 2006). There is a progressive transfer from predominantly lipid oxidation at rest, to the use of multiple fuel sources during exercise including blood glucose, muscle glycogen and NEFA (Hargreaves, 1995). This change in fuel utilisation provides both the aerobic and anaerobic pathways with the required substrates needed during periods of increased energy demand (Coyle *et al.,* 1991).

The metabolic reliance on blood glucose and muscle glycogen for ATP synthesis during exercise increases in an intensity dependent manner (Kjaer *et al.,* 1991; Hargreaves & Spriet, 2006). Contraction stimulated glucose uptake is known to be greater than that induced by maximal insulin stimulation (James *et al.,* 1985). It is widely accepted that fatigue is often associated with glycogen depletion (muscle and liver) and / or hypoglycaemia (Sahlin *et al.,* 1990; Coyle, 1995). Evidence for the importance of blood glucose to contracting skeletal muscle extends from the observation that the sole use of lipids as a substrate is unable to provide adequate energy at exercise intensities >50-60% $\dot{V}O_{2max}$ (Davies and Thompson, 1979; Coyle, 1991). The onset of exercise stimulates an increase in peripheral glucose uptake within skeletal muscle from ~15-20% during rest to ~80-85% of total blood glucose removal during moderate intensity exercise (50-60% $\dot{V}O_{2max}$) (Kjaer *et al.,* 1991). The reliance of skeletal muscle for blood glucose provides a clear rationale for the use of exercise in the prevention and treatment of hyperglycaemia in type 2 diabetics.

2.7.2 Acute Exercise & Glucose Metabolism

Acute exercise stimulates the transfer of glucose from capillary, by diffusion to the surface membranes of the muscle fibre, where it is transported into the cell via facilitative transport for the resynthesis of ATP. Exercise-induced stimulation of glucose transport activity is known to decrease hyperglycaemia and improve glucose tolerance in type 2 diabetics (Hayashi *et al.*, 2005; Bordenave *et al.*, 2008). Since insulin secretion is inhibited during exercise (Musi *et al.*, 2001), it is generally accepted that the reduction in glucose concentration can be attributed to the skeletal muscle's ability to promote glucose uptake independent of insulin (contraction stimulated glucose transport) (Rose & Richter, 2005). This insulin-independent pathway is thought to remain intact within type 2 diabetics (Azevedo *et al.*, 1995; Kennedy *et al.*, 1999). However, acute exercise seems to stimulate glucose transport by up-regulating both the insulin-dependent and -independent pathways (Dohm, 2002; Holloszy, 2005; Rose & Richter, 2005). The effects of exercise on insulin-stimulated glucose transport are discussed in more detail in section 2.7.4.

2.7.3 The Effects of a Single Bout of Exercise on Blood Glucose Concentrations in Type 2 Diabetes

Many studies have shown improvements in glucose tolerance during and / or following a single bout of physical activity (Devlin *et al*., 1987; Larsen *et al*., 1997a; Kang *et al*., 1999; Macdonald *et al*., 2004). Even moderate intensity exercise (60 min at 90% lactate threshold) has been shown to reduce blood glucose concentrations (-2.5 mmol/l) (Macdonald *et al*., 2004). Favourable changes in blood glucose concentrations have been reported in low-moderate (Larsen *et al.*, 1997a) and high intensity exercise (Larsen *et al.*, 1999). Recently, Sriwijitkamol *et al.* (2007) demonstrated decreases in blood glucose concentration during 40

min of both low [50% $\dot{V}O_{2max}$; 0.6 (0.3) mmol/l, $P < 0.05$] and moderate [70% % $\dot{V}O_{2max}$; 1.3 (0.3) mmol/l $P = 0.001$] intensity exercise. In agreement with these findings Colberg *et al.* (1996) reported that exercise intensities greater than 40% peak O_2 uptake ($\dot{V}O_{2peak}$) are minimum in bringing about improvements in glucose control. The current guidelines for physically activity are 30 min of moderate intensity exercise (50% $\dot{V}O_{2max}$) per day (Sigal *et al.,* 2006). Although, a recent review suggests that these recommendations are insufficient (Gill and Cooper, 2008) and that exercise durations should be increased to 60 min per day to assist weight loss and improve glycaemia (Haskell *et al.,* 2007). Baynard *et al.* (2005) recently found that 30 min of exercise at 60% $\dot{V}O_{2peak}$ had no influence on area under the curve for glucose (AUC_{Glu}) in type 2 diabetic females when compared to a non exercise control trial. These findings suggest that the current guidelines for general health and well-being are insufficient to promote positive changes in acute glucose control, and that obese / type 2 diabetic individuals may need to exercise for longer durations or at higher exercise intensities to produce favorable glycaemic responses.

Sriwijitkamol *et al.* (2007) suggested that type 2 diabetics may need to exercise at greater relative exercise intensities to produce similar glucose lowering effects than healthy controls. Previous work has shown that carbohydrate oxidation was significantly greater (68%; $P < 0.05$) during exercise at 70% $\dot{V}O_{2peak}$ [398 (67) kcal] when compared to 50% $\dot{V}O_{2peak}$ [237 (37) kcal] (Hayashi *et al.,* 2005). This data suggests that that the degree of glucose metabolism is intensity dependent.

Kang *et al*. (1999) compared the blood glucose response to two exercise conditions (50% and 70% $\dot{V}O_{2peak}$) in healthy control and type 2 diabetic individuals. Using isotope infusion method ([6,6$^2H_2$]glucose) these authors were able to show that the rate of glucose disappearance (R_d) was higher during exercise in the diabetic population when compared to the non diabetic control group ($P < 0.05$) (Figure 2.7). It is worth noting that hyperglycaemia can promote greater glucose removal during exercise in type 2 diabetics (DeFronzo *et al.,* 1981). This is highlighted in Kang *et al.* (1999) work which showed a significant reduction in blood glucose concentration in type 2 diabetics, which was not present health controls during 70 min of moderate intensity (50% $\dot{V}O_{2peak}$) exercise (Figure 2.7). Later work has demonstrated similar results to those published by Kang *et al.* (1999), which is consistent throughout the literature. Febbraio *et al.* (2003) showed that exercise lowers circulating blood glucose in type 2 diabetics ($P < 0.01$) and that this change can be attributed to an increased arterial-venous (a-v) glucose difference and exercising muscle blood flow. Suggesting that even moderate intensity short duration exercise [25 min; 60 (2) % $\dot{V}O_{2peak}$] can acutely improve glycaemic control (Febbraio *et al.,* 2003). The increase in glucose uptake can be attributed to NO induced vasodilation (De Filippis *et al.,* 2006) (increased muscle blood flow), glycogen depletion and increased content of glucose transporters in the plasma membrane (Lund *et al.,* 1997; Zinker *et al.,* 1993). Plasma membrane GLUT-4 content is highly correlated to glucose transport activity in human skeletal muscle (Lund *et al.,* 1997) with GLUT-4 sarcolemma content shown to increase 74% during acute exercise (45-60 min cycling exercise ~60-70% $\dot{V}O_{2max}$) in type 2 diabetic patients (Kennedy *et al.,* 1999). The ability of exercise to stimulate glycogen depletion seems to be the mechanism by which short-term improvements in glucose control are produced via insulin-dependent glucose transport (Houmard *et al.,* 2004; Richter *et al.,* 1982).

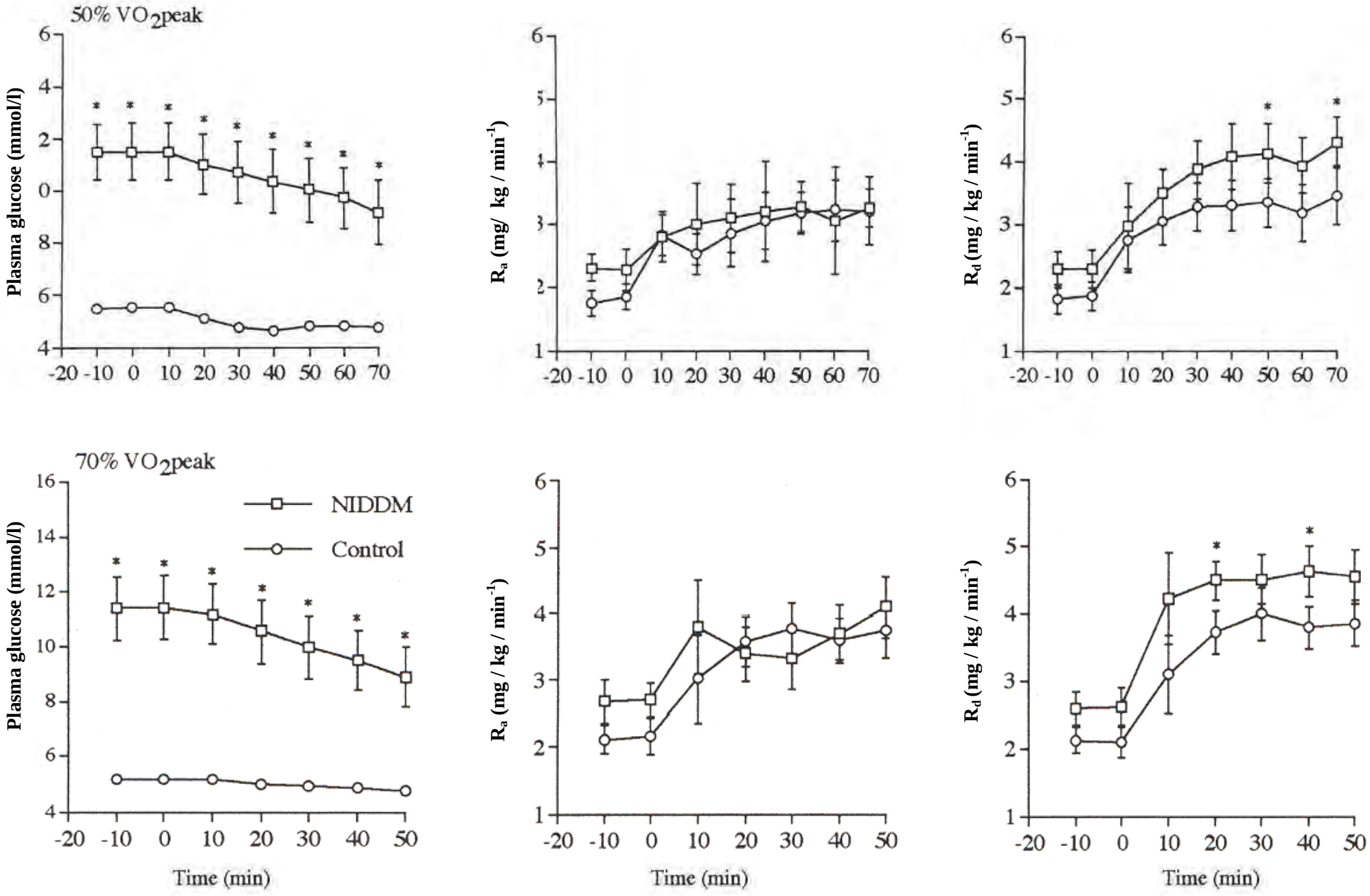

Figure 2.7 Plasma glucose concentrations, glucose rate of appearance (R_a) and disappearance (R_d) during Exercise at 50% and 70% $\dot{V}O_{2peak}$.
Subject groups are controls and type 2 diabetics. NIDDM; noninsulin dependent diabetes Mellitus. Actual data from Kang *et al.* (1999).

2.7.4 Effects of Acute Exercise: Post Exercise Insulin Sensitivity

As the acute effects of muscular activity wear off, insulin-independent (contraction-stimulated) glucose transport is replaced by an insulin-dependent pathway that provides the muscle cell with its substrate for glycogen synthesis. Garetto *et al.* (1984) was the first to describe that exercise-induced glucose metabolism occurs in two phases. The first phase, independent of insulin, provides glucose for glycolysis during exercise. As this mechanism returns to equilibrium, it is replaced by an increase in insulin-dependent transport, sometimes referred to as an increase in insulin sensitivity (Holloszy, 2005). There is a considerable body of literature demonstrating the acute effects of exercise on insulin sensitivity (Henriksen, 2002; Sakamoto & Goodyear, 2002; Wojtaszewski *et al.,* 2002; Zierath, 2002; Holloszy, 2003; Holloszy, 2005). Studies have demonstrated that acute exercise has the ability to activate insulin-stimulated glucose transport in both rodents (Gao *et al.,* 1994) and diabetic humans (Minuk *et al.*, 1981; Devlin *et al.*, 1987). That is, in addition to activating the intact contraction-stimulated pathway, in type 2 diabetics, exercise seems to stimulate insulin-dependent glucose disposal (insulin sensitivity) in the hours (20 hr) following exercise (Devlin *et al.,* 1987).

Whole body glucose metabolism can be assessed with oral (OGTT) and intravenous (IVGTT) forms of glucose tolerance tests. The latter can be divided into both the minimal (one-compartment) and two-compartment model following an intravenous glucose load. In 1982, Richter *et al.* not only demonstrated that insulin sensitivity in rat skeletal muscle was improved by prior exercise (45 min treadmill running; velocity ~ 18 m / min) but that increases in rates of glycogen synthesis were muscle fibre type dependent (Richter *et al.*,

1982). This work demonstrated that glycogen synthesis was eight times greater in type IIa gastrocnemius muscle fibres over control samples and that type IIb gastrocnemius muscle fibres, that were not glycogen depleted, showed no change from resting control. This highlighted the effect of fibre-type on the ability of exercise to improve insulin sensitivity via the glycogen synthesis pathway and that for insulin sensitivity to be improved following exercise; a reduction (~33%) in muscle glycogen content must take place (Richter *et al.*, 1982). The moderate intensity exercise performed within Richter *et al.*'s early work also resulted in a ~48% decrease in liver glycogen levels (Richter *et al.*, 1982). Hayashi *et al.* (2005) and more recently Bordenave *et al.* (2008) have shown improvements in glucose tolerance in type 2 diabetics following acute exercise. Using the minimal model analysis (IVGTT) Bordenave *et al.*'s (2008) showed significant improvements in insulin sensitivity (S_I^{2*}) [control; 0.62 (0.16) and exercise; 5.41 (1.59) x 10^{-4} · min^{-1} (μU/ml)] in the 3 hr following exercise (15 min at 50% followed by 5 min at 85% of age predicted HR).

Numerous studies have been published showing increased glucose disposal following various exercise intensities and durations. Using the frequent sampling IVGTT, Bloem and Chang (2007) concluded that short duration exercise (60 minutes at 60-70% HR reserve) improved insulin resistance (increase insulin sensitivity) and β-cell function in individuals with impaired glucose tolerance. Zhou and Dohm (1997) showed a 6-fold increase in insulin stimulated glucose transport in response to 60 minutes of treadmill running. In addition, muscular contractions are known to promote insulin mediated glucose transport for up to 16-18 hr (Devlin *et al.*, 1987; Cartee *et al.*, 1989) following exercise. This finding is supported by Bogardus *et al*. (1983) who demonstrated an increase in insulin-stimulated glucose transport in the ~16 hr following exercise at ~80-90% of age predicted HR_{max}.

The effects of acute exercise on insulin sensitivity seem to be limited to improvements in whole body insulin-stimulated glucose transport via GLUT-1 activation and GLUT-4 translocation (Phillips *et al.,* 1996), rather than an exercise mediated stimulation of insulin signalling proteins (IRS-1, PI-3kinase and PKB) (Henriksen, 2002). Despite this, rapid resynthesis of muscle glycogen is known to follow moderate intensity exercise (Bergström & Hultman, 1966). O'Gorman *et al.* (2006) assessed the impact of acute exercise on whole body insulin-stimulated glucose disposal and insulin signalling cascade in obese non-diabetic (n = 7) and obese type 2 diabetic (n = 8) subjects. Their conclusions seem to be similar to those drawn by Henriksen *et al.* (2002). That is, improvements in glucose transport are independent of functional alterations in the insulin-signalling cascade and related to increased muscle GLUT-4 protein content (O'Gorman *et al.,* 2006). Although later work would suggest that proteins involved in the insulin signalling pathway can be up-regulated by acute exercise, providing a relative threshold for stimulation has been achieved. In support of this Sriwijitkamol *et al.* (2007) suggests that type 2 diabetic subjects may need to exercise at higher relative intensities to stimulate insulin signalling proteins [Akt substrate 160 kDa (AS160)]. Kang *et al.* (1999) showed that a single bout of exercise improved glucose tolerance and insulin sensitivity in a dose-dependent manner, which would seem to agree with the cellular work of Sriwijitkamol *et al.* (2007). It seems that acute exercise can activate both the insulin- and the contraction- stimulated pathways. Although, the precise exercise intensity and duration required to improve insulin action requires additional work. Elsewhere, Praet & van Loon (2007) suggest that the energy equivalent for exercise is the major determinant of exercise-induced improvements in insulin sensitivity. Therefore, lower exercise intensity should be compensated for by an increase in exercise duration (Praet & van Loon, 2007).

2.7.5 Exercise Training & Peripheral Insulin Sensitivity

The positive effects of acute exercise on blood glucose removal have been discussed in the previous sections (2.7.3 and 2.7.4). The beneficial effects of exercise on insulin sensitivity are reversed within ~72 hr of the last bout of exercise with improvements lasting 24 hr (Holm *et al.,* 1978), 48 hr (Mikines *et al.,* 1988) and 72 hr (LeBlanc *et al.,* 1981). Regular physical activity is therefore imperative in maintaining exercise mediated improvements in glucose control [American College of Sports Medicine (ACSM), 2000] and reducing disease risk factors (Booth *et al.,* 2002). The benefits of exercise training on medium and long-term whole body glucose tolerance will now be discussed.

Increased insulin sensitivity is improved in most (Hansen *et al.,* 1998; Mari *et al.,* 2001; Houmard *et al.,* 2004), but not every study following low-moderate intensity exercise in healthy control subjects (Hayashi *et al.,* 2005). This may indicate that exercise needs to be at a higher intensity or that a single bout of exercise is not a great enough stimulus to achieve improvements in insulin sensitivity. Although the former would seem to be the case given the work of Bordenave *et al.* (2008) who demonstrated improvements in insulin-dependent glucose transport following high-intensity exercise. Becker-Zimmermann and colleagues (1982) showed that moderate intensity exercise training could decrease the exaggerated insulin responses to a glucose load, and improve whole body insulin sensitivity in obese Zucker rats. These findings were confirmed by Walberg *et al.* (1984) who demonstrated that exercise training improved glucose clearance in response to an intravenous insulin load in obese rodents. Becker-Zimmermann *et al.* (1982) also suggested that exercise training may prevent a genetic predisposition for reduced glucose tolerance and insulin sensitivity in

adolescent rodents. These findings have received support elsewhere (Fell *et al.,* 1982; Garetto *et al.* 1984; Cortez *et al.,* 1991).

The improvements in insulin sensitivity following exercise are not limited to animal models and can be identified in healthy, glucose intolerant and type 2 diabetic humans (Henriksen, 2002). Exercise training has been shown to have a persistent effect on insulin mediated glucose transport in these populations (Dela *et al.,* 1995). In non diabetic individuals, Nishida *et al.* (2001) showed that moderate intensity exercise training (60 min / day / 5 days a wk / 6 wks at a 100% of pre-training lactate threshold) improved both glucose effectiveness and insulin sensitivity with no decrease in body composition. These results suggest that the exercise-induced improvements in glucose tolerance were elicited via an increase in muscle permeability to glucose, rather than an increase in muscle mass. The work of Nishida *et al.* (2001) has received support with exercise training shown to induce a 36-62% improvement in insulin sensitivity (Kahn *et al.,* 1990; Houmard *et al.,* 1993). Nishida *et al.* (2001) also demonstrated significantly lower fasting glucose concentrations post exercise training [pre; 94.9 (1.8) vs. Post; 90.5 (2.2) mg / dL, $P < 0.05$] with no change in fasting insulin values, again suggesting improvements in insulin's action / sensitivity.

In insulin-resistant individuals as much as ~80% of glucose disposal during an OGTT can be attributed to glucose effectiveness (Section 2.5.4) (Best *et al.,* 1996). Interestingly Nishida *et al.* (2001) also showed that moderate intensity exercise training can promote glucose effectiveness and that these improvements can prevent the progression of type 2 diabetes (Nishida *et al.,* 2001). This work also demonstrated that glucose effectiveness was still

enhanced 1 wk after the last bout of exercise (Nishida *et al.,* 2001). The authors suggested that an increase in muscle content and expression of GLUT-4, under basal insulin concentrations, may have caused the increase in glucose effectiveness following training (Phillips *et al.,* 1996).

Similar to acute exercise, exercise training has been shown to increase phosphorylation and expression of specific proteins involved in insulin-dependent and –independent transport. Although, a review by Henriksen (2001), suggests that improvements in whole body insulin stimulated glucose disposal following exercise training does not always correspond with increases in phosphorylation or protein expression in signalling proteins in humans (PKB, PI-3 kinase and IRS-1). Although, this notion is not supported by others who observed exercise training to increase both IRS-1 (Chibalin *et al.,* 2000; Hevener *et al.,* 2000) and PI-3 kinase activity (Kim *et al.,* 1995; Kim *et al.,* 1999; Chibalin *et al.,* 2000). These discrepancies may reflect differences between measurement methods, site (i.e. gastrocnemius and soleus) and muscle fibre type sampling (glycotic and oxidative), as well as the degree of glycogen depletion induced by exercise. It is also suggested that increased GLUT-4 translocation may occur due to alterations, rather than increased activity of, specific signalling proteins involved in insulin-stimulated transport (Mu *et al.,* 2001).

2.7.6 β-Cell Response to Exercise Training

The previous section has focused on the effects of endurance training on the peripheral response to insulin. Little is known about training induced alteration in β-cell function following physical training. β-cell function (insulin secretion) is an important process contributing to the metabolic dysfunction of type 2 diabetes as the ability of β-cells to respond to a given glucose challenge progressively decreases (Stumvoll *et al.,* 2005) with the development of this condition. Hyperglycaemia, therefore becomes a product of both decreased insulin action (insulin resistance) as well as decreased insulin secretion (Stumvoll *et al.,* 2005).

Dela *et al.* (2004) proposes that impaired β-cell function may be heterogeneous within type 2 diabetics and that insulin secretion may be enhanced following exercise training in those individuals who have a moderate insulin secretor capacity prior to training. Exercise training has been shown to have little effect on β-cell response in type 2 diabetics with low insulin secretory capacity (Dela *et al.,* 2004). Work carried out in rodents demonstrated that exercise training improved β-cell function in mild conditions of diabetes but not within severe conditions of this disorder (Farrell *et al.,* 1991). It is therefore possible that the severity of the type 2 diabetes (habitual or prolonged hyperglycaemia) may determine the capacity for exercise to induce improvements in β-cell function and that those individuals in the early stages of this condition may respond better to exercise training.

β-cell glycolysis increases insulin secretion and could provide a link between impaired glucose disposal and impaired insulin secretion (Henquin, 2000). It is proposed that exercise training can enhance glucose-stimulated insulin secretion in humans (Bloem & Chang, 2008) and experimental animals with type 2 diabetes (Baron *et al.,* 1991; Reaven, 1991). The activity of GLUT-2 within pancreatic β-cells is largely proportional to the circulating glucose concentrations (Bouche *et al.,* 2004), a feature that allows GLUT-2 to act as a glucose sensor, stimulating insulin secretion during periods of elevated blood glucose. Exercise has been previously shown to increase pancreatic GLUT-2 and glucokinase expression in type 2 diabetic rodents while improving β-cell function (Park *et al.,* 2007). It is plausible that exercise induced improvements in insulin sensitivity are associated with a reduction in circulating insulin levels, which can result in a reversal of β-cell GLUT-2 and glucokinase down-regulation (Bouche *et al.,* 2004), a characteristic of diabetic rodents (Park *et al.,* 2007).

2.7.7 Endogenous Glucose Production (EGP) During Exercise

The role of endogenous glucose production (EGP) during exercise is to contribute to glucose availability for working muscle through both glycogenolysis (at the commencement of exercise) and gluconeogenesis (towards the end of exercise) pathways. These pathways are stimulated by the increased activity of epinephrine, cortisol and glucagon in response to lower concentrations of plasma insulin and glucose during exercise (Berger *et al.,* 1980). In type 2 diabetics, compensatory hyperinsulinaemia brought on by peripheral insulin resistance and elevated blood glucose may inhibit EGP. In experiments using type 2 diabetic humans the fall in blood glucose during exercise has been attributed to both increased muscle uptake (Martin

et al., 1995; Colberg *et al.,* 1996) and blunted glucose production (Minuk *et al.,* 1981; Kang *et al.,* 1996). Using isotope infusion technique Minuk *et al.* (1981) were able to show that glucose release is inhibited in type 2 diabetics during exercise when compared to controls. However, glucose utilisation (decrease in blood glucose concentrations) was increased within the diabetic group (Minuk *et al.,* 1981).

Giacca *et al.* (1998) have illustrated that moderate intensity exercise (45 min and 50% $\dot{V}O_{2max}$) increased glucose production in lean control, obese non diabetic and diabetic subjects to a similar extent (with no difference between groups). Although the moderate degree of basal hyperglycaemia [7.5 (0.8) mmol/l] along with decreasing insulin levels during exercise (Giacca *et al.,* 1998) may have prevented EGP inhibition in the diabetic group (Berger *et al.,* 1980). It seems clear that the ability of hepatocytes to respond to exercise by increasing glucose release is dependent on the severity of the disease in type 2 diabetes. That is, hyperglycaemia and hyperinsulinaemia may act to downregulate EPG (Zinman *et al.,* 1977) until a point in the disease has been reached when β-cells ability to secrete insulin is reduced and EGP increases (Staehr *et al.,* 2002). Although EGP is more likely controlled by changes in portal vein rather than whole body insulin levels (Sindelar *et al.,* 1998). Despite no improvement in insulin sensitivity, Segal *et al.* (1991) demonstrated that exercise training (12 wk, 4 hr / wk at 70% $\dot{V}O_{2max}$) significantly decreased EGP in type 2 diabetic subjects ($P < 0.05$; Figure 2.8). The decrease in EGP noted in both Devlin *et al.* (1987) and Segal *et al.* (1991) work could be attributed to a reduction in insulin production, as shown by lower C-peptide levels, and a decline in fasting glucose concentrations following exercise training. It seems likely that exercise training can decrease resting EGP in type 2 diabetics (Segal *et al.,*

1991) secondary to improvements in insulin sensitivity, although exercise-induced changes in EGP are dependent on the degree of hyperglycaemia and hyperinsulinaemia that accompany the progression of the disease (Giacca *et al.*, 1998).

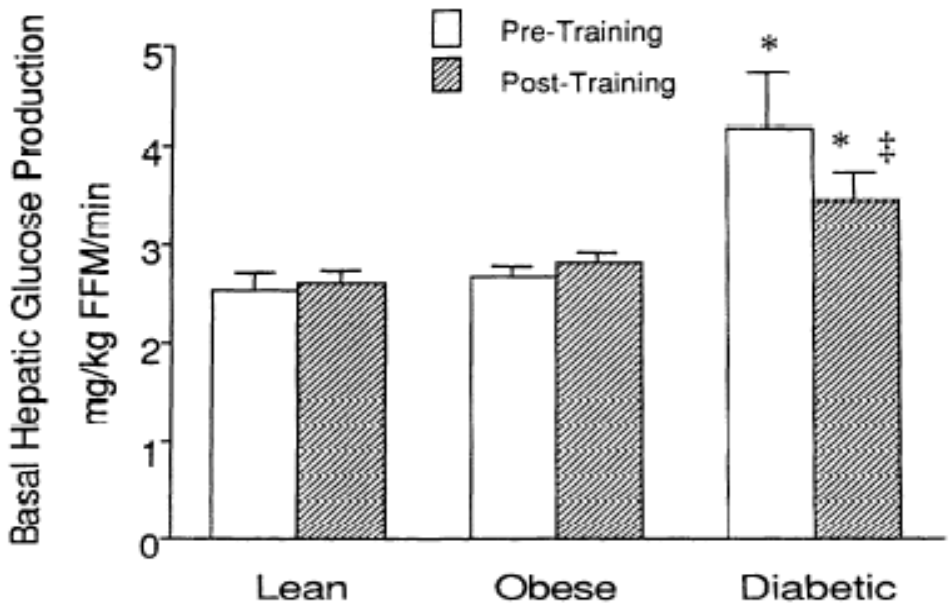

Figure 2.8 Resting hepatic glucose production (mg · kg fat free mass · min^{-1}) pre and post 12 wks of exercise training. Values are presented as mean (SEM). * denotes significant difference from lean or obese. ‡ $P < 0.05$ pre vs. post. Actual data from Segal *et al.* (1991)

2.8 Exercise as an Intervention for Type 2 Diabetes

Mitochondrial dysfunction (Kelley *et al.,* 2002), reduced capillary network, increased blood viscosity and the presence of vascular and neuropathic complications (ACSM, 2000) directly interfere with oxygen delivery and utilisation of the exercising limb in type 2 diabetic patients. Furthermore, accumulation of triglyceride depots have been suggested to impair mitochondrial lipid oxidation, decrease oxidative capacity and reduce mitochondrial size (Kelley *et al.,* 2002) all contributing to a decrease in exercise capacity noted in type 2 diabetics when compared to healthy controls (Fang *et al.,* 2005). Exercise training can promote changes in physiology including lowering resting heart rates, enhance oxygen delivery / extraction and reduced hypertension. In addition, physical activity has long been established as a useful component of type 2 diabetes management due to its ability to improve glucose tolerance (John, 2004) and reduce disease risk factors (Lee *et al*., 2005). There is support for this notion in that physical inactivity substantially increases the risk of developing type 2 diabetes and its secondary complications (Wei *et al.,* 1999). In a follow up study spanning 16 yrs, Hu *et al.* (2001) demonstrated a relationship between physical inactivity and obesity as predictors of diabetes and that this could be prevented by weight loss, regular exercise (≥7 hr / wk; moderate intensity) and dietary modification. The physical activity recommendations suggested by Hu *et al.* (2001) are somewhat ambitious, given that individuals with type 2 diabetes are known to be exercise intolerant (Green *et al.,* 2007).

Elsewhere, the current recommendations, as published by the Surgeon General, Centres for Disease Control and Prevention and the American Heart Association, are equivalent to 30 min of moderate physical activity per day (Larkin, 2001; Tuomilehto *et al.,* 2001; Booth et *al.,* 2002). Blair *et al*. (1992) and Fletcher *et al*. (1992) suggest individuals with type 2 diabetes should expend a minimum cumulative total of 1000 kcal/week in aerobic exercise or

walk for at least 2 hr per week (John, 2004). In addition, it has been shown that ~30-50% of all cases of type 2 diabetes and coronary heart disease can be prevented by 30 min (daily) of moderate intensity physical activity (Hu *et al.,* 2001; Manson *et al.,* 1999).

The primary goal of exercise in the treatment in type 2 diabetes is to acutely and chronically improve glycaemic control with the ultimate goal of preventing secondary complications and disease risk factors associated with type 2 diabetes (ACSM, 2000). The American College of Sports Medicine (2000) and The American Diabetic Association (ADA, 2004) have stated the clear therapeutic benefits of exercise with the latter describing them as substantial. Furthermore, large cohort studies have shown the link between low aerobic fitness and physical activity levels with increased risk of overall and CVD mortality in diabetic individuals (Sigal *et al.,* 2004).

There have been a number of large clinical studies assessing the effectiveness of exercise interventions on glycaemic control. In improving glucose tolerance acutely, exercise training can clinically and statistically reduce HbA_{1c} by 0.6 - 0.8% ($P < 0.05$) (Thomas *et al.,* 2006). A follow up study (12 month post exercise training) reported a 1.6% ($P < 0.03$) reduction in HbA_{1c} in the exercise group which was not apparent in the non exercise control and that the latter group demonstrated a 0.7% increase in HbA_{1c} (Thomas *et al.,* 2006). Stratton *et al.* (2000) reported that a 1% decline in HbA_{1c} resulted in a 33-41% fall in the relative risk of mircovascular complications, a 15-27% risk reduction in diabetic related deaths and an 8-21% decrease in risk of myocardial infarctions. These data emphasise the importance of exercise as a clinical intervention. There are two major goals of diabetes therapy; *1)* to reduce hyperglycaemia and *2)* decreased body fat (mass) (Boule *et al.,* 2001). The reduction in

HbA_{1c} witnessed in some studies (Agurs-Collins *et al.,* 1997; Dunstan *et al.,* 1997) has not been replicated in others (Vanninen *et al.,* 1992; Dunstan *et al.,* 1998). However, a meta-analysis (study number = 24) showed that exercise can significantly lower HbA_{1c} (-0.66%) (Boule *et al.,* 2001) indicating a clear role for exercise in diabetic management. This reduction of -0.66% in HbA_{1c} is very similar to differences noted in the UK Prospective Diabetes Study [UKPDS (33), 1998; UKPDS (38), 1998; UKPDS (49), 1999). Boule *et al.* (2001) suggested that some negative findings on the ability of exercise to lower HbA_{1c} may be due to the low subject number. The same meta-analysis also concluded that exercise training had no effect on body mass (Boule *et al.,* 2001). Reductions in body mass are regarded as a major goal in diabetes management (Boule *et al.,* 2001) as weight loss is aligned with decreased insulin resistance (Uusitupa *et al.,* 2003) and reduced inflammation (TNF-α and C-reactive protein) (Kelly *et al.,* 2004; Fischer *et al.,* 2007). An improvement in glucose tolerance with no change in body mass may suggest exercise promoted increases in fat free mass (Boule *et al.,* 2001), giving rise for a greater potential for insulin stimulated glucose disposal (Charron *et al.,* 2005).

A study spanning ~11 years concluded that life-style counselling (exercise and diet modifications) achieved a significant effect on measures of glucose tolerance, weight loss and alterations in dietary intake within a group of overweight impaired glucose tolerant (IGT) individuals (n = 522) [Finnish Diabetes Prevention Study (DPS), 2006]. The major findings of the DPS study were that the intervention group had a 43% reduction, compared to the control, in the relative risk of progression to type 2 diabetes. The authors attributed this reduction to a reduction in body mass [-4.5 (5.0) kg], decreased total calorific intake [-247 (438) kcal · day^{-1}] and saturated fat intake [-2.7 (4.6) % energy intake], increased fibre intake [2.5 (4.6) g/1000 kcal] and greater levels of physical activity [49 (interquartile range -41 to

140) min · wk^{-1}] (Finnish DPS, 2003 & 2006). Furthermore the reduction (36%) in the relative risk of diabetes progression was maintained for 3 years after the intervention period had ceased (Finnish DPS, 2006). A similar study conducted in China showed that individuals with IGT and type 2 diabetes responded positively to diet, exercise and exercise plus diet interventions with a 31%, 46% and 42% reduction in disease risk, respectively (Pan *et al.,* 1997). The Da Qing study also noted that there was no additive effect of the diet plus exercise group over the exercise and diet only groups which the authors attributed to each lifestyle modification influence the other in a positive manner (Pen *et al.,* 1997). Exercise can clearly improve glycaemic control by directly reducing blood glucose concentrations and HbA_{1c} (Stratton *et al.,* 2000) as well as improving insulin resistance via a reduction in adipose tissue and circulating adipocytokines (Esposito *et al.,* 2002; Schernthaner & Schernthaner, 2005). Defects in insulin-dependent glucose transport are a major cause of elevated blood glucose concentrations, type 2 diabetes and its associated complications (Abuissa *et al.,* 2005; Sesti, 2006).

2.9 Stimuli for Glucose transport – Contraction, Insulin and Hypoxia

Glucose transport across the plasma membrane provides much of the energy requirements for cellular respiration and / or glycogen synthesis. Muscle contractions (Holloszy, 2003), hypoxia (reductions in partial pressure or availability of oxygen) (Cartee *et al.*, 1991; Reynolds *et al.*, 1998) and insulin (Zierath, 2002) are known stimuli of glucose transport activity. In skeletal muscle the majority, but not all, research has concluded that muscle contraction and hypoxia stimulate glucose transport activity via the same signalling pathway, which is independent of the action of insulin (Fisher *et. al*, 2002; Holloszy, 2003). Nitric oxide (NO) and nerve / muscle-derived factors [neuregulins (NRGs)] are also known to induce glucose disposal using a distinct pathway (Zorzano *et al.*, 2005). The aim of this next section is to cover these pathways in more detail and in doing so, provide a rationale for the use of hypoxia in stimulating whole body glucose metabolism.

2.9.1 Insulin-Stimulated Glucose Transport

Insulin, a polypeptide hormone secreted by the islets of Langerhans is a potent anabolic hormone, promoting the synthesis and storage of carbohydrates, lipids and proteins and inhibiting their degradation and release back into circulation (Saltiel & Pessin, 2002). At the cellular level, insulin action is defined by its diverse complex effects, including changes in vesicle trafficking, activation and phosphorylation of various protein kinases, stimulation of cellular growth and differentiation and the activation or repression of transcription (Saltiel & Pessin, 2002). Saltiel & Pessin (2002) suggest that even individual effects of this hormone require multiple signalling inputs. Figure 2.9. shows a summary of the signalling proteins involved in insulin-stimulated glucose transport within skeletal muscle. The defects within

insulin signalling and their relationship to insulin resistance have been discussed in a previous section (2.5.1). The purpose of the next section is to provide additional detail and to highlight the difference to the separate contraction-stimulated pathway.

The mechanisms responsible for increases in cell permeability to insulin following exercise have been comprehensively reviewed (Zierath, 2002; Holloszy, 2005; Jenssen & Goodyear, 2005; Rose & Richter, 2005; Wright *et al.,* 2005). Insulin binding results in tyrosine phosphorylation of IRS-1 and the activation of phosphatidylinositol 3-kinase (PI-3Kinase) (Zhou & Dohm, 1997; Lajoie *et al.,* 2004), which generates the membrane-bound second messengers phosphatidylinositol 3,4-diphosphate [PI(3,4)P2] and PI(3,4,5)P3, leading to the membrane translocation of phosphoinositide-dependent kinase-1 (PDK1) and activation of protein kinase B (PKB, also known as Akt) (Harris, 2003; Mora *et al.,* 2004; reviewed in Sakamoto & Holman, 2008). Upon activation, PKB is hypothesized to phosphorylate the novel Akt substrate 160 kDa (AS160), which may decrease Rab GTPase-activating protein (Rab-GAP) activity, and result in GLUT-4 translocation (Howlett *et al.,* 2007; Sakamoto & Holman, 2008). It is suggested that Rab-GAP activity results in the conversion of GTP to GDP with the latter remaining bound on GLUT-4 intracellular storage sites and so blocking GLUT-4 movement to the cell membrane. Thus the current hypothesis is that PKB phosphorylation of AS160 leads to a reduction in Rab-GAP activity, resulting to GLUT-4 vesicle becoming loaded with GTP and the promotion of GLUT-4 trafficking (Kane *et al.,* 2002; Sakamoto & Holman, 2008) and glucose uptake.

PI-3kinase has long been established as an important mediator in insulin signalling and glucose transport. Bruss *et al.* (2005) recently showed that wortmannin inhibits insulin-stimulated phosphorylation of AS160 in a PI-3kinase dependent manner and that the introduction of 5-aminoimidazole carboxamide riboside (AICAR) increased phosphorylation of AS160 in an AMPK sensitive fashion in skeletal muscle. This work adds support to the notion that insulin-stimulated glucose transport functions independently to contraction stimulated pathway, as AICAR had no effect on PKB/Akt activity (Bruss *et al.,* 2005). PKB/Akt has been identified as playing a major role in insulin-stimulated glucose transport, via activation of insulin receptor substrate- (IRS-1) and PI-3Kinase (Farese *et al.,* 2005). This signalling protein has been shown to be defective in type 2 diabetic humans (Krook *et al.,* 1998) and animals (Beeson *et al.,* 2003). Protein kinase B (PKB)/Akt is also progressively downregulated as obesity develops into overt type 2 diabetes (for a review see Farese *et al.,* 2005). Furthermore, insulin-stimulated glucose uptake is inhibited in skeletal muscle in mice lacking the β isoform of PKB (Akt2) (Cho *et al.,* 2001). Therefore, it is no surprise that possible alterations in these signalling mechanisms in response to exercise has proven to be of great interest.

Using euglycaemic–hyperinsulinaemic clamp technique and muscle biopsies (vastus lateralis) O'Gorman *et al.* (2006) observed that exercise training increased whole-body insulin-mediated glucose disposal via increased GLUT-4 membrane protein content, independent of functional alterations in insulin-signalling (IRS-1, PI-3-kinase and AS160), in type 2 diabetic humans. Suggesting that other mediators, presumably allocated to the contraction-stimulated pathway, or unidentified proteins, may be responsible / involved in improved insulin-stimulated glucose transport. In support of this, reviews from Holloszy (2005) and Henriksen

(2002) have hypothesised that the increase in insulin-stimulated glucose uptake (following training) seems to be mediated by the translocation of more GLUT-4 to the cell surface in response to a given insulin dose. Wojtaszewski *et al.* (2002) has also proposed that exercise does not upregulate insulin signalling protein activity or phosphorylation but perhaps acts to change the arrangement of these proteins that allow these molecules a greater effectiveness or efficiency in stimulating GLUT-4 trafficking.

Although, using healthy subjects Frøsig *et al.* (2007) showed that insulin-stimulated glucose uptake was enhanced ~60% ($P < 0.01$) following 3 wks of one-legged knee extensor endurance training. This was coupled with increased activity of glycogen synthase, phosphorylation of AS160 and a 54 (19)% increase in GLUT-4 membrane content. PKB Ser^{473} and AS160 phosphorylation were increased 1.8-fold and 2.0-fold, respectively following endurance training (Deshmukh *et al.,* 2006). These findings are supported elsewhere (Treebak *et al.,* 2007). Sriwijitkamol *et al.* (2007) published data showing that exercise-induced AS160 phosphorylation is reduced in type 2 diabetics when compared to healthy controls. Moderate exercise intensity (70% $\dot{V}O_{2max}$; 40 min) performed in the same study, resulted in a significant increase in PKB/Akt Ser^{473} and Thr^{308} phosphorylation in the lean control group, but were unchanged in the type 2 diabetic group (Sriwijitkamol *et al.*, 2007). These data taken above suggest that type 2 diabetics may display a blunted insulin-signaling response following exercise and that type 2 diabetics may need to operate at a higher exercise intensity to stimulate these signalling mechanisms.

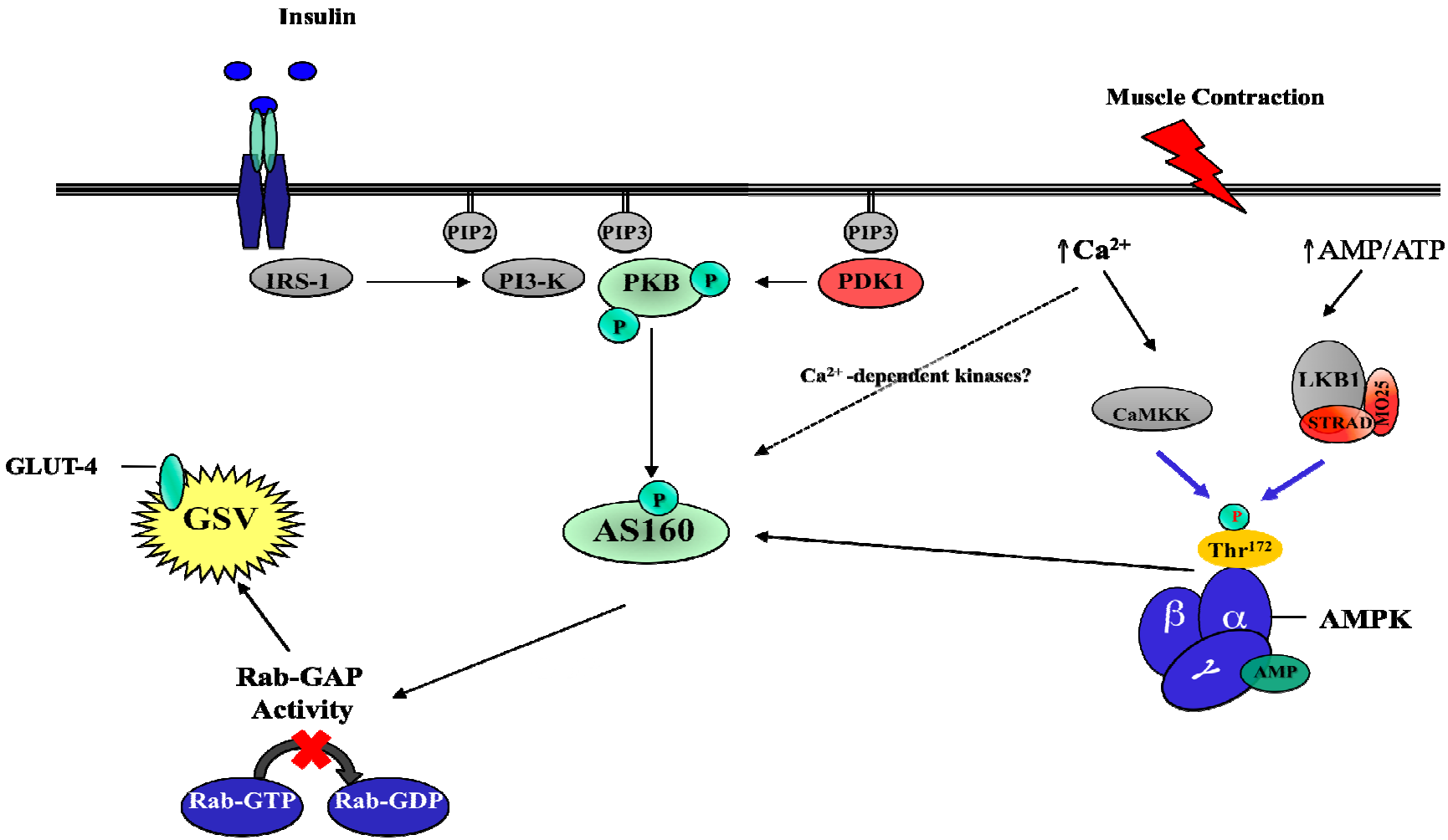

Figure 2.9 Signalling pathways involved in insulin and contraction-stimulated glucose transport. AMPK, AMP-activated protein kinase; CaMKK, calmodulin-dependent protein kinase; LKB1, a 50kDa serine/threonine kinase (STK 11); Thr^{172}, phosphorylated AMPKα at threonine172; IRS-1, insulin receptor substrate-1; PI-3kinase, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent kinase-1; PKB, protein kinase B/Akt; AS160; Akt substrate; Rab-GAP, Rab GTPase-activating protein GLUT-4, glucose transporter-4; GSV, glucose storage vesicles (Sakamoto & Holman, 2008).

2.9.2 Contraction Stimulated Glucose Transport

During contractile activity, ATP is hydrolyzed, resulting in the formation of ADP. ADP can then be utilised in the replenishment of ATP by donating a phosphate group to additional ADP molecule, resulting in ATP and AMP. This energy producing process leads to an increase the ratio of AMP:ATP and a decrease in creatine phosphate and inorganic phosphate (Corton *et al.,* 1994). It is these energy intermediates, as well as the accumulation of cytolytic Ca^{2+} that leads to the recruitment, phosphorylation and activation of protein kinases involved in contraction-stimulated glucose uptake. Indeed, the increase in AMP:ATP ratio results in the direct allosteric activation of AMP-Activated Protein Kinase (AMPK).

AMP-Activated Protein Kinase (AMPK)

Discovered over 36 years ago (Carlson & Kim, 1973) AMPK is an important protein kinase involved in the regulation of carbohydrate and fat metabolism. In mammalian tissues, AMPK is a heterotrimer consisting of a catalytic α subunit with two further regulatory subunits, β and γ (Koh *et al.,* 2008). Each of these subunits has two or more isoforms (Hardie *et al.,* 1998). The AMPKα1 isoform is widely expressed in the majority of tissue types while AMPKα2 is prominent in myocytes (Wood *et al.,* 1996). The AMPKβ subunit is thought to act as scaffolding unit for the complex and may be an important mediator in glycogen sensing via the glycogen-binding domain (McBride *et al.,* 2009). The γ subunit consists of 4-cystathionine-β-synthase (CBS) domains, which are required for the binding of ATP or AMP. The binding of two AMP to the γ subunit leads to conformational changes to the complex that cause the activation of AMPK through the exposure and phosphorylation of the Thr^{172} binding site on the catalytic α subunit (Figure 2.9) (Sanders *et al.,* 2007). Binding of ATP to

the γ subunit has been suggested to operate in an opposing manner by decreasing AMPK activity (Cheung *et al.,* 2000).

A working hypothesis is that upon stimulation, AMPK activates contraction-stimulated GLUT-4 translocation and glucose uptake in skeletal muscle via the phosphorylation of the Rab GTPase-activating protein (GAP), AS160, in a manner similar to insulin (Kramer *et al.*, 2006a; Sakamoto & Holman 2008). It is currently suggested that phosphorylation of AS160 results a decreased Rab-GTPase activity which leads to an increase in the loading of GTP on intracellular GLUT-4 storage sites promoting GLUT-4 translocation to the cell membrane (Kramer *et al.,* 2006a; reviewed by Sakamoto & Holman, 2008). Kramer *et al.,* (2006a), using a mutated mouse model with an *in vivo* electopration technique that prevents phosphorylation on four regulatory phospho-Akt-substrate sites on AS160 (4P), showed that contraction-stimulated glucose transport was impaired. In the same study, overexpression of mutant AS160 with abolished Rab-GAP activity showed elevations in contraction-stimulated glucose uptake in tibialis anterior muscle (Kramer *et al.*, 2006a). Furthermore, transgenic mice lacking AMPKα2 showed complete inhibition of AS160 phosphorylation and glucose transport on stimulation with AICAR. Take together, these data suggest a prominent role of AS160 in contraction-stimulated glucose transport (Kramer *et al.*, 2006b).

The central role of AMPK in contraction-stimulated glucose uptake has been consistently shown. Mu colleagues showed that glucose uptake is completely inhibited in whole body AMPKα2 knockout mice under 5-aminoimidazole carboxamide riboside (AICAR)

stimulation (Mu *et al.,* 2001). The AMPK activator, AICAR is a nucleoside that is taken up by cells and converted to ZMP, an AMP analog (Mu *et al.,* 2001). This finding is supported by more recent work showing that knockout of either the catalytic α2 or the regulatory γ3 subunits completely abolishes glucose transport when isolated skeletal muscle are incubated in 2 mM of AICAR (Jorgensen *et al.,* 2004).

The phosphorylation of the Thr^{172} site on the α subunits is linked to two upstream kinases, LKB1 and calmodulin-dependent protein kinases (CaMKK) (Stein *et al.,* 2000; Hawley *et al.,* 2003; Hawley *et al.,* 2005). LKB1 is a 50kDa serine/threonine kinase (STK 11) that requires the regulatory proteins STRAD and MO25 for it activation and intracellular localisation (Boudeau *et al.,* 2004). LKB1 has been identified as a prominent metabolic mediator in skeletal muscle by activating AMPK through the phosphorylation of a critical residue within the activation loop, Thr^{172} (Hawley *et al.,* 2005) leading to an increase in contraction-stimulated glucose uptake (Koh *et al.,* 2008). Sakamoto *et al.* (2005) suggests that once AMP binds with the γ subunit, AMPK phosphorylation occurs with alteration in the complex that stimulates phosphorylation of AMPKα via the LKB1 complex. Work carried out in Grahame Hardie's laboratory in 2003 showed that endogenous LKB1 and its two accessory subunits, STRAD and MO25 caused AMPK activation through the phosphorylation of the critical residue Thr^{172}, and Hawley *et al.* (2003) demonstrated that AICAR failed to activate AMPK in HeLa cells lacking the LKB1 complex. Although the latter finding must be interpreted with a degree of caution as HeLa cell lines may be lacking in other upstream kinases and therefore contribute to a decrease AMPK activation (Hawley *et al.,* 2003). In more recent work and using LKB1 knockout mice, Sakamoto *et al.,* (2005) demonstrated that basal AMPKα2 activity is decreased, and AMPK activity is not restored upon stimulation with

AICAR or muscle contraction. Highlighting the important role of LKB1 in AMPK activity and glucose transport. However, basal levels of AMPK activity and Thr^{172} phosphorylation are known to exist in LKB-/- MEF and HeLa (LKB1 deficient cell lines), suggesting that a separate and distinct kinases may phosphorylate Thr^{172}, independent to LKB1, at least in these cells (Hawley *et al.*, 2003).

Increasing intracellular levels of Ca^{2+} moderately [i.e. at levels unable to activate contractile activity or result in a decrease in high-energy phosphates (~P)] has been shown to stimulate glucose transport in skeletal muscle. Thus, providing a link between increased Ca^{2+} concentrations and glucose transport activity (Youn *et al.*, 1991). The ability of contractile activity to stimulate Ca^{2+} release from the SR and initiate glucose uptake in isolated frog muscle was noted over 40 years ago (Hollozy & Narahara, 1967). The activation of protein kinases, including protein kinase C (PKC) by elevated intracellular Ca^{2+} levels (Nishizuka, 1995) has been a major research interest in establishing the pathways involved in contraction-stimulated glucose transport. Although a developing and potentially important candidate for contraction stimulated (LKB1-independent) AMPK phosphorylation is the Ca^{2+}-dependent kinase, CaMKK (Wright *et al.*, 2004). Indeed, in vitro work in rat epitroclearis muscle using the CaMKK inhibitor KN62/93, resulted in a decrease in contraction-stimulated glucose uptake (Wright *et al.*, 2004). The involvement of CaMKK in contraction stimulated glucose uptake extends into whole body research. Rose and Hargreaves (2003) showed that acute exercise (40 min and ~76% $\dot{V}O_{2peak}$) resulted in ~50% increased in Thr^{287} phosphorylation of CaMKK that can be attributed to generation of Ca^{2+} spikes and the binding and activation of calmodulin (CaM) to the Ca^{2+}-dependent proteins, CaMKKs (De Koninck & Schulman, 1988). However, Rose and Hargreaves failed to measure variables related to glucose transport

activity. Although these authors hypothesised that the transient increase in CaMKK phosphorylation would result in AMPKα2 activation, GLUT-4 translocation and glucose uptake. Although, data presented elsewhere can validate these conclusions (Ojuka *et al.*, 2002; Hawley *et al.*, 2005).

2.9.3 Nitric Oxide (NO) Induced Glucose Transport

Previous work has suggested that skeletal muscle derived nitric oxide (NO) plays an important role in glucose transport. Although NO stimulation of glucose transport is thought to occur via a different mechanism and therefore, independent to both the insulin and contraction signalling pathways (Jessen & Goodyear, 2005). A rapid communication reported that nitric oxide synthase (NOS) inhibition downregulates glucose transport during exercise and that endurance training increased expression of endothelial NOS (eNOS) in male Sprague-Dawley rats (Balon & Nadler 1997). Furthermore, NO treatment stimulates glucose transport activity in isolated skeletal muscle by increasing GLUT-4 translocation (Etgen *et al.*, 1997). Recently Lira *et al.* (2007) reported that NO increases GLUT-4 mRNA and expression via a AMPK dependent mechanism which was reduced by ~50% when a NO inhibitor [1-(2-triflurormethyl-phenyl)-imidazole] was added, suggesting a role for NO in glucose transport activity.

2.9.4 Hypoxia Stimulates Glucose Transport - Contraction Pathway

Hypoxia is known to stimulate glucose transport in skeletal muscle using the contraction-stimulated pathway, increasing the activation of GLUT-1 pre-existing in the cell membrane (Behrooz, & Ismail-Beigi, 1999) while stimulating translocation of intracellular GLUT-1 and -4 to the sarcolemma (Cartee *et al.*, 1991; Behrooz, & Ismail-Beigi, 1999). In support of this, is the observation that glucose transport is additive when either hypoxia or contractile activity are coupled with insulin, whereas hypoxia and muscle contractions are not (Cartee *et al.*, 1991; Azevedo *et al.*, 1995). Lee *et al.* (1995) showed that wortmannin, a potent inhibitor of PI-3kinase, completely blocked insulin-stimulated glucose transport but had no effect on contraction or hypoxic mediated 2-Deoxy-D-glucose uptake in rat skeletal muscle. Taken together, this evidence suggests that hypoxia and contraction operate independently to insulin to induce glucose transport (Nesher *et al.*, 1988).

Hypoxia represents a potent stimulus for glucose transport activity. A reduction in O_2 availability is proposed to lower mitochondrial respiration and ATP synthesis while stimulating glucose transport (Behrooz, & Ismail-Beigi, 1999). Oxygen acts as an electron acceptor during aerobic metabolism to provide the majority ATP during oxidative level phosphorylation. Hypoxia has been shown to cause an increase in AMP:ATP ratio (Steenbergen *et al.*, 1987), resulting in an increase in cytolytic AMP availability, greater AMP binding capacity to the γ regulatory subunit, activation of AMPK (Wright *et al.*, 2004) and stimulation of glucose transport (Cartee *et al.*, 1991). Research has also demonstrated that hypoxia increases intracellular phosphate concentrations while decreasing phosphocreatine, which would potentially lead to a hypoxic-induced stimulation of glucose transport in an AMPK-dependent manner (Holloszy & Narahara, 1967; Cartee *et al.*, 1991).

In 1958 Randle and Smith published data showing that hypoxia, and other substances that inhibited oxidative metabolism, result in a loss of potassium (K^+) and an increase in cellular glucose uptake in isolated rat diaphragm muscle (Randle & Smith, 1958). It is suggested that K^+ channels are activated as an early responsive mechanism to hypoxia by activating K^+ loss, coupled with the inhibition of active K^+ cellular uptake (Reeves & Shah, 1994). Stimulation of ATP-sensitive K^+ channels by a hypoxic-induced decreases cytolytic ATP results in an increase in extracellular level of K^+. These ion changes lead to membrane depolarisation, opening of voltage-gated Ca^{2+} channels and an increase in SR Ca^{2+} release. Evidence suggests that Ca^{2+} can activate glucose uptake in a CaMKK / AMPK dependent manner (Hawley *et al.,* 2005). Although elevations in intracellular Ca^{2+} levels may also provoke AMPK-independent glucose transport. Wright *et al.* (2004) demonstrated that glucose transport activity is increased by a sub-contraction increase in Ca^{2+} levels when stimulated with caffeine and that the effects of AICAR and caffeine where additive. These results suggest that glucose transport can be activated by Ca^{2+} and AMPK-dependent. Although the increase in Ca^{2+} would also be expected to increase AMPK activation via CaMKK phosphorylation at the activation loop of the α subunit (Hawley *et al.,* 2005). However, there seems little doubt that hypoxia can promote glucose uptake via the activation of AMPK (Fisher *et al.,* 2002).

Hypoxia is considered a potent stimulus for glucose transport by activating specific protein kinases linked to the contraction-stimulated pathway (Cartee *et al.,* 1991). Similar to exercise (contraction), the mechanisms proposed to encourage hypoxic-induced glucose transport are an increase in AMPK activity (Fisher *et al.,* 2002) and Ca^{2+}-dependent mechanisms reliant on the activation of CaMKK (Wright *et al.,* 2005). In 2001 Mu and colleagues demonstrated that

AMPK is required for hypoxic-induced glucose transport (Mu *et al.,* 2001). Using AMPKα2 deficient rodents (Tg-KD1), Mu *et al.* (2001) showed that glucose transport was completely blocked under hypoxia when compared to wild-type counter-parts. Furthermore, the hypoxic-induced increase in membrane bound GLUT-4 content was reduced in the same Tg-KD1 mice (Mu *et al.*, 200). Wadley *et al.* (2006) extended these findings in an exercise study using sedentary humans. These authors found that AMPKα2 activity and AMPKα Thr^{172} phosphorylation was significantly increased during exercise in hypoxia when compared to the same relative exercise intensity in normoxic conditions. The rate of glucose disappearance was also found to be significantly higher in the hypoxic trial, suggesting that hypoxia when combined with exercise has a greater effect on AMPK activity and glucose transport (Wadley *et al.,* 2006).

2.10 Glucose Tolerance & Metabolism in High Altitude Natives

It is generally recognised that altitude natives have a reduced prevalence of type 2 diabetes (Picon-Reategui, 1963, Garmendia *et al.*, 1973; Santos *et al.*, 2001). In addition, Castillo *et al.* (2007) reported that altitude natives display lower glucose profiles [50.6 (3.7) mg / dL] than sea-level residents [73.4 (4.0) mg / dL] when monitored during a ~12 hr period. Despite having a high prevalence of obesity (BMI $\geq$ 30kg / m^2) rural Aymara natives (living at altitudes 2050-4250 m) are known to be at a reduced risk of developing type 2 diabetes (Santos *et al.*, 2001). These authors contributed this finding to near normal insulin values [mean (SD); 9.3 (10.2) µU/ml] and low levels of insulin resistance [$HOMA_{IR}$ 1.8 (2.4)] (Santos *et al.*, 2001). Although these data should be read with caution as the Aymara population are known to have a predominantly agriculture led (active) life-style. Furthermore, the use of BMI, as an indication of obesity can be misleading as it does not distinguishing between muscle and adipose mass in its measurement (Santos *et al.*, 2001). It was proposed that high levels of physical activity contributed to the lower frequency of type 2 diabetes noted in the Aymara population (Santos *et al.*, 2001). The evidence for this conclusion seems to be anecdotal as no data was published on the physical capacity or physical activity levels for the Aymara population (Santos *et al.*, 2001).

2.10.1 Glucose Response of Sea-level Natives at Altitude

Decreased O_2 delivery / availability (Cartee *et al.*, 1991) and capacity for utilisation (Gregg *et al.*, 1989) have been shown to alter the manner by which glucose is transported into peripheral tissue in animal models. A study by Forbes in 1936 was one of the first to suggest that altitude could alter the manner in which glucose is handled by sea-level residents (Forbes, 1936). This work showed that blood glucose clearance was increased during an

OGTT administrated at high altitude. Following this work, research has not only confirmed Forbes (1936) conclusions, but looked to extend it by showing that long-term exposure to simulated, or actual altitude results in; *1)* reduced fasting plasma glucose concentrations (Picon-Reategui, 1962; Picon-Reategui, 1963; Calderon & Llerena, 1965; Calderon *et al.,* 1966), and *2)* elevated glucose clearance rates during an intravenous glucose load (Picon-Reategui, 1962; Calderon and Llerena, 1965).

Humans visiting high altitude are also subject to distinct changes in metabolic pathways. Brooks *et al.* (1991) concluded that high altitude acclimatisation (4300 m) increases glucose rates of disappearance (R_d) and glucose metabolic clearance rates (MCR) during both exercise and resting states compared to sea-level values. Interestingly, insulin concentrations were unchanged from sea level values, suggesting an increase in contraction stimulated glucose transport or improved insulin sensitivity at altitude. In support Johnson *et al*. (1974) demonstrated that acute altitude exposure (2-40 hr) resulted in progressive hypoglycaemia, which was attributed to increased glucose clearance and oxidation. This conclusion was confirmed by Cooper *et al.* (1986). Although this work lacked a normoxic (sea-level) control. Using an oral glucose tolerance test, Lee *et al.* (2003) suggested that high altitude exposure (3 days) significantly improved glucose tolerance in sea-level natives. This group of authors also concluded that physical activity (habitual tasks) at altitude further enhanced the positive effects of hypoxia on glycaemic control (Lee *et al.*, 2003).

2.10.2 Hypoxia & Insulin Sensitivity

While the effects of hypoxia on insulin sensitivity remain controversial, Brooks *et al.* (1991) have reported elevations in insulin concentration upon arrival to altitude (4,300 metres), suggesting that hypoxia may cause a decrease in insulin sensitivity. Although, the increase in circulating insulin may have been due to hypoxic-induced stimulation of insulin synthesis and secretion by pancreatic β-cells (Kolesnik *et al.*, 1995), rather than a decrease in insulin-stimulated glucose clearance. The former would seem logical given that the rate of glucose disappearance (R_d) was found to be higher during hypoxia when compared to sea level values [3.59 (0.08) and 1.80 (0.02) mg / kg / min^{-1}, respectively $P < 0.05$] (Brooks *et al.*, 1991). However, insulin resistance has been known to increase due to hypoxia in both genetically obese mice (Polotsky *et al.*, 2003) and healthy human (Braun *et al.*, 2001). Although the increase in insulin resistance noted in Polotsky *et al*. (2003) study, which used obese, leptin deficient mice, was completely abolished by acute leptin replacement. Leptin, a peptide hormone produced by adipose tissue is able to interact with skeletal muscle (Kellerer *et al.*, 1997), increasing fatty acid oxidation and so reducing intramuscular stores of triglycerides (Shimabukuro *et al.*, 1997). It has been suggested that this reduction in intramuscular lipids may in part be responsible for improvements in insulin sensitivity (Yaspelkis, 1999). Using the gold standard assessment of glucose tolerance (euglycaemic – hyperinsulinaemic clamp) Larsen *et al.* (1997) found that insulin sensitivity decreased significantly in response to 2 days of altitude exposure (4559 m; ~12% O_2) with a reduction in glucose infusion rates, to achieve euglycaemia, from 9.8 (1.1) to 4.5 (0.6) mg / kg / min^{-1} ($P < 0.05$). The same work did however show improvements in insulin action with altitude acclimatisation (7 day exposure) (Larsen *et al.*, 1997).

Studies using animal models have demonstrated that hypoxia increases insulin action and encourages glucose transport activity (Fisher *et al.,* 2002; Holloszy, 2003; Chiu *et al.,* 2004). A study using rodents demonstrated that 4 weeks of intermittent hypoxic exposure (12 hr / day) with and without endurance training significantly lowered fasting glucose levels and improved glucose tolerance, with the greatest improvements seen in the hypoxic exercise group (Chiu *et al.,* 2004). Of interest, the same work showed that insulin sensitivity was also markedly improved due to hypoxia (Chiu *et al.,* 2004). These results suggest the possible value of intermittent hypoxia with and without exercise in the prevention or correction of metabolic defects associated with type 2 diabetes.

There is a wealth of literature investigating glucose metabolism in both animals (Cartee *et al.*, 1991; Holloszy, 2003; Wright *et al.,* 2005) and humans (Brooks *et al.,* 1991; Brooks *et al.*, 1992; Azevedo *et al.,* 1995) in response to hypoxia or altitude. However much of this work has been confined to whole body glucose disposal during high altitude exposure (Brooks *et al.,* 1991; Braun *et al.,* 2001) or *in vitro* analysis in human (Azevedo *et al.,* 1995) and animal models (Cartee *et al.*, 1991; Fisher *et al.,* 2002; Holloszy, 2003; Chiu *et al.,* 2004). The reason for the discrepancy between studies on insulin sensitivity and hypoxia remains unclear, although they may reflect differences in length of exposure (i.e. acute vs. chronic), which may in turn induce a biphasic hormonal response. Oltmanns *et al.,* (2004) demonstrated that cortisol, known to induce insulin resistance and increase EGP (Rooney *et al.,* 1993), increases during prolonged hypoxia but not during acute bouts. Thus, the effects of prior hypoxia on normoxic insulin sensitivity warrants further investigation.

2.11 Adipokines

Adipocytes are regulated by various hormonal, neural and nutrient stimuli. In fasting states, the process of lipolysis causes adipocytes to release NEFAs. The increase in circulating glucose that follows feeding promotes lipogenesis and decreases lipolysis in the presence of insulin. In addition to their role in fuel storage, adipocytes have also been shown to secrete a number of bioactive proteins, collectively termed adipokines. Adipokines are extremely diverse in both protein structure and physiological function (Trayhurn *et al.*, 2006). Adipokines are made up of cytokines, growth factors as well as proteins implicated in the control of blood pressure, vascular haemostasis and lipid and glucose metabolism (Trayhurn *et al.*, 2006). Adiponectin, leptin, interleukin-1α (IL-1α), interleukin-6 (IL-6) and tumour necrosis factor-*α* (TNF-*α*) are among the best characterized (Mahadik *et al.*, 2008). The latter of these two mentioned cytokines (IL-6 and TNF-*α*) have been linked to many chronic diseases (Plomgaard *et al.*, 2005). Sometimes referred to as low-grade systemic inflammation, elevated levels of IL-1*α*, IL-6 and TNF-*α* are a feature of obesity, insulin resistance (Dandona *et al.*, 2004) and type 2 diabetes (Duncan *et al.*, 2003; Tilg and Moschen, 2008).

2.12 Regulatory Role of Inflammatory Cytokines in Type 2 Diabetes

2.12.1 Interleukin 6 (IL-6) & Type 2 Diabetes

The human interleukin 6 protein (IL-6; molecular weight 21-28 kDa) is made up of 212 amino acids with a signal peptide of 27 amino acids and two NH_2-linked glycosylation sites (Kamimura *et al.,* 2004). IL-6 induces signalling in all cell types expressing the ubiquitous gp130 receptors, including most cells of the immune system, endothelial cells, adipose tissue (Mohamed-Ali *et al.,* 1997), skeletal and smooth muscle cells (Steensberg *et al.,* 2001) and hepatocytes (Kamimura *et al.,* 2004). High levels of IL-6 are often noted in type 2 diabetics, which has lead some to suggest a pro inflammatory role for IL-6 (Pradhan *et al.,* 2001). Although Kamimura *et al.* (2004), along with other research groups (Starkie *et al.,* 2003; Kristiansen & Mandrup-Poulsen, 2005; Petersen & Petersen, 2005) suggest that this view is a little simplistic given that IL-6 has been implicated in both increasing insulin resistance (Senn *et al.,* 2002) and fuel metabolism (Kelly *et al.,* 2004). A comprehensive review argues that circulating IL-6 levels may or may not be associated with insulin resistance (Petersen & Petersen, 2005). IL-6 should therefore not be viewed as either pro- or anti-inflammatory, but as both, for the role of IL-6 seems to be dependent upon its target tissue (Kamimura *et al.,* 2004).

Low-grade inflammation can precede the development of type 2 diabetes (Spranger *et al.,* 2003). Indeed, elevated circulatory concentrations of IL-6 have been shown to predict the onset of type 2 diabetes (Kristiansen & Mandrup-Poulsen, 2005; Pradhan *et al.,* 2001). Although elevated IL-6 concentrations in this population does not propose causation. There is little evidence to suggest a mechanistic role of IL-6 in insulin resistance. However glucose-

stimulated insulin secretion has been shown to be down regulated by IL-6 in rodents (Sandler *et al.,* 1990). More recently Senn *et al.* (2002) demonstrated a direct mechanism by which IL-6 interferes with insulin-dependent phosphorylation of IRS-1. Suggesting that insulin action in peripheral tissue is down regulated by IL-6 (Senn *et al.,* 2002).

Elsewhere, IL-6 has been implicated in encouraging glucose transport rather than causing insulin resistance. Carey *et al.* (2006) demonstrated increased insulin-stimulated glucose uptake, AMPK activation and GLUT-4 plasma membrane translocation with IL-6 infusion in healthy humans. Further, Wallwnius *et al.* (2002) proposed two interesting points; *1)* IL-6 knockout mice developed impaired glucose tolerance and *2)* this was reversed with administration of IL-6. The work of Carey *et al.* (2006) and Wallwnius *et al.* (2002) suggest that IL-6 plays a role in glucose metabolism by activating glucose uptake and improving glycaemic control.

2.12.2 IL-6 Concentrations & Exercise

The change in IL-6 during exercise has been the subject of many reviews (Febbraio & Pedersen, 2002; Febbraio *et al.,* 2003; Febbraio *et al.,* 2004; Petersen & Petersen, 2005). There is little doubt that circulating IL-6 levels increase during exercise and return rapidly to near baseline levels once exercise ceases (Steensberg *et al.,* 2002). The confusion seems to be centred upon what role IL-6 plays during and following the metabolic stresses imposed by exercise. In addition to its function as an inflammatory cytokine, IL-6 has also been linked to energy metabolism (Febbraio *et al.,* 2003). IL-6 expression is known to increase with progressive increases in glucose uptake during exercise (Keller *et al.,* 2005). Recently, mRNA expression and transcription rates for IL-6 have been shown to increase when there is a decrease in muscle glycogen (Keller *et al.,* 2005), which has led some to suggest an energy sensing role for IL-6, at least in skeletal muscle (Petersen *et al.,* 2001; Kelly *et al.,* 2004). Kelly *et al.* (2004) have recently provided evidence that proposes a role for IL-6 in AMPK activation. These authors demonstrated that incubation with high physiological concentrations of IL-6 (30 and 120 pg / ml) during exercise increased AMPK activation in adipose and extensor digitorum longus muscle tissue. Previous data suggests that IL-6 may apply an anti inflammatory effect following exercise (Petersen & Petersen, 2005) by inhibiting tumour necrosis factor-α (TNF-α) (Starkie *et al.,* 2003). The direct role of IL-6 in the inflammatory processes is hard to determine as its actions may not only be tissue specific (Kamimura *et al.,* 2004), but may also be dependent upon its interaction with other cytokines, including IL-1, IL-10 and TNF-α (Petersen & Petersen, 2005).

2.12.3 Tumour necrosis factor-α (TNF-α) & Type 2 Diabetes

TNF-*α* is a cell surface transmembrane protein that is produced by a variety of tissues types, including adipocytes (Wajan *et al.,* 2003) and skeletal muscle (Saghizadeh *et al.,* 1996). TNF-*α* functions in an autocrine, paracrine and endocrine manner and has various roles, incorporating the promotion of apoptosis, systemic inflammation, insulin resistance as well as the stimulation of other cytokines (Plomgaard *et al.,* 2005).

Dandona *et al.* (2004) has inferred that type 2 diabetes should be considered as an inflammatory disease, as the pro inflammatory cytokine TNF-α has been closely associated with states of insulin resistance. Cell culture work has shown that TNF-α reduces insulin action through direct inhibition of the insulin signalling cascade (Hotamisligil *et al.,* 1994; Plomgaard *et al.,* 2005). Direct evidence that TNF-α causes insulin resistance comes from animal models in which insulin-stimulated phosphorylation of insulin receptors (IR), phosphorylation of insulin receptor substrate-1 (IRS-1) and GLUT-4 translocations are decreased by TNF-α (Hotamisligil & Spiegelman, 1994). Furthermore, TNF-α-deficient (TNF-$\alpha^{-/-}$) obese mice displayed decreased insulin resistance and increased insulin signalling when compared to TNF-$\alpha^{+/+}$ rodents (Uysal *et al.,* 1997). A recent study conducted with human subjects suggested that TNF-α causes skeletal muscle insulin resistance by inhibiting Akt substrate (AS160) phosphorylation (Plomgaard *et al.,* 2005) and so GLUT-4 exocytosis to the plasma membrane (Zeigerer *et al.,* 2004). The data provided by Plomgaard *et al.* (2005) shows a direct link between systemic inflammation (elevated TNF-α concentrations) and molecular insulin resistance.

2.12.4 TNF-α Response to Exercise

Contracting skeletal muscle has been shown to release IL-6 with no change in TNF-α in type 2 diabetic patients (Steensberg *et al.,* 2002; Febbraio *et al.,* 2003). Although TNF-α has also been known to be elevated after prolonged intense running (Starkie *et al.,* 2001). These differences may be the product of differences in the level of stress imposed by short duration (25 min of supine cycling exercise 60% $\dot{V}O_{2peak}$) (Steensberg *et al.,* 2002) and long-term [marathon; completed in 169 (20) min] (Starkie *et al.,* 2001b) exercise. Interestingly, Zoppini *et al.* (2006) found no change in TNF-α following 6 months of moderate intensity exercise in type 2 diabetics. Although the training intensity (based on 40-50% of heart rate reserve) and frequency (110 minutes; 2 times per week) did not produce any decrease in fasting glucose, HbA_{1c} or BMI, suggesting that the exercise regime used was sufficient to produce improvements in glucose tolerance or have an anti inflammatory effect.

Despite this work, aerobic exercise has been shown to inhibit the production of TNF-α (Petersen & Petersen, 2005). This review (Petersen & Petersen, 2005) further suggests that exercise has an anti inflammatory effect which may be mediated by TNF-α suppression and improved peripheral insulin action. This view has received support by Keller *et al.* (2004) who demonstrated normalisation of TNF-α levels in knock-out mice (TNF-α over expressive) with exercise. The literature generally suggests that TNF-α has a pro inflammatory role, and in terms of metabolic diseases, is involved in promoting insulin resistance. Furthermore, exercise, known to improve insulin sensitivity (Richter *et al.,* 2001) can also suppress TNF-α production (Keller *et al.,* 2004).

2.13 Proposed Aims of the Thesis

Glycaemic control can be proportioned into indices of insulin-dependent and -independent glucose uptake, endogenous glucose production and β–cell function. Findings within the literature suggest that type 2 diabetics show defects in some, if not all, of these pathways. It is also widely known that exercise has the ability to acutely and moderately improve insulin sensitivity and encourage glucose transport activity in a wide range of populations. Therefore exercise is considered the cornerstone treatment in type 2 diabetic management.

It seems, at least in animal models, that hypoxia stimulates glucose transport activity via an insulin-independent pathway while the ability of the same environmental stress to activate insulin-dependent glucose transport is more controversial. Acute hypoxia, if proven to promote glucose disposal and improve glucose tolerance, may warrant further investigation as a preventive or therapeutic treatment in type 2 diabetes. Particularly, in type 2 diabetic sufferers, who are unable to exercise regularly for extended periods of time. Complications, such as obesity, foot ulcers, low fitness levels and low self esteem often mean that sufferers are unable to activate the exercise/contraction stimulated pathway for glucose uptake.

Using the labelled intravenous glucose tolerance test (IVGTT), the aims of the current thesis are *1)* to measure the effects of prior hypoxia on glucose effectiveness (S_G^{2*}), insulin sensitivity (S_I^{2*}) and endogenous glucose production (EGP) and to *2)* assess the influence of a single bout of moderate intensity exercise with and without hypoxia on glucose metabolism in individuals with type 2 diabetes. It is hypothesised that hypoxic exposure, at rest will mimic the effects of exercise on glucose metabolism and that exercise combined with

hypoxia may have an additive effect on S_G^{2*} and S_I^{2*} over exercise alone. The research aims allied to the latter part of this thesis are to *3)* identify to what extent blood glucose clearance is influenced by the total work (exercise) under hypoxic conditions, and to establish whether exercise intensity influences glucose clearance in a dose-dependent fashion in type 2 diabetics. The final (*4*) objective of this thesis is to identify whether intermittent exercise with or without hypoxia can improve glucose tolerance in the same population. To accomplish these latter aims, glucose metabolism was assessed during exercise using constant infusions of [6,6$^2H_2$]glucose, providing data on rates of glucose appearance (R_a) and disappearance (R_d).

CHAPTER 3

GENERAL METHODS

3.0 General Methods

The following section provides details of the methods common to more than one study of this thesis. Additional techniques and methods used in separate studies are described in the relevant chapters.

3.1 Laboratory Location

All experimental trials were carried out in the British Association of Sport and Exercise Science (BASES) accredited Welkin Laboratories, University of Brighton, (Eastbourne). All samples and analytical methods were stored and performed, respectively at the same location.

3.2 Subjects & Ethics

3.2.1 Subject Recruitment

Individuals with type 2 diabetes, diagnosed within the last 5 years by a general practitioner (physician) were recruited for this investigation via a specialist diabetic clinic in the Eastbourne area. All subjects were screened prior to contact by a consultant physician based at the same diabetic unit.

3.2.2 Informed Consent

Subjects were invited to visit the Welkin laboratories so that clinical records (i.e. medication), and information on their ability to complete moderate intensity exercise was obtained. Subjects were provided with written and verbal explanations of the structure, aims, commitments, possible benefits and risks of this research and of their right to withdraw from the study at any stage and without providing a reason. Medical questionnaires were completed before written informed consent was obtained. Subject adherence to this research programme was considered good at 95%. All data was stored on password-protected computers and held in a locked filing cabinet. Subjects were assured of complete anonymity following the release of data into the public domain.

3.2.3 Research Ethics

The experimental protocol was approved by the East Sussex Local Research Ethics Committee (LREC). All experimental designs used met the specific requirements of the World Medical Association for Medical Research Involving Humans (World Health Organisation Declaration of Helsinki, 2000). Volunteers treated with diet, exercise and medication were included within this thesis. Participants treated with hypoglycaemic agents such as metformin and glycazide agreed to avoid medication in the 48 hours prior to experimental conditions. In such cases, individual physician's (GP's) were consulted. The half-life of metformin and glycazide are ~17.6 and ~10 hours, respectively (Courtois *et al.*, 1999; Heller, 2007).

3.2.4 Exclusion Criteria

Only subjects diagnosed with type 2 diabetes within the last 5 years by a general practitioner (GP) were included in this study. The specialist diabetic clinic used to recruit diagnosed subjects used the criteria set out by The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (Table 3.0) (2002). Individuals with known secondary complications associated with diabetes (i.e. neuropathy, peripheral vascular and cardiovascular disease) were automatically excluded prior to contacted. Additional exclusion criteria included:

- HbA_{1c} values >10%
- Body Mass Index (BMI) of >35kg / m^2
- Pregnancy
- Blood pressure of >180mmHg systolic / and 110mmHg diastolic
- Disseminated cancer
- Any indication of muscle wasting
- Current smokers
- Volunteers requiring insulin treatment

Table 3.0 The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2002)

Diabetes Mellitus	• Fasting ≥ 7.0 mmol/l • Glucose concentration ≥ 11.1 mmol/l two hrs post 75g OGTT • Symptoms of diabetes + casual glucose concentrations 11.1 mmol/l
Impaired Glucose Tolerance	• Fasting <7.0 mmol/l and two hrs post OGTT ≥ 7.8 mmol/l and <11.1 mmol/l
Impaired fasting glucose	• Fasting ≥ 6.1 mmol/l and < 7.0 mmol/l and 2 hr post OGTT < 7.8 mmol/l

Fasting*; Defined as no caloric intake for at least 8 hours;* ***Casual****; Random blood glucose test without regard to time of day or feeding****; OGTT****; 75g Oral Glucose Tolerance Test. Values represent Glucose concentrations in venous plasma (mmol/l).*

3.3 Experimental Procedures

3.3.1 Experimental Controls

Participants were instructed to mirror dietary habits 24 hrs prior, abstain from exercise (bar habitual walking) in the 48 hrs leading up to, and refrain from caffeine and alcohol 24 hrs before experimental trials. Monitoring of dietary intake allowed for nutritional patterns to be replicated prior to subsequent trials. Dietary intake was analysed using Compeat (version 6; Visual Information Systems Ltd, UK). All trials were conducted under controlled ambient conditions [temperature; 20 (0.9)ºC, relative humidity; 41 (25)%, barometric pressure 745 (18)mmHg]. Subjects were required to attend the laboratory at ~08:00 following an overnight fast (12 hrs). This controlled for the diurnal variations in glucose metabolism, insulin secretion and insulin resistance (Jayagopal *et al.*, 2002).

3.3.2 Preliminary Measurements

On arrival at the laboratory each subject's baseline clinical and physiological characteristics were obtained. Standing barefoot with minimal clothing, no jewellery and in a Frankfort plane position, subject's height was recorded using a Harpenden stadiometer. Body mass was then measured using calibrated scales (Detecto, Webb City, USA) to a precision of 0.1 kg. Blood pressure was assessed in duplicate with automatic oscillometric blood pressure monitor (Omron Healthcare, Hamburg, Germany). Percentage of body fat and lean body mass were estimated using Bio Electrical Impedance analysis (Bodystat, Isle of Man, UK). A capillary blood sample (~5 µl) was then drawn for determination of HbA_{1c} using a boronate affinity assay (Axis-Shields Diagnostics, United Kingdom). This has been shown to be a reliable measure of glycaemic control as confirmed by the European Reference Laboratory (r = 0.995 and 95% confidence intervals) (ESRL, 2007).

3.3.3 Blood Glucose & Lactate Concentration

Blood glucose and lactate concentrations, drawn from capillary (fingertip), venous and arterialised samples (~25 µl) were determined in duplicate using an YSI STAT PLUS Glucose and Lactate analyser (YSI 2300 STAT Yellow Springs Instruments, Yellow Springs, USA). An electrode is used to detect the production of H_2O_2 from oxidation of the substrate. The further oxidation of H_2O_2, with the resulting electron flow, is linearly proportional to the steady-state H_2O_2, and is used to determine the concentration of the substrates (glucose and lactate) (Pyne *et al.*, 2000)

3.3.4 Reliability of YSI 2300 STAT (Glucose & Lactate) analyser

Default settings allowed for continuous calibration of the YSI lactate / glucose analyser using standardised solutions (lactate; 5 mmol/l and glucose; 10 mmol/l). Calibration was automatically completed after every 5 samples. Coefficient of variation (CV) for blood [La], assessed from 10 measures of a single fingertip capillary sample at rest (CV = 3.2%) and exercise (CV = 4.7%) were calculated. The technical error of measurement (TEM) for blood glucose values were calculated as follows: values were log-transformed and the standard deviation (SD) determined. Values were divided by square rooted and back transformed to obtain CV and used to determine TEM. These calculations were carried out using an excel spreadsheet desgined for this analysis (Hopkins, 2003; Gore and Hopkins, 2005). The resultant TEM for the experimental chapters was 4.2% with a unit value of 0.38 mmol/l (Figure 3.0).

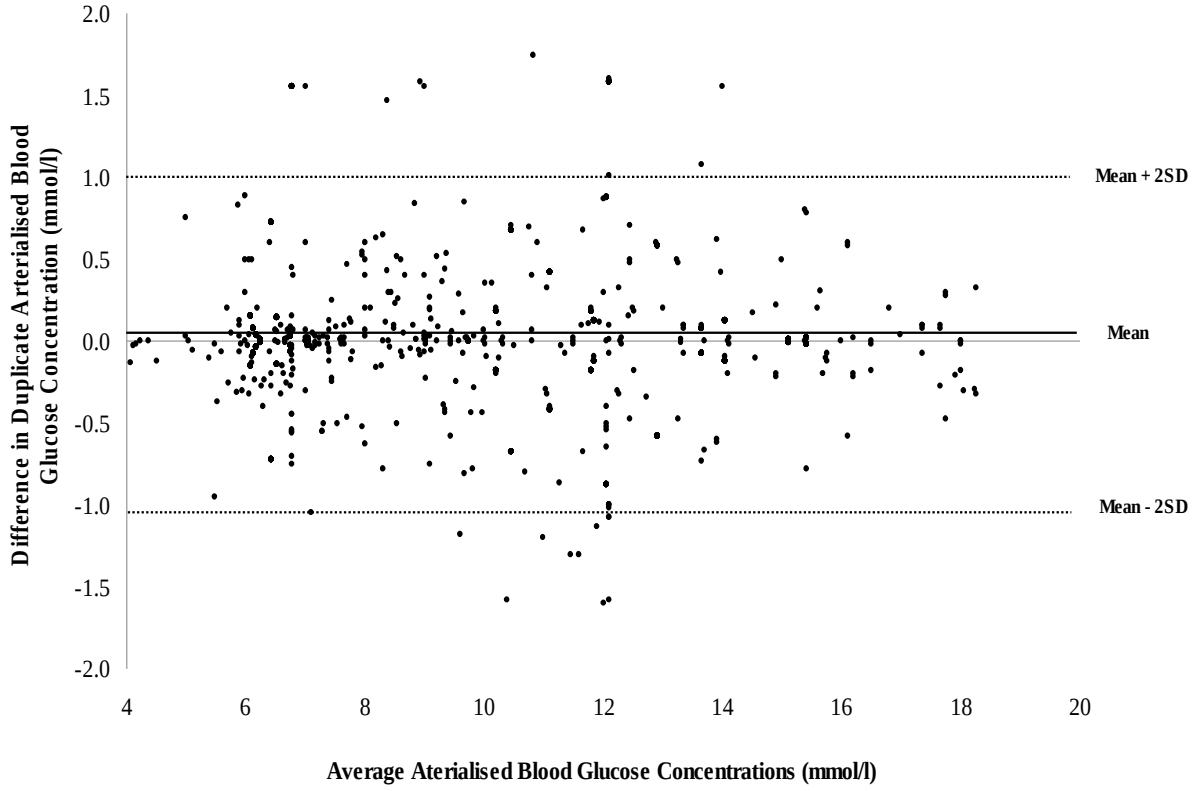

Figure 3.0 Limits of agreement (LOA) for differences between duplicate arterialised blood glucose samples. Glucose concentrations, sampled during the labelled IVGTT were used to calculate LOA.

3.3.5 Measurement of the Lactate Threshold

The lactate threshold (LT) is a submaximal exercise intensity that may be used to demonstrate the transition from moderate to hard exercise domain. The point at which LT occurs reflects an imbalance between lactate appearance and clearance or oxidation. LT represents an exercise intensity (power output; watts) that indicates a greater reliance on anaerobic energy sources. The subsequent (main) exercise trials used a work load (power output) equivalent to 90% of this LT (~50-55% $\dot{V}O_{2max}$). This exercise intensity has been shown to improve acute glycaemic control in individuals with type 2 diabetes (MacDonald *et al.*, 2004).

Sub-maximal LT tests were performed on an electrically-braked Jaeger cycle ergometer (Lode B.V. Medical Technology, Netherlands). Saddle height was adjusted for comfort before exercise began. Maintaining a constant pedal frequency (cadence ~60 rpm), subjects performed an incremental cycle ergometry protocol starting at 0 watts and increasing 10 watts every 3 minutes. Fingertip blood samples were immediately analysed (section; 3.5.1) for determination of blood lactate [La]. Heart rate (Polar Accurex monitor; Kemple, Finland) and rating of perceived exertion (RPE) (Borg, 1982) were recorded at the end of every 3 min stage. LT was defined as the power output preceding a sudden and sustained increase in [La] (≥ 1 mmol/l above the previous stage) above resting levels as described previously by (Jones & Carter, 2000) (Figure 3.1). Individual LT were confirmed independently by at least two experienced exercise physiologists.

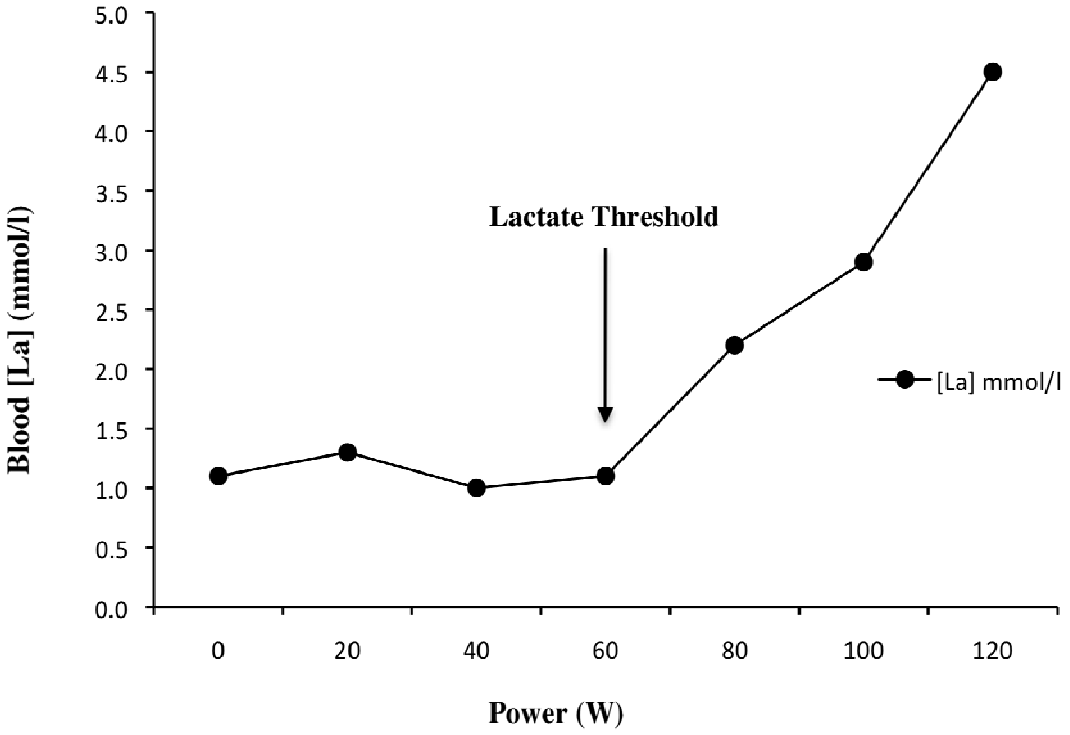

Figure 3.1 Typical determination of lactate threshold (LT) in a type 2 diabetic patient.

3.4 Main Experimental Trials

3.4.1 Exercise Intervention (Main Experimental trials)

All exercise trials were conducted on the same electrically-braked Jaeger cycle ergometer (Lode B.V. Medical Technology, Netherlands) throughout this thesis. Exercise intensities were based on values obtained during LT test and were therefore, individualised. Specific exercise intensities and durations are described within each chapter. Subjects remained in a seated position throughout each exercise condition and pedalled at a constant rate of ~60 rpm. Seat heights and handle bars were adjusted to ensure subject's comfort.

3.4.2 Hypoxic Treatment

Whole body hypoxia was induced using a closed environmental chamber with a continuous delivery of hypoxic gas mixture (O_2 ~14.6%). This system of normobaric hypoxia was generated using air-processing units (King Size, Air units SQ-10; Colorado Altitude Training, Boulder, USA), allowing for a steady flow of nitrogen (N_2; ~40 L / min) (Figure 3.2). The inflow of N_2 was managed to maintain the required O_2 percentage. Subjects remained in the environment until the completion of the each protocol. Oxygen concentrations were continuously measured throughout hypoxic exposures using Gasman *II* (Crowcon, Oxfordshire, UK).

Figure 3.2 Normobaric Hypoxic Environment

3.4.3 Heart Rate (HR), Oxyhaemoglobin (S_pO_2) and Rating of Perceived Exertion (RPE)

Heart rate (HR), oxyhaemoglobin (S_pO_2) and rating of perceived exertion (RPE) were all collected at 10 minute stages during exercise and hypoxic interventions. Heart rate was sampled using short distance telemetry (Polar sports tester PE400, Polar Electro Inc, Finland). S_pO_2% was measured indirectly using pulse oximetery (Nonin 2500, Minneapolis, USA). Pulse oximetery uses a light source and photodiode light detector to measure the amount of light passing through an arteriolar bed. Percentage of S_pO_2 can be estimated non-invasively

due to the difference in light-absorbing properties of oxyhaemoglobin and deoxyhaemoglobin. Safety levels for S_pO_2 were set at ≥ 70% for hypoxic exposures, whereby subjects and experimenters were removed from the environment. Rating of perceived exertion (RPE) was assessed using the Borg scale detailed earlier (Borg, 1982) (section 3.3.5).

3.5 Blood Sampling

The experimental design detailed within the general methods and in individual chapters required participants to provide frequent arterialised blood samples (section 3.5.2). Venous punctures were made by a trained phlebotomist using a prominent antecubital vein following localised cleaning of the surface site. All experimenters handling blood samples had been *vaccinated against Hepatitis B.* Protective gloves were used at all time and sharps were disposed off immediately after use.

3.5.1 Fingertip Blood Sampling

All lactate concentrations were measured from capillary fingertip samples. Puncture sites were cleaned using alcohol wipes (Alcowipes, Seton Health Group Plc, Oldham, England) before sampling. Baseline samples were warmed to ensure the collection of arterialised blood. Skin sites were punctured (~2 mm in depth) using an automated lancet (Softclix Pro, Accu-Chek, Manheim, Germany). The first drops of blood were wiped away before approximately 100µl of capillary blood was collected in a 300µL microvette (Sarsedt, Numbrecht, Germany) containing 1mg fluoride/ml and 15 I.U. heparin/ml of blood. Samples were mixed before

immediate analysis. All subjects were familiar with this protocol due to the nature of their disorder.

3.5.2 Arterialised Blood Sampling

In terms of metabolism, the difference between samples drawn from arterial and venous sites can be significant (Mcguire *et al.,* 1976). These differences can be attributed to the variation in transit times of glucose and other metabolites through the circulatory system between the two sampling sites and the rate of removal of the described substances to intervening tissue (Mcguire *et al.,* 1976). The extent of this difference will depend on both the cellular demands of the tissue, the influence of hormonal signalling, including insulin, as well as vascular tone. Although difficult, arterial sampling provides a more accurate measure of whole body metabolism. In understanding the thermoregulatory properties of the body, Mcguire *et al.* (1976) were able to show that heating the hand (> 60ºC) while sampling from a dorsal vein provides estimates of arterialised blood (~98%).

3.5.3 Arterialised Blood Sampling Procedure

On arrival to the laboratory (~08:00) subjects hands were placed into a hot box (~60ºC) for localised warming. Following ~30 min of heating an 18-gauge cannula was positioned into a dorsal hand vein to allow for frequent sampling of arterialised blood. To ensure effective blood collection, cannulae inserted into sampling hands were continuously infused with standard saline (~250 ml; 0.9% NaCl; Baxter Healthcare Ltd, Thetford, England). Arterialised blood samples (~10 ml) were drawn using a sterile syringe (10 ml; BD medical, Singapore) at baseline (-30 minutes) and every 10 minutes during experimental protocols.

Blood samples were immediately dispensed into ethylenediametetraacetic Acid (EDTA; ~5 ml) and lithium/heparin (~5 ml) coated tubes. Using the same sample ~100µl of whole blood was dispensed into microvettes (Sarsedt, Numbrecht, Germany) for the immediate analysis of blood glucose and lactate concentrations (section; *3.3.3*).

3.5.4 Centrifuge & Sample Storage

After ensuring blood was saturated with the relevant coating (EDTA or lithium/heparin), arterialised samples were promptly stored at ~4ºC until the end of each experimental protocol. Samples were then centrifuged at 6000 rpm for ~10 min (~4ºC). The resulting plasma was dispensed into three separate microtubes. Lithium/heparin plasma samples were stored in duplicate (2 x 1.5ml) with the third used to store 1.5ml of EDTA coated plasma. Lithium/heparin samples were collected in duplicate for the determination of insulin. Plasma samples were then held at -80 ºC until further analysis.

3.6 Measurement of Insulin - Hormone Analysis by Enzyme Linked ImmunoSorbent Assay (ELISA) method

Insulin was measured using a commercial availably enzyme linked immunoSorbent assay (ELISA), based on the sandwich principle (DRG diagnostics, Boldon, UK). Plasma taken from Lithium/heparin samples was thawed and vortexed before 25µL was added in duplicate to 84 of the mouse monoclonal coated wells (B1-H12). Wells marked as A1-A2 were used as blanks and A3-A12 were used for known insulin standards (6.25, 12.5, 25, 50 and 100 µU/ml). Known concentrations were used to plot the standard curve (Figure 3.3) by detection of light absorbance (450nm; Universal Microplate reader ELx800, IBL inc., Minneapolis, USA) and analysis using KC junior software (Bio-Tek instruments, Winooski, Vermont, Canada). The inter- and intra-assay coefficient of variation and technical error of measurement for insulin ELISA's are detailed within individual chapters.

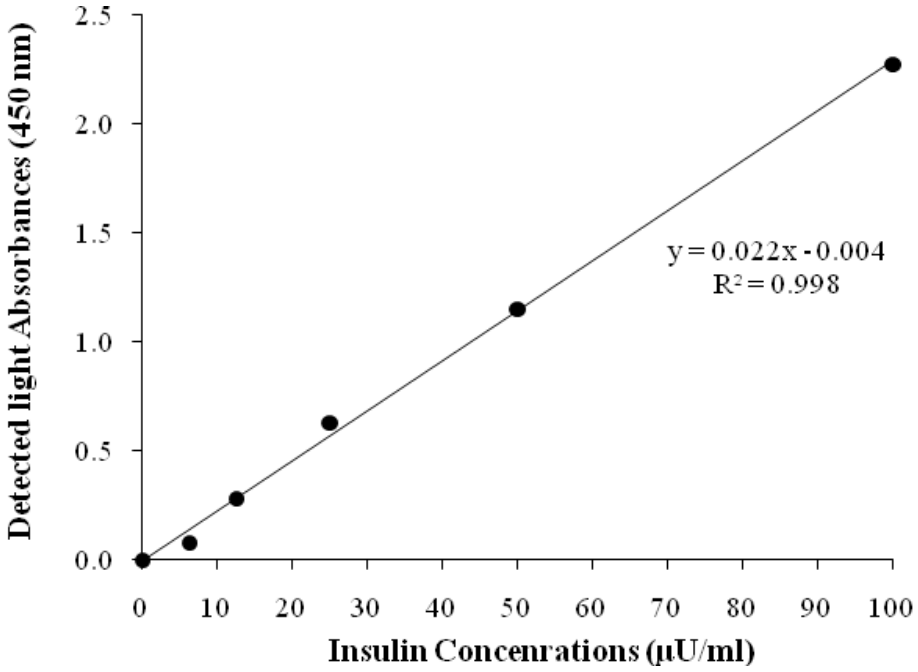

Figure 3.3 Data from absorbencies detected from known insulin standards (DRG diagnostics) obtained using KC junior software.

3.7 Determination of Labelled [6,6$^2H_2$]glucose

Experimental procedures required the injection and / or infusion of the stable isotope [6,6$^2H_2$]glucose (98% enrichment; Cambridge Isotope Laboratories Inc., Andover, USA), for the quantification of glucose turnover and kinetics during exercise and hypoxia. Analysis of enriched [6,6$^2H_2$]glucose was carried out using ~20µl of each plasma sample. Samples were deproteinized with 100µl of ethanol before the plasma / ethanol mixtures were centrifuged at 6000 rpm for 5 min. The supernatants that resulted were evaporated to dryness using a rotary evaporator. Hydroxylamine Hydrochloride (25mg) was dissolved in Pyridine (1 ml) before 100µl of the resultant oxime reagent was added to each microtube, converting samples to oxime-TMS. After a 60 min incubation period (70ºC) 100µl of 99% Bis(trimethyl)trifluroacetamide (BSTFA): 1% TMCS (Sigma-Aldrich, Exeter, UK) was added

before a further incubation period (45 min; 70ºC). Samples were then transferred to autosampler vials. Using gas chromatography mass spectrometer (GCMS; Hewlett Packard) glucose derivatives were measured for peaks of 319 (unlabelled glucose; trace) and 321 ([6,6$^{2}H_{2}$]glucose; tracer). A sample from the injected glucose bolus (~20 µl) was also analysed for determination of labelled and unlabelled glucose ratios.

3.8 Statistical Analyses

All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS, version 15) software. Results are expressed as mean and standard error of the mean (SEM). Statistical analysis specific to individual studies are described within each chapter. The level of significance was set as $P < 0.05$).

EXPERIMENTAL CHAPTERS

CHAPTER 4

Glucose Disposal During & Following Acute Hypoxia in Individuals with Type 2 Diabetes

4.0 Introduction

Research has consistently shown the therapeutic benefits of exercise on glycaemic control in type 2 diabetic patients (ADA, 2002; ADA, 2004; Sigal *et al.*, 2004; Thomas *et al.*, 2006; Sigal *et al.*, 2006). The mechanisms by which exercise stimulates glucose transport are complex and have been described in an earlier section (Literature Review, 2.9). The primary goal of exercise in diabetic sufferers is to reduce resting blood glucose concentration in an attempt to reduce disease risk factors.

Insulin and contractile activity (exercise) stimulate glucose disposal in skeletal muscle using separate, independent signalling pathways (Holloszy, 2003). Hypoxia appears to activate glucose transport via a similar signalling pathway to that of contractile activity (Cartee *et al.*, 1991). Glucose transport has been shown to be additive when either hypoxia or contractile activity are coupled with insulin, whereas hypoxia and contractile activity are not (Cartee *et al.*, 1991; Azevedo *et al.*, 1995). The ability of hypoxia to stimulate glucose disposal, independent of contractile activity has been documented in both animal (Cartee *et al,*. 1991; Chiu *et al*., 2004) and in *vitro* work using isolated human muscle tissue (Azevedo *et al.*, 1995; Holloszy, 2003). Experimental evidence suggests that hypoxia induces glucose transport activity via the translocation of GLUT-4 to the plasma membrane (Cartee *et al.*, 1991) which may require the activation of AMPK via decreases in phosphocreatine, ATP and increased AMP concentrations (Winder *et al.*, 1999). In vivo treatment with the AMP analog 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) is known to activate AMPK (Merrill *et al.*, 1997) and increase glucose transport (Musi *et al.*, 2001). Elsewhere, activation of the AMPK-dependent pathway stimulates glucose transport via increased cell surface content of GLUT-4 in type 2 diabetic muscle tissue (Koistinen *et al.*, 2003).

The literature surrounding hypoxia and glucose tolerance in humans is both minimal and confusing due to the nature of field-based studies and differences in measurement techniques (Literature Review 2.10.2). Although it provides a useful insight, work from animal models must be read with some degree of caution due to differences between rodent and human physiology. Insulin release is known to be stimulated from mouse islets of Langerhans at concentration of 2 mmol/l but not in humans at the same concentrations (Johnson *et al.*, 2007). Furthermore, the effects of acute hypoxia on whole body glucose metabolism (S_G^{2*} and S_I^{2*}) in type 2 diabetics has yet to be investigated.

Although the mechanisms by which hypoxia is able to stimulate glucose transport activity have received much attention, the possible use of resting hypoxic exposure in improving glycaemic control in type 2 diabetic humans is novel. Any intervention that may improve whole body glucose control through changes in S_G^{2*} and S_I^{2*} could be an important strategy in the treatment of type 2 diabetics who may be unable to activate their contraction-stimulated pathway due to diabetic related mobility problems such as peripheral vascular disease, lower extremity amputations and weight bearing problems. The aim of this study was to assess the effects of moderate hypoxia [O_2 ~14.6 (0.4)%], at rest on whole body glucose metabolism in type 2 diabetics.

Hypothesis – Acute hypoxia will improve insulin sensitivity (S_I^{2*}) in individuals with type 2 diabetes.

4.1 Methods

4.1.1 Subjects

Six sedentary males, diagnosed with type 2 diabetes within the last 5 years were recruited for this investigation. Subject's clinical and metabolic characteristics are detailed in Table 4.0. Ethical approval and subject exclusion criteria for this study are set out within the general methods (section 3.2.3). Three subjects were diet-treated, and therefore free from glycaemic altering drugs. The remaining three subjects were treated with metformin (n = 2 metformin 150 mg 3 x day and n = 1, metformin 150 mg 1 x day). Three individuals were also being treated for hypertension, taking moderate doses of calcium channel blockers (5-10 mg twice daily). Those subjects taking metformin were asked to abstain from this medication in the 48 hours prior to main experimental trials. Approximately 90% of metformin is removed from the body in urine within 24 hr (plasma specific half-life ~6.2 hr and whole blood half-life ~17.6 hr) (Heller, 2007).

Table 4.0 **Subjects' Clinical, Physiological and Metabolic Characteristics**

	Diabetic (n = 6)
Age (yr)	57.7 (3.6)
Height (cm)	167.3 (9.0)
Mass (kg)	80.7 (13.2)
Body Mass Index (BMI) (kg / m^2)	29.2 (6.7)
Body Fat (%)	37.0 (10.7)
Systolic Blood pressure (mmHg)	138.0 (17.4)
Dystolic Blood pressure (mmHg)	81.0 (8.1)
HbA_{1c} (%)	7.8 (2.3)
Fasting Arterialised Glucose (mmol/l)	8.4 (1.8)
$HOMA_{IR}$	7.3 (2.8)

Values are means (SEM). Glycosylated Haemoglobin A1C (HbA_{1c}); Homeostasis Model Assessment of Insulin Resistance ($HOMA_{IR}$)

4.1.2 Experimental Design & Procedures

The experimental protocol consisted of three visits. The first visit acted as a familiarisation, with visits two and three forming the main trial. The latter two laboratory visits required subjects to undergo a 60 min resting exposure to both normoxic [Nor Rest; O_2 = 20.93%] and hypoxic [Hy Rest; O_2 = 14.6 (0.4)%] environments. Trials were carried out in a randomised fashion with a minimum of seven days between each visit.

On arrival, height and body mass measurements were recorded. Subsequently, one 18-gauge cannula was placed into a dorsal hand vein for frequent sampling of arterialised blood and one 18-gauge cannula was placed into an antecubital vein in the opposite arm for injection of the labelled glucose bolus. The sampling hand remained within a hot box (~60ºC) throughout the described protocol to ensure the collection of arterialised blood (Arterialised Blood Sampling Procedure; 3.5.3) (Mcguire *et al.,* 1976). Baseline (-30 min) venous blood (~10ml) was collected and dispensed into lithium-heparin (~5ml) and EDTA (~5ml) coated tubes. A sample (~20µl) of whole blood was used to determine blood glucose concentrations, measured in duplicate (YSI 2300 STAT Yellow Springs Instruments, Yellow Springs, Ohio, USA).

4.1.3 Hypoxic Intervention

Subjects were then required to rest in a seated position within a normoxic [Nor Rest; O_2 = 20.93%] or hypoxic [Hy Rest; O_2 ~ 14.6 (0.4)%] environment for 60 min on two separate occasions. The method for generating hypoxia has been detailed in section 3.4.2 (General Methods). Arterialised blood samples, heart rate (HR; Polar Accurex monitor; Oy, Kemple, Finland) and oxhaemoglobin saturation (S_pO_2; pulse oximetry, Nonin 2500, Minneapolis, USA) were collected every 10 min during the 60 min trials. Symptoms of Acute Mountain Sickness were assessed using an adapted version of the Lake Louise Questionnaire. This was completed at the half-way point (30 min) of the protocol. Subjects displayed no AMS symptoms' during hypoxic exposure. Immediately following normoxic and hypoxic exposure a 4 hr labelled Intravenous Glucose Tolerance Test (IVGTT) was preformed (Vicini *et al.*, 1997).

4.1.4 Labelled (Hereafter Hot) Intravenous Glucose Tolerance Test (IVGTT) Protocol

The labelled IVGTT, as measured by the disappearance of unlabeled and labelled [6,6$^2H_2$]glucose is an effective means of assessing glucose disposal in *vivo* (Caumo & Cobelli, 1993). This relatively non invasive method generates a set of metabolic variables that differentiate glucose disposal into $S_G^{2*}$and S_I^{2*}. It also allows the estimation of endogenous glucose production (EPG) from the measured unlabeled and labelled glucose concentrations (Caumo & Cobelli, 1993).

4.1.5 Stable Isotope ([6,6$^2H_2$]glucose) Preparation and Administration

The stable isotope [6,6$^2H_2$]glucose (98% enrichment; Cambridge Isotope Laboratories Inc., Andover, USA) was used to enable quantification of glucose turnover during both trials. All solutions were prepared on the morning of the experiment under sterile conditions. Following the measurement of subjects' body mass ~28.4 mg / kg of [6,6$^2H_2$]glucose was dissolved and filtered, using a 0.22 µm Millipore filter (Bedford, MA), into ~250 mg / kg of unlabelled glucose (500 ml; 50% Glucose, Baxter Health Care, UK). On completion of the each trial (~10:00 hr), glucose solutions were immediately injected into the contralateral antecubital vein over a period of 40-60 sec. Subjects remained in a supine position during the glucose administration and the subsequent IVGTT. A small amount of each glucose solution (~20 µl) was kept for analysis of tracer-to-tracee ratios (General Methods 3.7).

Arterialised samples (~10ml) were then drawn at 0, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 25, 30, 35, 40, 60, 70, 80, 100, 120, 140, 160, 180, 210 and 240 min following glucose injection. A small amount (~20µl) from each sample was dispensed into microcuvettes for immediate determination of blood glucose concentrations in duplicate. The remaining blood samples were then dispensed into lithium/heparin (~5ml) and EDTA (~5ml) as described in section 3.5.3. Insulin concentrations, measured in plasma separated from whole blood were detected using a commercially available insulin ELISA kit (General Methods 3.6). The intra-assay and inter-assay Coefficient of Variation for all insulin ELISA's related to this chapter were <3.9%.

4.1.6 Glucose Kinetics & Modelling

Plasma insulin, endogenous glucose concentrations and [6,6$^2H_2$]glucose enriched values (General Methods 3.7) were modelled to obtain metabolic indices of *endogenous* and peripheral glucose metabolism [insulin sensitivity (S_I^{2*}), glucose effectiveness (S_G^{2*}) and *endogenous* glucose production (EGP)] (Vicini *et al.,* 1997). The use of [6,6$^2H_2$]glucose during the labelled IVGTT allows for the effects of glucose and insulin, on glucose disappearance (R_d), to be distinguished from each other, whilst providing plausible estimations on EGP (Caumo & Cobelli, 1993). Simulation Analysis and Modelling software (SAAMII; Institute, Seattle, WA) was used to carry out the two-compartmental minimal modelling of data.

4.2 Statistical Analyses

All results are expressed as mean with standard error of the mean (SEM). Statistical significance was set at the level $P < 0.05$. Differences over time for arterialised blood glucose and plasma insulin were evaluated by two way repeated measures ANOVA. Tukey's post-hoc tests were used when statistical significance was found. Paired t-tests were used to test for statistical differences in S_G^{2*}, S_I^{2*} and EGP between Nor Rest and Hy Rest trials. The area under the curve for both glucose (AUC_{Glu}) and insulin (AUC_{Ins}) were calculated using the trapezoidal rule (Matthews *et al.,* 1990).

4.3 Results

Self recorded nutritional intake was not different in total calories consumed [Nor Rest; 1788 (72) and Hy Rest; 1685 (24) kcal] (P = 0.193) and total carbohydrate intake [Nor Rest; 243.0 (12.5) and Hy Rest; 228.7 (24.1) g] (P = 0.071) in the 24 hr prior to each trial.

Heart rate recorded during the 60 min of hypoxia (Hy Rest) and normoxia (Nor Rest) showed no differences between trials (71 (3) vs 70 (3) beats · min^{-1}, respectively) (P = 0.989). Values for oxygen saturation (S_pO_2) showed reductions on exposure to environmental hypoxia and were lower during the Hy Rest trial (P = 0.006), with no difference seen in the control condition (Nor Rest) (Figure 4.0). Hy Rest showed a significant reduction in arterialised blood glucose concentrations, decreasing by 0.74 (0.14) mmol/l (P = 0.002) during 60 min of exposure (Figure 4.1). No change was found for the same variable during Nor Rest [-0.23 (0.16) mmol/l; P = 0.181].

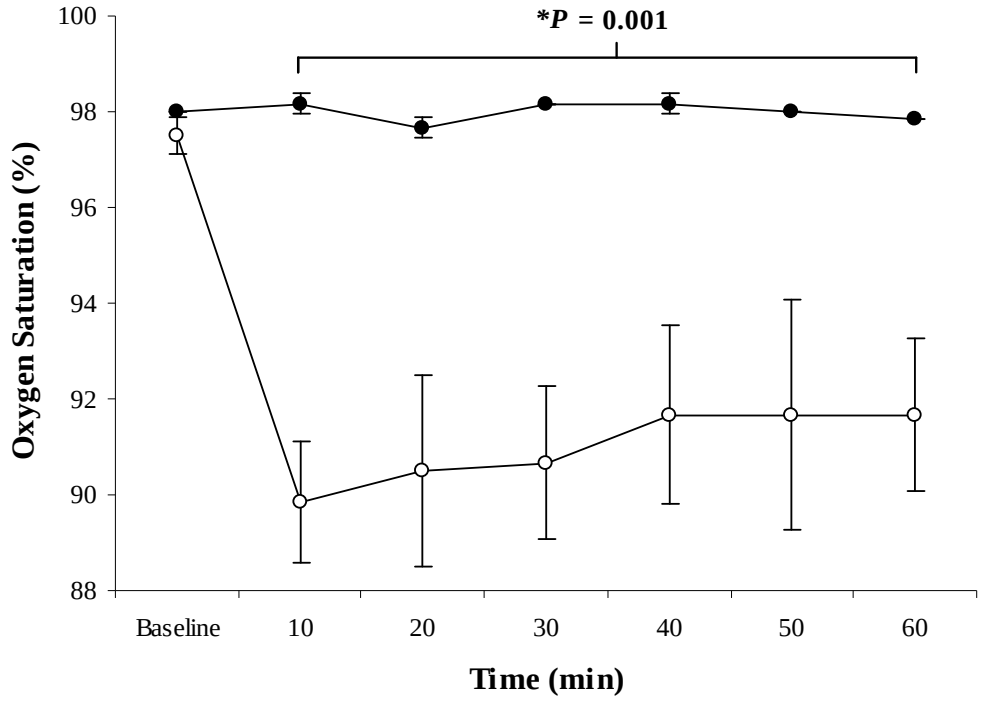

Figure 4.0 Oxygen saturation (S_pO_2) response at baseline and during normobaric hypoxia (Hy Rest; O_2 ~14.6%) (open symbols) and normoxia (Nor Rest; O_2 ~20.93%) (black symbols). * denotes significant difference between conditions (*P* = 0.001).Values are means (SEM).

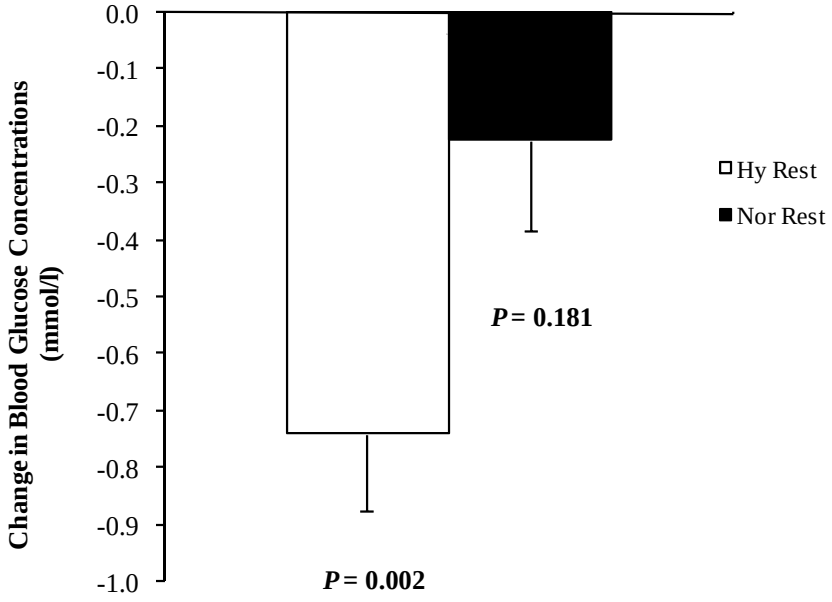

Figure 4.1 Changes in arterialised blood glucose concentrations from baseline to the end of 60 min rest in normobaric hypoxia (Hy Rest; O_2 ~14.8%) and normoxia (Nor Rest; O_2 ~20.93%). Values are mean (SEM).

Hypoxia had no effect on arterialised plasma insulin concentrations and was not changed from baseline during the 60 min exposure (Hy Rest) (P = 0.507). The resting control trial was also unchanged (Nor Rest) (P = 0.591). Furthermore, insulin values were not different between condition (P = 0.256) (Figure 4.2). Total area under the curve for arterialised blood glucose (AUC_{Glu}) and plasma insulin (AUC_{In}) are displayed in Table 4.1. Although AUC_{Glu} was lower following Hy Rest this difference was not found to be significant when compared to Nor Rest (P = 0.23). AUC_{Ins} was statistically lower during the 4 hr IVGTT (P = 0.03). Figure 4.3 shows arterialised blood glucose values during the 4 hr IVGTT for Nor Rest and Hy Rest. As expected, glucose concentrations firstly increased then fell over time following the intravenous glucose load which was administered at time point 0. Blood glucose values were found to be significantly lower in this period for Hy Rest when compared to Nor Rest (P = 0.001). Despite AUC_{Ins} being 24.4% (P = 0.03) lower in the Hy Rest trial, no difference was noted when an ANOVA was carried out to compare between conditions (Nor Rest vs. Hy Rest; P = 0.208; Figure 4.4).

The two-compartment minimal model analysis showed insulin sensitivity (S_I^{2*}) to be significantly higher in the 4 hr period immediately following Hy Rest as compared with the Nor Rest condition [Nor Rest; 1.39 (0.08) and Hy Rest; 2.25 (0.50) $x10^{-4} \cdot min^{-1}(\mu U/ml)$] ($P$ = 0.047). However, S_G^{2*} was not different between conditions (Figure 4.5; P = 0.178). Hypoxia at rest had no effect on endogenous glucose production with no difference between Nor Rest and Hy Rest (P = 0.472).

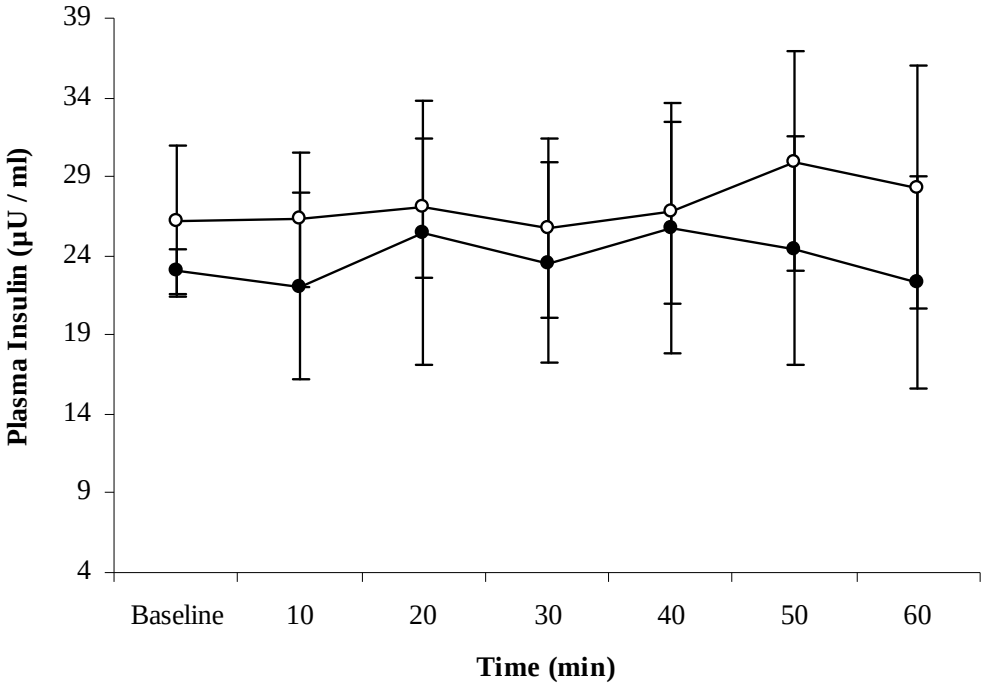

Figure 4.2 Insulin response during 60 min of rest in either a hypoxia and normoxia. Values showed no difference between conditions (P = 0.256). Values showed no change over time within conditions [Hy Rest (open symbols): P = 0.507 and Nor Rest (closed symbols): P = 0.591].

Table 4.1 The integrated area under the curve for arterialised blood glucose (AUC_{Glu}) and plasma insulin (AUC_{Ins}) concentrations following Nor Rest and Hy Rest

	Nor Rest	**Hy Rest**	***P* Value**
AUC_{Glu} (mmol · l^{-1} · min)	1867 (340)	1663 (171)	P = 0.23
AUC_{Ins} (µU · ml^{-1} · min)	7635 (590)	5774 (918)	P = 0.03

Values are means (SEM).

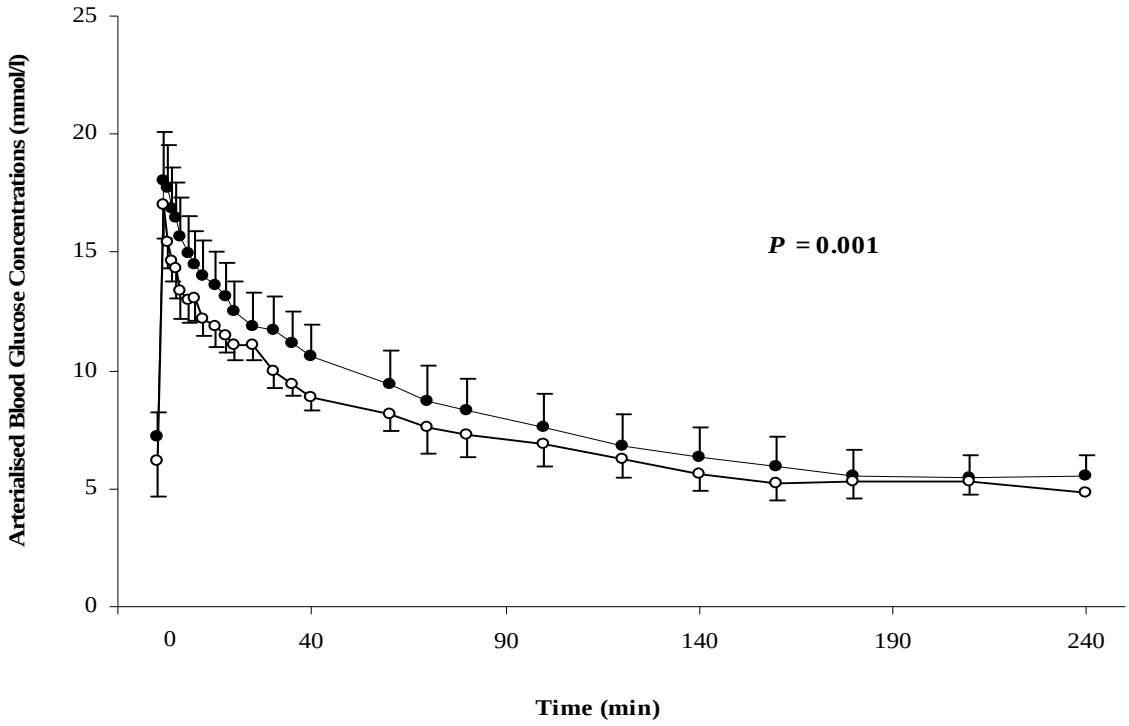

Figure 4.3 Glucose decline following an intravenous glucose challenge (IVGTT). A statistical difference was noted between Hy Rest (open symbols) and Nor Rest (closed symbols) ($P = 0.001$).

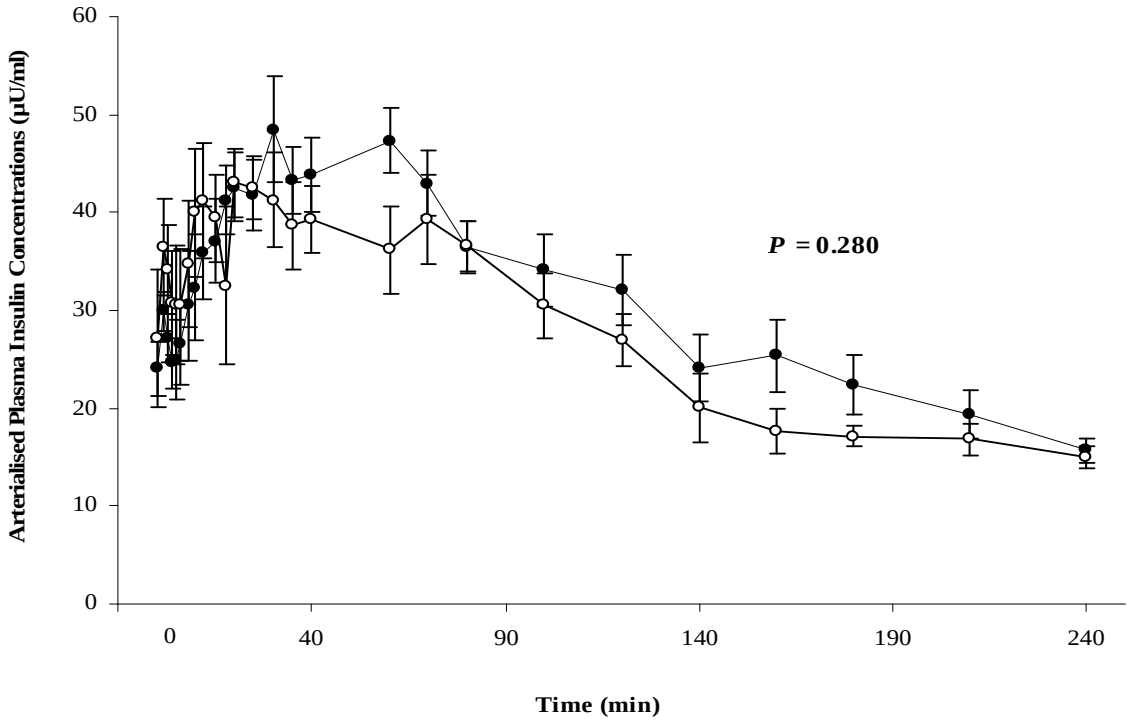

Figure 4.4 Insulin response during a 4 hr IVGTT following 60 min of Hy Rest (open symbols) and Nor Rest (closed symbols). No significant difference was noted between conditions.

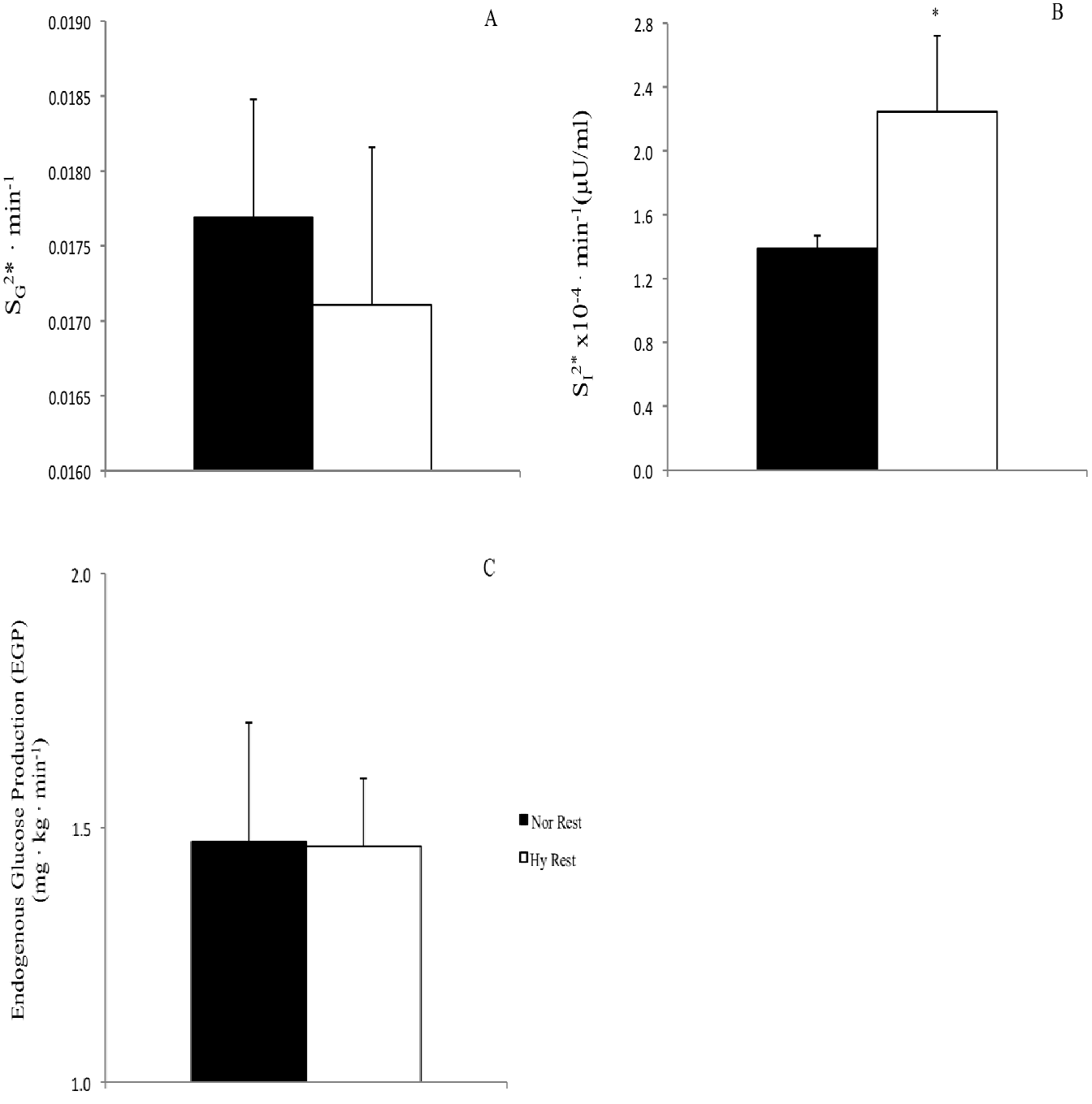

Figure 4.5 Glucose Effectiveness (S_G^{2*}) (A), Insulin Sensitivity (S_I^{2*}) (B) and Endogenous Glucose Production (EGP) (C) following Nor Rest and Hy Rest during a 4 hr IVGTT. A significant difference was noted between Nor Rest and Hy Rest for S_I^{2*} (P = 0.047), but not S_G^{2*} (P = 0.178) or EGP (P = 0.472)

4.4 Discussion

The aim of this study was to assess changes in whole body glucose metabolism during and following acute hypoxic exposure in individuals with type 2 diabetes. These results indicate that hypoxia increases blood glucose removal, shown by a decrease in arterialised blood glucose concentrations during, and in the 4 hours following, resting hypoxic exposure (Hy Rest). These values were significantly lower when compared to the control condition (Nor Rest). The current investigation also demonstrated that insulin sensitivity (S_I^{2*}) was 39% higher following hypoxic exposure, indicating that prior hypoxia improves insulin-stimulated glucose disposal in type 2 diabetics.

4.4.0 Hypoxia / Contraction-Stimulated Pathway

Insulin (Wardzala & Jeanrenaud, 1981) and contractile activity (Douen *et al.*, 1990) are known to activate glucose transport into skeletal muscle by encouraging the translocation of the glucose transporter-4 (GLUT-4) to the sarcolemma of muscle fibres. Environmental hypoxia or a reduction in oxygen availability has also been shown to stimulate glucose transport in a similar fashion as the exercise/contraction pathway, by increasing the GLUT-4 content in the plasma membrane (Cartee *et al.*, 1991). There is a wealth of literature investigating glucose metabolism both in animals (Cartee *et al.*, 1991; Holloszy, 2003; Wright *et al.*, 2005) and humans (Brooks *et al.*, 1991; Brooks *et al.*, 1992; Azevedo *et al.*, 1995) during exposure to hypoxia or altitude. In contrast there is little research investigating the effects of acute hypoxia on subsequent glucose disposal in type 2 diabetics. Azevedo *et al.* (1995) did, however evaluate the effects of hypoxia on glucose transport in insulin-resistant and type 2 diabetic rectus abdominis human muscle tissue. By removing muscle tissue and

incubating (*in vitro*) it under hypoxic and normoxic conditions, Azevedo *et al.* (1995) was able to show a 2-3 fold increase in glucose transport rates during and following hypoxia, which was not present during normoxic incubation. Furthermore, Cartee *et al.* (1991) demonstrated that 3-O-Methylglucose[3] uptake was increased from 0.21 (0.02) (normoxic control) to 1.18 (0.10) μmol · ml · 10 min^{-1} during incubation of rat epitrochlearis muscle in severe hypoxia. The current work is novel in that it studied *in vivo* analysis of whole body glucose metabolism during and following moderate (O_2 ~14.6%) rather than severe hypoxia (O_2 ~0.0%) (Cartee *et al.*, 1991; Azevedo *et al.*, 1995).

The increase in glucose disposal / transport demonstrated by both Azevedo *et al.* (1995) and the current study may be largely attributed to the well documented increase in the contraction (hypoxic) stimulated activation and translocation of GLUT transporters (Cartee *et al.*, 1991; Holloszy, 2003). As the subjects in this study were at rest, it is likely that the increase in glucose disposal was partly due to the contraction-stimulated translocation of GLUT-1 (Zhang, 1999) and to a larger extent GLUT-4 (Reynolds *et al.*, 1998; Zorzano *et al.*, 2005), as well as the activation of transporters pre-exiting in the plasma membrane (GLUT-1) of skeletal muscle (Zhang, 1999). In addition, adipocyte GLUT-4 translocation may have also been up regulated on exposure to hypoxia (Wood *et al.*, 2007). Offering the potential for improved glucose clearance in the subject cohort examined [Percentage body fat; 37.1 (10.7)%]. Further, GLUT known to be present in other tissue types [GLUT-6 (spleen and brain), GLUT-8 (liver, adipose and testis), GLUT-10 (liver and pancreas) and GLUT-12 (heart and adipose)] may have also been activated during hypoxic exposure (Wood *et al.*, 2007) and contributed to an increase in whole body (not just skeletal muscle) glucose disposal.

4.4.1 Hypoxia Stimulates Glucose Transport using an Insulin-Dependent Mechanism

Type 2 diabetes is primarily characterised by defects in insulin mediated glucose transport. Indeed, deficiencies in glucose disposal, glucose phosphorylation and glycogen synthesis may all contribute to reduced glucose transport activity in diabetes and obesity (Del Prato *et al.*, 1993; Pendergrass *et al.*, 1998). Glycogen synthesis is considered to be the pathway most affected, although glycolysis and glucose oxidation are also impaired (Del Prato *et al.*, 1993; Pendergrass *et al.*, 1998). However, the contraction stimulated pathway is known to remain largely intact within the same population (Azevedo *et al.*, 1995).

Previous work has indicated that exercise (hypoxia) and insulin stimulate glucose transport by different pathways in muscle as suggested by an additive and maximal effect when the two stimuli are combined (Cartee & Holloszy, 1990). Work taken from exercise studies show that once the acute effects of exercise on glucose transport wears off it is replaced by a separate and distinct pathway, mediated by insulin (Garetto *et al.*, 1984; Cartee *et al.*, 1990; Richter *et al.*, 2004). The current study showed no change in insulin concentrations from baseline values which was aligned with a hypoxic-induced reduction in blood glucose concentrations during hypoxic exposure. This suggests that a mechanism, independent of insulin was responsible for the decrease in blood glucose. Similar to the effects of exercise, it would appear that hypoxia alters insulin mediated transport in the hours following hypoxic exposure. As demonstrated by lower blood glucose concentrations (P = 0.001), significantly lower AUC_{Ins} (P = 0.03) and improved S_I^{2*} (P = 0.047) in the 4 hr following acute hypoxic treatment.

Although the labelled IVGTT allows for powerful estimation of glucose metabolism, muscle tissue samples would have provided a clearer indication of possible alterations in intracellular signalling proteins involved in the insulin-stimulated pathway. Data interpreted from the literature suggests that insulin receptor substrate (IRS) and specific proteins sitting downstream (AMPK and ∝PKC) of the insulin-stimulated pathway may have been activated due to hypoxia (Mu *et al.,* 2001; Perrini *et al.,* 2004; Jessen & Goodyear, 2005). Enhanced insulin action following exercise has been shown to be independent of any changes in signalling proteins, and therefore attributed to increased GLUT-4 translocation (Henriksen, 2002). This may reflect that post exercise increases in glucose disposal are a result of more efficient recruitment of GLUT-4 for a given dose of insulin. Although, differences between studies may reflect variations in measurement methods as others have reported exercise-induced increases in IRS-1 and PI3-kinase (Chibalin *et al.,* 2000; Hevener *et al.,* 2000).

AMPK's role in glucose transport activity has been well defined, both within the contraction-(hypoxic) (Jessen & Goodyear, 2005) and insulin-stimulated pathways (Fisher *et al.,* 2002). In the hours following exercise, the insulin pathway is the primary mechanism whereby muscle glycogen is restored. Muscle glycogen concentrations have previously been shown to decrease (~66%) during severe hypoxia (Cartee *et al.,* 1991). Therefore, it is at least plausible to suggest that the increase insulin mediated glucose disposal and S_I^{2*} following hypoxia may be partly attributed to hypoxic-induced glycogen depletion (Wadley *et al.,* 2006).

4.4.2 Can Hypoxia Cause Glucose Intolerance?

Larsen *et al.* (1997b) demonstrated that insulin action was decreased at 4,559 m on the second day of a moderate term exposure (7 days). Although, this was partly restored, returning to near normal (baseline sea-level) values on the final day (Larsen *et al.,* 1997b). It is entirely possible that the measured increase in plasma cortisol in the study of Larsen *et al.* (1997b) contributed to peripheral insulin resistance (Rooney *et al.,* 1993). Unfortunately, the current work did not measure cortisol. Cortisol treatment for ~24 hr is known to induce insulin resistance (Rooney *et al.,* 1993) due to a reduction in glycogen synthase enzyme activity in skeletal muscle (Holmäng *et al.,* 1992). However, Oltmanns *et al.* (2004) showed no difference from normoxic baseline values for cortisol during a 30 min hypoxic bout. Similar to Larsen *et al.* (1997b), the work of Oltmanns *et al.* (2004) suggested that hypoxia acutely decreases glucose tolerance shown through a significant reduction in dextrose infusion rates (Hypoxic ~250 ml / hr and Normoxic ~280 ml / hr; $P = 0.01$).

It is not clear why the results from Oltmanns *et al.* (2004) work differ from those of the current study. Disparities may be associated with differences in the population studied and or the method for assessing glucose tolerance. Oltmanns *et al.* (2004) used non-obese healthy control subjects and a more severe bout of hypoxia (S_pO_2 ~75%), although, the precise level of hypoxia was not published. The S_pO_2 measured during Oltmanns *et al.* (2004) study were higher than the values obtained within the current chapter (S_pO_2 ~90%). This work also showed a significant elevations in epinephrine concentration (Oltmanns *et al.,* 2004), which is known to stimulate glucose availability and utilisation (Saha *et al.,* 1968; Howlett *et al.,* 1999). Activation of the sympathoadrenal release of epinephrine was suggested by Oltmanns *et al.* (2004) to be the only mechanism causing hypoxic mediated glucose intolerance, via

increased endogenous glucose release and decreasing glucose disposal. However, data published by Brooks *et al.* (1991) have shown that despite an increase in circulating epinephrine concentrations, hypoxia still reduced blood glucose concentrations. This work may contradicts Oltmanns *et al*. (2004) conclusion and suggests that hypoxia increases glucose disposal, independent of epinephrine.

4.5 Conclusion

These results showed that hypoxia (O_2 ~14.6%) caused a significant increase in glucose disposal, both during and following acute hypoxic exposure. Although neural and hormonal variables were not measured, this study is the first to conclude that acute hypoxia can improve whole body glucose tolerance in type 2 diabetics via insulin-dependent glucose transport, as shown by increased S_I^{2*}. Hypoxia may therefore, be of clinical importance to type 2 diabetics by improving acute and short-term glucose control.

CHAPTER 5

Can the Blood Glucose Lowering Effect of Exercise in Individuals with Type 2 Diabetes be Further Stimulated by Acute Hypoxia?

5.0 Introduction

Normal blood glucose concentration can be maintained in healthy subjects despite an exercise-induced increase in glucose demand. This is attributed to an increase in blood glucose appearance via gluconeogenesis and endogenous glucose production (Berger *et al.,* 1980; Kjaer *et al.,* 1990; Howlett *et al.,* 1999**).** Endogenous glucose production is known to be of a comparable size when comparing healthy non- and type 2 diabetic subjects during exercise (Giacca *et al.,* 1998). A review of the literature shows that acute exercise stimulates glucose transport activity and improves glycaemic control in type 2 diabetics via increased glucose uptake (Devlin *et al*. 1987; Larsen *et al*., 1997a; Kang *et al*., 1999; Musi *et al.,* 2001; Macdonald *et al*., 2004; Thomas *et al*., 2006; Hordern *et al.,* 2008). Exercise is therefore, an effective tool in the management of hyperglycaemia in type 2 diabetes (for a review see Thomas *et al*., 2006). The reduction in circulating blood glucose concentration in diabetic patients has been attributed to greater glucose utilization when comparisons are made with healthy controls (Giacca *et al.,* 1998). Blood glucose concentrations are known to decline by ~2.5 mmol/l (13%) following a single bout of moderate intensity exercise (90% predetermined lactate threshold) (Macdonald *et al.,* 2006). Since insulin secretion is inhibited during exercise this effect may be attributed to contraction stimulated glucose uptake (Rose & Richter, 2005).

It is widely accepted that insulin (Dohm, 2002; Wojtaszewski *et al.,* 2002), exercise (Jessen & Goodyear, 2005; Rose & Richter, 2005) and hypoxia (Cartee *et al.,* 1991) are potent stimuli for glucose transport. Exercise is clinically recognised as an alternative pathway for glucose transport activity in states of insulin resistance and overt type 2 diabetes (Rose & Richter, 2005). This contraction stimulated pathway (Mu *et al.,* 2001; Wojtaszewski *et al.,*

2002; Richter *et al.*, 2004; Jessen & Goodyear, 2005; Jensen *et al.*, 2007) requires the activation and translocation of glucose transporters (GLUT 1 and GLUT 4, respectively) to the sarcolemma of muscle fibres (Winder & Hardie, 1999). Plasma membrane GLUT 4 content is highly correlated to glucose transport activity in human skeletal muscle (Lund *et al.*, 1997) with sarcolemmal GLUT 4 content shown to increase by 74% in type 2 diabetics following an acute bout of exercise (45-60 min cycling exercise ~60-70% $\dot{V}O_{2max}$), (Kennedy *et al.*, 1999). Central to exercise-induced glucose transport is the metabolic master switch AMPK (Wojtaszewski *et al.*, 2000; Mu *et al.*, 2001; Hardie *et al.*, 2006). AMPK has also been implicated in hypoxic-induced glucose disposal (Mu *et al.*, 2001; Wadley *et al.*, 2006).

The first experimental study from the present thesis showed that hypoxia at rest increased whole body glucose tolerance during and acutely following exposure in type 2 diabetic humans. Exercise and hypoxia are thought to stimulate glucose transport using the same signalling mechanisms independent of insulin (Cartee *et al.*, 1991). Although, an opposing notion is that hypoxia and exercise may stimulate glucose disposal independently of each other and that when combined could have an additive effect (Wojtaszewski *et al.*, 1998; Henriksen *et al.*, 2002). Using rodent models, Chiu *et al.* (2004) have shown that hypoxia and exercise increase GLUT-4 translocation and improve glucose control to a greater extent than exercise alone. Although, it remains to be determined if the same occurs in type 2 diabetic humans.

It seems reasonable to suggest that exercise and hypoxia may stimulate whole body glucose removal to a greater extent than exercise alone given the evidence in the literature (Wojtaszewski *et al.,* 1998; Henrikson *et al.,* 2002; Chui *et al.,* 2004) and the conclusions drawn in Chapter 3. Furthermore hypoxia may have the potential to activate GLUT transporters known to be present in cell types other than skeletal muscle, including liver and kidney (GLUT-1 & GLUT-10) (Dawson *et al.,* 2001), heart and adipose tissue (GLUT-4 & GLUT-12) (Lisinski *et al.,* 2001), spleen, leukocytes and brain (GLUT-6) (Doege *et al.,* 2001).

Interlukin-6 has been implicated in inflammatory processes (Pradhan *et al.,* 2001), causing insulin resistance (Senn *et al.,* 2002), and regulating energy metabolism (Febbraio *et al.,* 2003). Carey *et al.* (2006) published data showing increased AMPK activation, GLUT-4 plasma membrane translocation and insulin-stimulated glucose uptake during IL-6 infusion in healthy humans. The present chapter was designed to establish if hypoxia and exercise have an additive effect on whole body glucose metabolism in type 2 diabetics over either stimulus alone and to measure plasma IL-6 concentrations during exercise with and without hypoxia.

Hypothesis: Hypoxia and exercise will have an additive effect on insulin sensitivity (S_I^{2*}) over exercise alone, in individuals with type 2 diabetes.

5.1 Methods

5.1.0 Subjects

The type 2 diabetic individuals recruited for study 1 formed the subject cohort for the current investigation. Subject's clinical, physiological and metabolic characteristics are presented in Table 3.0 (Chapter 3.).

5.1.1 Experimental Design

The experimental protocol consisted of three visits. The first visit was used to collect physiological, metabolic and individual lactate responses to incremental exercise (Lactate Threshold; LT). Visits two and three acted as the main experimental trials and required subjects to exercise at 90% of their individual LT for 60 min in *1)* normoxia (Nor Ex; O_2 = 20.93%) and *2)* hypoxia [Hy Ex; O_2 ~ 14.9 (0.9)%] on separate occasions. Trials were carried out in a randomised order with a minimum of seven and a maximum of fourteen days between each trial.

5.1.2 Incremental Exercise - Lactate Threshold

On arrival at the laboratory, subjects blood pressure, measured in duplicate using an automatic oscillometric blood pressure monitor (Omron Healthcare, Hamburg, Germany) was obtained before body mass, subject height and percentage of body fat (Bodystat, Isle of Man, UK) were recorded (General Methods 3.3.2). Following localised cleaning, a fingertip blood sample (~5 µl) was then drawn for determination of HbA_{1c}, using a boronate affinity assay (Axis-Shields Diagnostics, United Kingdom). The protocol described within the General Methods (Measurement of the Lactate Threshold 3.3.5) was used to obtain individual lactate response to incremental exercise.

5.1.3 Main experimental Trials – Normoxic (Nor Ex) and Hypoxic Exercise (Hy Ex)

On arrival, height and body mass measurements were recorded. Subsequently, one 18-gauge cannula was placed into a dorsal hand vein for frequent sampling of arterialised blood and one 18-gauge cannula was placed into an antecubital vein in the opposite arm for injection of the labelled glucose bolus. Arterialised basal blood samples (~10 ml) were taken 30 min prior to exercise (-30 min). The sampling hand remained within a thermoregulated hotbox (~60ºC) throughout the protocol to allow for collection of arterialised blood (Arterialised Blood Sampling Procedure 3.5.2).

Subjects then performed 60 min of cycle ergometer exercise (90% LT) in the environmental conditions [Nor Ex; O_2 = 20.93% and Hy Ex; O_2 ~ 14.9 (0.9)%] on two separate occasions. Arterialised blood samples, heart rate, rating of perceived exertion (RPE) (Borg, 1982) and oxyhaemoglobin saturation (S_pO_2) were collected every 10 min. Symptoms associated with Acute Mountain Sickness were monitored at 30 min using the Lake Louise Questionnaire (LLS), (Roach *et al.,* 2003). This questionnaire was adapted to exclude specific questions relating sleep deprivation. No symptoms of AMS were reported during hypoxic exposure.

A 4 hr labelled IVGTT (section 4.1.5) was performed immediately following each trial. Arterialised blood samples (~10 ml) drawn during exercise and IVGTT's were immediately analysed for determination of whole blood glucose concentrations (~20µl) in duplicate. The remaining blood samples (lithium/heparin; ~5ml and (EDTA; ~5ml) were centrifuged and plasma stored at -80ºC for later analysis of plasma insulin (General Methods 3.6) and enriched [6,6$^2H_2$]glucose samples. These variables were then used to calculate indices of

glucose effectiveness (S_G^{2*}), insulin sensitivity (S_I^{2*}) and endogenous glucose production (EGP) (section 4.1.6).

5.1.4 Interleukin-6 (IL-6) ELISA

Plasma interleukin-6 concentrations were determined using a commercially available enzyme linked immunoSorbent assay (ELISA) (Quantikine HS, R&D systems). Fasting plasma samples and samples drawn during exercise in both Nor Ex and Hy Ex were thawed and vortexed before 200 µl was added to polysterene wells pre-coated with an IL-6 antibody. Two wells remained blank with the remaining wells used for known IL-6 concentrations (standards; 6.25, 12.5, 25, 50, 100 & 200 pg/ml). Samples and standards were determined in duplicate. Known concentrations were used to plot the standard curve. Detection of light absorbance was carried out using a microplate reader (450nm; Universal Microplate reader (ELx800, IBL inc., Minneapolis, USA). The inter- and intra-assay coefficient of variation for this ELISA were 4.0% and 5.2%, respectively.

5.2 Statistical Analysis

Two-way (Time x Trial) Analysis of Variance (ANOVA) with repeated measures were carried out to make direct comparisons between Nor Ex and Hy Ex. Tukey's Post Hoc tests were used to highlight the point at which significance occurred. Paired t-tests compared the indices of insulin sensitivity (S_I^{2*}), glucose effectiveness (S_G^{2*}), endogenous glucose production (EGP) and glucose (AUC_{Glu}) and insulin (AUC_{Ins}) integrated area under the curves. Data are expressed as mean with standard error of the mean (SEM). *P* values less < 0.05 were used to denote statistical significance.

5.3 Results

Analysis of self recorded nutritional diaries showed no difference in total kcal [Nor Ex; 1710.9 (68.2) and Hy Ex; 1718.8 (79.9) kcal] (P = 0.817) nor carbohydrate intake [Nor Ex; 225.9 (9.1) and Hy Ex; 217.9 (6.1) grams] (P = 0.635) in the 24 hr prior to exercise between trials.

Heart rate values increased from baseline throughout exercise in both Nor Ex [baseline; 69 (2) beat · min^{-1} and exercise; 94 (1) beat · min^{-1}] (P = 0.013) and Hy Ex [baseline; 69 (1) beat · min^{-1} and exercise; 96 (1) beat · min^{-1}) (P = 0.005). Although no difference was noted between conditions for the same variable (P = 0.747). Rating of perceived exertion (RPE) was found to be elevated during Hy Ex [13 (1) units] when compared to Nor Ex [12 (1) units] (P = 0.000). Oxygen saturation (S_pO_2), measured by pulse oximetery remained unchanged from baseline during Nor Ex (P = 0.996). Figure 5.0 shows S_pO_2 during both normoxic and hypoxic exercise. During Hy Ex, S_pO_2 significantly decreased over time (P = 0.000) and was statistically lower than S_pO_2 values throughout when compared to Nor Ex (P = 0.000) (Figure 5.0). As expected, lactate concentrations [La] significantly increased from baseline during exercise and peaked at 10 min within the Nor Ex trial [2.07 (0.45) mmol/l; P = 0.016]. Blood [La] also increased during Hy Ex, peaking at the 30 min point [2.02 (0.44) mmol/l; P = 0.009]. Blood lactate concentrations were not different between conditions throughout exercise (Figure 5.1; P = 0.094).

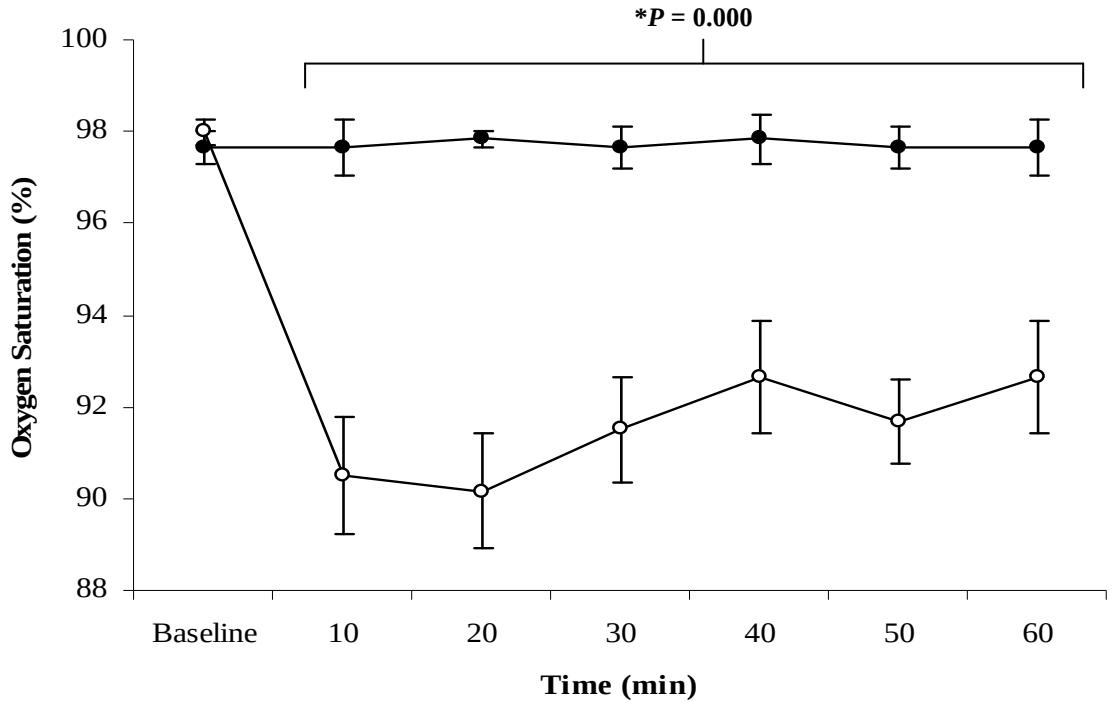

Figure 5.0 Oxygen saturation (S_pO_2) during Hy Ex (O_2 ~14.9%) (open symbols) and Nor Ex (O_2 ~20.93%) (closed symbols). *Denotes significantly different between conditions (*P* = 0.000). Values are means (SEM).

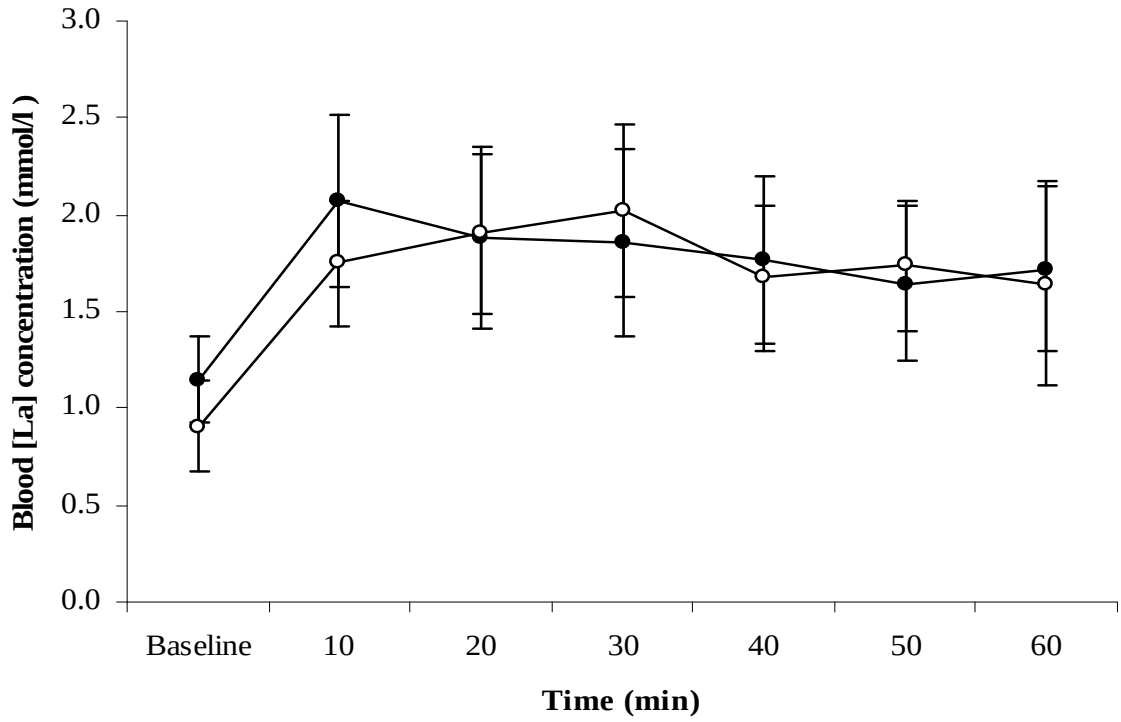

Figure 5.1 [La] response during 60 min of exercise (90% LT) in normoxic (closed symbols) and hypoxic (open symbols) conditions. No significant difference was noted between trials (*P* = 0.094). Values are means (SEM).

Both Nor Ex and Hy Ex demonstrated a blood glucose lowering effect during 60 min of moderate intensity exercise, with arterialised glucose concentrations decreasing by 0.91 (0.35) mmol/l (P = 0.037) and 1.80 (0.64) mmol/l (P = 0.026), respectively (Figure 5.2). The decrease in blood glucose concentrations was found to be significantly greater during Hy Ex when compared to Nor Ex (P = 0.031). Plasma insulin also decreased from baseline to the end of exercise in the hypoxia (Hy Ex; P = 0.028), with no difference in the Nor Ex condition (Figure 5.3; P = 0.208). Plasma insulin concentrations were not different between Hy Ex and Nor Ex (P = 0.10).

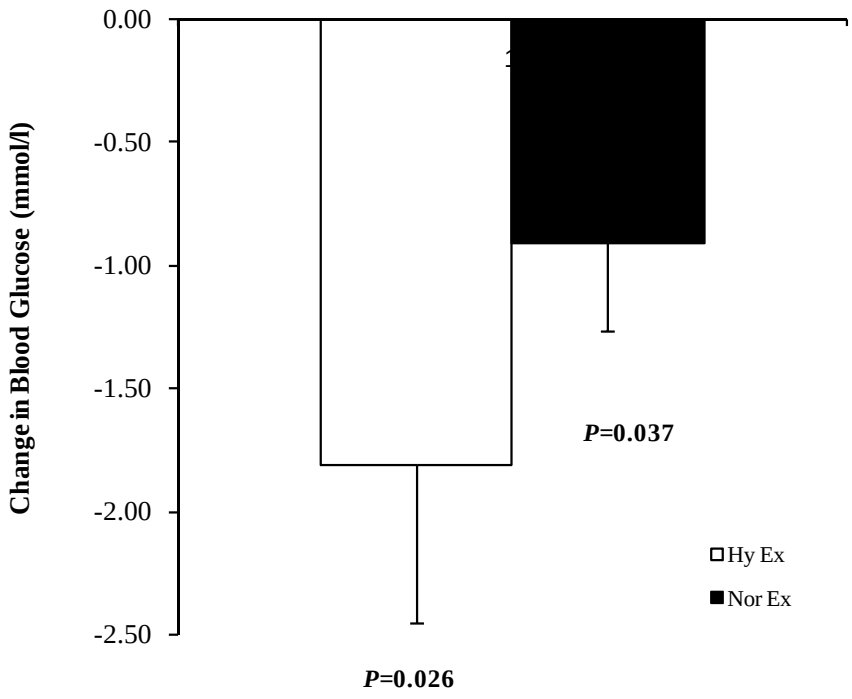

Figure 5.2 Change in arterialised blood glucose concentrations from baseline to the end of exercise in normobaric hypoxia [Hy Ex; O_2 ~ 14.9 (0.9)%] and normoxia (Nor Ex; O_2 = 20.93%). Values are mean (SEM).

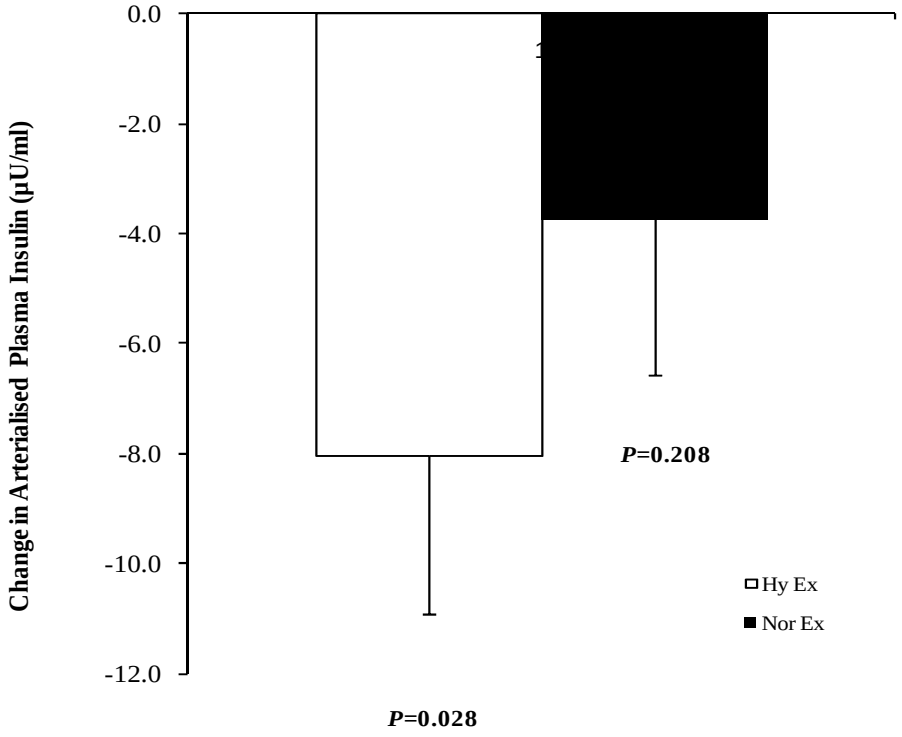

Figure 5.3 Plasma insulin decline during Nor Ex and Hy Ex from baseline to the end of exercise (90% LT). *P* values demonstrated the change within condition. No difference was found between Nor Ex and Hy Ex (*P* = 0.10). Values are mean (SEM) changes.

Area under the curve (AUC) for blood glucose and plasma insulin were calculated to estimate changes in glycaemic control in the 4 hr following each condition. Table 5.0 shows no difference between Hy Ex and Nor Ex for AUC_{Glu}. There was, however, a significant difference for AUC_{Ins} between conditions (*P* = 0.007). Indeed, Hy Ex demonstrated the lowest AUC_{Ins} value when comparing both within this study (Chapter 4) and with the results obtained in Chapter 3. Two way ANOVAs were conducted to assess differences between Hy Ex and Nor Ex over time. This demonstrated no difference in glucose values between conditions (Figure. 5.4; *P* = 0.268). Plasma insulin values were however, significantly lower following intravenous glucose injection in Hy Ex compared to Nor Ex (Figure 5.4; *P* =

0.005). Furthermore, two-compartmental minimal model analysis showed insulin sensitivity (S_I^{2*}) to be significantly higher following Hy Ex as compared to the Nor Ex [3.24 (0.51) and 4.37 (0.48) $x10^{-4} \cdot min^{-1}$(μU/ml), respectively] (*P* = 0.049). Endogenous glucose production (EPG) (*P* = 0.099) and glucose effectiveness (S_G^{2*}) (*P* = 0.123) were not different when comparisons were made between exercise in hypoxia and normoxia (Figure 5.5).

Table 5.0 The integrated area under the curve for arterialised blood glucose (AUC_{Glu}) and plasma insulin (AUC_{Ins}) during a 4 hr IVGTT, following Nor Ex and Hy Ex.

	Nor Ex	**Hy Ex**	***P* Value**
AUC_{Glu} (mmol · l^{-1} · min)	1742 (246)	1622 (154)	*P* = 0.30
AUC_{Ins} (μU · ml^{-1} · min)	5637 (820)	4334 (617)	*P* = 0.007

*Values are means (SEM)

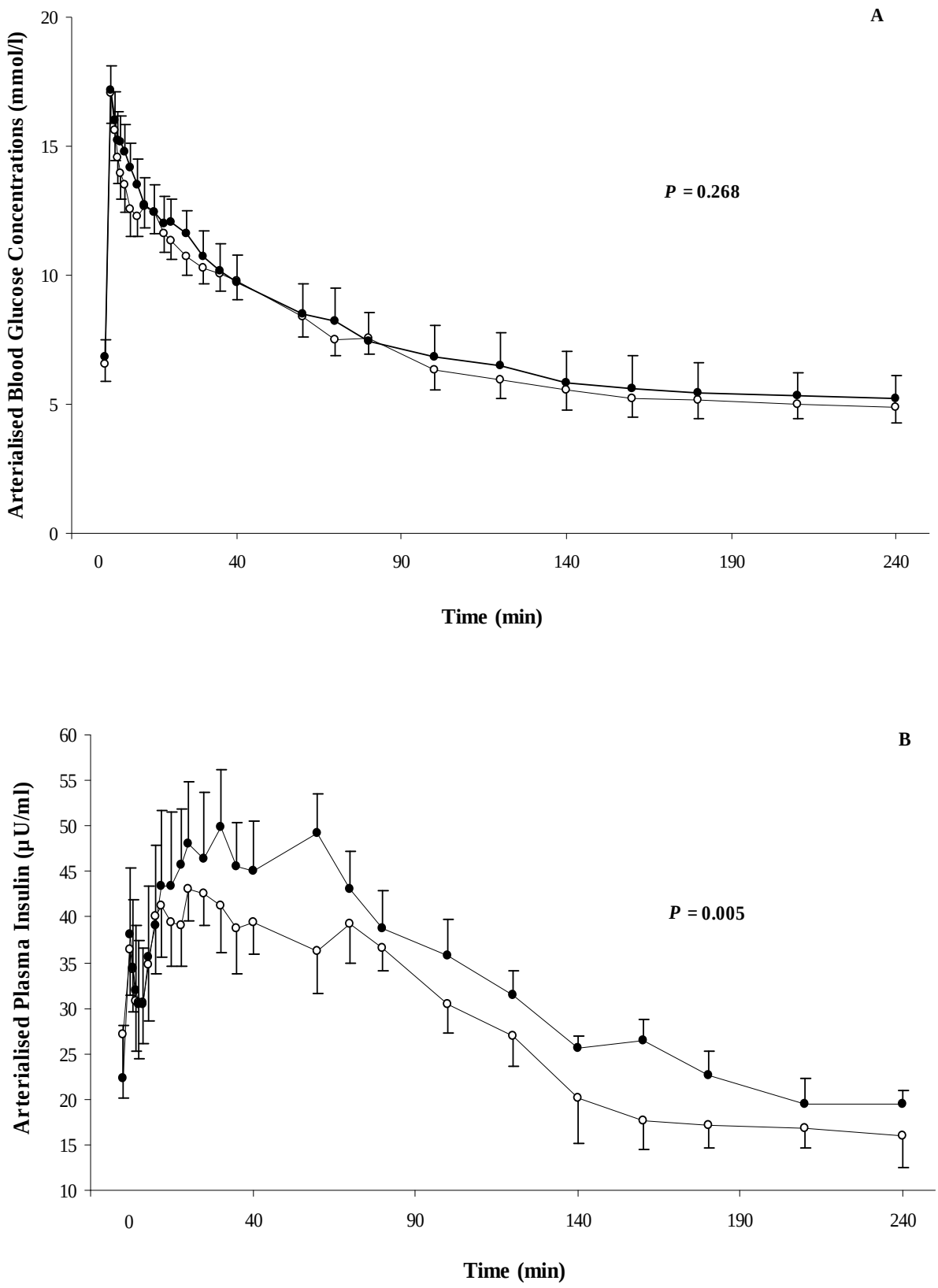

Figure 5.4 Glucose (A) and insulin (B) response during a 4 hr 'hot' IVGTT. Hy Ex (open symbols) and Nor Ex (closed symbols). No difference was found between trials for glucose concentrations. (P = 0.268). Plasma insulin values were lower following the intravenous glucose load (P = 0.005). Values are means (SEM).

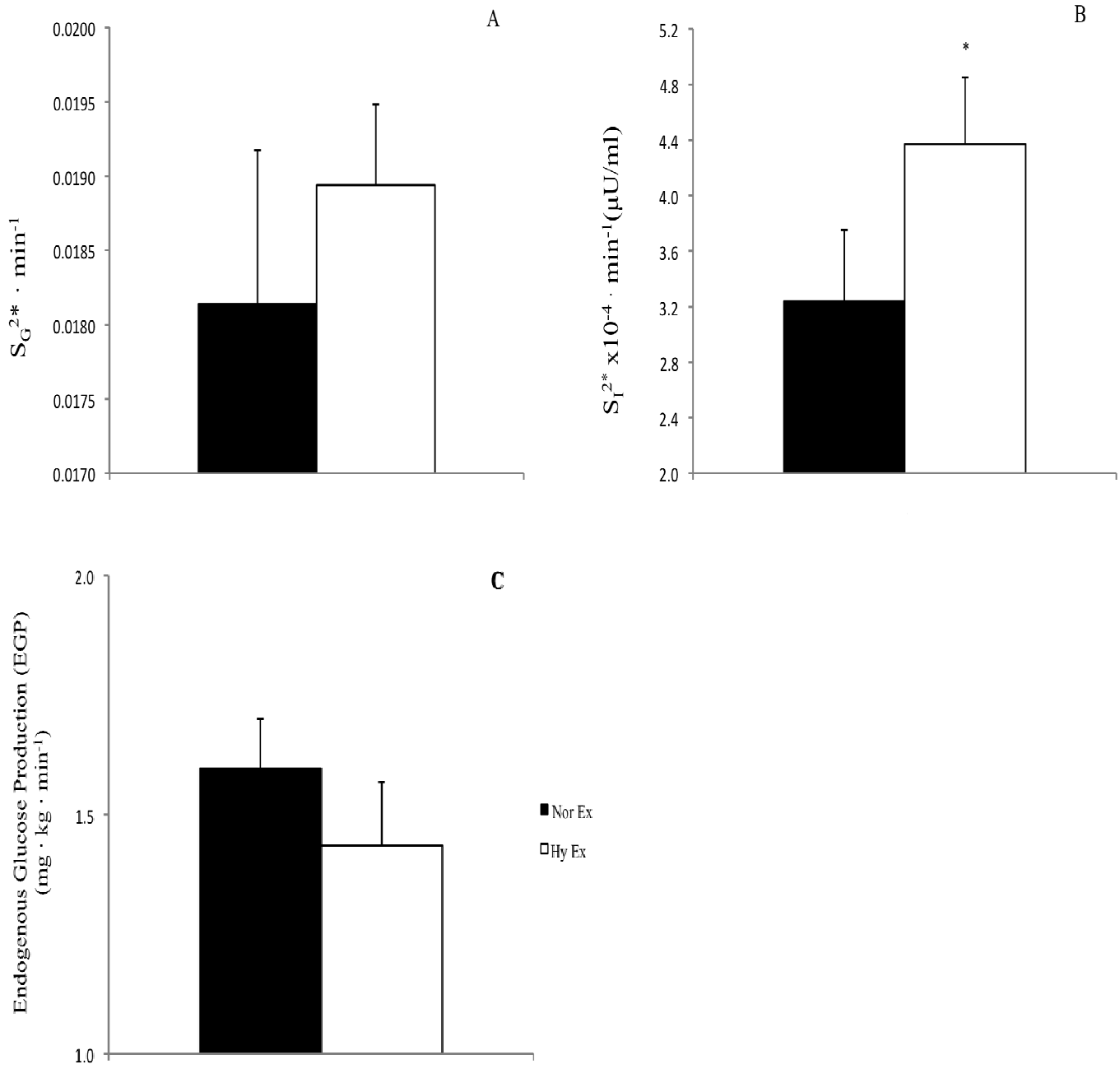

Figure 5.5 S_G^{2*} (A), S_I^{2*} (B) and EPG (C) following Nor Ex and Hy Ex estimated using the IVGTT. S_I^{2*} showed a significant difference between Hy Ex and Nor Ex (P = 0.049) with no statistical difference for S_G^{2*} (P = 0.123) and EGP (P = 0.099).

Figure 5.6 shows IL-6 response during 60 min of moderate intensity exercise. Both Nor Ex and Hy Ex demonstrated changes over time with Nor Ex showing a 58.7% (P = 0.001) and Hy Ex a 68.7% (P = 0.000) increase from baseline values for plasma IL-6 concentrations. Hypoxia and exercise caused circulating IL-6 concentrations to increase to a greater extent than exercise in normoxia and was statistically higher between min 50 to 60 (P = 0.022).

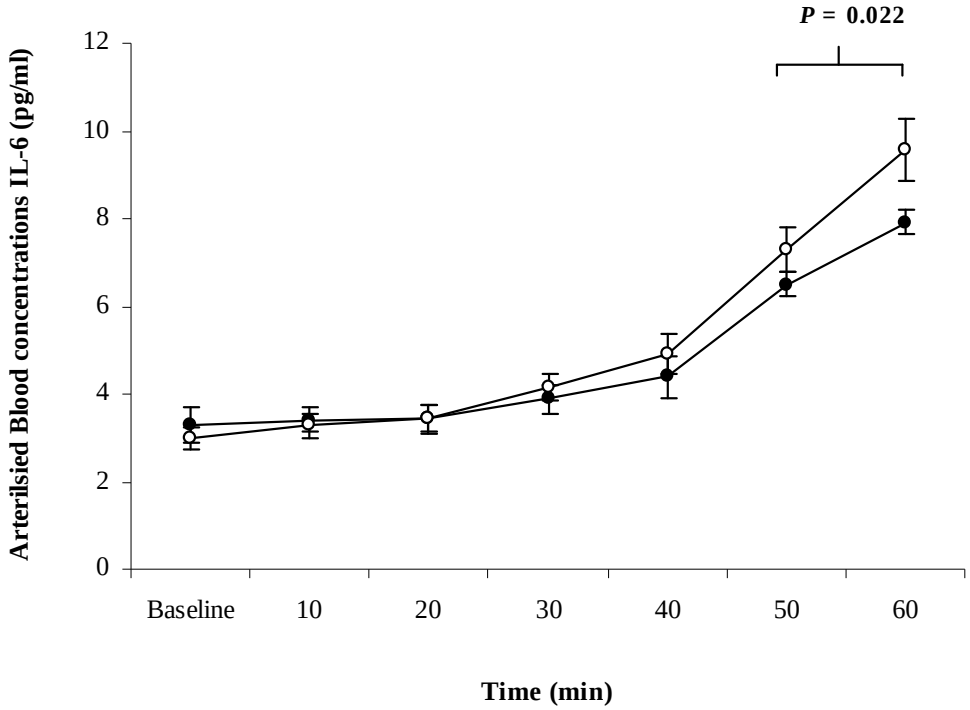

Figure 5.6 Interleukin-6 (IL-6) concentrations during Hy Ex (open symbols) and Nor Ex (closed symbols). A significant difference was noted between conditions (P = 0.022). Values are means (SEM).

5.4 Discussion

The previous chapter (Chapter 4) suggested a positive effect of moderate hypoxia on whole body glucose tolerance in type 2 diabetics. It was suggested that the contraction stimulated pathway may have been responsible for the acute improvements in glycaemic control (Cartee *et al.*, 1991; Winder & Hardie, 1999; Holloszy, 2003; Wright *et al.*, 2005) while hypoxic induced glycogen depletion may have up-regulated insulin simulated glucose transport (Cartee *et al.*, 1991; Wadley *et al.*, 2006) in the 4 hr following exposure as shown by an increase in S_I^{2*}. The aim of the current study was to ascertain if the glucose lowering effect of exercise, which has been clearly shown in the literature (for reviews see; Henriksen, 2002; Wright *et al.*, 2005), could be further stimulated by acute hypoxia.

5.4.0 Acute Response to Exercise & Hypoxia

The data demonstrates that exercise and hypoxia had an acute additive effect on glucose disposal. Circulating insulin concentrations are known to decrease during exercise (Wojtaszewski *et al.*, 2002). Arterialised insulin concentrations were 53.8% lower following Hy Ex when compared to Nor Ex in the current study. This finding supports the notion that the contraction stimulated pathway may be responsible for the decrease in blood glucose concentrations during hypoxic exercise through a greater effect of both stimulus on contraction stimulated glucose disposal (Fisher *et al.*, 2002; Chiu *et al.*, 2004; Holloszy, 2005), or that exercise and hypoxia acted upon separate distinct pathways to encourage glucose transport (Wojtaszewski *et al.*, 1998; Henrikson *et al.*, 2002). This is hard to interpret given the limitations of the current study (i.e. signalling proteins were not measured). Indeed, conclusions drawn from the literature are unclear with most suggesting that both muscular contraction and hypoxia activate the same signalling pathway (Cartee *et al.*, 1991; Reynolds

et al., 1998; Chiu *et al.,* 2005), with more recent work indicating that at some point exercise, hypoxia and insulin use similar signalling proteins (Mu *et al.,* 2001; Sakamoto & Goodyear, 2002). Interpretation of the literature would suggest that AMPK (Fisher *et al.,* 2002; Perrini *et al.,* 2004), ∝PKC (Jessen & Goodyear, 2005), AS160 (Howlett *et al.,* 2007) and presumably uncharacterised proteins (Mu *et al.,* 2001) can be recruited by more than one stimulus (Rose & Richter, 2005) and that exercise and hypoxia can have an additive effect on glucose disposal (Chiu *et al.,* 2004).

The additive effect of hypoxia and exercise on glucose disposal in type 2 diabetics would seem to occur without an increase in cardiovascular (heart rate) or metabolic ([La]) strain. In contrast, RPE values were found to be significantly higher during Hy Ex (P = 0.00), which could be attributed to an acute ventilatory response to hypoxia (hypoxic ventilatory response; HVR) rather than an increased physiological stress *per se*. Romer *et al.* (2002) have linked reduced ventilation rates with a downregulation in afferent messaging to the central nervous system (CNS) and an associated decreased in perception of effort. The data of Romer *et al.* (2002) may provide indirect evidence that the higher RPE's noted during Hy Ex may be due to an increase in ventilation, induced by hypoxia.

5.4.1 Post Exercise / Hypoxic Glucose Tolerance

Area under the curve for insulin (AUC_{Ins}) was found to be significantly lower in the 4 hr following Hy Ex when compared to the exercise only trial (Nor Ex). It is not clear from this data whether this is due to an increase in insulin stimulated post-receptor activity (i.e. PI-3kinase) at the site of peripheral tissue or a down regulation in β-cell (pancreas) insulin release. Although the significant increase in insulin sensitivity (S_I^{2*}) following Hy Ex suggest that insulin signalling, and so insulin-dependent glucose transport, may have been further up-regulated due to hypoxic exercise. This conclusion can be drawn given the short effects that both exercise and hypoxia have on glucose transport via the contraction (hypoxic) stimulated pathway (Holloszy, 2005) and that S_I^{2*}, derived from the use of labelled glucose, describes the effects of insulin on whole body (hepatic and peripheral) glucose disappearance (Cobelli *et al.*, 1986). However, the current work would benefit from the analysis of C-peptide (a peptide which forms when pro-insulin breaks down to produce insulin) to determine if hypoxia inhibits insulin release or encourages peripheral insulin action. During exercise there is known to be an up-regulation in the contraction-stimulated pathway, which is replaced (following exercise) by an up-regulation in the insulin-signalling pathway (Holloszy, 2005; Bordenave *et al.,* 2008). As glucose concentrations were not different and that AUC_{Ins} and S_I^{2*} were significantly lower and improved, respectively following Hy Ex, it would seem reasonable to suggest that the insulin signalling pathway may have been further up-regulated following hypoxic exercise compared to the exercise only trial (Holloszy, 2005).

The decrease in plasma insulin levels during and following hypoxic exercise are in disagreement with previous work (Braun *et al.*, 2001; Polotsky *et al.*, 2003). Braun *et al.* (2001) concluded that hypoxia (equivalent to ~4,300 m) actually increased insulin values and decrease insulin sensitivity. However, these values were obtained during hypoxic exposure in a rested state using the oral glucose tolerance test (OGTT) as a means of assessment. Although, the OGTT is a useful technique, particularly in clinical setting, the two compartment minimal model using labelled [6,6$^2H_2$]glucose during a 4 hr IVGTT remains a more precise method (Vicini *et al.*, 1997). Furthermore, the study of Braun *et al.* (2001) in non-diabetic humans must be read with some degree of caution as OGTT are known to stimulate incretin release (due to glucose ingestion), leading to elevated insulin secretion, when compared to the IVGTT (Muscelli *et al.*, 2006). This incretin effect occurs despite similar plasma glucose concentrations (Muscelli *et al.*, 2006). The comparison between moderate (Braun *et al.*, 2001) and acute exposure to hypoxia (current study) are hard to make, as the physiological and metabolic responses to acute hypoxia are somewhat different to altitude acclimatisation (Lopez-Barneo *et al.*, 2004).

5.4.2 Interleukin -6 (IL-6) Response to Acute Exercise & Hypoxia

IL-6 is an inflammatory-controlling cytokine which has been described as being both a pro- (Senn *et al.*, 2002) and an anti-inflammatory cytokine (Starkie *et al.*, 2003; Petersen & Petersen, 2005). IL-6 is consistently found to be higher in individuals with type 2 diabetes (Febbraio *et al.*, 2003), signifying a possible link between IL-6 and the pathophysiology of insulin resistance (Hamid *et al.*, 2004). The subjects recruited in the present study also showed elevated IL-6 concentrations [3.3 (0.41) pg/ml] when compared to healthy control data from previously published research (~1.5 pg/ml) (Febbraio *et al.*, 2004).

Petersen and colleagues have shown that plasma IL-6 levels increase following muscular contractions (Petersen & Pedersen, 2005). Additional work from the same group of researchers shows a clear link between low muscle glycogen and high IL-6 levels (Steensberg *et al.,* 2001). It has been shown that insulin-stimulated glucose uptake is impaired in type 2 diabetics, perhaps contributing to lower muscle glycogen concentrations (Hayashi *et al.,* 1997; Wojtaszewski *et al.,* 2002). Furthermore, Reynolds *et al.* (1998) suggests that a strong inverse relationship exists between hypoxic induced glucose disposal and muscle glycogen content. In metabolic terms, high levels of glycogen inhibit glucose transport activity during hypoxic exposure (Reynolds *et al.,* 1998). There is an opposing notion that IL-6 is a pro inflammatory cytokine, which causes insulin resistance (John *et al.*, 2000) and therefore, reduces glycogen synthesis. Circulating IL-6 concentrations, in the current study, increased during exercise and were further increased during exercise in hypoxia, when arterialised blood glucose underwent their greatest reduction. The same trial (Hy Ex) also demonstrated significant improvements in S_I^{2*}. These findings (increased IL-6 and improved S_I^{2*}) suggest that IL-6 may not be directly involved in insulin resistance, and therefore, may be acquired rather than a cause in type 2 diabetes. Therefore, high plasma IL-6 levels in type 2 diabetic patients may be related to low muscle glycogen content, known to be a metabolic characteristic of the same population (Reynolds *et al.,* 1998). Although the data presented in the current study is at best descriptive.

5.5 Conclusion

In conclusion, the present investigation has shown that a single bout of moderate intensity exercise enhances glucose disposal and that this effect is exaggerated when exercise is combined with hypoxic exposure in individuals with type 2 diabetes. As insulin values were reduced to a greater extent during hypoxic exercise, it would seem reasonable to suggest that the decrease in circulating glucose may be attributed to an up-regulation in the contraction stimulated pathway (Cartee *et al.*, 1991; Reynolds *et al.*, 1998; Holloszy, 2003). Furthermore, it would seem that the well documented post exercise-induced improvements in insulin sensitivity (Minuk *et al.*, 1981; Devlin *et al.*, 1987; Holloszy, 2005) are further improved by hypoxia as shown by a significant increase in S_I^{2*}. These results suggest that acute hypoxia alters insulin-dependent glucose transport or reduces insulin resistance in type 2 diabetics.

CHAPTER 6

The Effect of Work Intensity and Hypoxia on Glucose Kinetics and Glycaemic Control in Type 2 Diabetes

6.0 Introduction

The current recommendations to promote general health and well being for individuals with type 2 diabetes are equivalent to 30 minutes of moderate physical activity per day (Hu *et al.*, 2001; Larkin, 2001; Tuomilehto *et al.*, 2001). Although short bouts of medium intensity exercise have been shown to be ineffective in improving glucose control. Baynard *et al.* (2005) suggests that a single bout of moderate intensity exercise (30 min at 60% $\dot{V}O_{2peak}$) has no impact on glucose tolerance or insulin sensitivity the day after exercise. Earlier work by a group of authors including Holloszy (Rogers *et al.*, 1988) support Baynard *et al.* (2005) findings. High-intensity short duration exercise may provide a clinical alternative to low-moderate intensity exercise in providing improvements in glycaemic control. Sriwijitkamol and colleagues (2007) have suggested that individuals with type 2 diabetes may need to exercise at a higher relative intensity to stimulate AMPK and AS160 activity, with an assumed increase in glucose transport rates, to a similar level as healthy lean controls.

The previous chapter has shown that moderate intensity continuous exercise (60 min at 90% LT) in hypoxia acutely increases blood glucose removal and insulin sensitivity (S_I^{2*}) in type 2 diabetics when measured with a labelled IVGTT. This improvement was found to be 26% greater than exercise in normoxia. Short-term exercise interventions may offer the potential for close supervision and short-term success in improving glucose control and disease risk factors, with the likelihood of ongoing success in lifestyle changes (Boule *et al.*, 2003). Further, the combination of exercise and hypoxia may present a more palatable approach to exercise, maximising potential benefits while enhancing motivation and compliance (Hordern *et al.*, 2008).

A number of studies have investigated post-exercise glucose uptake immediately following continuous endurance exercise (Young *et al.*, 1989; Braun *et al.*, 1995; Houmard *et al.*, 1995; Bonen, 1998; Hildebrandt *et al.*, 2003). Young *et al.* (1989), Braun *et al*. (1995) and Bonen *et al.* (1998) all found no difference in glucose and insulin responses between high and low intensity exercise, although this has been contradicted elsewhere (Wahren *et al.*, 1978; Romijn *et al.*, 1993). In a study using non diabetic controls, Brambrink *et al.* (1997) demonstrated that the amount of muscle tissue recruited during exercise produced significantly lower values for AUC_{Ins}, without any change in C-peptide levels, 18 hr following exercise compared to a one legged bout of exercise that completed the same amount of work. They concluded that quantity of muscle mass recruited, rather than total work, was a more important factor in improving post exercise glucose tolerance via an insulin-dependent mechanism (Brambrink *et al.*, 1997). Hayashi *et al.* (2005) demonstrated that greater exercise intensities stimulate a greater degree of glucose transport in sedentary men. Although the work of Hayashi *et al.* (2005) used identical exercise durations (50% vs. 70% $\dot{V}O_{2max}$ for 20 min). Perhaps suggesting that total work and energy expenditure, and not exercise intensity *per se*, are key determinants in contraction stimulated improvements in glucose control. However, Kraniou *et al.* (2006) demonstrated that total work, and not exercise intensity or duration, was the significant contributing factor in glucose transport activity. Kraniou *et al.* (2006) found that a single bout of exercise at two different intensities and durations (~40% $\dot{V}O_{2max}$ for 60 min vs. ~80% $\dot{V}O_{2max}$ for ~27 min) increased GLUT-4 gene expression in human skeletal muscle to a similar extent [~40% $\dot{V}O_{2max}$; 1.6 (0.3) vs. 3.3 (0.7) arbitrary units (AU) and ~80% $\dot{V}O_{2max}$; 1.8 (0.5) vs. 2.9 (0.5) AU]. Analysis from the same work showed that high intensity exercise significantly lowered muscle glycogen content [pre exercise; 420 (43) vs. post exercise; 169 (35) mmol · kg dry mass] ($P < 0.05$) (Kraniou *et al.*, 2006). This latter finding indicates that high intensity exercise has a greater ability to

reduce muscle glycogen and perhaps to encourage (insulin-dependent) glycogen resynthesis. Although the study of Kraniou *et al.* (2006) was limited to analysis of muscle tissue so it is difficult to interpret the effects that exercise had on glucose disposal and tolerance.

The findings from the first and second chapters, that prior hypoxic exposure acutely encourages glucose disposal in type 2 diabetics is novel. The possibility that the same or even a greater effect (i.e. increased glucose clearance) would occur using shorter exercise durations, but of equal work would have clear clinical benefits. Therefore, the aim of this study is to identify if total work or exercise intensity has a greater influence on glucose metabolism during and following acute exercise in normobaric hypoxia in type 2 diabetic individuals. To accomplish this aim the total work completed (Joules) will be kept constant during three exercise / hypoxic trials with glucose control being measured both during and following (~48 hr) exercise.

Hypothesis – Short duration high intensity exercise will stimulate glucose metabolism to a greater extent than long-duration moderate intensity exercise in individuals with type 2 diabetes.

6.1 Methods

6.1.0 Subjects

Six individuals diagnosed with type 2 diabetes gave their written consent to participate in this study. Three volunteers were treated with diet and exercise. The remaining three subjects were treated with metformin (n = 2; 150 mg 3 x day and n = 1; 150 mg 2 x day). In keeping with this thesis, subjects taking metformin were required to abstain from this medication in the 48 hr prior to and in the 48 hr during each exercise / hypoxic trial.

Table 6.0 Subjects Clinical, Physiological and Metabolic Characteristics

Age (yr)	BMI (kg / m^2)	Waist Circumference (cm)	Body Fat (%)	HbA_{1c} (%)	Fasting Glucose (mmol/l)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}\%}$
57.5 (2.3)	29.2 (2.9)	113.6 (6.7)	37.2 (3.8)	7.3 (0.3)	7.5 (0.5)	5.0 (1.2)	72.5 (13.7)

Values are means (SEM). Body Mass Index (BMI); Glycosylated Haemoglobin (HbA_{1c}), Homeostasis Model Assessment of Insulin Resistance ($HOMA_{IR}$); β-Cell function ($HOMA_{\beta\text{-Cell}}$).

6.1.1 Experimental Design

The study design was based on four visits. The first visit enabled the collection of preliminary data and to obtain individual lactate threshold (LT) values. Clinical and physiological characteristics (Table 6.0) were also obtained during this visit. Procedures detailing lactate threshold determination and subject characteristics can be found in General Methods (3.3.5 and 3.3.2, respectively). Thereafter, subjects returned to the laboratory to complete 3 exercise trials in hypoxia [O_2 ~14.7 (0.2)%]. Each exercise trial was separated by a minimum of seven and a maximum of fourteen days. Exercise intensities were set using the data obtained in the

preliminary visit and were conducted on an electrically-braked Jaeger cycle ergometer (Lode B.V. Medical Technology, Netherlands). After each exercise trial (Day 1) subjects returned to the laboratory 24 hr (Day 2) and 48 hr (Day 3) later, to enable the measurement of glycaemic control over a medium time period. Subjects were required to refrain from exhaustive exercise (bar habitual walking) and maintain similar lifestyles activities throughout the experimental protocol. Nutritional intake (Compeat version 6; Visual Information Systems Ltd, UK) and calorie expenditure were self recorded over the three days of each experimental trial. Calorie (kcal) expenditure was estimated using pedometers (Sports-Tech; Fitness, UK), which has been shown to give reliable estimation of energy expenditure as determined by the simultaneously recorded 24-hr heart rate (Kashiwazaki *et al.,* 1986). Instructions were given to avoid caffeine and alcohol in the 24 hr preceding and in the days during experimental trials.

6.1.2 Determination of Exercise intensity

All three exercise trials were conducted under hypoxic conditions, each lasting a total of 60 minutes. The first trial required subjects to exercise continuously for 60 min at 90% of predetermined LT [Hy Ex^{60} mean (SEM); 48.7 (3.7) Watts]. Total work [Joules = time (sec) x power (Watts)] completed during this visit was then calculated and used to determine the intensity and duration, allowing for an equal amount of work to be completed in the next two exercise trials. Subsequently, subjects completed two further exercise bouts lasting 40 [Hy Ex^{40}; 70.1 (5.8) Watts] and 20 min [Hy Ex^{20}; 140.4 (11.6) Watts] on two separate occasions and in a randomised order.

6.1.3 Experimental Protocol (Day 1)

Subjects reported to the laboratory at ~08:00 having fasted for 12 hr, abstained from caffeine and alcohol for 24 hr, and exhaustive exercise for 48 hr. Each exercise trial (Hy Ex^{60}, Hy Ex^{40} & Hy Ex^{20}) acted as Day 1. On arrival to the laboratory one 18-gauge cannula was positioned into a dorsal hand vein to allow for frequent sampling of arterialised blood, using a thermoregulated hot box (~60ºC) (General Method 3.5.2). A second 18-gauge cannula was placed into a prominent contralateral antecubital vein for steady rate infusion of [6,6$^2H_2$]glucose (98% enrichment; Cambridge Isotope Laboratories Inc., Andover, USA).

6.1.4 Stable Isotope ([6,6$^2H_2$]glucose) Preparation & Infusion

All isotope solutions were prepared on the morning prior to a primed constant infusion under sterile conditions. 1.9g of [6,6$^2H_2$]glucose was dissolved in ~10 ml of standard saline solution (0.9% NaCl; Baxter Healthcare Ltd, Thetford, England) before being injected into a further ~240 ml of identical saline using a 0.22 µm Millipore filter (Bedford, MA). A glucose bolus (40 ml; 304 mg [6,6$^2H_2$]glucose) was then injected over a 45 sec period using a prominent antecubital vein. A 30 min primed constant isotope infusion period immediately followed using syringe pump method (VP 5000; Medical Systems, Arcomedical Infusions Ltd, Essex, UK) at a rate of 40 ml / hr. Therefore, the rate of [6,6$^2H_2$]glucose infusion was 5.1 mg/min (± 3%; Medical Systems, Arcomedical). Arterialised samples (~10 ml) where then drawn every 5 min during 30 min of rest and stored as previously described (General Methods 3.5.3). Basal blood samples (-30 min) were used to determine endogenous isotopic (background) enrichment of glucose (Brooks *et al.*, 1991). Whole blood (~100 µl) taken at each time point was dispensed into microvettes for analysis of blood glucose and lactate (YSI 2300 STAT

Yellow Springs Instruments, Yellow Springs, Ohio, USA) in duplicate. Subjects remained in a supine position throughout the resting infusion period.

6.2 Exercise / Hypoxic Trials

Following resting infusion, subjects were immediately exposed to hypoxia where they performed exercise (Hy Ex^{60}, Hy Ex^{40} & Hy Ex^{20}) on three different occasions. All exercise trials were performed on an electrically-braked Jaeger cycle ergometer (Lode B.V. Medical Technology, Netherlands). Infusion of [6,6$^2H_2$]glucose (described in section 6.1.4) was increased to a rate of 160 ml / hr (20 mg / min) during exercise (Brooks *et al.,* 1991). Subjects remained in this environment [O_2 ~ 14.7 (0.2)%] for a total of 60 min during each trial. Arterialized blood samples (~10 ml) were drawn every 10 min during this period. Heart rate (HR; Polar Electro Oy, Kemple, Finland), oxygen saturation (S_pO_2), indirectly measured using pulse oximeter (Nonin 2500, Minneapolis, USA) and Rating of Perceived Exertion (RPE; Borg, 1982) were recorded at 10 minutes intervals during exercise. Exercise trials were stopped and subjects removed from hypoxic environment if S_pO_2 reached 70%.

6.2.1 24 hr post Exercise / Hypoxic Protocol (Day 2)

Following a second consecutive overnight fast, subjects arrived at the laboratory ~ 24 hr (~ 10:00) following exercise (Day 1). This visit represented Day 2 for trials Hy Ex^{60}, Hy Ex^{40} and Hy Ex^{20}. As detailed earlier (section 6.1.1), subjects were instructed to record their dietary intake and calorific expenditure in the 24 hr between Day 1 and Day 2. Subjects were again asked to abstain from exhaustive exercise during this period. The cannulation (6.1.3)

and infusion (6.1.4) procedures described above were repeated during Day 2. Isotope saline mixtures consisted of 700 mg of [6,6$^2H_2$]glucose and 250 ml of standard saline solution (0.9% NaCl). The sampling hand remained heated (~60ºC) with basal arterialised samples collected 30 min (-30 min) prior to [6,6$^2H_2$]glucose infusion. ~125 ml of [6,6$^2H_2$]glucose / saline solution was administered intravenously as a priming bolus over a ~60 sec period. The remaining solution was infused (125 ml / hr) using a prominent contralateral antecubital vein over 60 minutes, representing a rate of 6 mg / min. Subjects remained rested throughout. Arterialised samples (~10 ml) were drawn at 10 min intervals and immediately analysed in duplicate, as previously described for blood glucose concentrations. The remaining blood samples were dispensed into ethylenediametetraacetic Acid (EDTA; ~5ml) and lithium/heparin (~5ml) collection tubes before being centrifuged (4ºC and 6000 rpm) and the resulting plasma stored at -80ºC for later analysis.

6.2.2 48 hr post Exercise / Hypoxic Protocol (Day 3)

Control procedures set out for Day 2 were repeated during this visit (Day 3). Subjects subsequently reported to the laboratory (~10:00) 48 hr following the hypoxic / exercise trials where a venous blood sample (~10 ml) was drawn under sterile conditions for analysis of whole blood glucose (3.3.3) and plasma insulin. Insulin concentrations were determined in duplicate using a commercially availably enzyme linked immunosorbent assay (ELISA; DRG diagnostics, Boldon, UK).

6.3 Data Analyses

6.3.0 Derived Indices of Insulin Sensitivity, Insulin Resistance & β-Cell Function

Fasting blood glucose and plasma insulin concentrations were used to estimate homeostasis model of insulin resistance [$HOMA_{IR}$; fasting insulin (μU/ml) x fasting glucose (mmol/l) / 22.5], HOMA of β-cell function [$HOMA_{\beta\text{-Cell}}$; 20 x fasting insulin (μU/ml) / fasting glucose – 3.5 (mmol/l)], Quantitative Insulin Sensitivity Check Index [QUICKI; 1/(log fasting insulin (μU/ml) + log glucose (mg/dl)] (Uwaifo *et al.,* 2002) and Fasting Insulin Resistance Index [FIRI; fasting glucose (mmol/L) x fasting insulin (mU/L)/25] (Duncan *et al.,* 1995; Bastard *et al.,* 2003). Fernandez-Real *et al.* (2001) have demonstrated a significant correlation between FIRI and insulin sensitivity determined using the one-compartment model (IVGTT; r = 0.79; $P < 0.0001$). $HOMA_{IR}$ (Katsuki *et al.,* 2001), $HOMA_{\beta\text{-Cell}}$ and QUICKI (Uwaifo *et al.,* 2002) have all been validated against the euglycaemic-hyperinsulinemic clamp technique, considered the gold standard assessment of insulin sensitivity and secretion (DeFronzo *et al.,* 1979; Uwaifo *et al.,* 2002).

6.3.1 Rate of Glucose Appearance (R_a), Disappearance (R_d) and Metabolic Clearance Rates (MCR)

Enrichment of [6,6$^2H_2$]glucose was determined using gas chromatography mass spectrometer (GCMS; Hewlett Packard) (Section 3.7). Radioisotope tracers are readily distinguishable from the nonradioactive background. This is not the case with stable isotopes. For this reason all isotopic enrichment values were corrected by subtracting the background enrichments as determined from pre infusion blood samples (Rosenblatt *et al.,* 1992). The resulting values were used to calculate rates of blood glucose appearance (R_a), disappearance (R_d) and metabolic clearance rates (MCR), using a non-steady-state one-compartment model adapted

from the work of Steele (1959). This was later modified for use with stable isotopes (Gastaldelli *et al.,* 1993). To compensate for a known inadequacy of the one-compartment model the current investigation increased the tracer infusate content of [6,6$^{2}H_{2}$]glucose above that previously used by Brooks *et al.* (1991) altitude study. Finegood *et al.* (1992) have shown that increasing the tracer amount in the exogenous glucose infusate minimises the fluctuations in enriched (APE) plasma samples.

$$\mathbf{C = Cm / (1 + IE)} \tag{1}$$

$$\mathbf{R_a\ (\mathit{t})} = \frac{\mathbf{\mathit{f}\ (\mathit{t}) - V \cdot C(\mathit{t}) \cdot \dfrac{dIE\ (\mathit{t})}{d\mathit{t}}}}{\mathbf{IE\ (\mathit{t})}} \tag{2}$$

$$\mathbf{R_d = R_a - V\ [(C_2 - C_1)\ /\ (t_2 - t_1)]\ (body\ wt^{-1})} \tag{3}$$

$$\mathbf{MCR = R_d\ /\ [(C_1 + C_2) / 2]} \tag{4}$$

where *f* is isotope infusion rate. V is the volume of distribution assumed to be equal and constant (145 ml / kg for glucose) **(**Gastaldelli *et al.,* 1993). The concentration (C) of the tracee at time (*t*) can be calculated from the endogenous measured concentration (Cm) of glucose and the enrichment at that time. C_1 and C_2; glucose concentrations and time points *1* (t_1) and *2* (t_2) (Brooks *et al.,* 1991). IE tracer enrichment expressed as atoms percent excess

(APE) at sampling times minus the background enrichment value determined from basal plasma samples (i.e. pre bolus and infusion) (Rosenblatt *et al.,* 1992).

6.4 Statistical Analyses

Statistical significance was set at the level $P < 0.05$. Differences over time were evaluated by two way repeated measures ANOVA. Tukey's post-hoc tests were used to identify exact differences in the event of statistical significance. All data are expressed as mean and standard error of the mean (SEM). Statistical tests were performed using the statistical software package SPSS (version 15).

6.5 Results

6.5.0 Nutritional Analysis & Calorie Intake

Subjects were asked to self record dietary intake in the 24 hr prior to and in the 48 hr post each hypoxic / exercise trial. No difference was found over the 3 days for Hy Ex^{60}, Hy Ex^{40} and Hy Ex^{20} in total calories (P = 0.182) and total carbohydrates consumed (P = 0.384). Total energy expenditure, as estimated by pedometer showed no difference over the 3 days for any of the experimental trials (P = 0.163) (Figure 6.0). Further analysis showed no difference between total energy intake and energy expenditure within and between trials (P = 0.098).

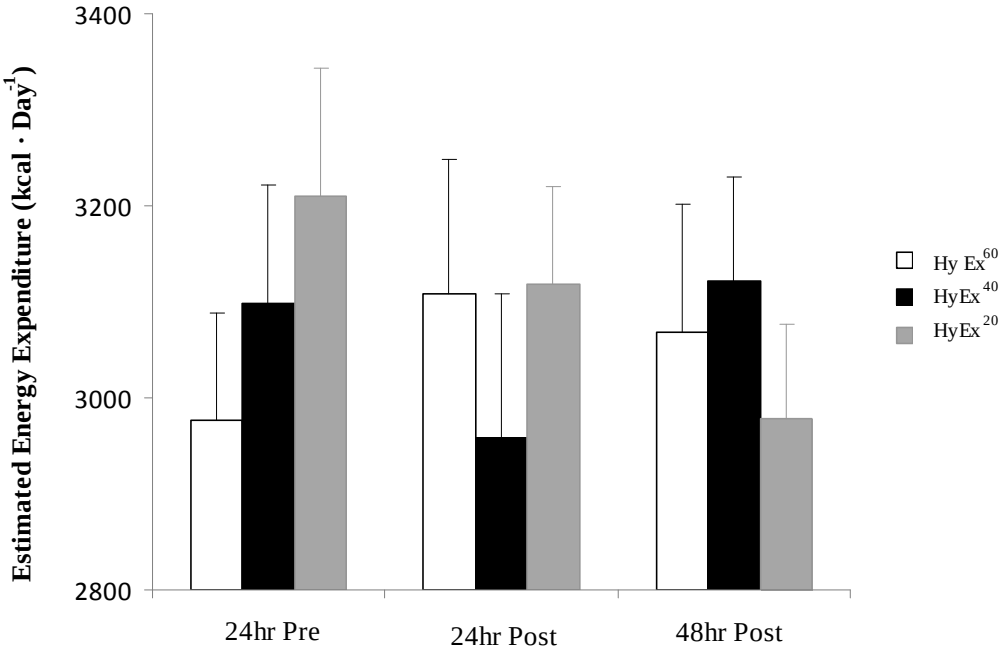

Figure 6.0 Estimated energy expenditure for Hy Ex^{60}, Hy Ex^{40} and Hy Ex^{20}. Values are means (SEM).

6.5.1 Oxygen Saturation (S_pO_2), Heart rate (HR) & Rating of Perceived Exertion (RPE)

S_pO_2 showed no difference between condition [Hy Ex^{60}; 89 (2)%, Hy Ex^{40}; 90 (2) Hy Ex^{20}; 89 (1)%]. Heart rate values increased with exercise from baseline and peaked at 40 mins for Hy Ex^{60} [113 (6) beats · min^{-1}] and Hy Ex^{40} [134 (7) beats · min^{-1}] (Figure 6.1). Heart rate peaked earlier during Hy Ex^{20} at 20 min [159 (6) beats · min^{-1}]. Heart rate was found to be significantly higher in Hy Ex^{40} (P = 0.049) and Hy Ex^{20} (P = 0.012) when comparisons were made with Hy Ex^{60} during the exercising part of each trial. Heart rate was also significantly higher from 0-20 min in Hy Ex^{20} compared to Hy Ex^{40} (P = 0.004). Rating of perceived exertion (RPE) showed a similar pattern to HR and was found to be highest in Hy Ex^{20} at 18 (1) units and was significantly different from both Hy Ex^{60} and Hy Ex^{40} (P = 0.001). RPE values in Hy Ex^{60} were not found to be different from Hy Ex^{40} (P = 0.345). Rating of perceived exertion values peaked at 60 min during Hy Ex^{60} 15 (1), 40 min for Hy Ex^{40} 15 (1) and 20 min for Hy Ex^{20} 18 (1) units.

6.5.2 Blood Lactate Response to Exercise

Comparisons between trials showed lactate to be significantly higher during Hy Ex^{20}, peaking at 8.50 (0.87) mmol/l. Arterialised [La] peaked at 2.49 (0.19) and 4.69 (0.60) mmol/l for Hy Ex^{60} and Hy Ex^{40}, respectively. Main effect difference for arterialised [La] was noted between trials (Hy Ex^{60} vs. Hy Ex^{40}; P = 0.019, Hy Ex^{60} vs. Hy Ex^{20}; P = 0.002 and Hy Ex^{40} vs. Hy Ex^{20}; P = 0.046) (Figure 6.2).

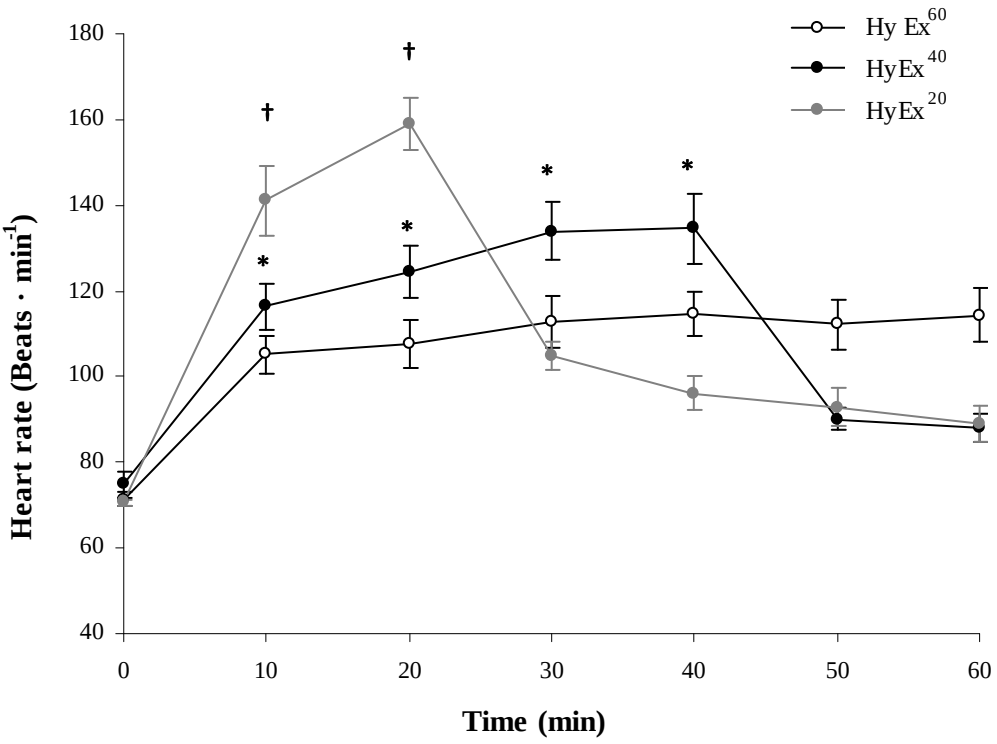

Figure 6.1 Heart rate response during exercise. * denotes a significant difference from Hy Ex^{60} ($P < 0.05$). † denotes significant difference from Hy Ex^{40} ($P < 0.05$). Values are means (SEM).

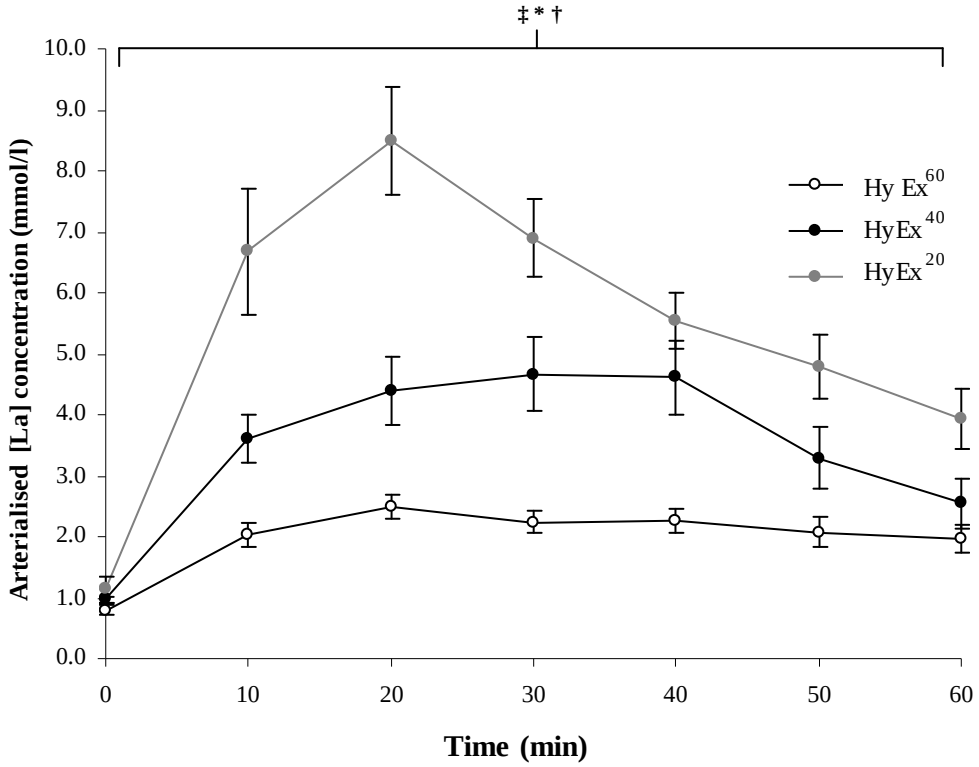

Figure 6.2 Arterialised blood [La] during exercise. Main effects differences between ‡ Hy Ex^{60} vs. Hy Ex^{40}; $P = 0.019$. * Hy Ex^{60} vs. Hy Ex^{20}; $P = 0.002$. † Hy Ex^{40} vs. Hy Ex^{20}; $P = 0.046$. Values are means (SEM).

6.5.3 Acute Blood Glucose Concentrations & Glucose Kinetics During Hypoxic Exercise

Figure 6.3 shows arterialised blood glucose concentration during exercise in hypoxia. Hy Ex^{20} demonstrated no difference in blood glucose concentration (-0.39 mmol/l; $P = 0.118$) over the 60 min exposure. Both Hy Ex^{60} and Hy Ex^{40} caused reductions in blood glucose at -1.60 mmol/l ($P = 0.001$) and -0.84 mmol/l ($P = 0.005$), respectively.

Rate of glucose appearance (R_a) was found to be greater in Hy Ex^{20} than Hy Ex^{60} during exercise at 10, 20 and 30 min ($P = 0.037$). R_a peaked at 8.89 (0.56) mg · kg · min^{-1} within the first 10 min of exercise in Hy Ex^{20}. Peak R_a values for Hy Ex^{60} [6.26 (0.30) mg · kg · min^{-1}] and Hy Ex^{40} [6.66 (0.84) mg · kg · min^{-1}] occurred at the 20 min point during exercise and were found to be 29.6% ($P = 0.020$) and 25.1% ($P = 0.041$) lower than Hy Ex^{20}, respectively. Glucose rate of appearance was significantly higher at the beginning of exercise in Hy Ex^{20} (10 min; $P = 0.001$) when comparisons were made with Hy Ex^{40}. This was reversed towards the end of exercise as R_a in Hy Ex^{20} fell below that recorded during Hy Ex^{40} ($P = 0.02$) (Figure 6.4 A). R_a was also higher at the end of exercise for Hy Ex^{40} as compared to Hy Ex^{60} ($P = 0.013$).

Rate of glucose disappearance (R_d) during hypoxic exercise was similar to that of R_a. The highest glucose R_d was noted in Hy Ex^{20} during the first 10 min of exercise [8.35 (0.60) mg · kg · min^{-1}] and remained elevated above both Hy Ex^{60} ($P = 0.001$) and Hy Ex^{40} ($P = 0.040$) until 40 min of exercise / hypoxia (Figure 6.4 B). Rate of glucose disappearance peaked

within 20 min for both Hy Ex^{60} [6.44 (0.32) ml · kg · min^{-1}] and Hy Ex^{40} [6.70 (0.79) ml · kg · min^{-1}] with no difference found between conditions (P = 0.123). Metabolic clearance rate (MCR) showed no main effect difference between Hy Ex^{60} [4.89 (0.30) ml · kg · min^{-1}], Hy Ex^{40} [5.00 (0.26) ml · kg · min^{-1}] and Hy Ex^{20} [5.04 (0.70) ml · kg · min^{-1}] (P =0.64).

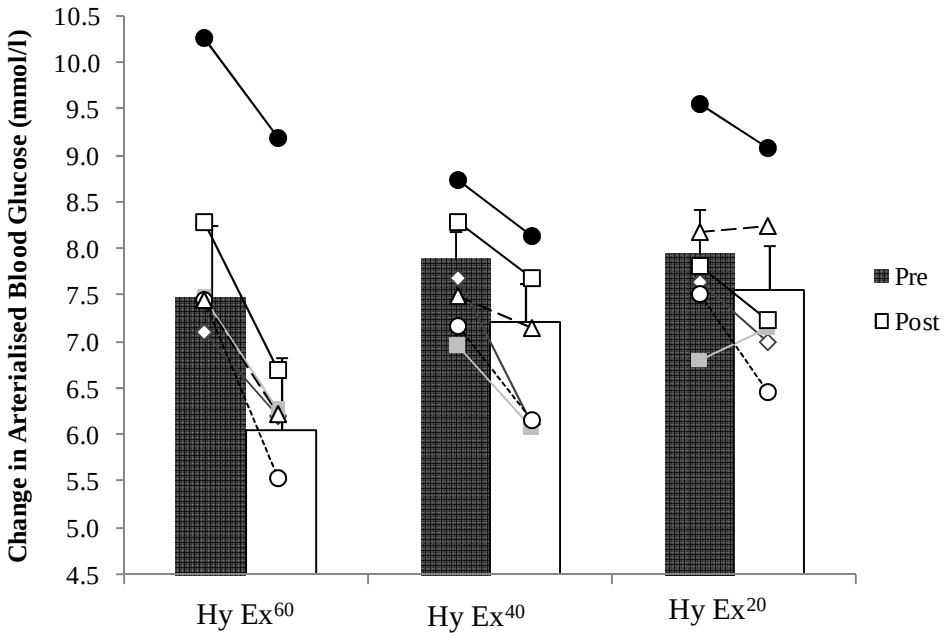

Figure 6.3 Changes in arterialised blood glucose concentrations from baseline to the end of exercise. Significant differences were noted in Hy Ex^{60} (P = 0.001) and Hy Ex^{40} (P = 0.005). Bar plots represent means (SEM) values. Individual changes are also presented.

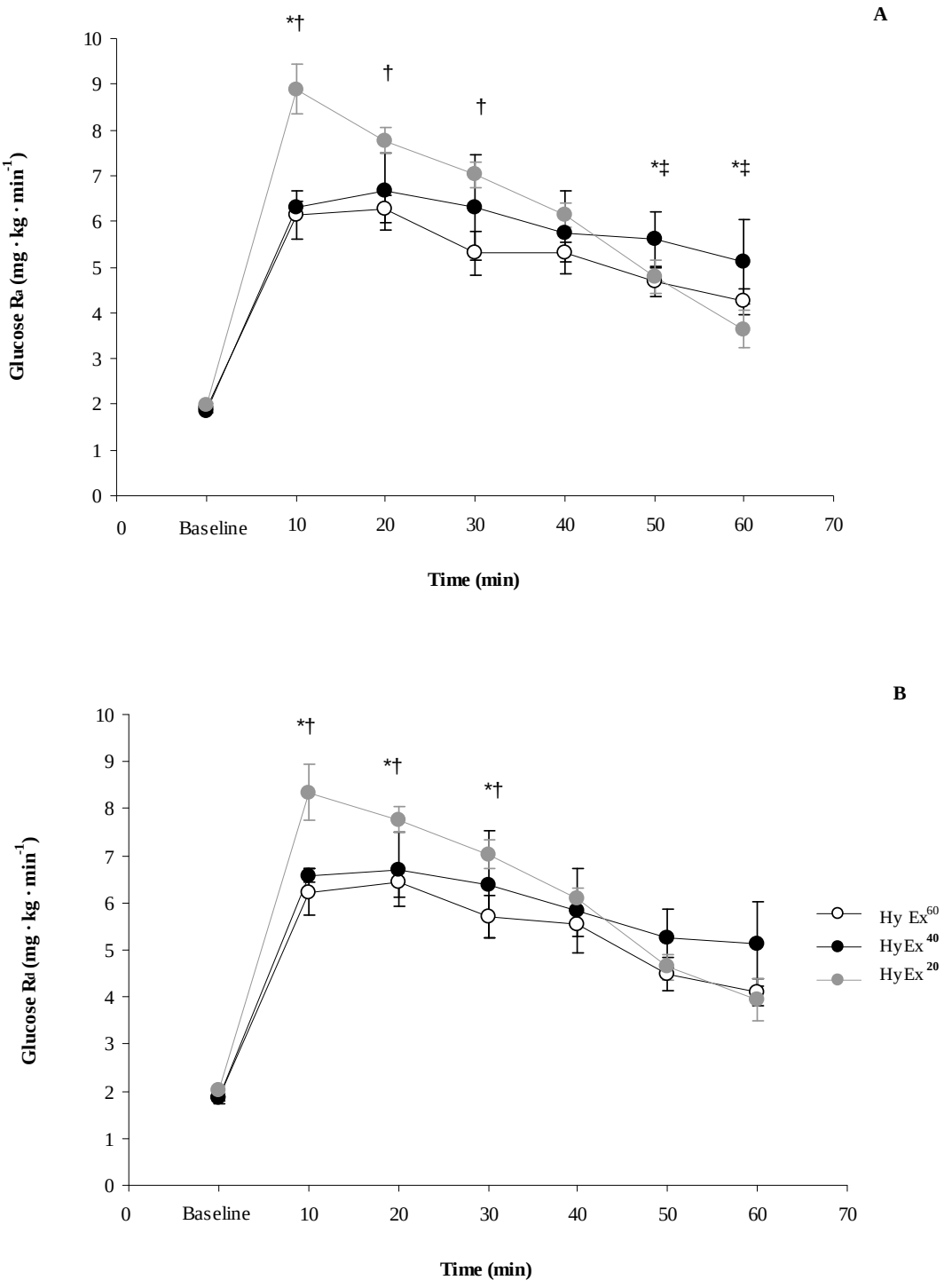

Figure 6.4 Rate of glucose appearance (R_a) (A) and disappearance (R_d) (B) during each hypoxic / exercise trial. ‡denotes significant difference between Hy Ex^{60} vs. Hy Ex^{40} ($P < 0.05$), † Hy Ex^{60} vs. Hy Ex^{20} ($P < 0.05$) and *Hy Ex^{40} vs. Hy Ex^{20} ($P < 0.05$). Values are means (SEM).

6.5.4 Indices of Insulin Sensitivity, Resistance and Glucose R_a, R_d and MCR

Hy Ex^{60}

Fasting blood glucose concentrations were significantly lower in the 24 hr (P = 0.025) and 48 hr (P = 0.048) following 60 min of moderate intensity exercise in hypoxia (Hy Ex^{60}; Table 6.1). Arterialised plasma insulin samples were also reduced 24 hr (P = 0.010) and 48 hr (P = 0.036) post Hy Ex^{60}. Indices of insulin resistance and insulin sensitivity also showed significant improvements in the 48 hr after Hy Ex^{60}. HOMA estimates of β-cell function ($HOMA_{\beta\text{-cell}}$) remained unchanged (Table 6.1).

Glucose rate of appearance (R_a) was not different from baseline values taken on Day 1 when compared to data from Day 2 for Hy Ex^{60} [Day 1; 1.93 (0.11) and Day 2; 1.87 (0.10) mg · kg · min^{-1}] (P = 0.245). R_d was higher in Hy Ex^{60} following exercise, although this was not found to be significant [Day 1; 1.80 (0.11) and Day 2; 1.89 (0.12) mg · kg · min^{-1}] (P = 0.098). Metabolic clearance rates demonstrated a similar pattern [Day 1; 1.49 (0.06) and Day 2; 1.51 (0.11) ml · kg · min^{-1}] (P = 0.101) to that of R_d.

Hy Ex^{40}

Fasting blood glucose concentrations were also lower 24 hr (P = 0.037) and 48 hr (P = 0.02) post Hy Ex^{40} (Table 6.2). $HOMA_{IR}$ (P = 0.05) and Fasting Insulin Resistance Index (FIRI; P = 0.047) were reduced, whereas Quantitative Insulin Sensitivity Check Index (QUICKI) increased (P = 0.037) in the 48 hr following Hy Ex^{40}. Glucose R_a was not altered from baseline values following Hy Ex^{40} [Day 1; 2.08 (0.14) and Day 2; 2.01 (0.13) mg · kg · min^{-1}] (P = 0.168). Although higher, R_d was not significantly different from values recorded on Day

1 within the same trial [Day 1; 1.99 (0.07) and Day 2; 2.10 (0.25) mg · kg · min^{-1}] (*P* = 0.101). Similar to Hy Ex^{60}, MCR remained unchanged in the 24 hr following Hy Ex^{40} [Day 1; 1.52 (0.00) and Day 2; 1.55 (0.04) ml · kg · min^{-1}] (*P* = 0.198).

Hy Ex^{20}

Hy Ex^{20} also showed improvements in fasting glucose, $HOMA_{IR}$ and FIRI in the 24 hr post exercise (Day 2). Unlike both Hy Ex^{60} Hy Ex^{40}, these improvements were not apparent on Day 3 (Table 6.3). Rates of glucose appearance [R_a Day 1; 1.99 (0.06) and Day 2; 1.91 (0.13) mg · kg · min^{-1}] (*P* = 0.125), disappearance [R_d Day 1; 1.93 (0.08) and Day 2; 1.91 (0.09) mg · kg · min^{-1}] (*P* = 0.432) and metabolic clearance rates [MCR Day 1; 1.78 (0.04) and Day 2; 1.75 (0.10) ml · kg · min^{-1}] (*P* = 0.235) were not changed in the 24 hr following Hy Ex^{20}.

Table 6.1 ***Fasting indices of glucose tolerance, insulin secretion, insulin sensitivity and resistance for the 60 min hypoxic exercise trial (Hy Ex^{60})***

Hypoxic Exercise (Hy Ex^{60})

	Glucose (mmol/l)	Insulin (µU/ml)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}}$	QUICKI	FIRI
Day 1	8.39 (0.39)	14.58 (1.1)	4.96 (0.97)	68.7 (6.5)	0.303 (0.005)	4.73 (0.78)
Day 2	7.32 (0.67)†	8.57 (1.3)†	2.48 (0.26)*	49.2 (13.9)	0.334 (0.006)†	2.47 (0.30)†
Day 3	7.38 (0.69)*	11.91 (0.9)*	3.00 (0.39)*	68.6 (14.3)	0.317 (0.005)†	3.48 (0.40)*

Values are mean (SEM). *Significantly difference from Day 1 ($P < 0.05$); † Significantly different from Day 1 ($P < 0.01$)

Table 6.2 ***Fasting indices of glucose tolerance, insulin secretion, insulin sensitivity and resistance for the 40 min hypoxic exercise trial (Hy Ex^{40})***

Hypoxic Exercise (Hy Ex^{40})

	Glucose (mmol/l)	Insulin (µU/ml)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}}$	QUICK	FIRI
Day 1	8.53 (0.43)	14.4 (1.0)	5.51 (0.67)	57.7 (3.1)	0.300 (0.004)	4.96 (0.60)
Day 2	7.37 (0.33)*	10.8 (0.5)*	3.53 (0.18)*	57.6 (6.5)	0.317 (0.002)*	3.17 (0.16)*
Day 3	7.66 (0.23)*	12.0 (0.7)	4.04 (0.28)*	60.3 (5.2)	0.312 (0.003)*	3.63 (0.26)*

Values are mean (SEM). *Significantly difference from Day 1 ($P < 0.05$); † Significantly different from Day 1 ($P < 0.01$)

Table 6.3 ***Fasting indices of glucose tolerance, insulin secretion, insulin sensitivity and resistance for the 20 min hypoxic exercise trial (Hy Ex^{20})***

Hypoxic Exercise (Hy Ex^{20})

	Glucose (mmol/l)	Insulin (µU/ml)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}}$	QUICKI	FIRI
Day 1	8.44 (0.56)	14.1 (1.8)	5.24 (0.66)	60.5 (10.9)	0.302 (0.005)	4.72 (0.59)
Day 2	7.41 (0.55)*	11.6 (1.3)	3.76 (0.35)*	64.4 (11.0)	0.373 (0.010)	3.39 (0.32)*
Day 3	7.98 (0.58)	13.9 (1.7)	5.06 (0.93)	63.7 (7.8)	0.305 (0.015)	4.55 (0.84)

Values are mean (SEM). *Significantly difference from Day 1 ($P < 0.05$); † Significantly different from Day 1 ($P < 0.01$).

6.6 Discussion

The current study was undertaken to identify whether work or exercise intensity had a greater influence on glucose metabolism during and following acute exercise in hypoxia. The main findings from this chapter were that exercise in hypoxia for 60 (Hy Ex^{60}) and 40 min (Hy Ex^{40}) acutely increased glucose disposal. That Hy Ex^{60} decreased circulating blood glucose is consistent with the findings of the previous experimental chapter (study 2). In addition to this, Hy Ex^{60} was shown to improve indices of insulin sensitivity and resistance in the 48 hr following exercise. This study also demonstrated that shorter duration exercise in hypoxia of equal work (Hy Ex^{40}) can improve moderate-term glucose tolerance in type 2 diabetics. Although, acute improvements in glucose control were greater in Hy Ex^{60} when comparisons were made with the other trials.

6.6.0 Acute Blood Glucose Response to Hypoxic Exercise

Exercise increases blood glucose clearance via augmented muscle blood flow, increased muscle capillary and GLUT recruitment, an increase substrate requirement of the active muscle and a post exercise increase in insulin sensitivity (DeFronzo *et al.,* 1981; Dela *et al.,* 1994; Rose & Richer, 2005). Blood glucose removal is also dependent on fibre type recruitment as increased recruitment of type II muscle fibers, with a high glycolytic capacity, during high intensity exercise, creates a greater potential for glucose disposal (Roy *et al.,* 1997). Data analyzed from muscle tissue has shown that exercise affects AMPKα2 activity in a dose-dependent manner (Chen *et al.,* 2003), resulting in a greater potential for glucose transport activity.

The current study showed that moderate intensity exercise [48.7 (3.7) Watts] in hypoxia demonstrated the greatest decrease in blood glucose concentrations. Although R_d was significantly higher during the exercise period of Hy Ex^{20} [143.2 (10.8) watts], this was matched by a similar elevation in endogenous glucose output (R_a). This would suggest that the inability of Hy Ex^{20} to reduce circulating blood glucose can be attributed to a significant elevation in glucose R_a resulting from high intensity exercise (Wadley *et al.,* 2006). Indeed, two subjects demonstrated increases in blood glucose concentrations (figure 6.3) during Hy Ex^{20}. Hy Ex^{40} demonstrated an acute reduction in blood glucose during exercise although this was 47% less than the reduction noted in Hy Ex^{60}. Given that R_d and MCR were similar in Hy Ex^{60} and Hy Ex^{40}, the smaller change noted in the latter trial would seem to be due to a higher glucose R_a towards the end of exercise. This can be supported by the work of Kang *et al.* (1999) which showed higher R_a with similar declines in blood glucose during greater intensity exercise (50% vs. 70% $\dot{V}O_{2max}$) in type 2 diabetics. The results presented within the current study suggest that higher intensity exercise in hypoxia has the capacity to encourage glucose transport. However, glucose concentrations remain unchanged, or decrease moderately during high intensity bouts, which can be attributed to an increase in endogenous glucose production (Wadley *et al.,* 2006).

There are numerous stimulatory and inhibitory mechanisms involved in regulating glucose R_d during exercise. In addition, hypoxia *pre se* is known to influence glucose metabolism independent of exercise (Brooks *et al.,* 1991; Rowell *et al.,* 1984). In terms of glucose transport activity, exercise intensity is known to have a dose-dependent effect on the signalling proteins (AMPKα2) (Sriwijitkamol *et al.,* 2007) involved in GLUT-1 and -4 activation and translocation to the cell membrane (Chen *et al.,* 2003). A greater muscle

glycogen utilisation during intense exercise has been shown to raise glucose-6-phophate (G-6-P) content (Katz and Sahlin, 1989; Parolin *et al.,* 2000) which may provide a reason why blood glucose concentrations were unchanged during higher intensity exercise in hypoxia. It seems plausible that exercise and hypoxia may raise glucose transport until intracellular levels of G-6-P reach a threshold after which glucose disposal achieves a steady state or declines. Katz and Sahlin (1989) have reported that inhibition of hexokinase during hypoxic exercise was greater than during normoxic conditions. Given that Katz and Sahlin (1989) used the same absolute exercise intensity during both Nor and Hy conditions, these findings would suggest that the higher relative intensity of exercise used in the present study may have increased cytosolic G-6-P, resulting in the inhibition of hexokinase (Rose & Ritcher, 2005) levels and prevented a greater decline in blood glucose concentrations during intense exercise. Although the mechanism describe may reflect a contributor factor to the lack of change in blood glucose concentrations, as endogenous glucose production was found to be elevated during Hy Ex^{20}.

Epinephrine infusion during moderate intensity exercise has been shown to downregulate glucose transport activity independent of G-6-P muscle content (Watt & Hargreaves, 2002). Epinephrine concentrations are also known to increase during exercise (Howlett *et al.,* 1999) with a further increase during hypoxic exercise (Roberts *et al.,* 1996). Intense exercise is also known to further stimulate epinephrine release (Howlett *et al.,* 1999; Wadley *et al.,* 2006). Interpretation from the work of Watt & Hargreaves (2002) and Wadley *et al.* (2006) would suggest that the inability of higher intensity exercise in hypoxia (Hy Ex^{20}) to lower blood glucose concentrations may be due to the inhibitory effect of epinephrine on glucose disposal via inhibition of GLUT-4 translocation (Watt & Hargreaves, 2002). Elsewhere, epinephrine has been shown to have no effect on exercise induced glucose transport (Aslesen and Jensen,

1998). Although, epinephrine clearly plays a significant role in glucose availability and utilisation (Howlett *et al.,* 1999).

6.6.1 Moderate-Term Effects of Exercise in Hypoxia

Fasting blood glucose, $HOMA_{IR}$ and FIRI were all improved in the 24 hr following Hy Ex^{20}, suggesting that the combined stress of high-intensity short-duration exercise and hypoxia can increase insulin-mediated glucose transport and insulin sensitivity. Although the current study did not measure muscle glycogen content, it is reasonable to hypothesise that exercise, hypoxia and a ~12 hr fast caused glycogen depletion. Indeed, net glycogen use increases during exercise (Wadley *et al.,* 2006) and hypoxia (Cartee *et al.,* 1991). Furthermore, glycogen synthase activity is increased following 60 min of moderate intensity exercise (90% anaerobic threshold) (Koval *et al.,* 1998). This effect seemed to be reversed within 48 hr of Hy Ex^{20} as variables reflecting glycaemic control returned to near baseline values (Table 6.3). Exercise lasting 40 min in hypoxia also demonstrated significant improvements in $HOMA_{IR}$, QUICKI and FIRI. Although these improvements extended to 48 hr post exercise. The decrease in insulin resistance shown in the Hy Ex^{60} trial was greater when compared to Hy Ex^{40} (Table 6.1 and 6.2). Taken together these finding propose that exercise duration in hypoxia and not total work, provide more obvious improvements in moderate-term glucose control in type 2 diabetics.

The mechanisms responsible for improvements in post exercise glucose tolerance have been discussed previously within this thesis (Literature review 2.7.4), although they are likely to include increased content of plasma membrane GLUT-4s and increased activity of proteins involved in the insulin signalling pathway for glucose transport. By completing the same amount of work in a shorter duration, the exercise intensities within the current experimental chapter progressively increased from Hy Ex^{60} [48.7 (3.7) Watts], Hy Ex^{40} [74.5 (6.4) Watts] and Hy Ex^{20} [143 (10.8) Watts]. Chen *et al.* (2003) reported that AMPK2α activity increases in an intensity dependent manner during exercise. AMPK activity is thought to be tightly regulated by a number of factors including circulating blood glucose (Akerstrom *et al.,* 2006) and insulin (Witters *et al.,* 1992) concentrations, free AMP content (Winder & Hardie, 1999) and muscle glycogen (Wojtaszewski *et al.,* 2003). Given the collective findings above it might be expected that the improvements in post exercise insulin sensitivity would have been greater with the increasing exercise intensity. Although the findings from the current work do not support this notion, particularly as the total amount of work completed between trials was equal.

Kraniou *et al.* (2006) suggested that high- and low-intensity exercise stimulate GLUT-4 activity to a similar level and that low-intensity (longer duration) exercise progressively increases AMPK activity. Kraniuo *et al.* (2006) work may provide a cellular mechanism for the findings of the current investigation in that longer duration exercise may further stimulate AMPK activity and promote a greater potential for glucose transport activity. In a more recent study, Treebak *et al.* (2007) demonstrated that AS160 (distal insulin-signalling protein linked to GLUT4 translocation) (Arias *et al.,* 2007) phosphorylation increased during moderate intensity exercise which was not evident in a shorter-high intensity bout. Suggesting again, that exercise duration is perhaps the key determining factor for stimulating

signalling mechanisms involved in GLUT-4 translocation, with the potential for increased glucose disposal. It is therefore hypothesised that both Hy Ex^{60} and Hy Ex^{40} decrease muscle glycogen and increased post exercise glucose uptake through an increased activity in signalling proteins involved in the insulin-stimulated pathway, resulting greater improvements in acute and moderate-term glycaemic control in type 2 diabetics.

6.7 Conclusion

In summary, the major findings were that moderate-intensity exercise in hypoxia stimulates acute- and moderate-term improvements in insulin sensitivity. It appears that high-intensity exercise of shorter durations and of equal work does not result in a decline in acute blood glucose concentrations which reflects a higher glucose R_a during Hy Ex^{20}. Although Hy Ex^{20} did cause improvements in glucose tolerance post exercise, these improvements were more apparent during Hy Ex^{60} and Hy Ex^{40}, showing that exercise duration and not total work has a greater influence on acute (during exercise) and moderate-term (48 hr post exercise) glucose tolerance in type 2 diabetics. The current data provides support to the findings obtain within Chapter 5, that longer duration exercise in hypoxia (Hy Ex^{60} and Hy Ex^{40}) is a more effective treatment in type 2 diabetes management.

CHAPTER 7

Can Intermittent Exercise with and without Hypoxia Improve Glucose Tolerance in Individuals with Type 2 Diabetes?

7.0 Introduction

Exercise has been proven to improve acute glycaemic control in type 2 diabetics (Devlin *et al.,* 1987; Larsen *et al.,* 1997a; Kang *et al.,* 1999; Kennedy *et al.,* 1999). Much of the literature has focused on continuous aerobic based exercise (Kennedy *et al.,* 1999; Macdonald, 2004; Hayashi *et al.*, 2005; Lee *et al.*, 2005; Zoppini *et al.*, 2006) with more recent work assessing the benefits of resistance type exercise (Rice *et al.,* 1999; Castaneda *et al.*, 2002; Howlett *et al.*, 2007). Comparisons have also been made between continuous, aerobic and resistance exercise (Andrew *et al.*, 2002; Snowling & Hopkins, 2006). Surprisingly, little work is available assessing the effects of acute intermittent exercise on glucose tolerance in type 2 diabetics.

During low intensity aerobic exercise the body relies predominately on lipid oxidation for fuel metabolism (Romijn *et al.,* 1993). As exercise intensity increases, glucose uptake and glycogen depletion increase in a dose dependant manner (Romijn *et al.,* 1993). This suggests that high intensity exercise may provide a greater potential for glucose disposal and post exercise glycogen resynthesis (Kjaer *et al.,* 1990). Intense exercise is also known to increase the rate of glucose appearance from endogenous stores (R_a), possibly causing acute hyperglycaemia in type 2 diabetics (Kjaer *et al.,* 1990). This seems to be reversed within 24 hrs post exercise, as shown by an increase in insulin-stimulated glucose disposal (Kjaer *et al.,* 1990). Although the work described above (Kjaer *et al.,* 1990; Romijn *et al.,* 1993) reflects glucose response to high intensity and not intermittent exercise.

Early work by Essen *et al.* (1977) showed that the contribution of glucose and lipids to energy expenditure was similar in both continuous and intermittent exercise of equal power outputs and oxygen uptake ($\dot{V}O_2$). Christmass *et al.* (1999) later demonstrated that intermittent exercise resulted in a higher rate of carbohydrate oxidation when compared to continuous exercise [264 (5) and 229 (6) μmol · kg · min^{-1}, respectively]. The increase in glucose oxidation and glycogen depletion that accompanies intermittent exercise (Saltin & Essen, 1971; Christmass *et al.,* 1999) may provide a useful intervention in diabetic management. The Surgeon General, Centres for Disease Control and Prevention and the American Heart Association recommend that individuals should exercise at moderate intensities for 30 min per day. The same governing bodies also propose that this exercise can be accumulated throughout the day (Pate *et al.,* 1995). However, little evidence is available to either support or refute the latter recommendation.

A recent study compared the effects of continuous exercise (30 min; 60% $\dot{V}O_{2max}$) with multiple short duration exercise (3 x 10 min; 60% $\dot{V}O_{2max}$) training over a 5 week period in type 2 diabetic patients (Eriksen *et al.,* 2007). These authors demonstrated that both fasting plasma glucose concentrations (P = 0.01) and glucose area under the curve (AUC_{Glu}) (P = 0.04) were lower during an oral glucose challenge post exercise training (Eriksen *et al.,* 2007). These variables remained unchanged in the continuous exercise group (Eriksen *et al.,* 2007), suggesting that intermittent exercise separated with periods of passive recovery may have the ability to stimulate glucose transport to a greater extent than continuous exercise. Measures of insulin sensitivity index (ISIcomposite) and insulin secretion rates (ISR) were unaffected by both training regimes (Eriksen *et al.,* 2007), indicating that glucose control was

possibly improved via an insulin-independent mechanism. These conclusions can not be supported elsewhere. Baynard *et al.* (2005) showed that repeated bouts of exercise (3 x 10 min; 60% $\dot{V}O_{2peak}$) had no effect on AUC_{Glu} [2579 (280) mmol/l · min] in type 2 diabetic patients when comparisons were made with a non exercise control [2828 (177) mmol/l · min]. The authors concluded that the exercise intensity or duration used was not great enough to produce improvements in glycaemic control (Baynard *et al.,* 2005).

TNF-α, a well defined pro inflammatory cytokine, has been implicated in insulin resistance and type 2 diabetes (Dandona *et al.,* 2004) through a direct inhibition of insulin signalling and reduced phosphorylation of IRS-1 and GLUT-4 translocation (Hotamisligil & Spiegelman, 1994). Exercise has been shown to improve insulin sensitivity (Braun *et al.,* 1995) while reducing TNF-α production (Keller *et al.,* 2004). Therefore, the current chapter aimed to assess the effects of intermittent exercise on circulating TNF-α concentrations and correlate potential changes with measures of insulin resistance.

There is a lack of published data assessing the effects of intermittent exercise on glucose control in type 2 diabetic patients. The major findings from the first two chapters of this thesis were that resting hypoxic exposure can significantly stimulate blood glucose removal and that exercise and hypoxia have an additive effect on whole body glucose metabolism. Intermittent exercise, if proven to be effective in lowering blood glucose concentrations, may provide a more palatable alternative to potentially laborious continuous exercise.

Furthermore, if intermittent exercise can encourage blood glucose removal then it would be reasonable to speculate that intermittent exercise in hypoxia may have an additive effect given that the passive recovery phase in hypoxia may further stimulate glucose uptake. The aim of this study was to assess the effectiveness of intermittent exercise with and without hypoxia on acute- and moderate-term glucose control in individuals with type 2 diabetes.

Hypothesis – Intermittent exercise with and without hypoxia will stimulate acute- and moderate-term improvements in glucose control in individuals with type 2 diabetes.

7.1 Methods

7.1.0 Subjects

Seven type 2 diabetic individuals were recruited for this investigation using the same recruitment strategy and exclusion criteria presented within the General Methods (Section 3.2). Subjects requiring diabetic controlling medication (n = 2; metformin) were asked to abstain from this in the 48 hrs prior to and throughout each trial. Subject clinical, physiological and metabolic characteristics are detailed in Table 7.0.

7.1.1 Experimental Design

The study design was centred on 4 four laboratory visits. The first visit allowed for the collection of clinical and physiological data (Table 7.0) before individual lactate threshold (LT; detailed General Methods 3.3.5) values were determined. The remaining visits formed the basis of the experimental design and included; *1)* 60 min of continuous exercise at 90% LT in hypoxia [Hy Ex60; O_2 ~ 14.8 (0.4)%] *2)* intermittent exercise at 120% LT, separated by periods of passive recovery (5:5 min) in hypoxia [Hy 5:5; O_2 ~ 14.8 (0.4)%] and *3)* intermittent exercise (5:5 min) at 120% LT in normoxia (Nor 5:5; O_2 ~ 20.93%). Days on which subjects performed exercise are described as Day 1 and were conducted in a randomised fashion. Subjects returned to the laboratory 24 hrs (Day 2; ~10:00) and 48 hrs (Day 3; ~10:00) post exercise to allow for glycaemic control to be assessed. Subjects were instructed to avoid all forms of exercise (bar habitual walking) and replicate all lifestyle activities throughout each trial (Section 6.1.1).

Table 7.0 Subjects Clinical, Physiological and Metabolic Characteristics

Age (yr)	BMI (kg/m^2)	Body Fat (%)	HbA_{1c} (%)	Fasting Glucose (mmol/l)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}}$	TNF-α (pg / ml)
58.7 (2.2)	28.3 (2.1)	36.0 (8.8)	7.8 (0.4)	8.1 (0.7)	6.0 (0.8)	74.3 (8.2)	60.0 (7.6)

Values are means (SEM). Body Mass Index (BMI); Glycosylated Haemoglobin (HbA_{1c}), Homeostasis Model Assessment of Insulin Resistance ($HOMA_{IR}$); *β*-Cell function ($HOMA_{\beta\text{-Cell}}$); Tumor necrosis factor-α (TNF-α)

7.1.2 Experimental Trials (Day 1)

Subjects arrived at the laboratory at ~08:00 following an overnight fast (~12 hrs). Once in a supine position the sampling hand was placed in a thermoregulated hotbox (~60°C) for localised warming using a method previously described. Subsequently one 18-gauge cannula was placed into a dorsal vein of the heated hand to allow for frequent sampling of arterialised blood. Basal blood samples (-30 min) were then drawn prior to a separate 18-gauge cannula being positioned into a contralateral antecubital vein to allow for isotope infusion.

7.1.3 Resting Stable Isotope Infusion [6,6$^2H_2$]glucose

Solutions containing 7.6 mg / ml of [6,6$^2H_2$]glucose in standard saline were infused at a rate of 5 mg / min at rest. A priming dose of 40 ml was administered intravenously over a ~45 sec period. Isotope infusion immediately followed (40 ml / hr) over a 30 min period. Basal blood samples (-30 min) were drawn prior to infusion and used to determine endogenous isotopic (back ground) enrichment of glucose (Brooks *et al.*, 1991). Arterialised blood samples were collected every 5 min during this 30 min rest period. Whole blood glucose and lactate were determined in duplicate using ~100µl from each sample (YSI 2300 STAT Yellow Springs Instruments, Ohio, USA). Insulin concentrations were measured in plasma (ELISA, General Methods 3.6). Intra- and inter-assay coefficient of variation for insulin were < 3.4%.

7.1.4 Exercise Trials

Immediately following infusion at rest, subjects performed three exercise trials (Hy Ex60, Nor 5:5 and Hy 5:5) separate by a minimum of seven and a maximum of fourteen days. Hy Ex60 required subjects to exercise at 90% LT continuously for 60 min under hypoxic conditions [O_2 ~ 14.8 (0.4)%] and therefore, replicated exercise protocols described in Chapters 4 and 5. The remaining trials required subjects to exercise for 5 min at 120% LT, separated by 5 min of passive recovery (5:5 min) for a total of 60 min in both normoxic (Nor 5:5) and hypoxic [Hy 5:5 O_2 ~ 14.8 (0.4)%] environments. Exercise intensities were set using the data obtained in the preliminary visit and were performed on an electrically-braked Jaeger cycle ergometer (Lode B.V. Medical Technology, Netherlands) consistent with the previous experimental chapters (4 and 5). The infusion rate of [6,6$^2H_2$]glucose increased to 160 ml / hr (20 mg / min) during exercise (Roberts *et al.*, 1996). Arterialized blood samples (~10ml) were drawn every 10 min during exercise. Heart rate (HR; Polar Electro Oy, Kemple, Finland), oxygen saturation (S_pO_2; pulse oximeter, Nonin 2500, Minneapolis, USA) and rating of perceived exertion (RPE; Borg, 1982) were recorded at 10 min intervals.

7.1.5 Profile of Mood States (POMS) Questionnaire

Profile of mood states (POMS) questionnaires have been used previously to assess changes in mood state within exercise based research (Markoff *et al.*, 1982; Cramer *et al.*, 1991). This questionnaire is formed of a 65-item mood adjective checklist in which each adjective is scored from 0 (absent) to 4 (very much). The POMS is designed to assess the following six mood scales: tension-anxiety, depression-dejection, confusion-bewilderment, vigour-activity,

anger-hostility and fatigue-inertia (Daniel *et al.*, 1992). POMS was used to obtain data immediately following exercise, completed on Day 1. Subjects remained in a seated position in the same laboratory environment until the questionnaire had been completed. With the exception of vigour, which reflects a positive mood, the remaining subscores represent a negative mood state. A total mood-disturbance score was also calculated as the sum of the scores; tension, depression, anger, fatigue and confusion minus the score for vigour [UKPDS (37), 1999]. Therefore, greater scores reflect a greater mood disturbance.

7.1.6 Post Exercise measures of Glycaemic Control (Day 2 and 3).

Subjects returned to the laboratory 24 hrs (Day 2) and 48 hrs (Day 3) at ~10:00 post Hy Ex60, Nor 5:5 and Hy 5:5 to allow for assessment of glucose tolerance between baseline (Day 1), Day 2 and Day 3. Day 2 required subjects to undergo a 60 min resting [6,6$^{2}H_{2}$]glucose isotope infusion at a rate of 6 mg / min. Volunteers then returned 48 hrs following exercise to provide a venous blood sample for the estimation of insulin sensitivity (QUICKI), insulin resistance ($HOMA_{IR}$ and FIRI) and β-cell function ($HOMA_{\beta\text{-Cell}}$). The calculations for these indices are detailed in section 6.3. Methodological procedures for Day 2 and Day 3 are identical to those employed in the previous chapter (Sections 6.2.1 and 6.2.2).

7.2 Data Analysis

7.2.0 Determination of Labelled [6,6$^2H_2$]glucose

Plasma samples taken from isotope infusion during rest and exercise (Day 1 and Day 2) were used to determine isotopic enrichment using the methodology described in section 3.7 (GCMS; Hewlett Packard). Rate of glucose appearance (R_a), disappearance (R_d) and metabolic clearance rates (MCR) were estimated from the resulting atom percentage excess values and the measured endogenous glucose concentrations (Section 6.3.1).

7.2.1 Tumour Necrosis Factor-α (TNF-α) ELISA

TNF-α concentrations were determined in fasting blood samples taken on Day 1, 2 and 3 using commercial availably enzyme linked immunoSorbent assay (ELISA) kits (BioSupply Limited, Bradford, UK). Plasma taken from samples were thawed and vortexed before 200 µL was added to pre-coated monoclonal antibody wells specific to TNF-α (B5-H12). TNF-α concentrations were determined in duplicate. Two wells remained blank with the remaining wells being used for known TNF-α concentrations (standards; 31.25 – 2000 pg / ml). Detection of light absorbance followed (450nm; Universal Microplate reader (ELx800, IBL inc., Minneapolis, USA) and the standard curve plotted (31.25 – 2000 pg / ml). The minimum detectable dose of TNF-α for the ELISA described is 4 pg / ml (BioSupply Imited, Bradford, UK). The intra- and intra-assay coefficient of variation for the ELISA were 3.2% and 5.0%, respectively.

7.3 Statistical Analysis

Differences over time during exercise and between days (Day 1, 2 and 3) were analyzed using two way repeated measures ANOVA and Tukey's post-hoc tests when significant differences were detected at $P < 0.05$. Friedman two-way ANOVA's were used to test for differences in non parametric variables, RPE and POMS. All results are expressed as mean and standard error of the mean (SEM). Where appropriate, pearson correlation coefficients were calculated to allow for comparisons between variables. Statistical analysis was performed using the statistical software package SPSS (version 15).

7.4 Results

7.4.0 Nutritional Intake

Subjects were instructed to refrain from physical activity (bar habitual walking) and record and replicate dietary intake through each experimental trial. Adherence to these instructions was deemed to be high with analysis of variance showing no difference within or between each exercise condition for total calories (P = 0.254) nor carbohydrates consumed (P = 0.102). Total energy expenditure, measured using pedometers also showed no significant difference within or between Hy Ex60, Nor 5:5 and Hy 5:5. (P = 0.190). Analysis also showed no significant differences between total energy intake and energy expenditure within and between trials (P = 0.254).

7.4.1 Oxygen Saturation (S_pO_2), Heart Rate (HR) & Rating of Perceived Exertion (RPE)

Heart rate (HR) was not different between trials at 107 (1), 102 (1) and 104 (2) beats · min^{-1} for Hy Ex60, Nor 5:5 and Hy 5:5, respectively (Main effect (P = 0.168). Oxygen Saturation (S_pO_2) was lower during Hy Ex60 when compared to Nor 5:5 [90 (1)% and 98 (1)%, respectively] (P = 0.023). Although lower in Hy 5:5 [93 (1)%] than during Nor 5:5, S_pO_2 was not found to be significantly different (P = 0.612). Again S_pO_2 was not different between Hy Ex60 and Hy 5:5 (P = 0.123). Ratings of perceived exertion (RPE) was at its highest during Hy Ex60 and found to be significantly greater than both Nor 5:5 (P = 0.000) and Hy 5:5 (P = 0.001). Nor 5:5 was also shown to be lower than Hy 5:5 (P = 0.001) (Figure 7.0). As expected, arterialised [La] concentrations increased during exercise and were highest within Hy 5:5. Although no differences were noted between Hy Ex60 [2.09 (0.31) mmol/l], Nor 5:5 [2.16 (0.44) mmol/l] and Hy 5:5 [2.74 (0.50) mmol/l] (P = 0.163) (Figure 7.1).

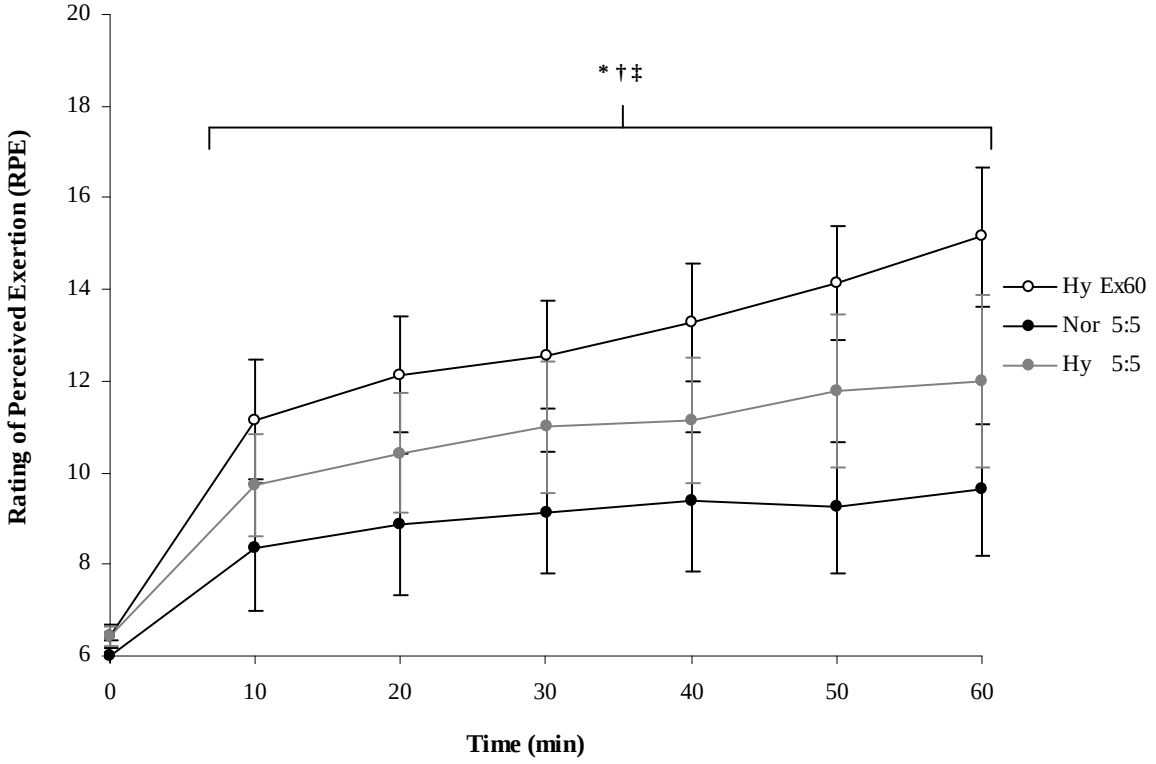

Figure 7.0 RPE during Hy Ex60, Nor 5:5 and Hy 5:5. Values are represented as means (SEM). Significant differences; * Hy Ex60 vs. Nor 5:5 (P = 0.000), † Hy Ex60 vs. Hy 5:5 (P = 0.001) and ‡ Nor 5:5 vs. Hy 5:5 (P = 0.001).

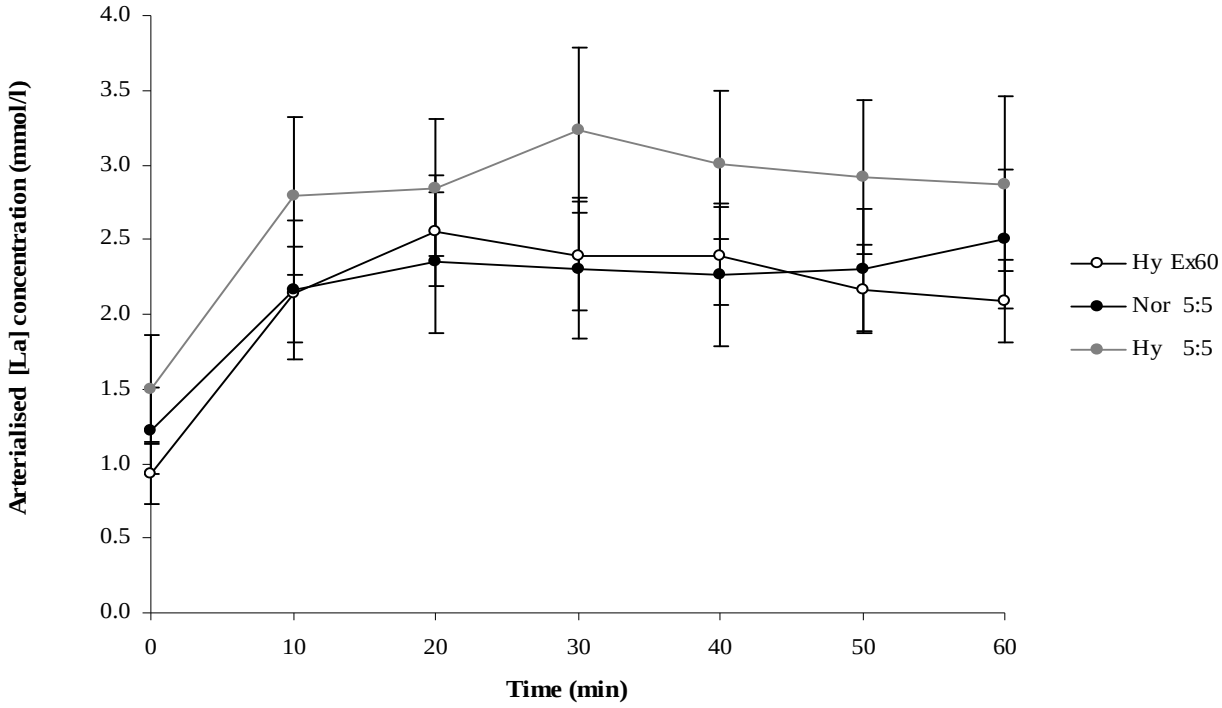

Figure 7.1 Arterialised [La] responses during three separate exercise trials. Values are represented as means (SEM). No statistical difference was found between trials (P = 0.163).

7.4.2 Blood Glucose Concentrations and Kinetics

Arterialised blood glucose concentrations decreased from baseline to end of exercise for each condition; Hy Ex60 [pre; 8.15 (0.52) post; 6.56 (0.54) mmol/l] (P = 0.000), Nor 5:5 [pre; 8.28 (0.51) post; 7.23 (0.79) mmol/l] (P = 0.008) and Hy 5:5 [pre; 8.39 (0.60) post; 7.38 (0.53) mmol/l] (P = 0.000) (Figure 7.2). A significant difference was noted between Hy Ex60 and Hy 5:5 (P = 0.049) but not between Hy Ex60 vs. Nor 5:5 (P = 0.186) and Nor 5:5 vs. Hy 5:5 (P = 0.926).

Rate of glucose appearance (R_a) and disappearance (R_d) increased from resting values during exercise in Hy Ex60 (P = 0.001), Nor 5:5 (P = 0.002) and Hy 5:5 (P = 0.010). Differences between R_a and R_d were calculated during exercise for each trial (Figure 7.3). All three comparisons demonstrated negative values showing that the R_d was greater than R_a. Although, Nor 5:5 [-0.10 (0.02) mg · kg · min^{-1}] was not different from Hy 5:5 [-0.11 (0.01) mg · kg · min^{-1}] (P = 0.256). Differences between R_a and R_d were higher during Hy Ex60 [-0.21 (0.04) mg · kg · min^{-1}] when compared to Nor 5:5 (P = 0.022) and Hy 5:5 (P = 0.002) (Figure 7.3). Nor 5:5 showed no difference between resting values for Day 1 and Day 2 for R_a (P = 0.839), R_d (P = 0.385) and MCR (P = 0.102) (Table 7.1). This was similar for Hy 5:5 (R_a; P = 0.163, R_d; P = 0.064 and MCR; P = 0.098). R_d was significantly higher during resting isotope infusion on Day 2 when comparisons were made with Day 1 for Hy Ex60 (P = 0.031), although R_a (P = 0.076) and MCR (P = 0.08) were unchanged.

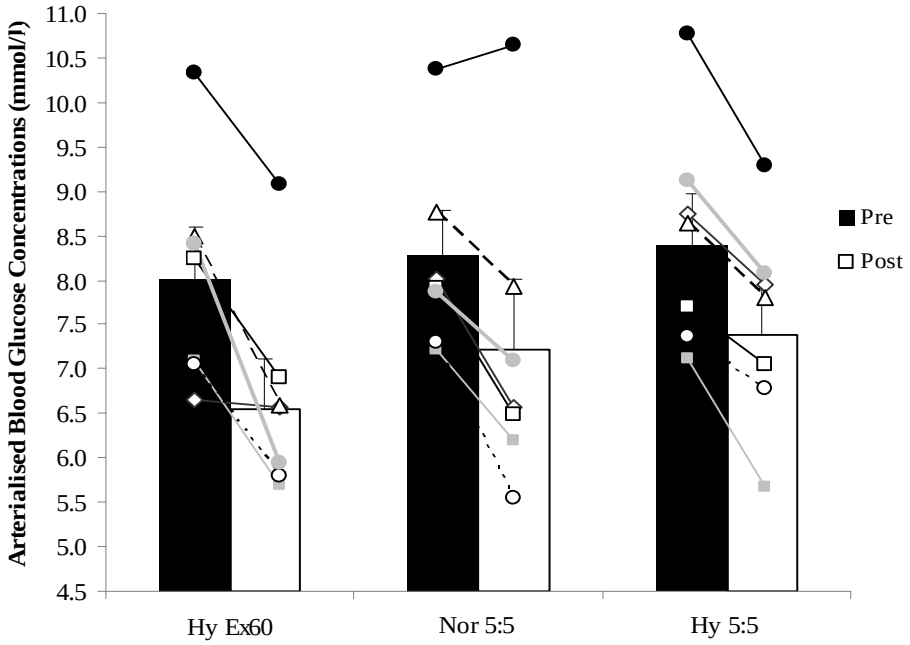

Figure 7.2 Mean (SEM) and individual changes in arterialised blood glucose concentrations from pre to post exercise. Significant decreases were found in Hy Ex60 (P = 0.000), Nor 5:5 (P = 0.008) Hy 5:5 (P = 0.000).

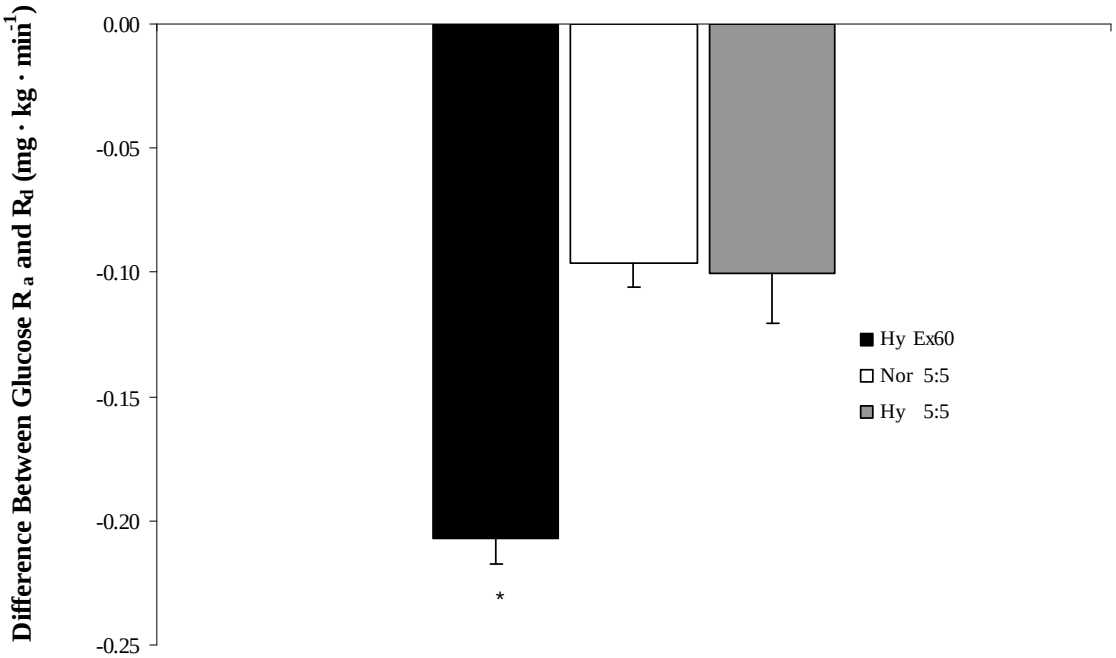

Figure 7.3 Mean (SEM) changes in rate of glucose appearance (R_a) and disappearance (R_d). * denotes significant difference; Hy Ex60 vs. Nor 5:5; P = 0.022. Hy Ex60 vs. Hy 5:5; P = 0.002. Nor 5:5 vs. Hy 5:5; P = 0.256.

Table 7.1 ***Rates of Glucose Appearance (R_a), Disappearance (R_d) and Metabolic Clearance Rates (MCR) at baseline (Rest Day 1), during Exercise and for resting isotope infusions 24 hrs following Hy Ex60, Nor 5:5 and Hy 5:5 (Rest Day 2).***

		Rest (Day 1)	Exercise	Rest (Day 2)
HyEx60	R_a ($mg \cdot kg \cdot min^{-1}$)	1.91 (0.11)	5.42 (0.28)*	1.82 (0.11)
	R_d ($mg \cdot kg \cdot min^{-1}$)	1.85 (0.11)	5.63 (0.29)*	2.01 (0.12)†
	MCR ($ml \cdot kg \cdot min^{-1}$)	1.70 (0.05)	5.24 (0.30)*	1.79 (0.12)
Nor 5:5	R_a ($mg \cdot kg \cdot min^{-1}$)	1.96 (0.07)	6.11 (0.68)*	1.98 (0.07)
	R_d ($mg \cdot kg \cdot min^{-1}$)	1.93 (0.14)	6.18 (0.69)*	2.03 (0.06)
	MCR ($ml \cdot kg \cdot min^{-1}$)	1.73 (0.05)	5.78 (0.42)*	1.81 (0.10)
Hy 5:5	R_a ($mg \cdot kg \cdot min^{-1}$)	1.90 (0.15)	6.23 (0.84)*	2.07 (0.13)
	R_d ($mg \cdot kg \cdot min^{-1}$)	1.83 (0.17)	6.33 (0.88)*	2.09 (0.12)
	MCR ($ml \cdot kg \cdot min^{-1}$)	1.69 (0.07)	5.67 (0.42)*	1.88 (0.20)

Values are means (SEM). * Significant difference within trial from Rest (Day 1) ($P < 0.05$); † Significant difference from Rest Day 1 and Rest Day 2, within trial ($P < 0.05$). No difference was found between trials

7.4.2 Homeostasis Model (HOMA) - Insulin Sensitivity, Insulin Resistance & β-Cell function

Hypoxic Exercise (Hy Ex60)

Fasting arterialised blood glucose concentrations were lower in the 24 hrs (Day 2; $P = 0.001$) but not 48 hrs (Day 3; $P = 0.164$) following 60 min of continuous exercise in hypoxia (Figure 7.4, A). Plasma insulin values were lower from baseline measures (Day 1) at 24 hrs ($P = 0.001$) and 48 hrs ($P = 0.025$) post Hy Ex60. Homeostasis model for insulin resistance ($HOMA_{IR}$) and fasting insulin resistance index (FIRI) improved in the 24 hrs ($HOMA_{IR}$; $P =$

0.002 and FIRI; P = 0.002), and remained reduced at 48 hrs post ($HOMA_{IR}$; P = 0.028 and FIRI; P = 0.028) Hy Ex60 (Figure 7.4 B & C).

Normoxic Intermittent Exercise (Nor 5:5)

Fasting insulin values were unchanged from pre exercise [Day 1; 17.5 (1.6) μU/ml] values for Day 2 [16.9 (1.2) μU/ml; P = 0.868] and Day 3 [17.7 (2.1) μU/ml; P = 0.627]. Arterialised blood glucose concentrations were lower 24 hrs following Nor 5:5 (P = 0.016) and returned to near baseline values at Day 3 (P = 0.128) (Figure 7.4 A). $HOMA_{IR}$, FIRI, QUICKI and $HOMA_{\beta\text{-Cell}}$ were not altered in the 48 hrs following Nor 5:5 (Figure 7.5 B, C, E, and F).

Hypoxic Intermittent Exercise (Hy 5:5)

Intermittent exercise in hypoxia (Hy 5:5) caused a reduction in blood glucose concentrations in the 24 hrs (Day 2) following exercise [-1.00 (0.19) mmol/l; P = 0.001]. No difference was noted for the same variable on Day 3 (P = 0.052). Homeostasis model for insulin resistance ($HOMA_{IR}$) was lower 24 hrs post Hy 5:5 (P = 0.013) as was FIRI (P = 0.013). Hy 5:5 also resulted in an increase in QUICKI on Day 2 (P = 0.018). β-cell function, as assessed with $HOMA_{\beta\text{-Cell}}$ was found to be significantly higher on Day 3 (P = 0.005) (Figure 7.6 A-F).

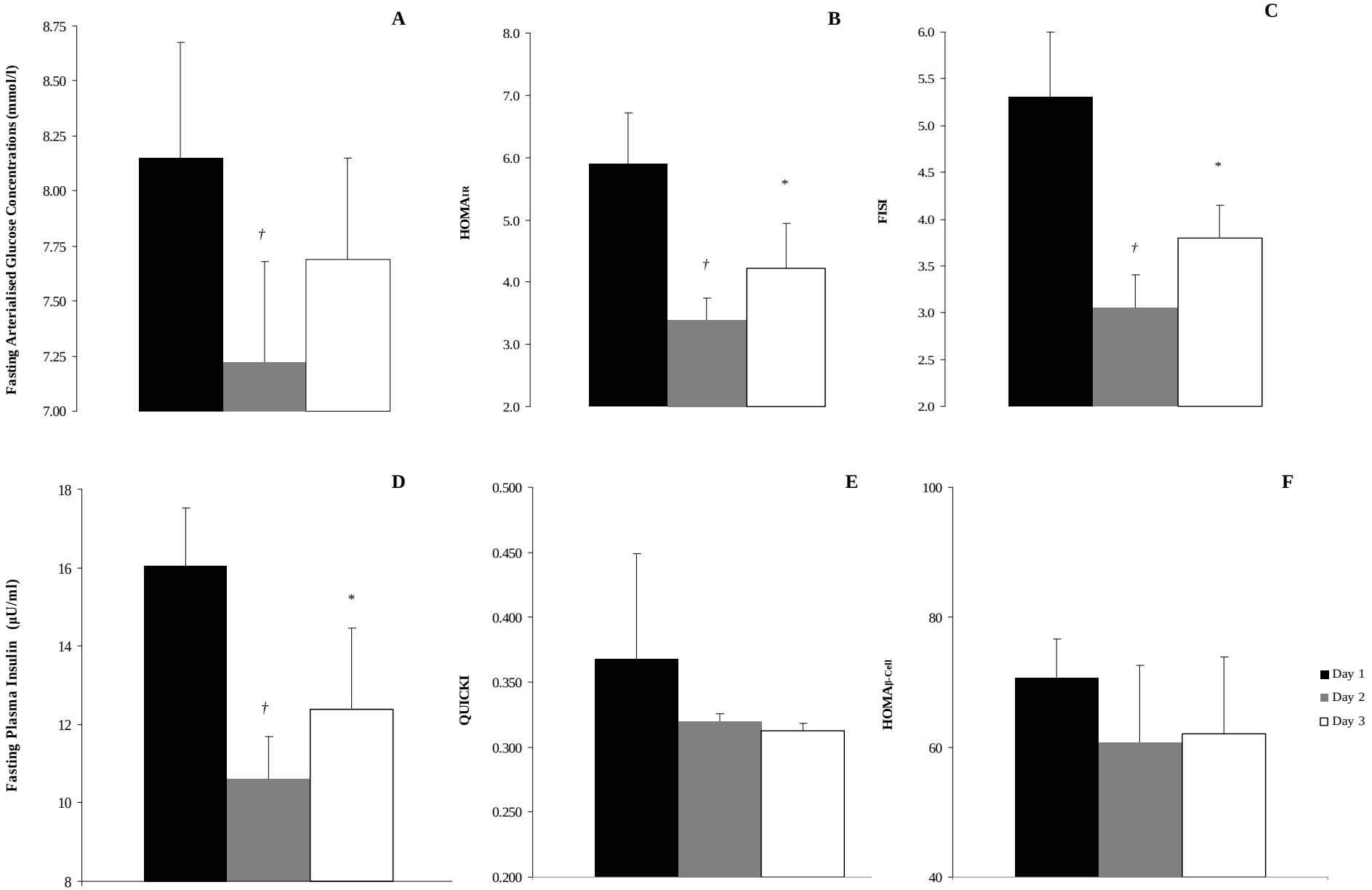

Figure 7.4 Values are presented as means (SEM) for ***Hy Ex60***. Fasting blood glucose (A), Homeostasis model for insulin resistance ($HOMA_{IR}$; B), fasting insulin resistance index (FIRI; C), plasma insulin (D), quantitative insulin sensitivity check index (QUICKI; E) and HOMA of β-cell function ($HOMA_{\beta\text{-Cell}}$; F). *Denotes significant difference from Day 1 at $P < 0.05$. † Denotes significant difference from Day 1 at $P < 0.01$.

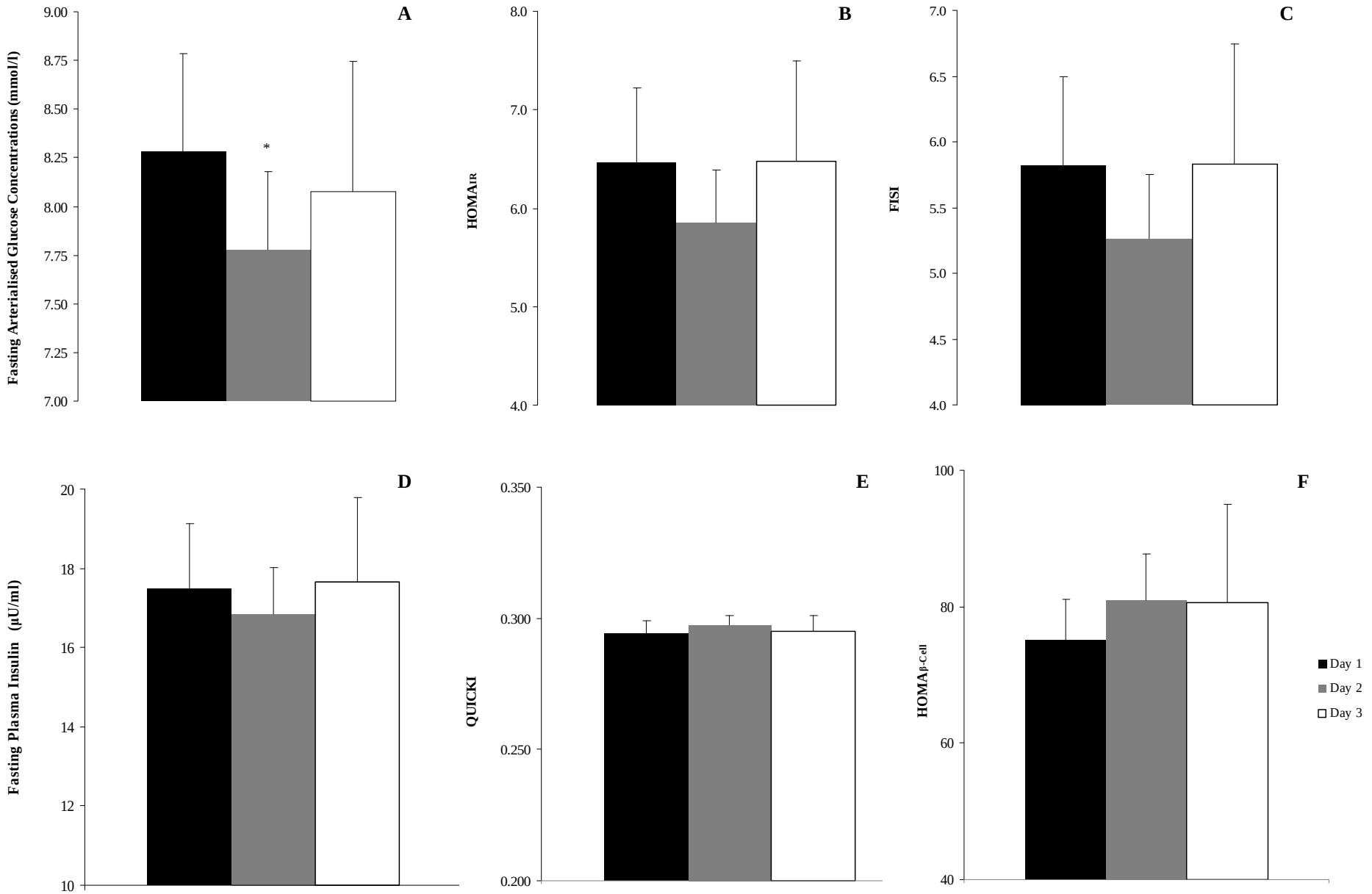

Figure 7.5 Values are presented as means (SEM) for ***Nor 5:5***. Fasting blood glucose (A), Homeostasis model for insulin resistance ($HOMA_{IR}$; B), fasting insulin resistance index (FIRI; C), plasma insulin (D), quantitative insulin sensitivity check index (QUICKI; E) and HOMA of β-cell function ($HOMA_{\beta\text{-Cell}}$; F). *Denotes significant difference from Day 1 at $P < 0.05$.

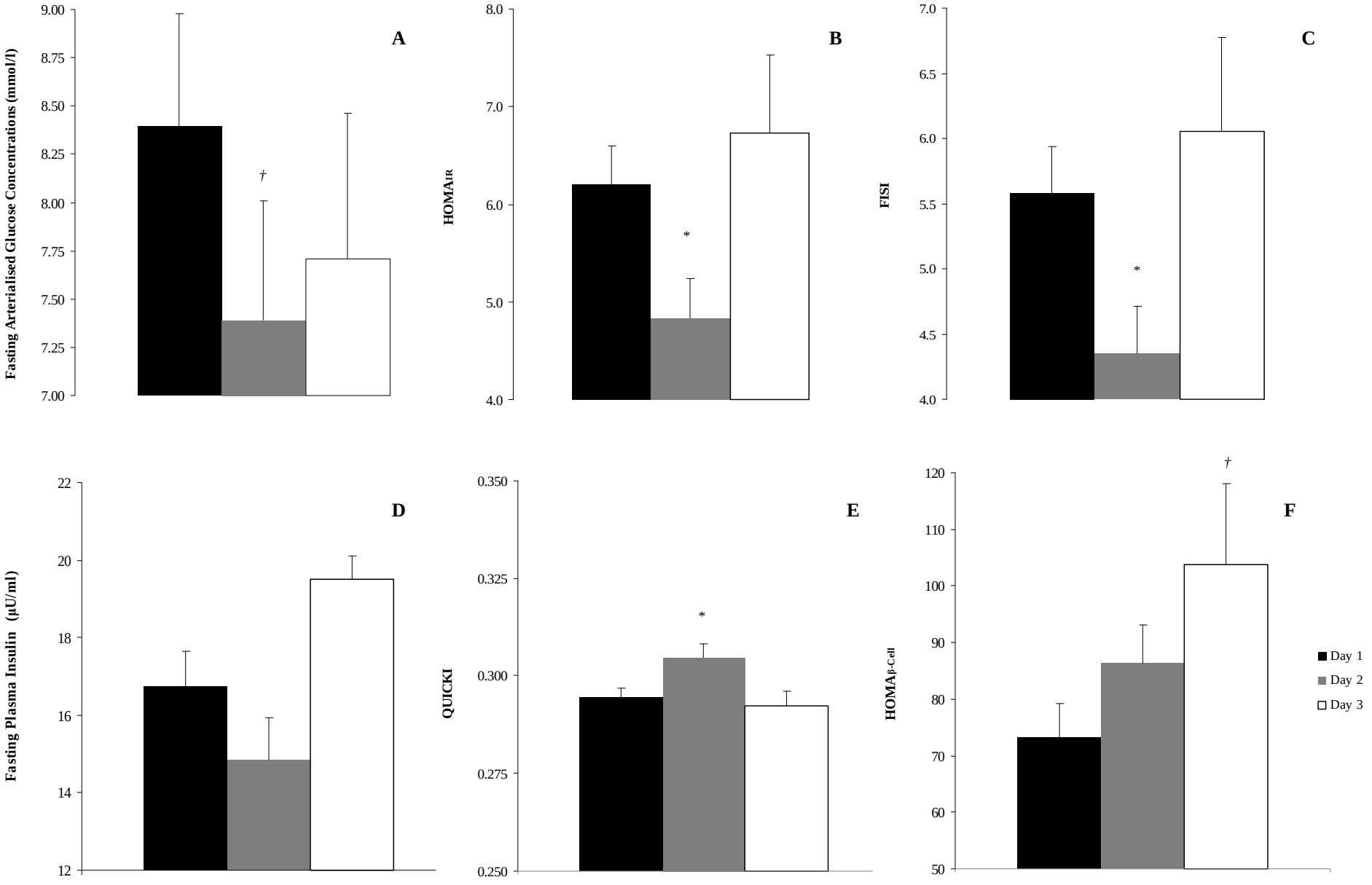

Figure 7.6 Values are presented as means (SEM) for ***Hy 5:5***. Fasting blood glucose (A), Homeostasis model for insulin resistance ($HOMA_{IR}$; B), fasting insulin resistance index (FIRI; C), plasma insulin (D), quantitative insulin sensitivity check index (QUICKI; E) and HOMA of β-cell function ($HOMA_{\beta\text{-Cell}}$; F). *Denotes significant difference from Day 1 at $P < 0.05$. † Denotes significant difference from Day 1 at $P < 0.01$.

7.4.4 Tumour Necrosis Factor-α (TNF- α)

Figure 7.7 shows TNF-α concentrations drawn from fasting blood samples on Day 1, Day 2 and Day 3. Comparisons between trials showed a significant difference in baseline samples drawn on Day 1 between Nor 5:5 and Hy 5:5 [55.8 (8.8) and 67.1 (7.9) pg/ml, respectively] (P = 0.04). No difference was noted between Hy Ex60 and Nor 5:5 (P = 0.772) and Hy Ex60 and Hy 5:5 (P = 0.064). Hy Ex60 caused a significant reduction in TNF-α 24 hrs (Day 2; P = 0.016) and 48 hrs (Day 3; P = 0.023) after exercise. No change was noted for Nor 5:5 (P = 0.064). Circulating TNF-α concentrations were lower 24 hrs following Hy 5:5 (Day 2; P = 0.022) which returned to near baseline values on Day 3 (P = 0.08).

Homeostasis model for insulin resistance ($HOMA_{IR}$) and fasting insulin resistance index (FIRI) decreased significantly from Day 1 post Hy Ex60 and showed the greatest magnitude of change within the variables used to estimate post exercise glucose tolerance (data presented above; Section 7.4.3). Changes in circulating TNF-α values from Day 1 were therefore correlated with $HOMA_{IR}$ and FIRI to assess the relationship between these variables. No significant correlations were noted between delta TNF-α and delta $HOMA_{IR}$ (Day 2, r = 0.45; P = 0.308) (Day 3; r = 0.11; P = 0.810) and delta FIRI (Day 2, r = 0.46; P = 0.304) (Day 3; r = 0.15; P = 0.906).

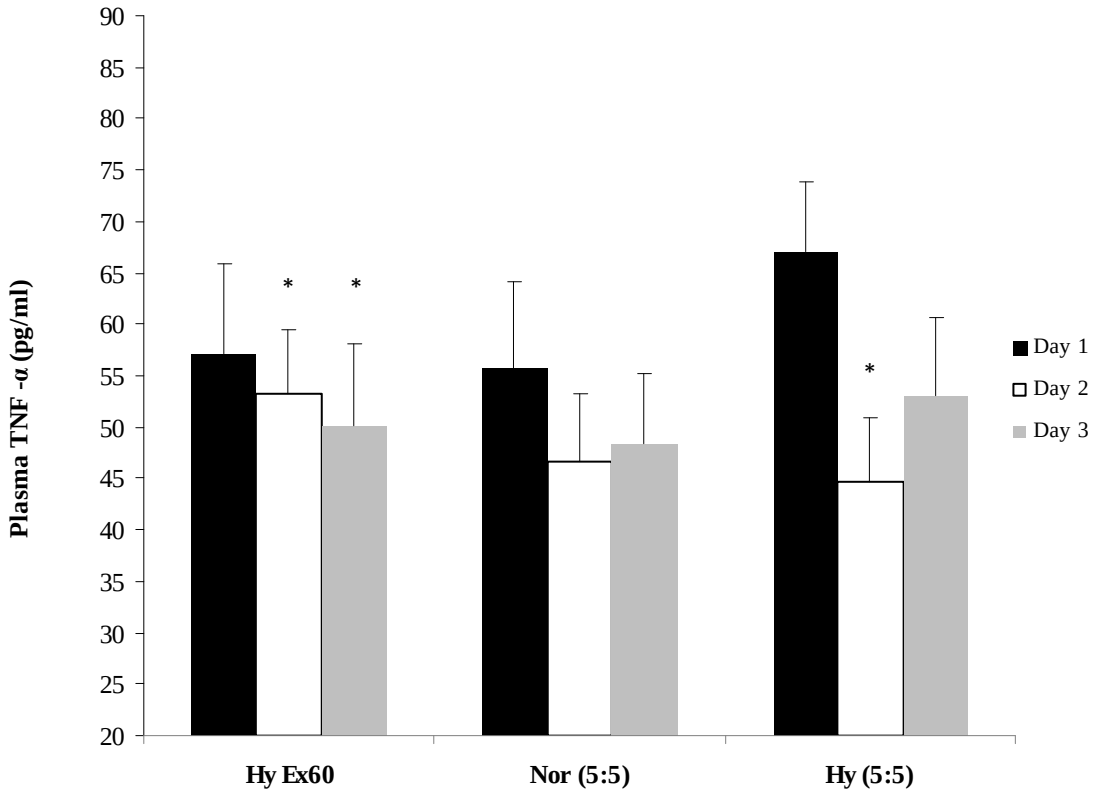

Figure 7.7 TNF-α concentrations at baseline (Day 1) and 24 hrs (Day 2) and 48 hrs (Day 3) following exercise. * Denotes significant difference from Day 1 within trial ($P < 0.05$).

7.4.5 Profile of Mood State

Table 7.2 shows POMS response to the three different exercise conditions. No differences were observed for any of the six mood state scales between conditions. Total Mood-Disturbance was at its highest immediately following Hy 5:5. Although this was not found to be significant ($P = 0.385$). The score for vigour was also noted to be lower in the Hy 5:5 trial, although again, this was not significant ($P = 0.247$). The subscales reflecting Fatigue within the POMS were higher following Hy Ex60 (NS; $P = 0.101$) (Table 7.2).

Table 7.2 ***Comparison of Mood State (POMS) immediately following each exercise condition***

Variables (POMS)	**Nor 5:5**	**Hy Ex60**	**Hy 5:5**	***P* Value**
Tension-Anxiety	8.7 (1.7)	11.6 (3.1)	11.2 (3.2)	*P* = 0.956
Depression-Dejection	18.8 (2.8)	16.2 (1.1)	17.8 (1.5)	*P* = 0.779
Anger-Hostility	14.7 (1.4)	13.4 (1.4)	15.5 (2.3)	*P* = 0.584
Vigor-Activity	24.7 (2.9)	24.8 (2.8)	21.2 (3.2)	*P* = 0.247
Fatigue-Inertia	11.3 (1.6)	18.8 (4.6)	16.0 (3.7)	*P* = 0.101
Confusion-Bewilderment	5.3 (1.6)	5.4 (1.8)	8.3 (2.3)	*P* = 0.127
Total Mood-Disturbance	**34.2 (8.9)**	**41.4 (13.4)**	**47.7 (14.5)**	***P* = 0.385**

Values are mean (SEM). P values are comparisons between condition for individual variables.

7.5 Discussion

Previous chapters have demonstrated that a single bout of moderate intensity exercise had a positive effect on blood glucose metabolism both during and following exercise in individuals with type 2 diabetes. The improvements caused by exercise can be attributed to insulin-independent and insulin-dependent (post exercise) glucose transport. This is the first study to determine whether intermittent exercise (120% LT and passive recovery; 5:5) with and without hypoxia can alter acute- and moderate-term glucose metabolism in type 2 diabetics. The significant findings of this study were that 60 min of moderate intensity exercise in hypoxia provided the greatest improvements in glucose tolerance when compared to intermittent exercise in either normoxia or hypoxia. Intermittent exercise in normoxia also stimulated acute blood glucose removal in type 2 diabetics. Although improvements in glucose tolerance were less apparent in the 24 to 48 hrs following Nor 5:5 (Figure 7.5). In contrast to normoxic intermittent exercise, Hy 5:5 augmented insulin sensitivity to a greater degree in the 24 and 48 hrs following exercise.

7.5.0 Acute Response of Glucose Kinetics to Exercise & Hypoxia

Similar to the previous experimental chapters moderate intensity exercise in hypoxia increased acute glucose disposal in type 2 diabetics. The mechanisms and literature supporting this finding has been detailed previously (section 6.6.0). It would seem that continuous exercise in hypoxia is a more potent stimulus for glucose disposal over intermittent exercise in either environment (Nor 5:5 and Hy 5:5). The present data showed that continuous exercise caused a 19.5% decline in blood glucose concentrations. This observation is supported by the significant difference between R_a and R_d during Hy Ex60 [-0.20 (0.04) mg · kg · min^{-1}; Figure 7.3]. No difference was found when the same calculations

were carried out for both intermittent exercise trials (Figure 7.3). These data suggest that the trend for higher rates of glucose disposal (R_d) were matched with a subsequent increase in endogenous glucose production (R_a) during intermittment exercise (Table 7.1), resulting in smaller changes in blood glucose concentrations when compared to Hy Ex60.

It is recognised that glucose uptake (R_d) and production (R_a) increase with exercise intensity as a result of a greater reliance of skeletal muscle on glucose oxidation (van Loon *et al.*, 2001). It is also known that R_a increases at a greater rate than R_d during sustained high intensity exercise (> 80% $\dot{V}O_{2max}$) (Purdon *et al.*, 1993; Sigal *et al.*, 1994). Christmass *et al.* (1999) previously found glucose utilisation to be 1.2 times higher during intermittent compared to continuous exercise in healthy controls. The current study does not support this notion as there was no difference between glucose R_a and R_d between conditions during exercise (Table 7.1), which may reflect differences in the measurement methods used. Christmass *et al.* (1999) measured substrate oxidation using indirect calorimetery which has been shown to overestimate carbohydrate, while underestimating fat oxidation during high intensity exercise via the depletion of the HCO_3^- pool and non-metabolic production of CO_2 (Bairstow *et al.*, 1990). Furthermore, the calculation of substrate oxidation rates using this technique is based on the assumption that respiration at a cellular level is accurately represented in the measurement of pulmonary gas exchange.

Comparisons between continuous exercise and intermittent exercise in hypoxia and normoxia in type 2 diabetics are difficult given the lack of available literature. The data presented showed that both Nor 5:5 and Hy 5:5 have the ability to reduce circulating blood glucose levels in type 2 diabetics to a similar degree. This finding was surprising given that hypoxic

exposure was found to stimulate glucose transport independent of exercise within the first study of this thesis (chapter 4). It was proposed that intermittent exercise in hypoxia, separated with passive (hypoxic) recovery would stimulate glucose transport activity to a greater degree than the same exercise in normoxia. A mechanistic stand point would support such a proposal in that as the exercise-induced stimulus for GLUT-4 activation / translocation (Brooks *et al.,* 1991) wears off, it would be replaced by a hypoxic-induced stimulus known to cause the same glucose transport effect (Cartee *et al.,* 1991; Chiu *et al.,* 2004; Chiu *et al.,* 2005). As glucose R_a, R_d and arterialised blood glucose concentrations changed to a similar extent, and that there was no statistical difference between the two trials (Nor 5:5 and Hy 5:5) for these variables, it was concluded that intermittent exercise in hypoxia had no additive effect on whole body glucose metabolism in type 2 diabetics.

Glucose availability, to the contracting muscle, increases during exercise via glycogenolysis (at the commencement of exercise) and gluconeogenesis (towards the end of exercise) pathways. These pathways are stimulated by the increased activity of epinephrine, cortisol and glucagon in response to lower concentrations of plasma insulin and glucose during contractile activity (Berger *et al.,* 1980). High physiological concentrations of epinephrine and reduced plasma insulin can enhance R_a in exercising humans **(**Kjaer *et al.,* 1993). Furthermore, it is thought that intermittent exercise suppresses insulin secretion over continuous exercise (Kjaer *et al.,* 1993). Although neither hormone was measured during exercise in the current study, data presented in chapter 4 (study 2) showed that exercise in hypoxia suppresses insulin secretion and possibly increases glucose R_a to a greater level than exercise in normoxia whilst generating parity with any hypoxic-induced increase in glucose R_d.

7.5.1 Exercise-Hypoxia Improve Insulin Sensitivity

Exercise increases insulin-dependent glucose transport (Cuff *et al.,* 2003; O'Gorman *et al.,* 2006; Frøsig *et al.,* 2007) and insulin sensitivity (Zierath, 2002; Holloszy, 2005; Jenssen & Goodyear, 2005; Rose & Richter *et al.,* 2005) in type 2 diabetic and healthy control subjects. The mechanisms surrounding this have been discussed elsewhere (Section 2.7.4) and are thought to include increased insulin-stimulated glucose transport via augmented plasma membrane content of GLUT-4 (Henriksen, 2002) as well as exercise induced stimulation of IRS-1 (Chibalin *et al.,* 2000), PI-3kinase (Kim *et al.,* 1995; Kim *et al.,* 1999) and AMPK (Musi *et al.,* 2001). The main finding from the current study is that Hy Ex60 had the most prominent effect on post exercise measures of insulin sensitivity and insulin resistance. Showing, with the support of the previous study (experimental chapter 5), that continuous exercise at 90% LT for 60 min is an effective intervention for improving glucose control in type 2 diabetics.

Although blood glucose concentrations decreased and glucose R_d increased to a similar degree during both Nor 5:5 and Hy 5:5, the latter trial increased insulin sensitivity (estimated; QUICKI) and improved insulin resistance ($HOMA_{IR}$ and FIRI) in the 24 hrs following exercise. This may be explained by the ability of hypoxia to increase S_I^{2*} independent of contractile activity (Chapter 3) (Cartee *et al.,* 1991). These data demonstrate that Hy 5:5 is an effective intervention in improving glucose control. The post-exercise improvements in insulin sensitivity may be attributed to the degree of glycogen depletion during exercise (Bogardus *et al.,* 1983) and hypoxia (Cartee *et al.,* 1991; Wadley *et al.,* 2006), improvements in post exercise vasodilatory function (De Filippis *et al.,* 2006) and increased muscle GLUT-4 content (Hensen *et al.,* 1998).

Hy 5:5 also increased β-cell function ($HOMA_{\beta\text{-cell}}$) in the 48 hrs after the end of exercise. The reasons for this improvement are not clear and were not shown during Hy Ex60. β-cell dysfunction play an important part in the progression from impaired glucose tolerance to overt type 2 diabetes. In the early stages of type 2 diabetes insulin resistance is met by a compensatory increase in β-cell mediated insulin secretion (Weyer *et al.,* 1999). Furthermore, the development of type 2 diabetes is associated with a reduction in insulin sensitivity, a decrease in insulin secretory capacity and progressive hyperglycaemia (Kahn, 2003; Stumvoll *et al.,* 2005). The finding that β-cell function was improved 48 hrs following Hy 5:5 is hard to interpret given that $HOMA_{\beta\text{-cell}}$ is merely an estimate of insulin secretion capacity. However, β-cell function has been reported to improve following exercise training in diabetic rodents (Farrell *et al.,* 1991) and humans (Dela *et al.,* 2004). It is possible that an acute bout of intermittent exercise in hypoxia may increase GLUT-2 membrane content within the β-cells thereby improving the glucose sensory and insulin secretory capacity of the pancreas (Baron *et al.,* 1991; Parks *et al.,* 2007; Yokoe *et al.,* 2008). Intermittent hypoxic exposure is also a stimulus for pancreatic β-cell replication in rodents (Yokoe *et al.,* 2008), suggesting an enhance insulin secretion capacity during intermittent hypoxia which may not be present following continuous exercise in hypoxia.

Insulin secretion is known to be influenced by insulin sensitivity and that a feedback loop exists between these two pathway (Kahn, 2003). Insulin resistance (estimated; $HOMA_{IR}$ and FIRI) and fasting insulin values increased from baseline values, suggesting insulin sensitivity decreased in the 48 hrs following Hy 5:5. The increase in β-cell function may reflect an error associated with this estimate ($HOMA_{\beta\text{-cell}}$). Jayagopal *et al.* (2002) has recently reported a large biological variation in the measures of HOMA in diabetic populations which would suggest limitations in the use of this measure in estimating insulin resistance and β-cell

function. However, $HOMA_{IR}$ (Katsuki *et al.,* 2001) and QUICKI (Uwaifo *et al.,* 2002) have been validated and closely correlated with clamped derived indices of insulin sensitivity and secretion. Furthermore, $HOMA_{\beta\text{-cell}}$ function was found to be poorly correlated with the gold standard estimates of insulin secretions, as measured using euglycaemic-hyperinsulinaemic clamp techniques (Uwaifo *et al.,* 2002). Given that diet was controlled throughout and that fasting glucose concentrations were reduced following each trial, it is proposed that the changes in glucose tolerance reported within the current study are reflective of real improvements in insulin sensitivity.

7.5.2 Circulating TNF-α Concentrations Following Exercise & Hypoxia

The TNF-α values reported within the current study seem to be higher than the majority of published data (Winkler *et al.,* 1998; Starkie *et al.,* 2003; Fischer *et al.,* 2005). Although, these disparities may be due to differences in the analytical procedures and methods (ELISA) used (Clanton *et al.,* 2001). In addition to its role in insulin resistance, TNF-α has also been implicated in eNOS and vascular function as well as having a role in apoptosis and microangiopathy in type 2 diabetics (Makino *et al.,* 2005). Therefore, circulating TNF-α concentrations are known to be elevated in type 2 diabetics compared to healthy controls (Winkler *et al.,* 1998) with a further rise in diabetic sufferers with secondary complications (Makino *et al.,* 2005). Perhaps signifying that the subjects in the current study may be in the early stages of endothelial dysfunction or suffering from secondary complications.

Exercise in hypoxia reduced TNF-α concentrations in the days following exercise. The reason for this may be associated with improvements in insulin sensitivity (resistance) witnessed in the same trials. TNF-α is widely implicated in insulin resistance and is known to be elevated in type 2 diabetics (Pedersen *et al.,* 2003; Zeigerer *et al.,* 2004; Plomgaard *et al.,* 2005). For this reasons changes in TNF-α and $HOMA_{IR}$ / FIRI in the days (Day 2 and Day 3) following hypoxic exercise were correlated to assess for possible relationships in the current work. The results obtained demonstrated no significant association between TNF-α and measures of insulin resistance. This is in direct contrast with Plomgaard *et al.* (2007) who found a significant relationship ($P < 0.01$) between TNF-α levels and $HOMA_{IR}$ in type 2 diabetics. Attempts to reduce insulin resistance in individuals with type 2 diabetes by suppressing TNF-α with TNF-α antibodies (Ofei *et al.,* 1996) has been shown to be ineffective (Plomgaard *et al.,* 2007). Proposing that the short-term reversal of insulin resistance seen with exercise

cannot be completely attributed to reductions in circulating TNF-α and that improvements in post-exercise insulin sensitivity in type 2 diabetics may be the result of increased peripheral blood flow and endothelium function (De Filippis *et al.,* 2006). The notion that exercise can lower TNF-α concentrations has been shown previously (Keller *et al.,* 2004) (literature review 1.12). Indeed exercise is considered to have an anti inflammatory effect (Petersen & Petersen, 2005). The finding that exercise with hypoxia can reduce circulating TNF-α levels is novel. Although the lack of a control trial (no exercise) within the current investigation makes it difficult to draw firm conclusions.

7.5.3 Rating of Perceived Exertion & Profile of Mood State

Rating of perceived exertion (RPE) was significantly higher during Hy Ex60 compared with both intermittent protocols, showing that the subjects within the current study perceived continuous exercise to be more demanding than intermittent exercise in either environmental condition. This can be supported by a study using obese type 2 diabetic females whereby RPE was reported to be significantly lower during intermittent [12.3 (2.3) units] versus continuous [13.7 (2.3) units] exercise (Coquart *et al.,* 2008).

It is worth pointing out that the lower RPE demonstrated during intermittent exercise may be due to the timing of data collection. RPE scores were measured in the last minute of each 10 min period. Therefore, the subjects were actually 4 min into a passive recovery phase during the intermittent protocol. Furthermore, that the subjects seemed to find continuous exercise more demanding was not supported by post exercise assessment of mood state, measured with POMS. The lack of change in mood state immediately post exercise may reflect the lack of

sensitivity of this measure to detect a change following acute exercise. Although POMS has previously been shown to not only highlight the positive effects in mood following acute exercise but is also able to reflect differences in mood disturbance following various exercise intensities (Steptoe & Cox, 1988). The lack of any difference in mood state within the current study may also be due to the small sample size used (Hall and Lane, 2001). A retrospective power analysis, calculated using the data obtained during the current study indicates that a further 13 subjects would be required to detect a significant difference with 0.95 power.

7.6 Conclusions

The conclusion from the current study are that continuous moderate intensity exercise in hypoxia offered the greatest improvements in acute and moderate-term glucose control in type 2 diabetics. Intermittent exercise also stimulated glucose disposal and improved post exercise insulin resistance which was further enhanced when exercise was combined with hypoxia (Hy 5:5). The improvements in insulin sensitivity following hypoxic exercise cannot be directly attributed to decreasing TNF-α concentration and may indicate changes in GLUT-4 membrane content and intracellular signalling mechanisms. A further conclusion was that perception of effort (RPE) was lower during Nor 5:5 and Hy 5:5, suggesting that intermittent exercise may provide a more palatable exercise intervention for type 2 diabetics. Although, the data presented shows that 60 min of continuous exercise in hypoxia provided the greatest improvement in indices of glycaemic control and suggest the possible use of hypoxic exercise in the treatment of type 2 diabetes and insulin resistance.

CHAPTER 8

General Discussion & Conclusions

Habitual hyperglycaemia in type 2 diabetes is the product of a singular or collective dysfunction(s) in insulin resistance, relative insulin secretion deficiency, elevated hepatic glucose production and impaired glucose effectiveness. It is the elevation in circulating blood glucose that appears to contribute to the severe secondary complications associated with this metabolic condition. Therefore, a primary goal in diabetes management is to achieve near normal blood glucose levels whilst improving associated disease risk factors. Current therapeutic treatments available include pharmaceutical, psychological interventions, diet and physical activity. Indeed, physical inactivity has been linked to many modern day chronic diseases (Booth *et al.,* 2000).

Despite these interventions physical inactivity, obesity and type 2 diabetes continues to rise, placing a staggering economic burden on society. A recent report estimates that the NHS spends in the region of £13.7 million a day on the treatment of diabetes (Diabetes UK, 2007). Given that physical inactivity plays a prominent role in obesity / diabetes progression and the relative low cost of exercise as an intervention, it is believed that this should be the first line of defence against diabetes and its health related complications (Booth *et al.,* 2000). Therefore, the manner in which the public view exercise may need to change. Interventions that maximise the potential for improvements in glycaemic control, while motivating and improving adherence should be investigated and implemented. The current thesis had two key aims; *1)* to measure the effects of acute hypoxia on whole body glucose metabolism and 2) assess the influence of a single bout of exercise on short- and medium-term glucose control in type 2 diabetics.

8.1 Hypoxia Stimulates Whole Body Glucose Metabolism

The data presented within this thesis lends support to the notion that hypoxia stimulates glucose disposal in insulin resistant humans. Comparisons with the general literature are hard given that this is the first study to measure *in vivo* insulin sensitivity, using the 4 hr labelled IVGTT, following hypoxic exposure in type 2 diabetic humans. It would appear that hypoxia activates glucose transport activity via the intact contraction-stimulating pathway in type 2 diabetics. The decrease in circulating blood glucose concentration seen during hypoxia (Chapter 4) may be attributed to an increase in contraction-stimulated translocation of GLUT-1, and to a larger extent GLUT-4, as well as the activation of transporters pre-existing in the plasma membrane (GLUT-1) of skeletal muscle (Zhang, 1999; Zorzano *et al.*, 2005). Furthermore, it seems reasonable to suggest that the hypoxic stimulus used, also has the potential to increase glucose transport via GLUT activation in numerous tissue types, including spleen (GLUT-6) (Doege *et al.*, 2001), liver and adipose (GLUT-8) (Lisinski *et al.*, 2001), and the pancreas (GLUT-10) (Dawson *et al.*, 2001). Cell culture work using human adipocytes showed that severe hypoxia (O_2 ~1%) increased GLUT-1 (9-fold), GLUT-3 (10-fold) and GLUT-5 (9-fold) mRNA level compared to a normoxic control condition (O_2 = 20.93%) with a 3.3-fold increase in 2-deoxy-D-glucose uptake (Wood *et al.*, 2007). The mechanisms responsible for this finding would seem to be an increased activation and / or phosphorylation of signalling proteins involved in the contraction-stimulated pathway. A lack of human studies using hypoxia as a model and the measurement limitations in the current thesis makes it hard to draw firm conclusions. Although, the likely candidates for increased contraction-stimulated glucose uptake would seem to include AMPK (Mu *et al.*, 2001), Ca^{2+}-dependent mechanisms (Wright *et al.*, 2005) and AS160 (Jing *et al.*, 2008).

8.1.0 Hypoxia Improves Short-Term Insulin Sensitivity (S_I^{2*})

Aerobic exercise positively affects whole body glucose tolerance and insulin-stimulated skeletal muscle glucose transport activity in insulin resistant individuals (Brooks *et al.*, 1991). An improvement in insulin sensitivity in insulin-resistant skeletal muscle has the potential to reduce progression rates to overt diabetes and perhaps lower cardiovascular mortality. At a cellular level the glucose lowering effect of exercise in type 2 diabetics is associated with an increase in GLUT-4 translocation (Kennedy *et al.*, 1999). Whether increased GLUT-4 membrane content is the result of increased activity in signalling proteins involved in insulin-stimulated glucose transport remains to be determined (Cusi *et al.*, 2000; Henriksen, 2002).

Of interest and seemingly challenging previous research, acute hypoxia improves short-term glucose control (~ 4 hrs), at least partly, via an insulin-dependent mechanism as shown by increased S_I^{2*} (Chapter 5). Cartee *et al.* (1991) previously presented data showing a decrease in muscle glycogen stores during hypoxia in rodents. Furthermore, exercise in hypoxia has been associated with greater rates of glycogen depletion compared to normoxic exercise [-253.6 (52.5) and -105.2 (35.4) mmol/kg dry wt, respectively] at the same absolute intensity in humans (Wadley *et al.*, 2006). Rapid muscle glycogen resynthesis has been shown to occur immediately following moderate aerobic exercise (Bergstrom & Hultman, 1966; Richter *et al.*, 1982; Houmard *et al.*, 2004). Therefore, the mechanism by which prior hypoxia increases insulin sensitivity may be linked to hypoxia's ability to provoke glycogen depletion to a greater extent than exercise alone.

Intense exercise (75 min at 75% $\dot{V}O_{2peak}$ followed by 5 x 1 min maximal sprints) has been reported to augment glycogen synthase activity while attenuating PI-3kinase response (O' Gorman *et al.,* 2000). This may suggest that increases in PI-3kinase activity are not required in post exercise muscle glycogen resynthesis. This finding, coupled with the increased S_I^{2*} noted in the current thesis may suggest that an increase in PI-3kinase activity may not be required to increase insulin-stimulated glucose transport (Wojtaszewski *et al.,* 2002) and the subsequent conversion of glucose into its polysaccharide form. An invited review by Wojtaszewski *et al.* (2002) concluded that prior exercise influences the insulin signalling cascade via alterations in the functional arrangement of the signalling proteins and / or draws upon unidentified intermediates. It may also be possible that insulin-dependent glucose transport requires the recruitment of signalling proteins that are generally accepted to be exclusive to the contraction-stimulated pathway. A working hypothesis is that, at some point, common effectors are involved in response to both glucose transport pathways, one of which maybe the Akt substrate AS160 (Treebak *et al.,* 2007). AS160 is implicated in insulin- (Eguez *et al.,* 2005) and contraction-stimulated (Kramer *et al.,* 2006; Treebak *et al.,* 2007) GLUT-4 trafficking to the cell membrane. Such a mechanism would suggest that insulin and contraction (hypoxia) may employ similar signalling proteins for GLUT-4 translocation.

8.2 Can Hypoxia cause Insulin Resistance?

There is some evidence to suggest that hypoxia causes insulin resistance in both human and animal models. Larsen *et al.* (1997b) reported decreased insulin sensitivity during a hyperinsulinaemic-euglyceamic clamp after 2 days at 4300 m. This may have been due to a greater fasting period before the commencement of clamp procedures. The authors reported that fasting times were ~6 hr greater during altitude when compared to per-exposure tests (Larsen *et al.,* 199b). Increasing the potential for a greater energy deficit prior to the hyperinsulinaemic-euglyceamic clamps performed at altitude. A shift towards fat oxidation and glycogen restoration is known to accompany periods of energy deficit (Braun, 2008) and was proposed by Larsen *et al.* (1997b) as a possible reason for reduced insulin action.

A potential mechanism, not discussed by Larsen *et al.* (1997b) for their results, may be associated with reduced activity of the insulin-stimulated pathway. Insulin-stimulated glucose transport is the primary mechanism for glucose uptake during periods of rest. At least in peripheral tissue (muscle), hypoxia is known to increase energy turnover resulting in increased intracellular AMP and Ca^{2+} levels (Mu *et al.,* 2001; Wright *et al.,* 2005). This response activates glucose uptake using the contraction- (hypoxic), rather than the insulin-stimulated pathway (Cartee *et al.,* 1991). Furthermore, an increase in circulating insulin and perceived insulin resistance (Larsen *et al.,* 1997b) may be due to the ability of hypoxia to encourage insulin synthesis and release by pancreatic β-cells (Kolesnik *et al.,* 1995), the introduction of exogenous insulin (hyperinsulinaemic clamp) and a decrease in insulin signalling activity (Yin *et al.,* 2009) due to a heavier reliance on contraction-stimulated glucose uptake. In addition, treatment with high levels of insulin has been shown to inhibit insulin binding capacity, cause a marked depletion of IRS-1 and -2 content and impair

sensitivity to insulin stimulation of PKB activity (Buren *et al.*, 2003). Taken together, this evidence may suggest that the use of the hyperinsulinaemic-clamp technique during hypoxic exposure carries distinct limitations.

However, Braun *et al.* (2001) also found a rise in circulating insulin and a corresponding increase in insulin resistance ($HOMA_{IR}$) on exposure to altitude (4300 m). Despite a reduction in energy intake (~84% of calculated energy requirements), the authors proposed that this energy deficit had no effect on insulin sensitivity (Braun *et al.*, 2001). The discrepancy between the current work and Braun *et al.* (2001) may be due to methodological differences. Braun *et al.* (2001) obtained measures of glucose tolerance during hypoxic exposure using an oral glucose tolerance test (OGTT). The OGTT is known to stimulate incretin release (due to glucose ingestion), leading to elevated insulin secretion, when comparisons are made with the IVGTT (Muscelli *et al.*, 2006). This incretin effect (i.e. increase in insulin with the OGTT) occurs despite no difference in glucose concentration (Muscelli *et al.*, 2006) and may potential mask the true effects of hypoxia on insulin sensitivity. Particularly in light of data published elsewhere in which glucose control has been shown to improve at an identical elevation. Brooks *et al.* (1991) reported an increase in glucose R_d during exposure to 4300 m in healthy humans when compared to sea level values [3.59 (0.08) and 1.80 (0.02) mg · kg · min^{-1}, respectively $P < 0.05$].

Animal models have also suggested decreased glucose control during hypoxic episodes (Polotsky *et al.*, 2003; Otlmann *et al.*, 2004). The work of Polotsky *et al.* (2003) and Otlmann *et al.* (2004) must be considered with a certain degree of caution. Polotsky *et al.* (2003)

reported two important findings *1)* insulin sensitivity was improved in lean insulin resistant tissue and *2)* insulin resistance in genetically obese / leptin deficient mice was completely reversed with leptin replacement (section 2.10.2). Activation of the sympathoadrenal release of epinephrine was proposed by Oltmanns *et al.* (2004) to be the mechanism responsible for hypoxic-induced decreases in glucose infusion rates. Brooks *et al.,* (1991) also demonstrated an increase in epinephrine during hypoxic exposure with a decrease in blood glucose concentrations. This finding may suggest that the hypoxic-induced dependency for glucose as an efficient source of fuel overrides any inhibitory effect of epinephrine on insulin-stimulated glucose uptake. The current thesis can not support this latter notion but did, however, show that blood glucose levels were reduced during resting hypoxia, with no difference in circulating insulin concentrations between Hy Rest and Nor Rest trials. Furthermore, stimulation of the sympathoadrenal system (i.e. sharp rise in plasma epinephrine level) on acute exposure to hypoxia is associated with a increase in glucose uptake (Mazzeo *et al.,* 1991; Roberts *et al.,* 1996; Mazzeo *et al.,* 2000).

This thesis has demonstrated that acute hypoxia decreases blood glucose concentrations and improves whole body insulin sensitivity in the hours following exposure in type 2 diabetics. There seems little doubt that glucose disposal is increased during hypoxic exposure (Johnson *et al*., 1974; Cooper *et al.,* 1986; Brooks *et al.,* 1991; Lee *et al.,* 2003) via the contraction-(hypoxic) stimulated pathway (Cartee *et al.,* 1991; Reynolds *et al.,* 1998). Comparisons between the current work and the literature are difficult, given that the hypoxic stress used in the present thesis was introduced prior to the principal measurement of glucose control (i.e. IVGTT). Therefore, insulin sensitivity was assessed under normoxic conditions in the hours following, rather than during, hypoxic exposure (Larsen *et al.,* 1997b; Bruan *et al.,* 2001). The

results published by these authors are therefore mixed parameters under the influence of hypoxia, insulin (Larsen *et al.*, 1997b) and glucose challenges (Bruan *et al.*, 2001), with the increase in S_I^{2*} post exposure in the current thesis being reflective of the effects of prior hypoxia on glucose metabolism.

8.3 Hypoxia & Exercise have an Additive Effect on Glucose Disposal

The literature has consistently shown hypoxia to be a potent stimulus for glucose transport by recruiting the exercise / contraction signalling pathway for GLUT translocation. Glucose transport rates have been shown to be additive when either hypoxia or contractile activity are coupled with insulin, whereas hypoxia and muscle contraction are not (Cartee *et al.*, 1991; Azevedo *et al.*, 1995). The data presented here conflicts with the latter notion but is consistent with *in vivo* research carried out in humans at high altitude (Brooks *et al.*, 1991). It is therefore, considered that exercise and hypoxia have an additive effect on contraction-stimulated GLUT-4 movement or that these stimuli draw upon similar, but not identical pathway for glucose transport (Mu *et al.*, 2001).

Mu *et al.* (2001) reported that AMPK inhibition completely blocked the ability of hypoxia to activate glucose uptake, while only partially reducing contraction-stimulated glucose uptake in skeletal muscle. Furthermore, exercise with or without hypoxia increases AMPK-α2 activity with only the former condition (exercise + hypoxia) found to be significantly different ($P < 0.05$) (Wadley *et al.*, 2006). Hypoxia alters AMPK activity in human skeletal muscle and the effects are additive when combined with exercise (Wadley *et al.*, 2006). It is possible that the additive effect witnessed in the current thesis may be the result of a non-maximal stimulation of the contraction pathway. For example, Cartee *et al.* (1991) used supramaximal

electrical stimulation in severe hypoxia (95% N_2 and 5% CO_2) not akin with the moderate conditions used in the present thesis. Taken together, these finding may suggest that hypoxia and exercise, or either stimulus alone can activate the contraction-stimulating pathway until a theoretical maximum level has been achieved, after which no further increase in glucose disposal is seen. Hypoxic-induced vasodilation represents a rapid and acute response to reduced O_2; the result is the maintenance of adequate blood-O_2 supply to peripheral tissue. Hypoxia is proposed to activate O_2-regulated Ca^{2+} channels, resulting in arterial vasodilation (Lopez-Barneo *et al.*, 2004). It is possible, therefore, that an increase in blood / glucose delivery caused by hypoxia-induced vasodilation (Miura *et al.*, 2003) may have contributed to the enhanced glucose disposal noted within this work.

8.3.0 Acute Hypoxic Exercise Enhances Insulin Sensitivity (S_I^{2*})

Results from studies 1 and 2 (chapters 4 and 5) show that exercise under hypoxic conditions has the greatest influence on S_I^{2*}. Exercise trained (insulin sensitive) individuals demonstrate increased insulin mediated PI-3 kinase activity and glucose disposal rates (Eguez *et al.*, 2000) over sedentary controls. PI-3 kinase stimulation is also known to be increased following short-term exercise training in non diabetic humans (Houmard *et al.*, 1999). However, exercise training does not seem to enhance insulin stimulated PI-3kinase or PKB activity in vastus lateralis muscle tissue in elderly men (~58 yr) (Tanner *et al.*, 2002). Cusi *et al.* (2000) showed that prior exercise increased phosphorylation of insulin receptor substrate-1 (IRS-1) 24 hrs post exercise. However, PI-3kinase activity was not altered within the same study (Cusi *et al.*, 2000). Wojtaszewski *et al.* (1998) has proposed that exercise does not enhance the activity of protein phosphorylation or insulin signalling, but offers improvements in

glucose transport activity via changes in the arrangement of insulin signalling proteins that allow these molecules a greater effectiveness or efficiency in stimulating GLUT-4 trafficking (Wojtaszewski *et al.,* 2002).

It is suggested that increased S_I^{2*} (chapter 5) and insulin-stimulated glucose disposal in insulin resistant states (including diabetes) post exercise, may be the result of increased (Kennedy *et al.,* 1999) or more efficient (Wojtaszewski *et al.,* 2002) GLUT-4 translocation to the plasma membrane. Kennedy *et al.* (1999) showed that acute exercise increased GLUT-4 plasma membrane content in vastus lateralis muscle by 74 (20)% in type 2 diabetics. The literature described above may provide the mechanism by which exercise in hypoxia improves short-term (4 hr) glucose control in type 2 diabetics. Although, it remains to be determined if acute hypoxia with or without exercise can alter insulin signalling proteins, or whether increased S_I^{2*} can be contributed to a more effective or efficient translocation of GLUTs in diabetic muscle tissue.

8.4 Continuous Hypoxic Exercise is an Effective Intervention in Improving Glycaemia in Type 2 Diabetics

The prominent finding throughout this thesis is that both exercise and exercise with hypoxia encourage acute glucose disposal and improve post exercise insulin sensitivity. However, the shortest duration of exercise used (Hy Ex^{20}) within chapter 6 did not demonstrate a blood glucose lowering effect on the day of exercise. The maintenance of blood glucose levels at pre-exercise concentrations may reflect the higher R_a, which was greater than the other exercise / hypoxic trials used throughout this thesis. Both $HOMA_{IR}$ and FISI were improved in the 24 hrs following Hy Ex^{20}, suggesting that the intensity of exercise (hypoxia) used caused an increase in insulin action, presumably via a glycogen depletion / resynthesis mechanism. This improvement was present 24 hrs, but not 48 hrs following hypoxic exercise. It would seem that completing the same amount of work over a longer duration (Hy Ex^{40}) further improves glucose tolerance in type 2 diabetics. This is shown by an acute decrease in circulating glucose concentrations as well as significant enhancements in $HOMA_{IR}$, QUICKI and FISI in the 48 hrs following the trial. It is worth noting that the estimates of insulin resistance and sensitivity used in the current thesis have been validated again the gold standard assessment (euglycaemic-hyperinsulinaemic clamp) (Katsuki *et al.*, 2001; Uwaifo *et al.,* 2002). These data therefore, suggest that exercise duration and not intensity, at least in hypoxia, is the important variable in improving glycaemic control in type 2 diabetics and that the benefits can extend for up to 2 days after exercise.

Moderate intensity exercise (60 min; 90% LT) combined with hypoxia increased glucose disposal to a greater extent than the other hypoxic / exercise conditions used throughout the experimental chapters. In the fourth experimental chapter (study 2) hypoxic exercise (60 min)

resulted in an acute decrease in blood glucose concentrations, which was found to be ~50% greater than Nor Ex. Furthermore, S_I^{2*} was significantly higher following Hy Ex^{60} when compared to Nor Ex. Study 3 (Experimental Chapter 6) compared three different exercise conditions of equal work in hypoxia; Hy Ex^{60}, Hy Ex^{40} and Hy Ex^{20}. The result from this study again demonstrated that the greatest reduction in blood glucose levels were noted in Hy Ex^{60} and that indices reflecting insulin sensitivity and resistance showed the greatest improvements in the 48 hrs following Hy Ex^{60} (Table 6.1, 6.2 and 6.3).

The results presented in study 4 suggest that insulin sensitivity showed the greatest improvements following Hy Ex60 and propose again, that continuous exercise at 90% LT for 60 min is an effective intervention for improving glucose control in type 2 diabetics. Kraniou *et al.* (2006) has reported that low-intensity prolonged exercise provides a greater increase in AMPK activity over high-intensity exercise of shorter durations. Treebak *et al.* (2007) also demonstrated augmented AS160 phosphorylation during moderate intensity exercise which was not detected during shorter duration-high intensity exercise. The findings within the current research programme and those of Treebak *et al.* (2007) and Kraniou *et al.* (2006) would indicate that exercise duration, and not total work or intensity, is the determining factor in stimulating glucose metabolism in type 2 diabetics.

8.5 Normoxic Exercise

The present thesis draws upon the normoxic rest trial from study 1 (chapter 3) to make comparison between exercise and a non-exercise control. These comparisons can be made given that five out of seven subjects completed each trial within this research programme. As expected the non-exercise control condition (Nor Rest) showed a non-significant reduction in blood glucose concentrations (0.23 mmol/l). Therefore, it can be concluded that both continuous (0.91 mmol/l) and intermittent (1.05 mmol/l) exercise in normoxia reduce blood glucose concentrations to a similar degree. The finding that continuous aerobic exercise decreases whole blood glucose concentrations is consistent with the majority of published work (Abuissa *et al.,* 2005; Booth & Winder, 2005). The current recommendations for physical activity are 30 min of moderate intensity exercise every day, which may or may not be accumulated over the course of a day (i.e. 3 x 10 min). However, in type 2 diabetes this recommendation is still not based on firm scientific evidence (Eriksen *et al.,* 2007). The present finding gives rise to the notion that discontinuous exercise, more indicative of modern day life-styles, may be a useful therapy in diabetic management.

8.5.0 Normoxic Exercise Improves Insulin Sensitivity

In employing the same subjects in studies 1 and 2, the current work was able to make direct comparisons between a non exercise trial and 60 min of moderate intensity exercise in normoxia (Nor Rest vs. Nor Ex). S_I^{2*} was 57% greater in the 4 hrs following moderate intensity exercise compared to Nor Rest [Nor Rest; 1.39 (0.08) and Nor Ex; 3.24 (0.51) $x10^{-4} \cdot min^{-1}$ (μU/ml); $P = 0.003$]. This data is in keeping with the published literature (Hansen *et al.,* 1998; Cuff *et al.,* 2003; Bloem & Chang, 2008) and supports the therapeutic benefits of exercise in insulin resistant and diabetic patients.

8.6 Glucose Effectiveness (S_G^{2*}) is unaltered by Hypoxic and Normoxic Exercise

Glucose effectiveness (S_G^{2*}) represents the ability of hyperglycaemia to encourage glucose disposal at basal insulin concentrations and is a key component of glucose tolerance (Higaki *et al.,* 1996). Following intravenous glucose administration, 50% of all blood glucose clearance can be attributed to the mass action effect of hyperglycaemia (i.e. S_G^{2*}). Prior exposure to low physiological concentrations of glucose activates skeletal muscle glucose uptake, whereas exposure to hyperglycaemia produces the opposite response, suggesting that habitual hyperglycaemia contributes to glucose resistance and impaired tolerance (Itani *et al.,* 2003). In support of this, glucose effectiveness is impaired in individuals with type 2 diabetes, suggesting glucose resistance in this population (Del Prato *et al.,* 1997).

Itani *et al.* (2003) have shown that phosphorylation of AMPKα2 is diminished in incubated rat extensor digitorum longus muscle when glucose availability is progressively increased and that the greatest decrement in AMPK activity was seen when glucose concentrations were elevated to 25 mmol/l (Itani *et al.,* 2003). Elsewhere, data indicates that the reduction in glucose uptake, caused by hyperglycaemia (20 mmol/l), is related to mechanisms responsible (partly mediated by AMPK) for increasing GLUT-4 membrane content in isolated rat hindlimbs lacking insulin (Mathoo *et al.,* 1999). Indeed, the increase in glucose availability may favour glycolytic energy producing pathways, increasing ATP and resulting in less AMP availability for AMPKγ binding. Although, data presented by Itani *et al.* (2003) demonstrated no change in metabolic intermediates (AMP/ATP ratio and creatine phosphate) known to be involved in AMPK Ser^{172} phosphorylation at varying glucose concentrations (0, 6 and 25 mmol/l) (Itani *et al.,* 2003), suggesting that reduced AMPK activity induced by

hyperglycaemia may be subject to mechanisms distinct to traditional pathways involved in AMPK regulation. One hypothesis is that high physiological concentrations of glucose could result in elevated intracellular levels of G-6-P until a theriretical threshold has been reach afterwhich G-6-P acts to inhibit hexokinase and reduce glucose uptake (Rose & Ritcher, 2005).

The work within this thesis does not support the benefits of acute exercise on S_G^{2*} in diabetic populations. Although S_G^{2*} was greatest following Hy Ex, this was not found to be significantly different from Nor Ex, indicating that hypoxia and exercise do not have an additive effect on S_G^{2*}. Further analysis, using paired t-tests, showed no difference between normoxic exercise (Nor Ex; P = 0.167) and hypoxic exercise (Hy Ex; P = 0.170) over the non-exercise control. A power analysis indicated a further nine subjects would be required to detect a significant difference in S_G^{2*} with 0.95 power (Riffenburgh, 1999; Hopkins, 2002), highlighting the need for a greater subject number in future studies assessing S_G^{2*} in type 2 diabetics in response to exercise. Glucose effectiveness (S_G^{2*}) represents the ability of glucose to promote its own transport by mass action (Bouche *et al.,* 2004). In contrast to the present thesis, a single bout of intense exercise (15 min) independent of hypoxia has been shown to enhance glucose effectiveness (Brun *et al.,* 1995). The view that exercise enhances glucose effectiveness is supported elsewhere (Sakamoto *et al.,* 1999; Nishida *et al.,* 2001; Nishida *et al.,* 2004; Hayashi *et al.,* 2005).

The reasons for the differences between the current work and others are unclear, although Sakamoto and colleagues (Sakamoto *et al.,* 1999) used an exercise intensity at 100% LT. This may suggest that higher exercise intensities have a positive dose dependant effect on post exercise S_G^{2*}. Hayashi *et al.* (2005) also showed S_G^{2*} to be significantly increased following exercise at 70% and not 50% $\dot{V}O_{2max}$. Additionally, the majority of research showing improvements in post exercise S_G^{2*} have been conducted in healthy (non diabetic) populations (Brun *et al.,* 1995; Sakamoto *et al.,* 1999; Nishida *et al.,* 2001; Nishida *et al.,* 2004; Boule *et al.,* 2005; Hayashi *et al.,* 2005). Hyperglycaemia itself is proposed to cause decreased glucose transport via insulin-independent mechanisms (Itani *et al.,* 2003; Bouche *et al.,* 2004). The ability of exercise to improve S_G^{2*} in healthy humans and not type 2 diabetics may suggest that the latter population exhibit a delayed response in glucose dependent (S_G^{2*}) transport following exercise. Resistance exercise has previously been shown to improve S_I^{2*} but not S_G^{2*} in presumably glucose intolerant (Henriksen, 2002; Tanner *et al.,* 2002) elderly males (Zachwieja *et al.,* 1996) thus providing support that S_G^{2*} is less responsive to change following exercise in insulin resistant populations. It is concluded that S_G^{2*} seems less sensitive to change following exercise in insulin resistant and diabetics patients. Or, for any change to be detected, type 2 diabetics may need to exercise at a higher relative intensity to stimulate a post-exercise increase in glucose-dependent transport activity (i.e. S_G^{2*}). Sriwijitkamol *et al.* (2007) have recently concluded that type 2 diabetics need to exercise at a higher relative intensity to stimulate the AMPK / AS160 axis in skeletal muscle than their lean counterparts.

8.7 Sample Size

The population and experimental methods employed within the current thesis presented inherent problems throughout. Prentice (2001) purposed that there are three main challenges to research in chronic disease; *1)* adequate sample size *2)* subject adherence and *3)* the associated costs. The last challenge can be broken down further into time commitments and the actual cost for experimental consumables. The research design not only required individuals (from a population predisposed to exercise intolerance) to exercise, but also required lengthy laboratory visits (≤ 7 hr) with frequent sampling of arterialised blood. It was observed that these two factors limited subject recruitment and retention. An additional limiting factor included the number of subjects that could be processed, not only in terms of time but also the cost associated with the data analyses.

As discussed earlier, S_G^{2*} accounts for ~50% of the total glucose clearance following an intravenous glucose load (Section 2.5.4). In assuming that no difference was found for S_G^{2*} due to a lack of statistical power rather than a delayed response of this mechanism to an acute bout of exercise / hypoxia (Section 8.5), it would seem prudent to highlight the effect size for this parameter. The data presented an effect size and observed power for S_G^{2*} to be 0.37 and 0.31, respectively. This indicates that the current research programme would have perhaps benefited from a greater subject number. Indeed, the retrospective power analysis discussed above indicated that a further nine subjects would be required to obtain a level of significance ($P < 0.05$).

However, insulin sensitivity (S_I^{2*}) and resistance ($HOMA_{IR}$) were shown to improve following moderate intensity exercise with and without hypoxia. The same analysis showed an effect size and observed power of 0.77 and 1.00 for S_I^{2*}, and 0.65 and 0.96 for $HOMA_{IR}$, respectively. These data suggest that the lack in a significant finding for S_G^{2*} may reflect that the reversal of glucose resistance would require a greater exercise / hypoxic stimulus in individuals with type 2 diabetes (Section 8.5).

8.8 Conclusions

The aims allied to this thesis were to assess and measure the glycaemic response to various exercise protocols with and without hypoxia in type 2 diabetics. Exercise is known to cause blood glucose declines in diabetic patients. A reduction in whole body O_2 availability reduces the relative energy contribution from oxidative phosphorylation, placing a heavier reliance on blood glucose and intracellular glycogen stores. It was rationalised that the introduction of hypoxia may enable insulin resistant individuals to activate the intact contraction-stimulate pathway for glucose transport thereby improving glycaemic control. It appeared that hypoxia, in a similar manner to exercise, positively influenced basal insulin action in type 2 diabetics.

A summary of the conclusions drawn within the General Discussion are six-fold; *1)* 60 min of moderate hypoxia has the ability to increase glucose disposal both during and in the 4 hrs following exposure via insulin-independent and -dependent mechanisms, respectively; *2)* continuous moderate-intensity exercise in normoxia improves S_I^{2*} in type 2 diabetics; *3)* intermittent exercise can acutely improve glucose control, although hypoxia enhances this effect in the 24 hrs following exercise; *4)* the glucose lowering effect of exercise is enhanced by hypoxia; *5)* hypoxic-induced improvements in post-exposure glucose control can not be explained by S_G^{2*} and are therefore, largely attributed to changes in insulin action (S_I^{2*}); and finally, *6)* the most potent effect on acute and moderate-term glycaemia was consistently evident following 60 min of continuous exercise in hypoxia. These novel findings suggest a possible use of hypoxia, with or without exercise, for the prevention and clinical management of type 2 diabetes.

The use of hypoxia in a clinical setting does present ethical (safety), financial and practical issues. The data presented may facilitate and promote the use of hypoxic exposure as a preventive or therapeutic treatment in improving medium- and possible long-term glucose intolerance in type 2 diabetics. Particularly, type 2 diabetic sufferers, who are unable to exercise regularly for extended periods of time. Complications, such as obesity, foot ulcers and low fitness levels often mean that sufferers are unable to activate the exercise/contraction-stimulated pathway for glucose uptake, but may be able to achieve some benefits through frequent hypoxic exposure with or without exercise.

8.9 Future Research

Beyond these results it will be critical to assess whether resting hypoxia produces a dose-dependent response on blood glucose disposal rates. Interpreting the data from exercise studies would at least make it plausible to hypothesise that AMPK activation would increase with a greater hypoxic stimulus while encouraging glucose uptake via the contraction-stimulated pathway. It would also seem equally important to measure the signalling proteins involved in glucose transport activity both during and following whole body hypoxia at rest in type 2 diabetic individuals. Any increase in hypoxic stimulus (i.e. decrease in O_2) may have negative effects associated with hypoxic illness and that these should be controlled for in future work.

Following on from the additive effects of exercise and hypoxia, the next logical research question would seem to be; can intermittent hypoxic exposure with or without exercise, administered over the course of days / weeks offer long-term improvements in glycaemic control within insulin resistant individuals? The labelled intravenous glucose tolerance test is a precise and useful method used in research to provide reasonable estimates of insulin S_I^{2*} and S_G^{2*}. Muscle tissue drawn from type 2 diabetic patients will offer direct measures of signalling proteins involved in both the contraction (hypoxic) and insulin-stimulated pathways and may help answer some of the questions remaining from this thesis. This is not to say that *in vivo* measurements that estimate whole body glucose metabolism are without scientific value.

Although, it is important to determine the mechanisms that regulate exercise and hypoxic-induced improvements in glucose removal acutely, short- (4 hrs) and medium-term (48 hrs). A number of signalling proteins have been implicated to the well defined pathways for glucose transport and the literature supports the notion that both the insulin- and contraction-stimulated pathways operate separately to each other (Holloszy, 2003) and that hypoxia activates glucose transport using the latter of these signalling mechanisms (Cartee *et al.*, 1991). The finding that hypoxia and exercise provoke a greater response in whole body glucose metabolism than either stimulus alone poses some interesting questions. Do exercise and hypoxia stimulate glucose transport activity via identical pathways and that the current results merely reflect a non-maximal stimulus on the signalling mechanisms. Alternatively, hypoxia and exercise use similar, but ultimately separate pathways to encourage glucose uptake. Although it must be pointed out that once GLUTs reach the cell surface to assist receptor and non-receptor mediate endocytosis of glucose, the nature of the stimulus or indeed the pathway involved becomes irrelevant. It does however, seem important to achieve a more detailed knowledge of glucose transport mechanisms in type 2 diabetes if a greater understanding of the cellular defects associated with insulin resistance is to be accomplished.

REFERENCES

References

Abuissa, H., Bel, D.S. and O'keefe, J.H. (2005). Strategies to prevent type 2 diabetes. *Current Med Res Opinion, 21*(7), 1107-1114.

Agurs-Collins, T. D., Kumanyika, S.K., Ten Have, T.R., & Adams-Campbell, L.L. (1997). A randomized controlled trial of weight reduction and exercise for diabetes management in older African-American subjects. *Diabet Care 21*(5), 875-877.

Akerstrom, T. C., Birk, J.B., Klein, D.K., Erikstrup, C., Plomgaard, P., Pedersen, B.K., Wojtaszewski, J. (2006). Oral glucose ingestion attenuates exercise-induced activation of 5'-AMP-activated protein kinase in human skeletal muscle. *Biochem Biophys Res Commun, 14*(342), 945-955.

Alberti, K. (1997). The costs of non-insulin-dependent diabetes mellitus. *Diabet Med., 14*(1), 7-9.

American College of Sports Medicine. (2000). Position stand on exercise and type 2 diabetes. *Medicine and Science in Sports and Exercise, 32*, 1346-1359.

American Diabetes Association. (2004). Physical Activity/Exercise and Diabetes. *Diabet Care, 27*(9), 58S-62.

American Diabetes Association. (2008). Economic Costs of Diabetes in the U.S. in 2007. *Diabet Care, 31*(3), 1-20.

American Diabetes Association. Position Statement. (2002). Diabetes Mellitus and Exercise. *Diabet Care, 25*(9), S64.

Andres, R., Swerdloff, R., Pozefsky, T., & Coleman, D. (1965). *Manual feedback technique for the control of blood glucose concentration.* New York: Mediad, Inc.

Andrew, M., Gerard, O. D., Carmel, G., Roger, T., & Daniel, G. (2002). Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. *Diabet Res Clin Pract, 56*(2), 115-123.

Arias, E. B., Kim, J., Funai, K., & Cartee, G. D. (2007). Prior exercise increases phosphorylation of Akt substrate of 160 kDa (AS160) in rat skeletal muscle. *Am J Physiol Endocrinol Metab, 292*(4), E1191-1200.

Armbruster, D. A. (1987). Fructosamine: structure, analysis, and clinical usefulness. *Clin Chem, 33*(12), 2153-2163.

Arnfred, J., Schmitz, O., Hother-Nielsen, O., Orskov, C., Beck-Nielsen, H., Hermansen, K., Christiansen, J.S., Alberti, K.G., & Orskov, H. (1988). Marked impairment of the effect of hyperglycaemia on glucose uptake and glucose production in insulin-dependent diabetes. *Diabet Med, 5*(8), 755-760.

Aslesen, R., & Jensen, J. (1998). Effects of epinephrine on glucose metabolism in contracting rat skeletal muscles. *Am J Physiol Endocrinol Metab, 275*(3), E448-456.

Atkinson, M. A. M., N.K. (1994). The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med, 24*(331), 1428-1436.

Avogaro, A., Toffolo, G., Kiwanuka, E., de Kreutzenberg, S. V., Tessari, P., & Cobelli, C. (2003). L-Arginine-Nitric Oxide Kinetics in Normal and Type 2 Diabetic Subjects: A Stable-Labelled 15N Arginine Approach. *Diabet, 52*(3), 795-802.

Azevedo, J. L., Carey, J. O., Pories, W. J., Morris, P. G., & Dohm, G. L. (1995). Hypoxia stimulates glucose transport in insulin-resistant human skeletal muscle. *Diabet, 44*(6), 695-698.

Azuma, K., Heilbronn, L. K., Albu, J. B., Smith, S. R., Ravussin, E., Kelley, D. E. (2007). Adipose tissue distribution in relation to insulin resistance in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab, 293*(1), E435-442.

Balon, T. W., & Nadler, J. L. (1994). Nitric oxide release is present from incubated skeletal muscle preparations. *J Appl Physiol, 77*(6), 2519-2521.

Balon, T. W., & Nadler, J. L. (1997). Evidence that nitric oxide increases glucose transport in skeletal muscle. *J Appl Physiol, 82*(1), 359-363.

Barnes, K., Ingram, J. C., Porras, O. H., Barros, L. F., Hudson, E. R., Fryer, L. G. D. (2002). Activation of GLUT1 by metabolic and osmotic stress: potential involvement of AMP-activated protein kinase (AMPK). *J Cell Sci, 115*(11), 2433-2442.

Baron, A. D., Laakso, M., Brechtel, G., & Edelman, S.V. (1991). Reduced capacity and affinity of skeletal muscle for insulin-mediated glucose uptake in noninsulin-dependent diabetic subjects. Effects of insulin therapy. *J Clin Invest, 87*(4), 1186-1194.

Baron, A. D., Zhu, J.S., Zhu, J.H., Weldon, H., Maianu, L., & Garvey, W.T. (1995). Glucosamine induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J Clin Invest, 96*(6), 2792-2801.

Barry, R. E., Allan, B. B., Cummins, E. P., Kattla, J. J., Giblin, A., Scally, N.(2007). Enhanced sensitivity of protein kinase B/Akt to insulin in hypoxia is independent of HIF1-α and promotes cell viability. *Eur J Cell Biol , 86*(7), 393-403.

Bastard, J. P., Rabasa-Lhoret, R., Maachi, M., Ducluzeau, P.H., Andreelli, F., Vidal, H,, & Laville, M. (2003). What kind of simple fasting index should be used to estimate insulin sensitivity in humans? *Diabet Metab, 29*(3), 285-288.

Barstow, T. J., Cooper, D. M., Sobel, E. M., Landaw, E. M., and Epstein, S. (1990) Influence of increased metabolic rate on [13C]bicarbonate washout kinetics. *Am J Physiol Regul Integr Comp Physiol* 259, R163-171

Basu, A., Caumo, A., Bettini, F., Gelisio, A., Alzaid, A., Cobelli, C., & Rizza, R.A. (1997). Impaired basal glucose effectiveness in NIDDM: contribution of defects in glucose disappearance and production, measured using an optimized minimal model independent protocol. *Diabet, 46*(3), 421-432.

Baynard, T., Franklin, R. M., Goulopoulou, S., Carhart, J. R., & Kanaley, J. A. (2005). Effect of a single vs multiple bouts of exercise on glucose control in women with type 2 diabetes. *Metab 54*(8), 989-994.

Baynes, J. W., & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabet, 48*(1), 1-9.

Becker-Zimmermann, K., Berger, M., Berchtold, P., Gries, F.A., Herberg, L., & Schwenen, M. (1982). Treadmill training improves intravenous glucose tolerance and insulin sensitivity in fatty Zucker rats. *Diabetologia, 22*(6), 468-474.

Beeson, M., Sajan, M. P., Dizon, M., Grebenev, D., Gomez-Daspet, J., Miura, A. (2003). Activation of Protein Kinase C-{zeta} by Insulin and Phosphatidylinositol-3,4,5-(PO4)3 Is Defective in Muscle in Type 2 Diabetes and Impaired Glucose Tolerance: Amelioration by Rosiglitazone and Exercise. *Diabet, 52*(8), 1926-1934.

Behrooz, A., & Ismail-Beigi, F. (1999). Stimulation of Glucose Transport by Hypoxia: Signals and Mechanisms. . *News Physiol Sci 14*(3), 105-110.

Bell, G. I., Burant, C. F., Takeda, J., & Gould, G. W. (1993). Structure and function of mammalian facilitative sugar transporters. *J. Biol. Chem., 268*(26), 19161-19164.

Belli, T., Ackermann, M. A., Ribeiro, L. F., Langeani, R., Galdino da Silva, R., & Baldissera, V. (2006). Lactate and ventilatory thresholds in type 2 diabetic women. *Diabet Research and Clinical Practice, 76*(1), 18-23.

Berger, M., Assal, J.P., & Jorgens, V. (1980). Physical exercise in the diabetic. The importance of understanding endocrine and metabolic responses. *Diabete Metab, 6*(1), 59-69.

Bergström, J., & Hultman, E. (1966). Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature, 16*(210), 309-310.

Best, J. D., Kahn, S.E., Ader, M., Watanabe, R.M., Ni, T.C., & Bergman, R.N. (1996). Role of glucose effectiveness in the determination of glucose tolerance. *Diabet Care, 19*(9), 1018-1030.

Blair, S. N. K., H.W. Gordon, N.F. and Paffenbarger, R.S. (1992). How much physical activity is good for health? *Annu Rev Public Health, 13*, 99-126.

Bloem, C. J., & Chang, A. M. (2008). Short-Term Exercise Improves β-Cell Function and Insulin Resistance in Older People with Impaired Glucose Tolerance. *J Clin Endocrinol & Metab, 93*(2), 387-392.

Boden, G. (1997). Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabet, 46*(1), 3-10.

Bogardus, C., Ravussin, E., Robbins, D.C., Wolfe, R.R., Horton, E.S., & Sims, E.A. (1984). Effects of physical training and diet therapy on carbohydrate metabolism in patients with glucose intolerance and non-insulin-dependent diabetes mellitus. *Diabet, 33*(4), 311-318.

Bogardus, C., Thuillez, P., Ravussin, E., Vasquez, B., Narimiga, M., & Azhar, S. (1983). Effect of muscle glycogen depletion on in vivo insulin action in man. *J Clin Invest, 72*(5), 1605–1610. .

Bonen, A., Ball-Burnett, M, Russel C. (1998). Glucose tolerance is improved after low- and high-intensity exercise in middle-age men and women. *Can J Appl Physiol., 23*(6), 583-593.

Booth, F. W., Chakravarthy, M. V., Gordon, S. E., & Spangenburg, E. E. (2002). Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. *J Appl Physiol, 93*(1), 3-30.

Booth, F. W., Gordon, S. E., Carlson, C. J., & Hamilton, M. T. (2000). Waging war on modern chronic diseases: primary prevention through exercise biology. *J Appl Physiol, 88*(2), 774-787.

Booth, F. W., & Winder, W. W. (2005). HIGHLIGHTED TOPIC: Role of Exercise in Reducing the Risk of Diabetes and Obesity. *J Appl Physiol, 99*(1), 3-4.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exer, 14*, 377-381.

Bouche, C., Serdy, S., Kahn, C. R., & Goldfine, A. B. (2004). The Cellular Fate of Glucose and Its Relevance in Type 2 Diabet. *Endocr Rev, 25*(5), 807-830.

Boudeau, J., Scott, J., Resta, N., Deak, M., Kieloch, A., Komander, D., Hardie, D. G., Prescott, A. R., van Aalten, D. M. & Alessi, D. R. (2004). Analysis of the LKB1-STRAD-MO25 complex. *J Cell Sci 117*: 6365-6375

Boule, N. G., Haddad, E., Kenny, G.P. Wells, G.A., & Sigal, R.J. (2001). Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA 286*, 1218-1227.

Boulé, N. G., Kenny, G. P., Haddad, E., Wells, G. A., & Sigal, R. J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia, 46*(8), 1071-1081.

Boule, N. G., Weisnagel, S. J., Lakka, T. A., Tremblay, A., Bergman, R. N., Rankinen, T. (2005). Effects of Exercise Training on Glucose Homeostasis: The Heritage Family Study. *Diabet Care, 28*(1), 108-114.

Brambrink, J. K., Fluckey, J. D., Hickey, M. S., & Craig, B. W. (1997). Influence of muscle mass and work on post-exercise glucose and insulin responses in young untrained subjects. *Acta Physiologica, 161*(3), 371-377.

Braun, B., Rock, P. B., Zamudio, S., Wolfel, G. E., Mazzeo, R. S., Muza, S. R. (2001). Women at altitude: short-term exposure to hypoxia and/or α1-adrenergic blockade reduces insulin sensitivity. *J Appl Physiol, 91*(2), 623-631.

Braun, B., Zimmermann, M. B., & Kretchmer, N. (1995). Effects of exercise intensity on insulin sensitivity in women with non-insulin-dependent diabetes mellitus. *J Appl Physiol, 78*(1), 300-306.

Brooks, G. A., Butterfield, G. E., Wolfe, R. R., Groves, B. M., Mazzeo, R. S., Sutton, J. R., (1991). Increased dependence on blood glucose after acclimatization to 4,300 m. *J Appl Physiol, 70*(2), 919-927.

Brooks, G. A., Wolfel, E. E., Groves, B. M., Bender, P. R., Butterfield, G. E., Cymerman, A. (1992). Muscle accounts for glucose disposal but not blood lactate appearance during exercise after acclimatization to 4,300 m. *J Appl Physiol, 72*(6), 2435-2445.

Brownlee, M., Vlassara, H. & Cerami, A. (1985). Nonenzymatic glycosylation products on collagen covalently trap low-density lipoprotein. *Diabet, 34*(9), 938-941.

Bruss, M. D., Arias, E. B., Lienhard, G. & Cartee, G. D. (2005) Increased Phosphorylation of Akt Substrate of 160 kDa (AS160) in Rat Skeletal Muscle in Response to Insulin or Contractile Activity. *Diabet 54*: 41-50

Buhl, E.S., Jessen, N., Pold, R., Ledet, T., Flyvbjerg, A., Pedersen, S.B., Pedersen, O., Schmitz, O. & Lund, S. (2002) Long-Term AICAR Administration Reduces Metabolic Disturbances and Lowers Blood Pressure in Rats Displaying Features of the Insulin Resistance Syndrome. *Diabet 51*: 2199-2206

Buren, J., Liu, H. X., Lauritz, J., and Eriksson, J. W. (2003). High glucose and insulin in combination cause insulin receptor substrate-1 and -2 depletion and protein kinase B desensitisation in primary cultured rat adipocytes: possible implications for insulin resistance in type 2 diabetes. *Eur J Endocrinol* 148, 157-167

Calderon, R., & Llerena, L.A. (1965). Carbohydrate Metabolism in People Living in Chronic Hypoxia. *Diabet, 14*, 100-105.

Calderón, R., Llerena, L.A., Munive, L., & Kruger, F. (1966). Intravenous glucose tolerance test in pregnancy in women living in chronic hypoxia. *Diabet, 15*(2), 130-132.

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G. (2006). Interleukin-6 Increases Insulin-Stimulated Glucose Disposal in Humans and Glucose Uptake and Fatty Acid Oxidation In Vitro via AMP-Activated Protein Kinase. *Diabet, 55*(10), 2688-2697.

Carling, D. & Hardie, D. G. (1989). The substrate and sequence specificity of the AMP-activated protein kinase. Phosphorylation of glycogen synthase and phosphorylase kinase. *Biochim Biophys Acta 1012*(1):81-6

Carlson, C.A. & Kim, K. (1973). Regulation of Hepatic Acetyl Coenzyme A Carboxylase by Phosphorylation and Dephosphorylation. *J Biol Chem 248*: 378-380

Cartee, G. D., Douen, A. G., Ramlal, T., Klip, A., & Holloszy, J. O. (1991). Stimulation of glucose transport in skeletal muscle by hypoxia. *J Appl Physiol, 70*(4), 1593-1600.

Cartee, G. D., & Holloszy, J. O. (1990). Exercise increases susceptibility of muscle glucose transport to activation by various stimuli. *Am J Physiol Endocrinol Metab, 258*(2), E390-393.

Cartee, G. D., Young, D. A., Sleeper, M. D., Zierath, J., Wallberg-Henriksson, H., & Holloszy, J. O. (1989). Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab, 256*(4), E494-499.

Carter, J., & Jeukendrup, A. (2002). Validity and reliability of three commercially available breath-by-breath respiratory systems. *Eur J Appl Physiol, 86*(5), 435.

Castaneda, C., Layne, J. E., Munoz-Orians, L., Gordon, P. L., Walsmith, J., Foldvari, M. (2002). A Randomized Controlled Trial of Resistance Exercise Training to Improve Glycemic Control in Older Adults With Type 2 Diabetes. *Diabet Care, 25*(12), 2335-2341.

Castillo, O., Woolcott, O.O., Gonzales, E., Tello, V., Tello, L., Villarreal, C., Méndez, N., Damas, L., Florentini, E. (2007). Residents at High Altitude Show a Lower Glucose Profile Than Sea-Level Residents Throughout 12-Hour Blood Continuous Monitoring. *High Alt Med Biol, 8*(4), 307-311.

Caumo, A., & Cobelli, C. (1993). Hepatic glucose production during the labeled IVGTT: estimation by deconvolution with a new minimal model. *Am J Physiol Endocrinol Metab, 264*(5), E829-841.

Chakravarthy, M. V., & Booth, F. W. (2004). Eating, exercise, and "thrifty" genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases (Vol. 96, pp. 3-10).

Chalkley, S. M., Hettiarachchi, M., Chisholm, D.J. & Kraegen, E.W. (1998). Five-hour fatty acid elevation increases muscle lipids and impairs glycogen synthesis in the rat. *Metab 47*(9), 1121-1126.

Charron, M. J., Gorovits, N., Laidlaw, J.S., Ranalletta,. M., & Katz, E.B. (2005). Use of GLUT-4 Null mice to Study Skeletal Muscle Glucose Uptake. *Clin Exper Pharm Physiol, 32*(4), 308-313.

Chen, Z.-P., Stephens, T. J., Murthy, S., Canny, B. J., Hargreaves, M., Witters, L. A. (2003). Effect of Exercise Intensity on Skeletal Muscle AMPK Signaling in Humans. *Diabet, 52*(9), 2205-2212.

Cheung, P. C., Salt, I. P., Davies, S. P., Hardie, D. G. & Carling, D. (2000). Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem J 346:* 659-669

Chibalin, A. V., Yu, M., Ryder, J. W., Song, X. M., Galuska, D., Krook, A. (2000). Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: Differential effects on insulin-receptor substrates 1 and 2. *Proceedings of the National Academy of Sciences of the United States of America, 97*(1), 38-43.

Chiu, L. L., Chou, S. W., Cho, Y. M., Ho, H. Y., Ivy, J. L., Hunt, D. (2004). Effect of Prolonged Intermittent Hypoxia and Exercise Training on Glucose Tolerance and Muscle GLUT4 Protein Expression in Rats. *J Biomedl Scien, 11*(6), 838-846.

Chiu, L.-L., Tsai, Y.-L., Lee, W.-C., Cho, Y.-M., Ho, H.-Y., Chen, S.-M. (2005). Acute Effect of Exercise-Hypoxia Challenge on GLUT4 Protein Expression in Rat Cardiac Muscle. *High Alt. Med. Biol, 6*(3), 256-262.

Cho, H., Mu, J., Kim, J. K., Thorvaldsen, J. L, Chu, Q., Crenshaw, E., Kaestner, K. H., Bartolomei, M. S., Shulman, G. I. & Birnbaum, M.J. (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). *Science 292*: 1728-1731

Christmass, M. A., Dawson, B., Passeretto, P., & Arthur, P. G. (1999). A comparison of skeletal muscle oxygenation and fuel use in sustained continuous and intermittent exercise. *Eur J Appl Physiol, 80*(5), 423-435.

Clanton, T. L., & Klawitter, P. F. (2001). Physiological and Genomic Consequences of Intermittent Hypoxia: Invited Review: Adaptive responses of skeletal muscle to intermittent hypoxia: the known and the unknown. *J Appl Physiol, 90*(6), 2476-2487.

Clark, C. M., & Lee, D. A. (1995). Prevention and Treatment of the Complications of Diabetes Mellitus. *N Engl J Med., 332*(18), 1210-1217.

Clore, J. N., Stillman, J., & Sugerman, H. (2000). Glucose-6-phosphatase flux in vitro is increased in type 2 diabetes. *Diabet, 49*(6), 969-974.

Cobelli, C., Pacini G, Toffolo G & Sacca L (1986) Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT. *Am J Physiol* 250, E591-598

Coghlan, M. P., Culbert, A. A., Cross, D. A., Corcoran, S. L., Yates, J. W., Pearce, N.J., Rausch, O.L., Murphy, G.J., Carter, P.S., Roxbee Cox, L., Mills, D., Brown, M. J., Haigh, D., Ward, R.W., Smith, D. G., Murray, K.J., Reith, A.D. & Holder, J.C. (2000) Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Che. & Biol 7*: 793-803

Colberg, S. R., Hagberg, J. M., McCole, S. D., Zmuda, J. M., Thompson, P. D., & Kelley, D. E. (1996). Utilization of glycogen but not plasma glucose is reduced in individuals with NIDDM during mild-intensity exercise. *J Appl Physiol, 81*(5), 2027-2033.

Colberg, S. R., Parson, H. K., Nunnold, T., Holton, D. R., & Vinik, A. I. (2006). Effect of a single bout of prior moderate exercise on cutaneous perfusion in type 2 diabetes (Brief Report: Pathophysiology/Complications)(Clinical report) *Diabet Care, 29*(10), 2316-2319.

Colberg, S. R., Somma, C. T., & Sechrist, S. R. (2008). Physical Activity Participation May Offset Some of the Negative Impact of Diabetes on Cognitive Function. *J of the Am Medical Directors Association, 9*(6), 434-438.

Cooper, D. M., Wasserman, D. H., Vranic, M., & Wasserman, K. (1986). Glucose turnover in response to exercise during high- and low-FIO2 breathing in man. *Am J Physiol Endocrinol Metab 251*(2), E209-214.

Coquart, J. B., Lemaire, C., Dubart, A.E., Luttembacher, D.P., Douillard, C., & Garcin, M. (2008). Intermittent versus Continuous Exercise: Effects of Perceptually Lower Exercise in Obese Women. *Med Sci Sports Exerc, 40*(8), 1546-1553.

Cortez, M. Y., Torgan, C. E., Brozinick, J. T., Jr., & Ivy, J. L. (1991). Insulin resistance of obese Zucker rats exercise trained at two different intensities. *Am J Physiol Endocrinol Metab, 261*(5), E613-619.

Corton, J.M., Gillespie, J.G. & Hardie, D.G. (1994) Role of the AMP-activated protein kinase in the cellular stress response. *Curr Biol 4:* 315-324

Courtois, P., Sener, A., Herbaut, C., Turc, A., & Malaisse, W.J. (1999). Pharmacokinetics of gliquidone, glibenclamide, gliclazide and glipizide in middle-aged and aged subjects. *Res Commun Mol Pathol Pharmacol, 103*(2), 211-222.

Coyle, E. F., Hamilton, M. T., Alonso, J. G., Montain, S. J., & Ivy, J. L. (1991). Carbohydrate metabolism during intense exercise when hyperglycemic. *J Appl Physiol, 70*(2), 834-840.

Cramer, S. R., Nieman, D.C., & Lee, J.W. (1991). The effects of moderate exercise training on psychological well-being and mood state in women. *J Psychosom Res., 35*(4-5), 437-449.

Cuff, D. J., Meneilly, G. S., Martin, A., Ignaszewski, A., Tildesley, H. D., & Frohlich, J. J. (2003). Effective Exercise Modality to Reduce Insulin Resistance in Women With Type 2 Diabetes. *Diabet Care, 26*(11), 2977-2982.

Cusi, K. J., Pratipanawatr, T., Koval, J., Printz, R., Ardehali, H., Granner, D. K. (2001). Exercise increases hexokinase II mRNA, but not activity in obesity and type 2 diabetes. *Metab 50*(5), 602-606.

Cusi, K. M. K., Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. (2000). Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest., 105*(3), 311-320.

Dabelea, D., Pettitt, D.J., Jones, K.L. & Arslanian, S.A. (1999). Type 2 diabetes mellitus in minority children and adolescents: an emerging problem. *Endocrinol Metab Clin North Am 28*, 709-729.

Dahl-Jorgensen, K., Brinchmann-Hansen, O., Bangstand, H.J. and Hanssen, K.F. (1994). Blood glucose control and microvascular complications—what do we know *Diabetologia, 37*, 1172-1177.

Dahlquist, G. (1998). The aetiology of type 1 diabetes: an epidemiological perspective. *Acta Paediatrica, 87*, 5-10.

Dandona, P., Aljada, A., & Bandyopadhyay, A. (2004). Inflammation: the link between insulin resistance, obesity and diabetes. *Trends in Immunology, 25*(1), 4-7.

Daniel, M., Martin, A.D., & Carter, J. (1992). Opiate receptor blockade by naltrexone and mood state after acute physical activity. *Br J Sports Med, 26*(2), 111–115.

Davidson, M. B., Schriger, D. L., & Lorber, B. (2001). HbA1c Measurements Do Not Improve the Detection of Type 2 Diabetes in a Randomly Selected Population. Diabet Care, 24, 2017-8.

Davies, C. T., & Thompson, M.W. (1979). Estimated aerobic performance and energy cost of severe exercise of 24 h duration. *Ergonomics, 22*(11), 1249-1255.

Dawson, P. A., Mychaleckyj, J. C., Fossey, S. C., Mihic, S. J., Craddock, A. L., & Bowden, D. W. (2001). Sequence and Functional Analysis of GLUT10: A Glucose Transporter in the Type 2 Diabetes-Linked Region of Chromosome 20q12-13.1. *Molecular Genetics and Metab 74*(1-2), 186-199.

De Filippis, E., Cusi, K., Ocampo, G., Berria, R., Buck, S., Consoli, A. (2006). Exercise-Induced Improvement in Vasodilatory Function Accompanies Increased Insulin Sensitivity in Obesity and Type 2 Diabetes Mellitus. *J Clin Endocrinol & Metab, 91*(12), 4903-4910.

DeFronzo, R. A., Ferrannini, E., Sato, Y., Felig, P., & Wahren, J. (1981). Synergistic interaction between exercise and insulin on peripheral glucose uptake. *J Clin Invest, 68*(6), 1468-1474.

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol Endocrinol Metab. 237, E214-E223.

De Koninck, P. & Schulman, H. (1998). Sensitivity of CaM Kinase II to the Frequency of Ca^{2+} Oscillations. *Science 279*: 227-230

Del Prato, S., Bonadonna R.C., Bonora E., Gulli G., Solini A., Shank M., & DeFronzo R.A. (1993). Characterization of cellular defects of insulin action in type 2 (noninsulin-dependent) diabetes mellitus, *J Clin Invest 81*, 484–494.

Del Prato, S., Matsuda, M., Simonson, D.C., Groop, L.C., Sheehan, P., Leonetti, F., Bonadonna, R.C., DeFronzo, R.A. (1997). Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. *Diabetologia., 40*(6), 687-697.

Dela, F., Larsen, J.J., Mikines, K.J., Ploug, T., Petersen, L.N., & Galbo, H. (1995). Insulin-stimulated muscle glucose clearance in patients with NIDDM. Effects of one-legged physical training. *Diabet, 44*(9), 1010-1020.

Dela, F., Mikines, K. J., Sonne, B., & Galbo, H. (1994). Effect of training on interaction between insulin and exercise in human muscle. *J Appl Physiol, 76*(6), 2386-2393.

Dela, F., von Linstow, M. E., Mikines, K. J., & Galbo, H. (2004). Physical training may enhance β-cell function in type 2 diabetes. *Am J Physiol Endocrinol Metab, 287*(5), E1024-1031.

Department of Health. (2002). *National Service Framework for Diabetes.*(Crown Copyrights), p. 45.

Deshmukh, A., Coffey, V. G., Zhong, Z., Chibalin. A. V., Hawley, J.A. & Zierath, J.R. (2006) Exercise-Induced Phosphorylation of the Novel Akt Substrates AS160 and Filamin A in Human Skeletal Muscle. *Diabet 55*: 1776-1782

Devlin, J. T., Hirshman, M., Horton, E. D., & Horton, E. S. (1987). Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabet, 36*(4), 434-439.

Diabetes: State of the Nations. Progress made in delivering the national diabetes frameworks A report from Diabetes UK. (2006).

Dinneen, S., Gerich, J. & Rizza, R. (1992). Carbohydrate metabolism in non-insulin-dependent diabetes mellitus. *N Engl J Med 327, 327*, 707-713.

Doege, H., Bocianski, A., Scheepers, A., Axer, H., Eckel, J., Joost, H. G. (2001). Characterization of human glucose transporter (GLUT) 11 (encoded by SLC2A11), a novel sugar-transport facilitator specifically expressed in heart and skeletal muscle 359, 443-449

Douen, A., Ramlal T., Rastogi S., Bilan P.J., Cartee G.D., Vranic M., Holloszy J.O., & Klip A. (1990) Exercise induces recruitment of the "insulin-responsive glucose transporter." *J Biol Chem* 265, 13427-13430

Du, X. L., Edelstein, D., Dimmeler, S., Ju, Q., Sui, C., & Brownlee, M. (2001). Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J Clin Invest., 108*(9), 1341-1348.

Duncan, B. B., Schmidt, M. I., Pankow, J. S., Ballantyne, C. M., Couper, D., Vigo, A. (2003). Low-Grade Systemic Inflammation and the Development of Type 2 Diabetes: The Atherosclerosis Risk in Communities Study. *Diabet, 52*(7), 1799-1805.

Duncan, M. H., Singh, B.M., Wise, P.H., Carter, G., & Alaghband-Zadeh, J. (1995). A simple measure of insulin resistance. *Lancet, 346*, 120-121.

Dunstan, D. W., Mori, T.A., Puddey, I.B., Beilin, L.J., Burke, V., Morton, A.R., & Stanton, K.G. (1997). The independent and combined effects of aerobic exercise and dietary fish intake on serum lipids and glycemic control in NIDDM. A randomized controlled study. *Diabet Care, 20*(6), 913-921.

Dunstan, D. W., Puddey, I. B., Beilin, L. J., Burke, V., Morton, A. R., & Stanton, K. G. (1998). Effects of a short-term circuit weight training program on glycaemic control in NIDDM. *Diabet Research and Clinical Practice, 40*(1), 53-61.

Dunstan, D. W., Salmon, J., Healy, G. N., Shaw, J. E., Jolley, D., Zimmet, P. Z. (2007). Association of Television Viewing With Fasting and 2-h Postchallenge Plasma Glucose Levels in Adults Without Diagnosed Diabetes. *Diabet Care, 30*(3), 516-522.

Eaton, S. B., Konner, M., & Shostak, M. (1988). Stone agers in the fast lane: Chronic degenerative diseases in evolutionary perspective. *The Am J of Medicine, 84*(4), 739-749.

Eguez , J. P., del Aguila, L. F., Hernandez, J. M., Williamson, D. L., O'Gorman, D. J., Lewis, R. (2000). Regular exercise enhances insulin activation of IRS-1-associated PI3-kinase in human skeletal muscle. *J Appl Physiol, 88*(2), 797-803.

Eguez, L., Lee, A., Chavez, J. A., Miinea, C. P., Kane, S., Lienhard, G. E. (2005). Full intracellular retention of GLUT4 requires AS160 Rab GTPase activating protein. *Cell Metab 2*(4), 263-272.

Ehrmann, D. A., Kasza, K. Azziz, R., Legro, R.S. & Ghazzi, M.N. (2005). Effects of Race and Family History of Type 2 Diabetes on Metabolic Status of Women with Polycystic Ovary Syndrome. J. Clin. Endocrinol. Metab. 90: 66-71.

Eriksen, L., Dahl-Petersen, I., Haugaard, S., & Dela, F. (2007). Comparison of the effect of multiple short-duration with single long-duration exercise sessions on glucose homeostasis in type 2 diabetes mellitus. *Diabetologia, 50*(11), 2245-2253.

Esposito, K., Nappo, F., Marfella, R., Giugliano, G., Giugliano, F., Ciotola, M. (2002). Inflammatory Cytokine Concentrations Are Acutely Increased by Hyperglycemia in Humans: Role of Oxidative Stress. *Circulation, 106*(16), 2067-2072.

Essen, B., Hagenfeldt, L., & Kaijser, L. (1977). Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man. *J Physiol, 265*(2), 489-506.

Etgen, G. J., Fryburg, D.A., & Gibbs, E.M. (1997). Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent pathway. *Diabet, 46*(11), 1915-1919.

Evans, P., Langley, P., & Gray, D. P. (2008) Diagnosing Type 2 diabetes before patients complain of diabetic symptoms--clinical opportunistic screening in a single general. Family Practice 25(5):376-381

Fang, Z. Y., Sharman, J., Prins, J. B., & Marwick, T. H. (2005). Determinants of Exercise Capacity in Patients With Type 2 Diabetes. *Diabet Care, 28*(7), 1643-1648.

Farese, R. V., Sajan, M. P., & Standaert, M. L. (2005). Insulin-Sensitive Protein Kinases (Atypical Protein Kinase C and Protein Kinase B/Akt): Actions and Defects in Obesity and Type II Diabetes. *Exp Biol Med, 230*(9), 593-605.

Farrell, P. A., Caston, A. L., & Rodd, D. (1991). Changes in insulin response to glucose after exercise training in partially pancreatectomized rats. *J Appl Physiol, 70*(4), 1563-1568.

Febbraio, M. A., Hiscock, N., Sacchetti, M., Fischer, C. P., & Pedersen, B. K. (2004). Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. *Diabet, 53*(7), 1643-1648.

Febbraio, M. A., & Pedersen, B. K. (2002). Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J., 16*(11), 1335-1347.

Febbraio, M. A., Steensberg, A., Starkie, R. L., McConell, G. K., & Kingwell, B. A. (2003). Skeletal muscle interleukin-6 and tumor necrosis factor-α release in healthy subjects and patients with type 2 diabetes at rest and during exercise. *Metab 52*(7), 939-944.

Federici, M., Menghini, R., Mauriello, A., Hribal, M. L., Ferrelli, F., Lauro, D. (2002). Insulin-Dependent Activation of Endothelial Nitric Oxide Synthase Is Impaired by O-Linked Glycosylation Modification of Signaling Proteins in Human Coronary Endothelial Cells. *Circulation, 106*(4), 466-472.

Fell, R. D., Terblanche, S. E., Ivy, J. L., Young, J. C., & Holloszy, J. O. (1982). Effect of muscle glycogen content on glucose uptake following exercise. *J Appl Physiol, 52*(2), 434-437.

Fernandez-Real, J.-M., Vayreda, M., Richart, C., Gutierrez, C., Broch, M., Vendrell, J.(2001). Circulating Interleukin 6 Levels, Blood Pressure, and Insulin Sensitivity in Apparently Healthy Men and Women. *J Clin Endocrinol & Metab, 86*(3), 1154-1159.

Fery, F. (1994). Role of hepatic glucose production and glucose uptake in the pathogenesis of fasting hyperglycemia in type 2 diabetes: normalization of glucose kinetics by short-term fasting. *J Clin Endocrinol Metab, 78*(3), 536-542.

Finegood, D. T., Miles, P. D., Lickley, H. L., & Vranic, M. (1992). Estimation of glucose production during exercise with a one-compartment variable-volume model. *J Appl Physiol, 72*(6), 2501-2509.

Fischer, C. P., Berntsen, A., Perstrup, L. B., Eskildsen, P., & Pedersen, B. K. (2007). Plasma levels of interleukin-6 and C-reactive protein are associated with physical inactivity independent of obesity. *Scand J Med Sci Sports, 17*(5), 580-587.

Fischer, C. P., Perstrup, L. B., Berntsen, A., Eskildsen, P., & Pedersen, B. K. (2005). Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. *Clin Immunol, 117*(2), 152-160.

Fisher, J. S., Gao, J., Han, D.-H., Holloszy, J. O., & Nolte, L. A. (2002). Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab, 282*(1), E18-23.

Fletcher, G. F., Blair, S. N., Blumenthal, J., Caspersen, C., Chaitman, B., Epstein, S.(1992). Statement on exercise. Benefits and recommendations for physical activity programs for all Americans. A statement for health professionals by the Committee on Exercise and Cardiac Rehabilitation of the Council on Clinical Cardiology, American Heart association. *Circulation, 86*(1), 340-344.

Forbes, W. H. (1936). Blood sugar and glucose tolerance at high altitudes. *Am J Physiol, 116*, 309-316.

Frosig, C., Rose, A. J., Treebak, J. T., Kiens, B., Richter, E. A., & Wojtaszewski, J. F. (2007a). Effects of Endurance Exercise Training on Insulin Signaling in Human Skeletal Muscle:

Interactions at the Level of Phosphatidylinositol 3-Kinase, Akt, and AS160. *Diabet, 56*(8), 2093-2102.

Frosig, C., Rose, A. J., Treebak, J. T., Kiens, B., Richter, E. A., & Wojtaszewski, J. F. P. (2007b). Effects of Endurance Exercise Training on Insulin Signaling in Human Skeletal Muscle: Interactions at the Level of Phosphatidylinositol 3-Kinase, Akt, and AS160. *Diabet, 56*(8), 2093-2102.

Gabbay, K. (1973). The sorbitol pathway and the complications of diabetes. *N Eng J Med 288*(16), 831-836.

Gabbay, K., Korff, J., Schneeberger, E. (1975) Vesicular binesis: glucose effect on insulin secretory vesicles. *Science 187*:177-179

Gao, J., Gulve, E. A., & Holloszy, J. O. (1994). Contraction-induced increase in muscle insulin sensitivity: requirement for a serum factor. *Am J Physiol Endocrinol Metab, 266*(2), E186-192.

Garetto, L. P., Richter, E. A., Goodman, M. N., & Ruderman, N. B. (1984a). Enhanced muscle glucose metabolism after exercise in the rat: the two phases. *Am J Physiol Endocrinol Metab, 246*(6), E471-475.

Garetto, L. P., Richter, E. A., Goodman, M. N., & Ruderman, N. B. (1984b). Enhanced muscle glucose metabolism after exercise in the rat: the two phases. *Am J Physiol Endocrinol Metab 246*(6), E471-475.

Garvey, W. T., Olefsky, J.M. & Marshall, S. (1985). Insulin receptor down-regulation is linked to an insulin-induced postreceptor defect in the glucose transport system in rat adipocytes. *J Clin Invest, 76*(1), 22-30.

Gastaldelli, A., Coggan, A. R., & Wolfe, R. R. (1999). Assessment of methods for improving tracer estimation of non-steady-state rate of appearance. *J Appl Physiol, 87*(5), 1813-1822.

Gastaldelli, A., Miyazaki, Y., Pettiti, M., Matsuda, M., Mahankali, S., Santini, E.(2002). Metabolic Effects of Visceral Fat Accumulation in Type 2 Diabetes. *J Clin Endocrinol Metab, 87*(11), 5098-5103.

Giacca, A., Groenewoud, Y., Tsui, E., McClean, P., & Zinman, B. . (1998). Glucose production, utilization, and cycling in response to moderate exercise in obese subjects with type 2 diabetes and mild hyperglycemia *Diabet, 47*, 1763-1770.

Gill, R. M., Braz, J. C., Jin, N., Etgen, G. J., & Shen, W. (2007). Restoration of impaired endothelium-dependent coronary vasodilation in failing heart: role of eNOS phosphorylation and CGMP/cGK-I signalling. *Am J Physiol Heart Circ Physio, 292l* (H2782-2790)

Gill, J. & Cooper A.R. (2008). Physical activity and prevention of type 2 diabetes mellitus. *Sports Med* 38, 807-824

Giugliano, D., Ceriello, A. & Paolisso, G. (1995). Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? *Metab 44*(3), 363-368.

Giugliano, D., Marfella, R., Coppola, L., Verrazzo, G., Acampora, R., Giunta, R.(1997). Vascular Effects of Acute Hyperglycaemia in Humans Are Reversed by L-Arginine: Evidence for Reduced Availability of Nitric Oxide During Hyperglycaemia. *Circulation, 95*, 1783-1790.

Green, S., Askew, C., & Walker, P. (2007). Effect of type 2 diabetes mellitus on exercise intolerance and the physiological responses to exercise in peripheral arterial disease. *Diabetologia, 50*(4), 859-866.

Greene, D. A., Lattimer, S.A., & Sima, A.A. (1988). Are disturbances of sorbitol, phosphoinositide, and Na+-K+-ATPase regulation involved in pathogenesis of diabetic neuropathy? *Diabet, 37*(6), 688-693.

Gregg, S. G., Willis, W. T., & Brooks, G. A. (1989). Interactive effects of anemia and muscle oxidative capacity on exercise endurance. *J Appl Physiol, 67*(2), 765-770.

Griffin, M. E., Marcucci, M. J., Cline, G. W., Bell, K., Barucci, N., Lee, D.(1999). Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signalling cascade. *Diabet, 48*(6), 1270-1274.

Groop, L. & Orho-Melander, M. (2008) New Insights into Impaired Muscle Glycogen Synthesis. *PLoS Med* 5: e25

Gugliucci, A. (2000). Glycation as the glucose link to diabetic complications. *J Am Osteopath Assoc, 100*(10), 621-634.

Hahn, R. A., Teutsch, S. M., Rothenberg, R. B., & Marks, J. S. (1990). Excess deaths from nine chronic diseases in the United States, 264, 2654-2659.

Hall, C. J., & Lane, A. M. (2001). Effects of rapid weight loss on mood and performance among amateur boxers. *Br J Sports Med, 35*(6), 390-395.

Halse, R., Bonavaud, S. M., Armstrong, J. L., McCormack, J.G.& Yeaman, S. J. (2001) Control of Glycogen Synthesis by Glucose, Glycogen, and Insulin in Cultured Human Muscle Cells. *Diabet 50*: 720-726

Hamburg, N. M., McMackin, C. J., Huang, A. L., Shenouda, S. M., Widlansky, M. E., Schulz, E. (2007). Physical Inactivity Rapidly Induces Insulin Resistance and Microvascular Dysfunction in Healthy Volunteers. *Arterioscler Thromb Vasc Biol, 27*, 2650-2656.

Hamid, Y. H., Urhammer, S. A., Jensen, D. P., Glumer, C., Borch-Johnsen, K., Jorgensen, T. (2004). Variation in the Interleukin-6 Receptor Gene Associates With Type 2 Diabetes in Danish Whites. *Diabet, 53*(12), 3342-3345.

Hansen, P. A., Nolte, L. A., Chen, M. M., & Holloszy, J. O. (1998). Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. *J Appl Physiol, 85*(4), 1218-1222.

Hardie, D. G., and D. Carling. (1997). The AMP-activated protein kinase. Fuel gauge of the mammalian cell? *Eur J Biochem, 246*, 259-273.

Hardie, D. G., Carling, D. & Carlson, M. (1998). The AMP-Activated/SNF1 Protein Kinase Subfamily: Metabolic Sensors of the Eukaryotic Cell? *Ann Rev Bioch 67*: 821-855

Hardie, D. G. (2004). AMP-Activated Protein Kinase: A Master Switch in Glucose and Lipid Metabolism. *Rev Endocr Metab Dis, 5*(2), 119-125.

Hardie, D. G., Hawley, S. A., & Scott, J. W. (2006). AMP-activated protein kinase - development of the energy sensor concept. *J Physiol (Lond), 574*(1), 7-15.

Hargreaves, M. & Spirit, L. (2006). Exercise Metabolism. *Champaign, IL : Human Kinetics*, 2 Ed.

Harvard Report on Cancer Prevention. Causes of human cancer. (1996). *Cancer Causes Control, 7* (1), S3-59.

Haskell, W., Lee, I.M., Pate, R.R., Powell, K.E., Blair, S.N., Franklin, B.A., Macera, C.A., Heath, G.W., Thompson, P.D. & Bauman, A. (2007). Physical Activity and Public Health: Updated Recommendation for Adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 39, 1423-1434

Hawkins, M., Hu, M., Yu, J., Eder, H., Vuguin, P., She, L. (1999). Discordant Effects of Glucosamine on Insulin-stimulated Glucose Metabolism and Phosphatidylinositol 3-Kinase Activity. *J Biol Chem, 274*(44), 31312-31319.

Hawley, S., Boudeau, J., Reid, J., Mustard, K., Udd, L., Makela, T., Alessi, D. & Hardie, D. G. (2003). Complexes between the LKB1 tumor suppressor, STRAD α/β and MO25 α/β are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2: 28

Hawley, S. A., Pan, D. A., Mustard, K. J., Ross, L., Bain, J., Edelman, A. M., Frenguelli, B. G. & Hardie, D. G. (2005). Calmodulin-dependent protein kinase kinase-β is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab 2*: 9-19

Hayashi, T., Wojtaszewski, J. F. P., & Goodyear, L. J. (1997). Exercise regulation of glucose transport in skeletal muscle. *Am J Physiol Endocrinol Metab, 273*(6), E1039-1051.

Hayashi, Y., Nagasaka, S., Takahashi, N., Kusaka, I., Ishibashi, S., Numao, S. (2005). A Single Bout of Exercise at Higher Intensity Enhances Glucose Effectiveness in Sedentary Men. *J Clin Endocrinol Metab, 90*(7), 4035-4040.

Heller, J. B. (2007). Metformin overdose in dogs and cats *Veterinary Medicine*, 231-233.

Henquin, J. C. (2000). Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabet, 49*(11), 1751-1760.

Henriksen, E. J. (2002). Exercise Effects of Muscle Insulin Signaling and Action: Invited Review: Effects of acute exercise and exercise training on insulin resistance. *J Appl Physiol, 93*(2), 788-796.

Henriksen, E. J., & Dokken, B. B. (2006). Role of Glycogen Synthase Kinase-3 in Insulin Resistance and Type 2 Diabetes. *Current Drug Targets, 7*, 1435-1441.

Herman, M. A., & Kahn, B. B. (2006). Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *J Clin Invest, 116*(7), 1767-1775.

Hevener, A. L., Reichart, D., & Olefsky, J. (2000). Exercise and thiazolidinedione therapy normalize insulin action in the obese Zucker fatty rat. *Diabet, 49*(12), 2154-2159.
Higaki, Y., Kagawa, T., Fujitani, J., Kiyonaga, A., Shindo, M., Taniguchi, A. (1996). Effects of a single bout of exercise on glucose effectiveness. *J Appl Physiol, 80*(3), 754-759.
Hildebrandt, A. L., Pilegaard, H., & Neufer, P. D. (2003). Differential transcriptional activation of select metabolic genes in response to variations in exercise intensity and duration. *Am J Physiol Endocrinol Metab, 285*(5), E1021-1027.
Hill, A. V., & Lupton, H. (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Quart J Med, 16*, 135.
Hochachka, P. (1992). Muscle enzymatic composition and metabolic regulation in high altitude adapted natives. *Int J Sports Med, 13*(1), S89-91.
Holloszy, J. O. & Narahara, H. T. (1967). Enhanced permeability to sugar associated with muscle contraction. Studies of the role of Ca^{++}. *J Gen Physiol 50*: 551-562
Holloszy, J. O. (2003). A forty-year memoir of research on the regulation of glucose transport into muscle. *Am J Physiol Endocrinol Metab, 284*(3), E453-467.
Holm, G., Bjorntorp, P., & Jagenburg, R. (1978). Carbohydrate, lipid and amino acid metabolism following physical exercise in man. *J Appl Physiol, 45*(1), 128-131.
Holmang, A., Bjorntorp, P., & Rippe, B. (1992). Tissue uptake of insulin and inulin in red and white skeletal muscle in vivo. *Am J Physiol Heart Circ Physiol, 263*(4), H1170-1176.

Hopkins, W.G. (2000). Measures of reliability in sports medicine and science. *Sport Med 30*, 1-15

Hopkins, W. (2003). A spreadsheet for analysis of straightforward controlled trials [Online]. Sport Science. http://www.sportsci.org/jour/03/wghtrials.htm.
Hordern, M. D., Cooney, L.M., Beller, E.M., Prins, J.B., Marwick, T.H., & Coombes, J.S. (2008). Determinants of changes in blood glucose response to short-term exercise training in patients with type 2 diabetes. *Clin Sci (Lond)*, 115, 273–281
Hotamisligil, G. S., & Spiegelman, B.M. (1994). Tumor necrosis factor-α: a key component of the obesity-diabetes. *Diabet, 43*(11), 1271-1278.
Hotamisligil, G. S., Budavari, A., Murray, D., & Spiegelman, B.M. (1994). Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. *J Clin Invest, 94*(4), 1543-1549.
Hother-Nielsen, O., Henriksen, J. E., Holst, J. J., & Beck-Nielsen, H. (1996). Effects of insulin on glucose turnover rates in vivo: Isotope dilution versus constant specific activity technique. *Metab 45*(1), 82-91.
Houmard, J. A., Hickey, M. S., Tyndall, G. L., Gavigan, K. E., & Dohm, G. L. (1995). Seven days of exercise increase GLUT-4 protein content in human skeletal muscle. *J Appl Physiol, 79*(6), 1936-1938.
Houmard, J. A., Shinebarger, M. H., Dolan, P. L., Leggett-Frazier, N., Bruner, R. K., McCammon, M. R. (1993). Exercise training increases GLUT-4 protein concentration in previously sedentary middle-aged men. *Am J Physiol Endocrinol Metab, 264*(6), E896-901.
Houmard, J. A., Tanner, C. J., Slentz, C. A., Duscha, B. D., McCartney, J. S., & Kraus, W. E. (2004). Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol, 96*(1), 101-106.
Houston, M.E. (2006). Biochemistry Primer for Exercise Science, 3rd Ed.
Howlett, K., Febbraio, M., & Hargreaves, M. (1999). Glucose production during strenuous exercise in humans: role of epinephrine. *Am J Physiol Endocrinol Metab, 276*(6), E1130-1135.
Howlett, K., Galbo, H., Lorentsen, J., Bergeron, R., Zimmerman-Belsing, T., Bulow, J. (1999). Effect of adrenaline on glucose kinetics during exercise in adrenalectomised humans. *J Physiol 519*(3), 911-921.
Howlett, K. F., Sakamoto, K., Garnham, A., Cameron-Smith, D., & Hargreaves, M. (2007). Resistance Exercise and Insulin Regulate AS160 and Interaction With 14-3-3 in Human Skeletal Muscle. *Diabet, 56*(6), 1608-1614.
Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G. (2001). Diet, Lifestyle, and the Risk of Type 2 Diabetes Mellitus in Women. *N Engl J Med, 345*(11), 790-797.

Itani, S. I., Saha, A. K., Kurowski, T. G., Coffin, H. R., Tornheim, K., & Ruderman, N. B. (2003). Glucose Autoregulates Its Uptake in Skeletal Muscle: Involvement of AMP-Activated Protein Kinase. *Diabet, 52*(7), 1635-1640.

James, D. E., Kraegen, E. W., & Chisholm, D. J. (1985). Muscle glucose metabolism in exercising rats: comparison with insulin stimulation. *Am J Physiol Endocrinol Metab, 248*(5), E575-580.

Jayagopal, V., Kilpatrick, E. S., Jennings, P. E., Hepburn, D. A., & Atkin, S. L. (2002). Biological Variation of Homeostasis Model Assessment-Derived Insulin Resistance in Type 2 Diabetes. *Diabet Care, 25*(11), 2022-2025.

Jenssen, T. E., Rose, A. J., Jorgensen, S. B., Brandt, N., Schjerling, P., Wojtaszewski, J. F. (2007). Possible CaMKK-dependent regulation of AMPK phosphorylation and glucose uptake at the onset of mild tetanic skeletal muscle contraction. *Am J Physiol Endocrinol Metab, 292*(5), E1308-1317.

Jessen, N., & Goodyear, L. J. (2005). Contraction signaling to glucose transport in skeletal muscle. *J Appl Physiol, 99*(1), 330-337.

Jing, M., Cheruvu, V. K., & Ismail-Beigi, F. (2008). Stimulation of Glucose Transport in Response to Activation of Distinct AMPK Signaling Pathways. *Am J Physiol Cell Physiol* 00040.02008.

Joffe, B., & Zimmet, P. (1998). The thrifty genotype in type 2 diabetes. *Endocrine, 9*(2), 139-141.

John, A. H. (2004). Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance. *Diabet/Metab Res Rev, 20*(5), 383-393.

John, S. Y., Meena, K., Steve, E. H., & Vidya, M. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis, 148*(2), 209-214.

Johnson, D., Shepherd, R. M., Gill, D., Gorman, T., Smith, D. M., & Dunne, M. J. (2007). Glucokinase activators: molecular tools for studying the physiology of insulin-secreting cells. *Biochem Soc Trans, 035*(5), 1208-1210.

Johnson, H. L., Consolazio, C.F., Burk, R.F., & Daws TA. (1974). Glucose-14 C-UL metabolism in man after abrupt altitude exposure (4,300 m). *Aerosp Med, 45*(8), 849-854.

Jones, A. M., & Carter, H. (2000). The Effect of Endurance Training on Parameters of Aerobic Fitness *Sport Med, 29*(6), 373-386.

Jorgensen, S. B., Viollet, B., Andreelli, F., Frosig, C., Birk, J. B., Schjerling, P., Vaulont, S., Richter, E. A., Wojtaszewski, J. F. (2004). Knockout of the α2 but Not α1 5'-AMP-activated Protein Kinase Isoform Abolishes 5-Aminoimidazole-4-carboxamide-1-β-4-ribofuranosidebut Not Contraction-induced Glucose Uptake in Skeletal Muscle. *J Biol Chem 279:* 1070-1079

Kahn, S. E. (2003). The relative contributions of insulin resistance and β-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia, 46*(1), 3-19.

Kahn, S. E., Larson, V. G., Beard, J. C., Cain, K. C., Fellingham, G. W., Schwartz, R. S. (1990). Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. *Am J Physiol Endocrinol Metab, 258*(6), E937-943.

Kamimura, D., Ishihara, K., & Hirano, T. (2004). IL-6 signal transduction and its physiological roles: the signal orchestration model. *Rev Physiol, Biochem Pharmacol* (1-38).

Kane, S., Sano, H., Liu, S., Asara, J. M., Lane, W., Garner, C. C. & Lienhard, G. E. (2002) A Method to Identify Serine Kinase Substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activation protein (GAP) domain. *J Biol Chem 277*: 22115-22118

Kang, J., Kelley, D.E., Roberton, E., Robert, J., Goss, F.L., Suminski, R., & Utter, A.C. (1999). Substrate utilization and glucose turnover during exercise of varying intensities in individuals with NIDDM. *Med Sci Sport Exer , 31*(1), 82-89.

Kang, J., Robertson, R.J., Hagberg, J.M., Kelley, D.E., Goss, F.L., DaSilva, S.G., Suminski, R.R., & Utter, A.C. (1996). Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabet Care., 19*(4), 341-349.

Karlsson, H. K., Zierath, J. R., Kane, S., Krook, A., Lienhard, G. E., & Wallberg-Henriksson, H. (2005). Insulin-Stimulated Phosphorylation of the Akt Substrate AS160 Is Impaired in Skeletal Muscle of Type 2 Diabetic Subjects. *Diabet, 54*(6), 1692-1697.

Katsuki, A., Sumida, Y., Gabazza, E. C., Murashima, S., Furuta, M., Araki-Sasaki, R. (2001). Homeostasis Model Assessment Is a Reliable Indicator of Insulin Resistance During Follow-up of Patients With Type 2 Diabetes. *Diabet Care, 24*(2), 362-365.

Katz, E. B., Stenbit, A.E., Hatton, K., DePinho, R., & Charron, M.J. (1995). Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature, 14*(377), 151-155.

Katzen, H. M., Soderman, D. D., & Wiley, C. E. (1970). Multiple forms of hexokinase. Activities associated with subcellular particulate and soluble fractions of normal and streptozotocin diabetic rat tissues. *J Biol Chem, 245*(16), 4081-4096.

Kearney, M. T., Duncan, E. R., Kahn, M., & Wheatcroft, S. B. (2008). Insulin resistance and endothelial cell dysfunction: studies in mammalian models. *Exp Physiol, 93*(1), 158-163.

Keller, C., Steensberg, A., Hansen, A. K., Fischer, C. P., Plomgaard, P., & Pedersen, B. K. (2005). Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J Appl Physiol, 99*(6), 2075-2079.

Kellerer, M., Koch, M., Metzinger, E., Mushack, J., Capp, E., & HÃ¤ring, H. U. (1997). Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia, 40*(11), 1358-1362.

Kelley, D. E., He, J., Menshikova, E.V., & Ritov, V.B. (2002). Dysfunction of Mitochondria in Human Skeletal Muscle in Type 2 Diabetes. *Diabet, 51*(10), 2944-2950.

Kelly, M., Keller, C., Avilucea, P. R., Keller, P., Luo, Z., Xiang, X. (2004). AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun, 320*(2), 449-454.

Kennedy, J. W., Hirshman, M. F., Gervino, E. V., Ocel, J. V., Forse, R. A., Hoenig, S. J. (1999). Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabet, 48*(5), 1192-1197.

Kim, Y.-B., Inoue, T., Nakajima, R., Shirai-Morishita, Y., Tokuyama, K., & Suzuki, M. (1999). Effect of Long-Term Exercise on Gene Expression of Insulin Signaling Pathway Intermediates in Skeletal Muscle. *Biochem Biophys Res Commun, 254*(3), 720-727.

Kim, Y. B., Inoue, T., Nakajima, R., Nakae, K., Tamura, T., Tokuyama, K. (1995). Effects of Endurance Training of Gene Expression on Insulin Signal Transduction Pathway. *Biochem Biophys Res Commun, 210*(3), 766-773.

King, H., Aubert, R. E., & Herman, W. H. (1998). Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabet Care 21*(9), 1414-1431).

King, T. D., Gandy, J. C., Bijur, G. N. (2006) The protein phosphatase-1/inhibitor-2 complex differentially regulates GSK3 dephosphorylation and increases sarcoplasmic/endoplasmic reticulum calcium ATPase 2 levels. *Experimental Cell Research 312*:3693-3700

Kjaer, M., Engfred, K., Fernandes, A., Secher, N. H., & Galbo, H. (1993). Regulation of hepatic glucose production during exercise in humans: role of sympathoadrenergic activity. *Am J Physiol Endocrinol Metab, 265*(2), E275-283.

Kjaer, M., Hollenbeck, C. B., Frey-Hewitt, B., Galbo, H., Haskell, W., & Reaven, G. M. (1990). Glucoregulation and hormonal responses to maximal exercise in non-insulin-dependent diabetes. *J Appl Physiol, 68*(5), 2067-2074.

Kjaer, M., Kiens, B., Hargreaves, M., & Richter, E. A. (1991). Influence of active muscle mass on glucose homeostasis during exercise in humans. *J Appl Physiol, 71*(2), 552-557.

Koh, H., Brandauer, J. & Goodyear, L. J. (2008). LKB1 and AMPK and the regulation of skeletal muscle metabolism. *Curr Opinion Clin Nutrit & Metab Care 11:* 227-232

Koistinen, H. A., Galuska, D., Chibalin, A. V., Yang, J., Zierath, J. R., Holman, G. D. (2003). 5-Amino-Imidazole Carboxamide Riboside Increases Glucose Transport and Cell-Surface GLUT4 Content in Skeletal Muscle From Subjects With Type 2 Diabetes. *Diabet 52*(5), 1066-1072.

Kolesnik, Y., Seredenko, M., & Abramov, A. (1995). Adaptation to hypoxia stimulates the pancreatic islet apparatus in intact and diabetic rats. *Bulletin of Exp Biol Med, 120*(6), 1183-1185.

Konstantinos, N., Faidon, M., Nikos, G., & Labros, S. (2005). Insulin sensitivity derived from oral glucose tolerance testing in athletes: Disagreement between available indices. *J Sport Sci, 23*(10), 1065-1073.

Koval, J. A., DeFronzo, R. A., O'Doherty, R. M., Printz, R., Ardehali, H., Granner, D. K. (1998). Regulation of hexokinase II activity and expression in human muscle by moderate exercise. *Am J Physiol Endocrinol Metab, 274*(2), E304-308.

Kraegen, E. W., Saha, A. K., Preston, E., Wilks, D., Hoy, A. J., Cooney, G. J. (2006). Increased malonyl-CoA and diacylglycerol content and reduced AMPK activity accompany insulin resistance induced by glucose infusion in muscle and liver of rats. *Am J Physiol Endocrinol Metab 290*(3), E471-479.

Kramer, H. F., Witczak, C. A., Taylor, E. B., Fujii, N., Hirshman, M. F. & Goodyear, L. J. (2006a). AS160 Regulates Insulin- and Contraction-stimulated Glucose Uptake in Mouse Skeletal Muscle. *J Biol Chem 281*: 31478-31485

Kramer, H. F., Witczak, C. A., Fujii, N., Jessen, N., Taylor, E.B., Arnolds, D. E., Sakamoto, K., Hirshman, M. F. & Goodyear, L.J. (2006b). Distinct Signals Regulate AS160 Phosphorylation in Response to Insulin, AICAR, and Contraction in Mouse Skeletal Muscle. *Diabet 55:* 2067-2076

Kraniou, G. N., Cameron-Smith, D., & Hargreaves, M. (2006). Acute exercise and GLUT4 expression in human skeletal muscle: influence of exercise intensity. *J Appl Physiol, 101*(3), 934-937.

Kristiansen, O. P., & Mandrup-Poulsen, T. (2005). Interleukin-6 and Diabetes: The Good, the Bad, or the Indifferent? *Diabet 54*(suppl 2), S114-124.

Krolewski, A. S., Kosinski, E. J., Warram, J. H., Stevens Leland, O., Busick, E. J., Cader Asmal, A. (1987). Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *Am J Cardiol, 59*(8), 750-755.

Krook, A., Bjornholm, M., Galuska, D., Jiang, X. J., Fahlman, R., Myers, M. G. (2000). Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabet 49*(2), 284-292.

Krook, A., Roth, R. A., Jiang, X. J., Zierath, J. R., & Wallberg-Henriksson, H. (1998). Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects. *Diabet 47*(8), 1281-1286.

Krotkiewski, M., Lönnroth, P., Mandroukas, K., Wroblewski, Z., Rebuffé-Scrive, M., Holm, G., Smith, U., & Björntorp, P. (1985). The effects of physical training on insulin secretion and effectiveness and on glucose metabolism in obesity and type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia, 28*(12), 881-890.

Kullmer, T. G. H., Jungmann E, Haak T, Morbitzer D, Usadel KH, Kindermann W. (1995). Increase of serum insulin and stable c-peptide concentrations with exhaustive incremental graded exercise during acute hypoxia in sedentary subjects. *Exp Clin Endocrinol Diabet 103*(3), 156-161.

Lajoie, C., Calderone, A., Trudeau, F., Lavoie, N., Massicotte, G., Gagnon, S. (2004). Exercise training attenuated the PKB and GSK-3 dephosphorylation in the myocardium of ZDF rats. *J Appl Physiol, 96*(5), 1606-1612.

Lange, A. J., Argaud, D., Elmaghrabi, M. R., Pan, W. S., Maitra, S. R., & Pilkis, S. J. (1994). Isolation of a cDNA for the Catalytic Subunit of Rat Liver Glucose-6-Phosphatase: Regulation of Gene Expression in FAO Hepatoma Cells by Insulin, Dexamethasone and cAMP. *Biochem Biophys Res Commun, 201*(1), 302-309.

Larkin, M. (2001). Diet and exercise delay onset of type 2 diabetes, say US experts. *Lancet, 358*, 565.

Larsen, J., Dela, F., Madsbad, S., & Galbo, H. (1999). The effect of intense exercise on postprandial glucose homeostasis in Type II diabetic patients. *Diabetologia, 42*(11), 1282-1292.

Larsen, J., Dela, F., Kjaer, M., & Galbo, H. (1997a). The effect of moderate exercise on postprandial glucose homeostasis in NIDDM patients. *Diabetologia, 40*(4), 447-453.

Larsen, J., Hansen, J.M., Olsen, N.V., Galbo, H., & Dela, F. (1997b). The effect of altitude hypoxia on glucose homeostasis in men. *J Physiol (Lond), 504*(1), 241-249.

LeBlanc, J., Nadeau, A., Richard, D., & Tremblay, A. (1981). Studies on the sparing effect of exercise on insulin requirements in human subjects. *Metab 30*(11), 1119-1124.

Lee, W.-C., Chen, J.-J., Ho, H.-Y., Hou, C.-W., Liang, M.-P., Shen, Y.-W. (2003). Short-term Altitude Mountain Living Improves Glycemic Control. *High Alt Med Biol, 4*(1), 81-91.

Lee, A. D., Hansen, P. A. & Holloszy, J. O. (1995). Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. *FEBS Letters 361*: 51-54

Lee, S., Kuk, J. L., Davidson, L. E., Hudson, R., Kilpatrick, K., Graham, T. E. (2005). Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. *J Appl Physiol, 99*(3), 1220-1225.

Lichtenstein, P., Holm, N. V., Verkasalo, P. K., Iliadou, A., Kaprio, J., Koskenvuo, M. (2000). Environmental and Heritable Factors in the Causation of Cancer -- Analyses of Cohorts of Twins from Sweden, Denmark, and Finland. *N Engl J Med 343(2*), 78-85.

Lindstrom, J., Ilanne-Parikka, P., Peltonen, M., Aunola, S., Eriksson, J. G., Hemio, K. (2006). Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet, 368*(9548), 1673-1679.

Lira, V. A., Soltow, Q. A., Long, J. H. D., Betters, J. L., Sellman, J. E., & Criswell, D. S. (2007). Nitric oxide increases GLUT4 expression and regulates AMPK signaling in skeletal muscle. *Am J Physiol Endocrinol Metab, 293*(4), E1062-1068.

Lisinski, I., SchÃrmann, A., Joost, H. G., Cushman, S. W., & Al-Hasani, H. (2001). Targeting of GLUT6 (formerly GLUT9) and GLUT8 in rat adipose cells. *Biochem J, 358*, 517-522.

Lopes-Virella, M. F. V., G. (1996). Cytokines, modified lipoproteins, and arteriosclerosis in diabetes. *Diabet 45*(3), S40-44.

Lopez-Barneo, J., del Toro, R., Levitsky, K. L., Chiara, M. D., & Ortega-Saenz, P. (2004). Regulation of oxygen sensing by ion channels. *J Appl Physiol, 96*(3), 1187-1195.

Lund, S., Holman, G. D., Zierath, J. R., Rincon, J., Nolte, L. A., Clark, A. E. (1997). Effect of insulin on GLUT4 cell surface content and turnover rate in human skeletal muscle as measured by the exofacial bis-mannose photolabeling technique. *Diabet 46*(12), 1965-1969.

Macdonald, A. L., Harrison, M., Bone, A.J. & Watt, P.W. (2004). Even Moderate Intensity Exercise Improves Glucose Utilisation in Type 2 Diabetes. *Diabet Med; J Diabetes UK, Abstract 223*.

Macdonald, A. L., Philp, A., Harrison, M., Bone, A.J. & Watt, P.W. (2006). Monitoring Exercise-Induced Changes in Glycemic Control in Type 2 Diabetes. *Med Sci Sport Exer 38*(2), 201-207.

Mahadik, S. R., Deo, S.S., & Mehtalia, S.D. (2008). Association of adiposity, inflammation and atherosclerosis: the role of adipocytokines and CRP in Asian Indian subjects.Mahadik SR, Deo SS, Mehtalia SD. *Metab Syndr Relat Disord, 6*(2), 2008.

Maiorana, A., O'Driscoll, G., Taylor, R., & Green, D. (2003). Exercise and the nitric oxide vasodilator system. *Sports Med, 33*(14), 1013-1035.

Makino, N., Maeda, T., Sugano, M., Satoh, S., Watanabe, R., & Abe, N. (2005). High serum TNF-α level in Type 2 diabetic patients with microangiopathy is associated with eNOS down-regulation and apoptosis in endothelial cells. *J Diabet Compl, 19*(6), 347-355.

Manson, J. E., Hu, F. B., Rich-Edwards, J. W., Colditz, G. A., Stampfer, M. J., Willett, W. C. (1999). A Prospective Study of Walking as Compared with Vigorous Exercise in the Prevention of Coronary Heart Disease in Women. *N Engl J Med, 341*(9), 650-658.

Mari, A., Pacini, G., Murphy, E., Ludvik, B., & Nolan, J. J. (2001). A Model-Based Method for Assessing Insulin Sensitivity From the Oral Glucose Tolerance Test. *Diabet Care, 24*(3), 539-548.

Markoff, R. A., Ryan, P., & Young, T. (1982). Endorphins and mood changes in long-distance running. *Med Sci Sports Exerc, 14*(1), 11-15.

Martin, B. C., Warram, J. H., Krolewski, A. S., Soeldner, J. S., Kahn, C. R., & Bergman, R. N. (1992). Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet, 340*(8825), 925-929.

Martin, I. K., Katz, A., & Wahren, J. (1995). Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am J Physiol Endocrinol Metab, 269*(3), E583-590.

Matthews, J. N. S., Altman, D.G., Campbell, M.J., & Royston, P. (1990). Analysis of serial measurements in medical research. *Brit Med J 27*, 230-235.

Mathoo, J. M., Shi, Z. Q., Klip, A. & Vranic, M. (1999) Opposite effects of acute hypoglycemia and acute hyperglycemia on glucose transport and glucose transporters in perfused rat skeletal muscle. *Diabet 48:* 1281-1288

Mazzeo, R. S., Bender, P. R., Brooks, G. A., Butterfield, G. E., Groves, B. M., Sutton, J. R., Wolfel, E. E., & Reeves, J. T. (1991) Arterial catecholamine responses during exercise with acute and chronic high-altitude exposure. *Am J Physiol Endocrinol Metab* 261, E419-424

Mazzeo, R. S., Child, A., Butterfield, G. E., Braun, B., Rock, P. B., Wolfel, E. E., Zamudio, S., & Moore, L. G. (2000) Sympathoadrenal responses to submaximal exercise in women after acclimatization to 4,300 meters. *Metabolism* 49, 1036-1042

McBride, A., Ghilagaber, S., Nikolaev, A. & Hardie, D. G. (2009) The Glycogen-Binding Domain on the AMPK-β Subunit Allows the Kinase to Act as a Glycogen Sensor. *Cell Metab 9*: 23-34

Mcguire, E. A., Helderman, J.H, Tobin, J.D, Anders, R. & Berman, M. (1976). Effects of arterial versus venous sampling on anaylsis of glucose kintetics in man. *J Appl Physiol, 41*(4), 565-573.

Merrill, G. F., Kurth, E. J., Hardie, D. G., & Winder, W. W. (1997) AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol Endocrinol Metab* 273, E1107-1112

Meyer, C., Dostou, J., Nadkarni, V., & Gerich, J. (1998). Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. *Am J Physiol Renal Physiol, 275*(6), F915-921.

Mikines, K. J., Farrell, P. A., Sonne, B., Tronier, B., & Galbo, H. (1988). Postexercise dose-response relationship between plasma glucose and insulin secretion. *J Appl Physiol, 64*(3), 988-999.

Milan, D., Jeon, J., Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronne, H., Reinsch, N., Gellin, J., Kalm, E., Roy, P., Chardon, P. & Andersson, L. (2000) A Mutation in PRKAG3 Associated with Excess Glycogen Content in Pig Skeletal Muscle. *Science 288*: 1248-1251

Minuk, H. L., Vranic, M., Marliss, E. B., Hanna, A. K., Albisser, A. M., & Zinman, B. (1981). Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. *Am J Physiol Endocrinol Metab, 240*(5), E458-464.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S. (1997). Subcutaneous Adipose Tissue Releases Interleukin-6, But Not Tumor Necrosis Factor-α, in Vivo. *J Clin Endocrinol Metab, 82*(12), 4196-4200.

Monauni, T., Zenti, M. G., Cretti, A., Daniels, M. C., Targher, G., Caruso, B. (2000). Effects of glucosamine infusion on insulin secretion and insulin action in humans. *Diabet 49*(6), 926-935.

Mu, J., Brozinick, J. J. T., Valladares, O., Bucan, M., & Birnbaum, M. J. (2001). A Role for AMP-Activated Protein Kinase in Contraction- and Hypoxia-Regulated Glucose Transport in Skeletal Muscle. *Molecular Cell, 7*(5), 1085.

Muscelli, E., Mari, A., Natali, A., Astiarraga, B. D., Camastra, S., Frascerra, S. (2006). Impact of incretin hormones on β-cell function in subjects with normal or impaired glucose tolerance. *Am J Physiol Endocrinol Metab, 291*(6), E1144-1150.

Musi, N. F., N. Hirshman, M.F, Ekberg, I. Froberg, S. Ljungqvist, O. Thorell, A. & Goodyear, L.J. (2001). AMP-Activated Protein Kinase (AMPK) is activated in muscle of subjects with Type 2 Diabetes During Exercise. *Diabet 50*(921-927).

Nathan, D. M., Singer, D.E., Hurxthal, K. & Goodson, J.D. (1984). The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med, 1984*(9), 310-316.

National Diabetes Data Group. (1979). Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabet 28*: 1039-1057.

National Institute for Health and Clinical Excellence (NICE). (2006). Obesity: the prevention, identification, assessment and management of overweight and obesity in adults and children. London: NICE. www.nice.org.uk/guidance/CG43.

Neel, J. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet, 14*, 353-362.

Neel, J., Weder AB, Julius S. (1998). Type II diabetes, essential hypertension, and obesity as "syndromes of impaired genetic homeostasis": the "thrifty genotype" hypothesis enters the 21st century. *Perspect Biol Med, 42*(1), 44-74.

Neel, J. V. (1999). The "thrifty genotype" in 1998. *Nutr Rev, 57(5)*(2-9.).

Nesher, R., Karl, I. E. & Kipnis, D. M. (1985). Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *Am J Physiol Cell Physiol 249:* C226-232

Nishida, Y., Higaki, Y., Tokuyama, K., Fujimi, K., Kiyonaga, A., Shindo, M. (2001). Effect of Mild Exercise Training on Glucose Effectiveness in Healthy Men. *Diabet Care, 24*(6), 1008-1013.

Nishida, Y., Tokuyama, K., Nagasaka, S., Higaki, Y., Shirai, Y., Kiyonaga, A. (2004). Effect of Moderate Exercise Training on Peripheral Glucose Effectiveness, Insulin Sensitivity, and

Endogenous Glucose Production in Healthy Humans Estimated by a Two-Compartment-Labeled Minimal Model. *Diabet 53*(2), 315-320.

Nishizuka, Y. (1995). Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J 9*: 484-496

Njoroge, F. G., & Monnier, V.M. (1989). The chemistry of the Maillard reaction under physiological conditions: a review. *Prog Clin Biol Res, 304*, 85-107.

O'Gorman, D., Karlsson HK, McQuaid S, Yousif O, Rahman Y, Gasparro D, Glund S, Chibalin AV, Zierath JR, Nolan JJ. (2006). Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. *Diabetologia, 49*(12), 2983-2992.

Obici S, W. J., Chowdury R, Feng Z, Siddhanta U, Morgan K, Rossetti L. (2002). Identification of a biochemical link between energy intake and energy expenditure. *J Clin Invest, 109*(12), 1599-1605.

Ofei, F., Hurel, S., Newkirk, J., Sopwith, M., & Taylor, R. (1996). Effects of an engineered human anti-TNF-α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabet 45*(7), 881-885.

Ojuka, E. O., Jones, T. E., Nolte, L. A., Chen, M., Wamhoff, B. R., Sturek, M. & Holloszy, J. O. (2002). Regulation of GLUT4 biogenesis in muscle: evidence for involvement of AMPK and Ca^{2+}. *Am J Physiol Endocrinol Metab 282:* E1008-1013

Oltmanns, K. M., Gehring, H., Rudolf, S., Schultes, B., Rook, S., Schweiger, U. (2004). Hypoxia Causes Glucose Intolerance in Humans. *Am J Respir Crit Care Med, 169*(11), 1231-1237.

Ouiddir, A., Planes, C., Fernandes, I., VanHesse, A., & Clerici, C. (1999). Hypoxia Upregulates Activity and Expression of the Glucose Transporter GLUT1 in Alveolar Epithelial Cells. *Am J Respir Cell Mol. Biol., 21*(6), 710-718.

Pan, X. R., Yang, W.Y., Li, G.W., & Liu, J. (1997). Prevalence of diabetes and its risk factors in China, 1994. National Diabetes Prevention and Control Cooperative Group. *Diabetes Care, 20(11)*, 1664-1669.

Park, S., Hong, S. M., Lee, J. E., & Sung, S. R. (2007). Exercise improves glucose homeostasis that has been impaired by a high-fat diet by potentiating pancreatic β-cell function and mass through IRS2 in diabetic rats. *J Appl Physiol 103*(5), 1764-1771.

Parolin, M. L., Spriet, L. L., Hultman, E., Hollidge-Horvat, M. G., Jones, N. L., & Heigenhauser, G. J. F. (2000). Regulation of glycogen phosphorylase and PDH during exercise in human skeletal muscle during hypoxia. *Am J Physiol Endocrinol Metab, 278*(3), E522-534.

Pate, R. R., Pratt. M., Blair, S.N., Haskell, W.L., Macera, C.A., Bouchard, C., Buchner, D., Ettinger, W., Heath, G.W., King, A.C. (1995). Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA, 273*(5), 402-407.

Pedersen, M., Bruunsgaard, H., Weis, N., Hendel, H. W., Andreassen, B. U., Eldrup, E. (2003). Circulating levels of TNF-α and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. *Mech Ageing Develop, 124*(4), 495-502.

Pendergrass, M., Koval, J., Vogt, C., Yki-Jarvinen, H., Iozzo, P., Pipek, R. (1998). Insulin-induced hexokinase II expression is reduced in obesity and NIDDM. *Diabet 47*(3), 387-394.

Perrini, S., Henriksson, J., Zierath, J. R., & Widegren, U. (2004). Exercise-Induced Protein Kinase C Isoform-Specific Activation in Human Skeletal Muscle. *Diabet 53*(1), 21-24.

Petersen, A. M., & Pedersen, K. (2005). The anti-inflammatory effect of exercise. *J Appl Physiol, 98*(4), 1154-1162.

Petersen, K. F., Dufour, S., Befroy, D., Garcia, R., & Shulman, G. I. (2004). Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes. *N Engl J Med, 350*(7), 664-671.

Phillips, S. M., Han, X. X., Green, H. J., & Bonen, A. (1996). Increments in skeletal muscle GLUT-1 and GLUT-4 after endurance training in humans. *Am J Physiol Endocrinol Metab, 270*(3), E456-462.

Picon-Reategui, E. (1962). Studies on the metabolism of carbohydrates at sea level and at high altitudes. *Metab 11*, 1148-1154.

Picon-Reategui, E. (1963). Intravenous glucose tolerance test at sea level and at high altitudes. *J Clin Endocrinol Metab 23*, 1256-1261.

Pierce, M., Keen H. & Bradley C. (1995). Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. *Diabet Med, 12*(1), 1:6-13.

Ploug, T., van Deurs, B., Ai, H., Cushman, S. W., & Ralston, E. (1998). Analysis of GLUT4 Distribution in Whole Skeletal Muscle Fibers: Identification of Distinct Storage Compartments That Are Recruited by Insulin and Muscle Contractions. *J Cell Biol, 142*(6), 1429-1446.

Polotsky, V. Y., Li, J., Punjabi, N. M., Rubin, A. E., Smith, P. L., Schwartz, A. R. (2003). Intermittent hypoxia increases insulin resistance in genetically obese mice. *J Physiol (Lond), 552*(1), 253-264.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *JAMA, 286*(3), 327-334.

Praet, S. F., & van Loon, L. J. C. (2007). Optimizing the therapeutic benefits of exercise in Type 2 diabetes. *J Appl Physiol 103*,1113-1120.

Prentice, R. L. (2001). Methodologic challenges in chronic disease population research. *Biostatistics, 2*(4), 365-381.

Purdon, C., Brousson, M., Nyveen, S. L., Miles, P. D., Halter, J. B., Vranic, M. (1993). The roles of insulin and catecholamines in the glucoregulatory response during intense exercise and early recovery in insulin-dependent diabetic and control subjects. *J Clin Endocrinol Metab, 76*(3), 566-573.

Pyne, D. B., Boston, T., Martin, D. T., & Logan, A. (2000). Evaluation of the Lactate Pro blood lactate analyser. *Eur J Appl Physiol, 82*(1), 112-116.

Randle, P. J. & Smith, G. H. (1958). Regulation of glucose uptake by muscle. The effects of insulin, anaerobiosis and cell poisons on the uptake of glucose and release of potassium by isolated rat diaphragm. *Biochem J 70:* 490-500

Reaven, G. M. (1991). Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension. Parallels between human disease and rodent models. *Diabet Care, 14*(3), 195-202.

Reeves, W. & Shah, S. V. (1994). Activation of potassium channels contributes to hypoxic injury in proximal tubules. *J Clin Invest 94:* 2289-2294

Reichard, G. A., Jacobs, A.G., Kimbel, P.H., Norman, J., & Weinhouse, S. (1961). Blood glucose replacement rates in normal and diabetic humans. *J Appl Physiol, 16*(5), 789-795.

Reitman, J. S., Vasquez, B., Klimes, I., & Nagulesparan, M. (1984). Improvement of glucose homeostasis after exercise training in non-insulin-dependent diabetes. *Diabet Care, 7*(5), 434-441.

Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (2002). *Diabet Care, 25*(90001), 5S-20.

Retnakaran, R., & Zinman, B. (2008). Type 1 diabetes, hyperglycaemia, and the heart. *Lancet, 371*(9626), 1790-1799.

Reynolds, T., Brozinick, J T., Rogers, M A. & Cushman, S W. (1998). Mechanism of hypoxia-stimulated glucose transport in rat skeletal muscle: potential role of glycogen. *Am J Physiol Endocrinol Metab, 274*(5), E773-778.

Rice, B., Janssen, I., Hudson, R., & Ross, R. (1999). Effects of aerobic or resistance exercise and/or diet on glucose tolerance and plasma insulin levels in obese men. *Diabet Care, 22*(5), 684-691.

Richter, E. A., Derave, W., & Wojtaszewski, J. F. (2001). Glucose, exercise and insulin: emerging concepts. *J Physiol (Lond), 535*(2), 313-322.

Richter, E. A., Garetto, L. P., Goodman, M. N., & Ruderman, N. B. (1984). Enhanced muscle glucose metabolism after exercise: modulation by local factors. *Am J Physiol Endocrinol Metab, 246*(6), E476-482.

Richter, E. A., Garetto, L.P., Goodman, M.N., & Ruderman, N.B. (1982). Muscle glucose metabolism following exercise in the rat. Increased sensitivity to insulin. *J Clin Invest, 69*, 785-793.

Richter, E. A., Hansen, B.F. & Hansen, S.A. (1988). Glucose-induced insulin resistance of skeletal-muscle glucose transport and uptake. *Biochem J, 252*(3), 733-737.
Richter, E. A., Nielsen, J.N., Jørgensen, S.B., Frøsig, C., Birk, J.B. & Wojtaszewski, J.F. (2004). Exercise signalling to glucose transport in skeletal muscle. *Proc Nutr Soc, 63*(2), 211-216.
Riffenburgh, R.H. (1999). Statistics in Medicine. *Harcourt Brace & Company, Publishers.*
Roach, J. A. (2003). Body Temperature, Autonomic Responses, and Acute Mountain Sickness. *High Alt Med Biol, 4*(4), 367-373.
Roach, P. J. (1990). Control of glycogen synthase by hierarchal protein phosphorylation. *FASEB J., 4*(12), 2961-2968.
Roberts, A. C., Reeves, J. T., Butterfield, G. E., Mazzeo, R. S., Sutton, J. R., & Wolfel, E. E. (1996). Altitude and beta-blockade augment glucose utilization during submaximal exercise. *J Appl Physiol, 80*(2), 605-615.
Roberts, C. K., Barnard, R. J., Jasman, A., & Balon, T. W. (1999). Acute exercise increases nitric oxide synthase activity in skeletal muscle. *Am J Physiol Endocrinol Metab, 277*(2), E390-394.
Robertson, R. P., Harmon, J., Tran, P. O. T., & Poitout, V. (2004). β-Cell Glucose Toxicity, Lipotoxicity, and Chronic Oxidative Stress in Type 2 Diabetes. *Diabet 53*(90001), S119-124.
Rockl, K. S. C., Hirshman, M. F., Brandauer, J., Fujii, N., Witters, L. A., & Goodyear, L. J. (2007). Skeletal Muscle Adaptation to Exercise Training: AMP-Activated Protein Kinase Mediates Muscle Fiber Type Shift. *Diabet 56*(8), 2062-2069.
Rogers, M. A., Yamamoto. C., King. D.S., Hagberg, J.M., Ehsani, A.A., Holloszy, J.O. (1988). Improvement in glucose tolerance after 1 wk of exercise in patients with mild NIDDM. *Diabet Care, 11*(8), 613-618.
Romer, L., McConnell, A.K., & Jones, D.A.. (2002). Effects of inpiratory muscle training on time-trial performance in trained cyclists. *J Sport Sci, 20*, 547-562.
Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Metab, 265*(3), E380-391.
Rooney, D. P., Neely, R. D., Cullen, C., Ennis, C. N., Sheridan, B., Atkinson, A. B. (1993a). The effect of cortisol on glucose/glucose-6-phosphate cycle activity and insulin action. *J Clin Endocrinol & Metab, 77*(5), 1180-1183.
Rooney, D. P., Neely, R. D., Cullen, C., Ennis, C. N., Sheridan, B., Atkinson, A. B. (1993b). The effect of cortisol on glucose/glucose-6-phosphate cycle activity and insulin action. *J Clin Endocrinol Metab, 77*(5), 1180-1183.
Rose, A. J. & Hargreaves, M. (2003). Exercise increases Ca^{2+}-calmodulin-dependent protein kinase II activity in human skeletal muscle. *J Physiol (Lond) 553*: 303-309
Rose, A. J. & Richter, E. A. (2005). Skeletal Muscle Glucose Uptake During Exercise: How is it Regulated? *Physiol, 20*(4), 260-270.
Rosenblatt, J., Chinkes, D., Wolfe, M., & Wolfe, R. R. (1992). Stable isotope tracer analysis by GC-MS, including quantification of isotopomer effects. *Am J Physiol Endocrinol Metab, 263*(3), E584-596.
Rosenbloom, A. L., Joe, J. R., Young, R. S., & Winter, W. E. (1999). Emerging epidemic of type 2 diabetes in youth. *Diabet Care 22*(2), 345-354.
Ross, R., Aru, J., Freeman, J., Hudson, R., & Janssen, I. (2002). Abdominal adiposity and insulin resistance in obese men. *Am J Physiol Endocrinol Metab, 282*(3), E657-663.
Rowell, L. B., Blackmon, J. R., Kenny, M. A., & Escourrou, P. (1984). Splanchnic vasomotor and metabolic adjustments to hypoxia and exercise in humans. *Am J Physiol Heart Circ Physiol, 247*(2), H251-258.
Roy, D., Johannsson, E., Bonen, A., & Marette, A. (1997). Electrical stimulation induces fiber type-specific translocation of GLUT-4 to T tubules in skeletal muscle. *Am J Physiol Endocrinol Metab, 273*(4), E688-694.
Rui, L., Yuan, M., Frantz, D., Shoelson, S., & White, M. F. (2002). SOCS-1 and SOCS-3 Block Insulin Signaling by Ubiquitin-mediated Degradation of IRS1 and IRS2. *J Biol Chem, 277*(44), 42394-42398.

Saghizadeh, M., Ong, J.M., Garvey, W.T., Henry, R.R.& Kern, P.A. (1996). The expression of TNF-α by human muscle. Relationship to insulin resistance. *J Clin Invest, 15*(97), 1111-1116

Saha, J., Lopez-Mondragon, R., & Narahara, H. T. (1968). Effect of Epinephrine on Permeability to Sugar and on the Production of Free Glucose in Skeletal Muscle. *J Biol Chem, 243*(3), 521-527.

Sahlin, K., Katz, A., & Broberg, S. (1990). Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *Am J Physiol Cell Physiol 259*(5), C834-841.

Sakamoto, K., & Goodyear, L. J. (2002). Exercise Effects on Muscle Insulin Signaling and Action: Invited Review: Intracellular signalling in contracting skeletal muscle. *J Appl Physiol, 93*(1), 369-383.

Sakamoto, M., Higaki, Y., Nishida, Y., Kiyonaga, A., Shindo, M., Tokuyama, K. (1999). Influence of mild exercise at the lactate threshold on glucose effectiveness. *J Appl Physiol, 87*(6), 2305-2310.

Saltiel, A. R., & Pessin, J. E. (2002). Insulin signaling pathways in time and space. *Trends Cell Biol, 12*(2), 65-71.

Sakamoto, K., McCarthy, A., Smith, D., Green, K.A., Hardie, G. D., Ashworth, A. & Alessi D. R. (2005). Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J 18;*24(10): 1810-1820

Sakamoto, K. & Holman, G. D. (2008). Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *Am J Physiol Endocrinol Metab 295:* E29-37

Saltin, B., & Essen., B. (1971). *Muscle glycogen, lactate, ATP, and CP in intermittent exercise. In: Saltin B, Pernow B (eds) Muscle metabolism during exercise*. New York.

Sanders, C. A., Levinson, G.E., Abelmann, W.H., & Freinkel, N. (1964). Effects of Exercise on the Peripheral Utilisation of Glucose in Man. *N Engl J Med, 30*(271), 220-225.

Sanders, M. J., Grondin, P. O., Hegarty, B. D., Snowden, M. A. & Carling, D. (2007). Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J 403*: 139-148

Sandler, S., Bendtzen, K., Eizirik, D.L., & Welsh, M. (1990). Interleukin-6 affects insulin secretion and glucose metabolism of rat pancreatic islets in vitro. *Endocrinol, 126*(2), 1288-1294.

Sano, H., Kane, S., Sano, E., Miinea, C. P., Asara, J. M., Lane, W. S. (2003). Insulin-stimulated Phosphorylation of a Rab GTPase-activating Protein Regulates GLUT4 Translocation. *J Biol Chem, 278*(17), 14599-14602.

Santos, J. L., Perez-Bravo, F., Carrasco, E., Calvillan, M., & Albala, C. (2001). Low prevalence of type 2 diabetes despite a high average body mass index in the aymara natives from chile. *Nutrit, 17*(4), 305.

Schernthaner, G. H., & Schernthaner, G. (2005). Insulin resistance and inflammation in the early phase of type 2 diabetes: potential for therapeutic intervention. *Scand J Clin Lab Invest Suppl.* (240), 30-40.

Schmidt, W., Eckardt, K.U., Hilgendorf, A., Strauch, S., & Bauer, C. (1991). Effects of maximal and submaximal exercise under normoxic and hypoxic conditions on serum erythropoietin level. *Int J Sports Med, 12*(5), 457-461.

Schneider, S., Khachadurian,AK, Amorosa,LF, Gavras,H, Fineberg,SE, Ruderman,NB. (1987). Abnormal glucoregulation during exercise in type II (non-insulin- dependent) diabetes. *Metab 36*, 1161-1166.

Segal, K. R., Edano, A., Abalos, A., Albu, J., Blando, L., Tomas, M. B. (1991). Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol, 71*(6), 2402-2411.

Senn, J. J., Klover, P. J., Nowak, I. A., & Mooney, R. A. (2002). Interleukin-6 Induces Cellular Insulin Resistance in Hepatocytes. *Diabet 51*(12), 3391-3399.

Sesti, G. (2006). Pathophysiology of insulin resistance. *Res Clin Endo Metab, 20*(4), 665-679.

Shetty, P. (1999). Adaptation to low energy intakes: the responses and limits to low intakes in infants, children and adults. *Eur J Clin Nutr, 53*, S14-33.

Shimabukuro, M., Koyama, K., Chen, G., Wang, M.-Y., Trieu, F., Lee, Y. (1997). Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *PNAS, 94*(9), 4637-4641.

Shimizu, S., Satoh, H., Yano, Y., Minokoshi, S.W., Cushman, T., & Shimazu, T. (1998). Effects of noradrenaline on the cell-surface glucose transporters in cultured brown adipocytes: novel mechanism for selective activation of GLUT1 glucose transporters. *Biochem J, 15*(330), 397-403.

Sigal, R. J., Kenny, G. P., Wasserman, D. H., & Castaneda-Sceppa, C. (2004). Physical Activity/Exercise and Type 2 Diabetes. *Diabet Care, 27*(10), 2518-2539.

Sigal, R. J., Kenny, G. P., Wasserman, D. H., Castaneda-Sceppa, C., & White, R. D. (2006). Physical Activity/Exercise and Type 2 Diabetes: A consensus statement from the American Diabetes Association. *Diabet Care, 29*(6), 1433-1438.

Sigal, R. J., Purdon, C., Bilinski, D., Vranic, M., Halter, J. B., & Marliss, E. B. (1994). Glucoregulation during and after intense exercise: effects of beta- blockade. *J Clin Endocrinol Metab, 78*(2), 359-366.

Sindelar, D. K., Chu, C. A., Venson, P., Donahue, E. P., Neal, D. W., & Cherrington, A. D. (1998). Basal hepatic glucose production is regulated by the portal vein insulin concentration. *Diabet 47*(4), 523-529.

Singh, L. P., Gennerette, D., Simmons, S., & Crook, E. D. (2001). Glucose-induced insulin resistance of phosphatidylinositol 3'-OH kinase and AKT/PKB is mediated by the hexosamine biosynthesis pathway. *J Diabet Complications, 15*(2), 88-96.

Sinha, R., Fisch, G., Teague, B., Tamborlane, W. V., Banyas, B., Allen, K. (2002). Prevalence of Impaired Glucose Tolerance among Children and Adolescents with Marked Obesity. *N Engl J Med, 346*(11), 802-810.

Snowling, N. J., & Hopkins, W. G. (2006). Effects of Different Modes of Exercise Training on Glucose Control and Risk Factors for Complications in Type 2 Diabetic Patients: A meta-analysis. *Diabet Care, 29*(11), 2518-2527.

Soop, M., Duxbury, H., Agwunobi, A. O., Gibson, J. M., Hopkins, S. J., Childs, C. (2002). Euglycemic hyperinsulinemia augments the cytokine and endocrine responses to endotoxin in humans. *Am J Physiol Endocrinol Metab 282*(6), E1276-1285.

Soulis-Liparota, T., Cooper, M.E., Dunlop, M. & Jerums, G. (1995). The relative roles of advanced glycation, oxidation and aldose reductase inhibition in the development of experimental diabetic nephropathy in the Sprague-Dawley rat. *Diabetologia, 38*(4), 387-394.

Spranger, J., Kroke, A., Möhlig, M., Bergmann, M. M., Ristow, M., Boeing, H. (2003). Adiponectin and protection against type 2 diabetes mellitus. *Lancet, 361*(9353), 226-228.

Sriwijitkamol, A., Coletta, D. K., Wajcberg, E., Balbontin, G. B., Reyna, S. M., Barrientes, J. (2007). Effect of Acute Exercise on AMPK Signaling in Skeletal Muscle of Subjects With Type 2 Diabetes: A Time-Course and Dose-Response Study. *Diabet 56*(3), 836-848.

Staehr, P., Hother-Nielsen, O., & Beck-Nielsen, H. (2002). Hepatic glucose production: therapeutic target in type 2 diabetes? *Diabet Obesity and Metab 4*(4), 215-223.

Stamler, J. S., & Meissner, G. (2001). Physiology of Nitric Oxide in Skeletal Muscle. *Physiol Rev, 81*(1), 209-237.

Stampfer, M. J., Hu, F. B., Manson, J. E., Rimm, E. B., & Willett, W. C. (2000). Primary Prevention of Coronary Heart Disease in Women through Diet and Lifestyle. *N Engl J Med, 343*(1), 16-22.

Starkie, R., Ostrowski, S. R., Jauffred, S., Febbraio, M., & Pedersen, B. K. (2003). Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α; production in humans. *FASEB J*, 02-0670fje.

Starkie, R. L., Arkinstall, M. J., Koukoulas, I., Hawley, J. A., & Febbraio, M. A. (2001). Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *J Physiol, 533*(2), 585-591.

Starkie, R. L., Rolland, J., Angus, D. J., Anderson, M. J., & Febbraio, M. A. (2001). Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-α levels after prolonged running. *Am J Physiol Cell Physiol, 280*(4), C769-774.

Steele, R. (1959). Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci, 82*(Current Trends in Research and Clinical Management of Diabetes), 420-430.

Steensberg, A., Febbraio, M. A., Osada, T., Schjerling, P., van Hall, G., Saltin, B. (2001). Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol (Lond), 537*(2), 633-639.

Steensberg, A. S., R.L. McConell, G.K. Kingwelln, B.A. and Febbraio, M.A. (2002). Contracting skeletal muscle releases IL-6, but not TNF-α, in healthy subjects and patients with type 2 diabetes. *J Physiol, 539P*, S095.

Steenbergen, C., Murphy, E., Levy, L. & London, R. (1987). Elevation in cytosolic free calcium concentration early in myocardial ischemia in perfused rat heart. *Circ Res 60*: 700-707

Stein, S. C., Woods, A., Jones, N. A., Davison, M. D. & Carling, D. (2000). The regulation of AMP-activated protein kinase by phosphorylation. *Biochem J 345*: 437-443

Steptoe, A., & Cox, S. (1988). Acute effects of aerobic exercise on mood. *Health Psychol, 7*(4), 329-340.

Stevens, V. J., Vlassara, H., Abati, A., & Cerami, A. (1977). Nonenzymatic glycosylation of hemoglobin. *J Biol Chem, 252*(9), 2998-3002.

Stratton, I. M., Adler, A. I., Neil, H. A. W., Matthews, D. R., Manley, S. E., Cull, C. A. (2000). Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ , 321, 405-412.

Stumvoll, M., Goldstein, B. J., & van Haeften, T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet, 365*(9467), 1333.

Stumvoll, M., Mitrakou, A., Pimenta, W., Jenssen, T., Yki-Jarvinen, H., Van Haeften, T. (2000). Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabet Care, 23*(3), 295-301.

Sutton, J. R. (1977). Effect of acute hypoxia on the hormonal response to exercise. *J Appl Physiol, 42*(4), 587-592.

Tanner, C. J., Koves, T. R., Cortright, R. L., Pories, W. J., Kim, Y.-B., Kahn, B. B. (2002). Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in middle-aged men. *Am J Physiol Endocrinol Metab, 282*(1), E147-153.

Thomas, D., Elliott, EJ, Naughton, GA. (2006). Exercise for type 2 diabetes mellitus. *Cochrane Database Syst Rev, 19*(3).

Tilg, H., & Moschen, A.R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med, 14*(3-4), 222-231.

Tomlinson, D. R. (1996). Aldose reductase inhibitors and the complications of diabetes mellitus. *Diabet Med, 10*(3), 214-230.

Trayhurn, P., Bing, C., & Wood, I. S. (2006). Adipose Tissue and Adipokines--Energy Regulation from the Human Perspective. *J Nutr, 136*(7), 1935S-1939.

Treebak, J. T., Birk, J. B., Rose, A. J., Kiens, B., Richter, E. A., & Wojtaszewski, J. F. (2007). AS160 phosphorylation is associated with activation of $\alpha_2\beta_2\gamma_1$- but not $\alpha_2\beta_2\gamma_3$-AMPK trimeric complex in skeletal muscle during exercise in humans. *Am J Physiol Endocrinol Metab, 292*(3), E715-722.

Tremblay, F., Lavigne, C., Jacques, H., & Marette, A. (2001). Defective Insulin-Induced GLUT4 Translocation in Skeletal Muscle of High Fat-Fed Rats Is Associated With Alterations in Both Akt/Protein Kinase B and Atypical Protein Kinase C ({zeta}/{lambda}) Activities. *Diabet 50*(8), 1901-1910.

Trout, K. K., Homko, C., & Tkacs, N. C. (2007). Methods of Measuring Insulin Sensitivity. *Biol Res Nursing, 8*(4), 305-318.

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P. (2001). Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. *N Engl J Med, 344*(18), 1343-1350.

Turner, R. C., Cull, C. A., Frighi, V. & Holman, R. R. (1999). Glycemic Control With Diet, Sulfonylurea, Metformin, or Insulin in Patients With Type 2 Diabetes Mellitus: Progressive Requirement for Multiple Therapies. *JAMA, 281*(21), 2005-2012.

U.K. Prospective Diabetes Study (UKPDS 37) Group. (1999). Quality of life in type 2 diabetic patients is affected by complications but not by intensive policies to improve blood glucose or blood pressure control *Diabet Care, 22*(7), 1125-1136.

UK Prospective Diabetes Study (UKPDS 33) Group. (1998a). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. *Lancet, 352*, 837–853.

UK Prospective Diabetes Study (UKPDS 33) Group. (1998b). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. *Lancet, 352*(9131), 837-853.

UKPDS. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). *Lancet, 352*(837-853).

Uusitupa, M., Lindi, V., Louheranta, A., Salopuro, T., Lindstrom, J., & Tuomilehto, J. (2003). Long-Term Improvement in Insulin Sensitivity by Changing Lifestyles of People with Impaired Glucose Tolerance: 4-Year Results From the Finnish Diabetes Prevention Study. *Diabet 52*(10), 2532-2538.

Uwaifo, G. I., Fallon, E. M., Chin, J., Elberg, J., Parikh, S. J., & Yanovski, J. A. (2002). Indices of Insulin Action, Disposal, and Secretion Derived From Fasting Samples and Clamps in Normal Glucose-Tolerant Black and White Children. *Diabet Care, 25*(11), 2081-2087.

Uysal, K. T., Wiesbrock, S. M., Marino, M. W., & Hotamisligil, G. S. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature, 389*(6651), 610-614.

van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H., & Wagenmakers, A. J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol, 536*(1), 295-304.

Vanninen, E., Uusitupa, M., Siitonen, O., Laitinen, J., & Länsimies, E. (1992). Habitual physical activity, aerobic capacity and metabolic control in patients with newly-diagnosed type 2 (non-insulin-dependent) diabetes mellitus: effect of 1-year diet and exercise intervention. *Diabetologia, 35*(4), 340-346.

Vicini, P., Caumo, A., & Cobelli, C. (1997). The hot IVGTT two-compartment minimal model: indexes of glucose effectiveness and insulin sensitivity. *Am J Physiol Endocrinol Metab, 273*(5), E1024-1032.

Vicini, P., & Cobelli, C. (2001). The iterative two-stage population approach to IVGTT minimal modeling: improved precision with reduced sampling. *Am J Physiol Endocrinol Metab, 280*(1), E179-186.

Viollet, B., Foretz, M., Guigas, B., Horman, S., Dentin, R., Bertrand, L. (2006). Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. *J Physiol, 574*(1), 41-53.

Vogt, C., Ardehali, H., Iozzo, P., Yki-Järvinen, H., Koval, J., Maezono, K. (2000). Regulation of hexokinase II expression in human skeletal muscle in vivo. *Metab 49*(6), 814-818.

Wadley, G. D., Lee-Young, R. S., Canny, B. J., Wasuntarawat, C., Chen, Z. P., Hargreaves, M. (2006). Effect of exercise intensity and hypoxia on skeletal muscle AMPK signaling and substrate metabolism in humans. *Am J Physiol Endocrinol Metab, 290*(4), E694-702.

Wahren, J., Felig P, Hagenfeldt L. (1978). Physical exercise and fuel homeostasis in diabetes mellitus. *Diabetologia, 14*(4), 13-22.

Wajant, H., Pfizenmaier, K., & Scheurich, P. (2003). Tumor necrosis factor signaling. *Cell Death Differ, 10*(1), 45-65.

Walaas, O., & Walaas, E. (1950). Effect of Epinephrine on rat Diaphragm. *J Biol. Chem, 187*(2), 769-776.

Walberg, J. L., Upton, D., & Stern, J.S. (1984). Exercise training improves insulin sensitivity in the obese Zucker rat. *Metab 33* (12), 1075-1079.

Wallace, T. M., & Matthews, D.R. (2002). Coefficient of failure: a methodology for examining longitudinal β-cell function in Type 2 diabetes. *Diabetic Med, 19*(6), 465-469.

Wallenius, K., Wallenius, V., Sunter, D., Dickson, S. L., & Jansson, J.-O. (2002). Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun, 293*(1), 560-565.

Wang, J., Liu, R., Hawkins, M., Barzilai, N., & Rossetti L. (1998). A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature, 18*(393), 684-688

Wardzala, L., & Jeanrenaud, B. (1981). Potential mechanism of insulin action on glucose transport in the isolated rat diaphragm. *J Biol Chem* 256: 7090-7093

Wasserman, D. H. (1995). Regulation of Glucose Fluxes During Exercise in the Postabsorptive State. *Ann Rev Physiol, 57*(1), 191-218.

Watt, M. J., & Hargreaves, M. (2002). Effect of epinephrine on glucose disposal during exercise in humans: role of muscle glycogen. *Am J Physiol Endocrinol Metab 283*(3), E578-583.

Wei, M., Kampert, J. B., Barlow, C. E., Nichaman, M. Z., Gibbons, L. W., Paffenbarger, R. S., Jr. (1999). Relationship Between Low Cardiorespiratory Fitness and Mortality in Normal-Weight, Overweight, and Obese Men. *JAMA, 282*(16), 1547-1553.

Wendorf, M., & Goldfine, I.D. (1991). Archaeology of NIDDM. Excavation of the "thrifty" genotype. *Diabet 40*(2), 161-165.

Weyer, C., Bogardus, C., Mott, D.M. & Pratley, R.E. (1999). The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest., 104*(6), 787-794.

White, M. F. (2002). IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab, 283*(3), E413-422.

Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabet Care, 27*(5), 1047-1053.

Williamson, J. R., Chang. K., Frangos, M., Hasan, K.S., Ido, Y., Kawamura, T., Nyengaard, J.R., van den Enden, M., Kilo, C. & Tilton, R.G. (1993). Hyperglycemic pseudohypoxia and diabetic complications. *Diabet 42*(6), 801-813.

Winder, W. W., & Hardie, D. G. (1999). AMP-activated protein kinase, a metabolic master switch: possible roles in Type 2 diabetes. *Am J Physiol Endocrinol Metab, 277*(1), E1-10.

Winkler, G., Salamon, F., Harmos, G., Salamon, D., Speer, G., Szekeres, O. (1998). Elevated serum tumor necrosis factor-α concentrations and bioactivity in Type 2 diabetics and patients with android type obesity. *Diabet Res Clin Pract, 42*(3), 169-174.

Witters, L. A., & Kemp, B. E. (1992). Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5'-AMP-activated protein kinase. *J Biol Chem, 267*(5), 2864-2867.

Wojtaszewski, J. F., Laustsen J.L., Derave W., Richter E.A. (1998). Hypoxia and contractions do not utilize the same signaling mechanism in stimulating skeletal muscle glucose transport. *Biochimica Biophysica Acta 1380*(3), 396-404.

Wojtaszewski, J. F., MacDonald, C., Nielsen, J. N., Hellsten, Y., Hardie, D. G., Kemp, B. E. (2003). Regulation of 5'AMP-activated protein kinase activity and substrate utilization in exercising human skeletal muscle. *Am J Physiol Endocrinol Metab, 284*(4), E813-822.

Wojtaszewski, J. F., Nielsen, P., Hansen, B. F., Richter, E. A., & Kiens, B. (2000). Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle. *J Physiol, 528*(1), 221-226.

Wojtaszewski, J. F., Nielsen, J.N., & Richter, E.A. (2002). Exercise Effects on Muscle Insulin Signaling and Action: Invited Review: Effect of acute exercise on insulin signaling and action in humans. *J Appl Physiol, 93*(1), 384-392.

Woods, A., Salt, I., Scott, J., Hardie, D. G. & Carling, D. (1996). The α1 and α2 isoforms of the AMP-activated protein kinase have similar activities in rat liver but exhibit differences in substrate specificity in vitro. *FEBS Letters 397*: 347-351

Wood, I. S., Wang, B., Lorente-Cebrián, S., & Trayhurn, P. (2007). Hypoxia increases expression of selective facilitative glucose transporters (GLUT) and 2-deoxy-d-glucose uptake in human adipocytes. *Biochem Biophys Res Commun, 361*(2), 468-473.

World Health Organization. (1985). Glucose tolerance test and glycosylated haemoglobin measurement for diagnosis of diabetes mellitus--an assessment of the criteria of the WHO Expert Committee on Diabetes Mellitus 1980. *Ann Clin Biochem, 22*, 67-73.

World Health Organization Expert Committee. (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation, part 1: diagnosis and classification of diabetes mellitus. *Geneva: World Health Organization*.

World Medical Association 2000 Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects Adopted by the 18th WMA General Assembly, H., Finland, June 1964. Amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th

WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, September1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000,.

Wright, D. C., Geiger, P. C., Holloszy, J. O., & Han, D.-H. (2005). Contraction- and hypoxia-stimulated glucose transport is mediated by a Ca^{2+}-dependent mechanism in slow-twitch rat soleus muscle. *Am J Physiol Endocrinol Metab, 288*(6), E1062-1066.

Wright, E. M. (2001). Renal Na+-glucose cotransporters. *Am J Physiol Renal Physiol, 280*(1), F10-18.

Wu, S., & Ren, J. (2006). Benfotiamine alleviates diabetes-induced cerebral oxidative damage independent of advanced glycation end-product, tissue factor and TNF-α. *Neuroscience Letters, 394*(2), 158-162.

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn B.B. & Kadowaki, T. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med, 8,* 1288-1295.

Yang, A. L., Tsai, S.J., Jiang, M.J., Jen, C.J. & Chen, H.I. (2002). Chronic Exercise Increases Both Inducible and Endothelial Nitric Oxide Synthase Gene Expression in Endothelial Cells of Rat Aorta. *J Biomed Sci, 9*(2), 149-155.

Yaspelkis, B. B., Ansari, L., Ramey, E.L., Holland, G.J., & Loy, S.F. (1999). Chronic leptin administration increases insulin-stimulated skeletal muscle glucose uptake and transport. *Metab 48*(5), 671-676.

Yin, J., Gao, Z., He, Q., Zhou, D., Guo, Z., & Ye, J. (2009). Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. *Am J Physiol Endocrinol Metab* 296, E333-342

Yokoe, T., Alonso, L. C., Romano, L. C., Rosa, T. C., O'Doherty, R. M., Garcia-Ocana, A. (2008). Intermittent hypoxia reverses the diurnal glucose rhythm and causes pancreatic β-cell replication in mice. *J Physiol, 586*(3), 899-911.

Youn, J. H., Gulve, E. A. & Holloszy, J. O. (1991). Calcium stimulates glucose transport in skeletal muscle by a pathway independent of contraction. *Am J Physiol Cell Physiol 260*: C555-561

Young, J. C., Enslin, J., & Kuca, B. (1989). Exercise intensity and glucose tolerance in trained and nontrained subjects. *J Appl Physiol, 67*(1), 39-43.

Zachwieja, J. J., Toffolo, G., Cobelli, C., Bier, D. M., & Yarasheski, K. E. (1996). Resistance exercise and growth hormone administration in older men: Effects on insulin sensitivity and secretion during a stable-label intravenous glucose tolerance test. *Metab 45*(2), 254-260.

Zeigerer, A., McBrayer, M. K., & McGraw, T. E. (2004). Insulin Stimulation of GLUT4 Exocytosis, but Not Its Inhibition of Endocytosis, Is Dependent on RabGAP AS160. *Mol Biol Cell, 15*(10), 4406-4415.

Zhang, J. Z., Behrooz, A., & Ismail-Beigi, F. (1999). Regulation of glucose transport by hypoxia. *Am J Kidney Dis, 34*(1), 189-202.

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M.F., Goodyear, L.J. & Moller, D.E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest, 108*, 1167–1174.

Zhou, Q., & Dohm, G. L. (1997). Treadmill Running Increases Phosphatidylinostol 3-Kinase Activity in Rat Skeletal Muscle. *Biochem Biophys Res Commun, 236*(3), 647-650.

Zick, Y. (2001). Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. *Trends Cell Biol, 11*(11), 437-441.

Zierath, J. R. (2002). Exercise Effects of Muscle Insulin Signaling and Action: Invited Review: Exercise training-induced changes in insulin signaling in skeletal muscle. *J Appl Physiol, 93*(2), 773-781.

Zimmet, P., Alberti, K. G., & Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature, 414*(6865), 782.

Zinker, B. A., Lacy, D. B., Bracy, D. P., & Wasserman, D. H. (1993). Role of glucose and insulin loads to the exercising limb in increasing glucose uptake and metabolism. *J Appl Physiol, 74*(6), 2915-2921.

Zinman, B., Murray, F.T., Vranic, M., Albisser, A.M., Leibel, B.S., Mc Clean, P.A., & Marliss, E.B. (1977). Glucoregulation during moderate exercise in insulin treated diabetics. *J Clin Endocrinol Metab*, *45*(4), 641-652.

Zoppini, G., Targher, G., Zamboni, C., Venturi, C., Cacciatori, V. & Moghetti, P. (2006). Effects of moderate-intensity exercise training on plasma biomarkers of inflammation and endothelial dysfunction in older patients with type 2 diabetes. *Nutr Metab Cardiovasc Dis*, *16*(8), 543-549.

Zorzano, A., Palacin, M., & Guma, A. (2005). Mechanisms regulating GLUT4 glucose transporter expression and glucose transport in skeletal muscle. *Acta Physiol Scand*, *183*(1), 43-58.

Website:

http://eatlas.idf.org/

Diabetes UK, Report (2007).

http://www.diabetes.org.uk /Aboutus/NewsLandingPage/Obesity-could-bankrupt-NHS/

www.who.int/diabetes/facts/world_figures/en