Short-term effects of a low glycemic index carob containing snack on energy intake, satiety and glycemic response in normal-weight, healthy adults. Results from two randomized-trials.

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EP conceptualized, designed the study, conducted statistical analysis and drafted the manuscript; NO collected the data; AK, IM, NG and PS created the carob snack and conducted all laboratory nutrient analyses; PF and MH served as scientific counselors to the project and critically revised the manuscript. All authors contributed to the writing and editing of this manuscript according to their area of expertise and all authors approved it.

Abstract

Background/Objectives:

The potential positive health effects of carob containing snacks are largely unknown. Therefore, two studies were conducted to 1.firstly determine the glycemic index (GI) of a carob-snack compared to chocolate cookie containing equal amounts of available carbohydrates and 2.compare the effects of a carob vs. chocolate cookie preload consumed as snack before a meal on (a) short-term satiety response measured by subsequent *ad libitum* meal intake, (b) subjective satiety as assessed by visual analogue scales (VAS), and (c) postprandial glycemic response.

Subjects/ Methods:

Ten healthy, normal-weight volunteers participated in GI investigation. Then, 50 healthy, normal-weight subjects consumed, cross-over, in random order, the preloads as snack, with one-week wash-out period. *Ad-libitum* meal (lunch and dessert) was offered. Capillary blood glucose samples were collected at baseline, 2h-after-breakfast-and-just-before-preload consumption, 2h-after-preload, 3h-after-preload-and-just-before-meal-(lunch-and-dessert),1h-after-meal and 2h-after-meal consumption.

Results

The carob snack was low and chocolate cookie high GI foods (40vs.78 on glucose scale). Consumption of the carob preload decreased the glycemic response to a following meal and subjects' feeling of hunger, desire to eat, preoccupation with food, and thirst between snack and meal, as assessed with the use of VAS. Subsequently, subjects consumed less amount of food (g) and had lower total energy intake at meal.

Conclusions:

The carob snack led to increased satiety, lower energy intake at meal and decreased post-meal glycemic response possibly due to its low GI value. Identifying foods that promote satiety and decrease glycemic response without increasing the overall energy intake may offer advantages to body weight and glycemic control.

Word Count: 250

Keywords: carob, blood glucose, satiety, energy intake, preload, snack

Introduction

Carob (Ceratonia siliqua) is a natural sweetener and may be used as a nutritious substitute for cocoa powder [1]. The effects of carob on glycemic responses and satiety are poorly understood. It is well known that consumption of high glycemic index (GI) foods is associated with increased chronic disease risk [2, 3]. The GI of foods is a method by which foods can be ranked on the basis of the glycemic impact in relation to the available carbohydrate within those foods [3]. One study that examined the GI of carob bars, containing 26 g of available carbohydrates, in seven healthy subjects, showed that they were low GI (GI = 39) foods [4]. A moderate improvement in glycemic control may be accomplished when low GI foods replace foods with higher GI [5]. In controlled feeding studies, energy intake with high GI meals was 29% higher compared to consumption of low GI meals with similar macronutrient composition [6]. Both meal type and snack patterns contribute significantly in energy intake and body weight management [7]. Several studies have examined the effects of a variety of preloads on satiety response in an effort to identify meal or snack patterns or foods that promote satiety and body weight control without increasing significantly the overall energy intake. The satiety response and consequent energy intake are affected by multiple factors including the type of food consumed, the macronutrient content, the energy density as well as the food's volume [8, 9].

Therefore, due to the lack of evidence on the effects of carob flour on glycemic responses, satiety and subjective appetite ratings, two studies were carried out. The aim of the first study was to determine the GI of a snack made with carob flour compared with a chocolate cookie. Then a second study tested the hypothesis that a preload which included the carob snack before a meal, compared to the chocolate cookie snack, would decrease: a) meal time energy intake, b) appetite for meal (lunch and dessert), c) energy intake for the next 24 h, and d) decrease postprandial capillary blood glucose concentrations.

Materials and Methods

Subjects and methods

Healthy, non-smoking, non-diabetic men and women participated in these studies. Subjects were recruited via notices posted at Agricultural University of Athens. The inclusion criteria for participation were a body mass index (BMI) between 18-and-25 kg/m^2 and an age between 18-and-50 years. Exclusion criteria included severe chronic disease (e.g. coronary heart disease, diabetes mellitus, kidney or liver conditions, endocrine conditions), gastrointestinal disorders, pregnancy, lactation, competitive sports and alcohol or drug dependency. All subjects gave their written consent. The protocol was approved by the Bioethics Committee of the Agricultural University of Athens and was carried out in accordance with the Declaration of Helsinki (1997). (ClinicalTrials.gov Identifier: NCT02935829).

Determination of glycemic index

Ten subjects participated in this randomized, single-blind, cross-over design study (male: 6, female: 4; Figure 1). Subjects were randomly enrolled to the interventions using a single allocation ratio. One person, not involved in data handling and analysis, was responsible for test and reference food allocation. All participants fasted for 10-14h before the test. Participants received the reference (white bread and glucose) and test (carob snack and chocolate cookie snack) foods, two times, in different weeks according to suggested GI methodology [10]. Each portion of the test products (65g carob snack, 269 kcal; 43g chocolate cookie, 174 kcal) or the reference food (42g white bread, 105 kcal; 25g glucose, 95 kcal) was equivalent to a 25g amount of available carbohydrates. The participants were instructed to consume the foods along with 250ml water within a maximum of 5 to 10min for the glucose beverage and 10 to 15min for solids. To determine blood glucose concentrations, fingertip capillary blood samples using an automatic lancet (FORA Comfort lux GD50, ATCARE ltd, Greece) were taken at baseline, 15, 30, 45, 60, 90 and 120 min. The first glucose sample was taken exactly 15min after the first bite of food or drink. Blood glucose was measured with Glucose Dehydrogenase – FAD test strips which show no reactivity to any sugars other than glucose and has better heat-resistance and oxygen-resistance. The allowed deviation limits of glucose meters for glucose results ≥ 100 mg/dl were within 15% of the reference method. The CV (%) was less than 5% both in intermediate precision and repeatability. The blood glucose value recorded was the mean of three measurements.

Effects of consuming a preload including the carob snack on blood glucose responses and subjective appetite ratings

Fifty healthy subjects (male: 22, female: 28) participated in this randomized, single-blind, crossover design study (Figure 1). Subjects were randomly enrolled to the interventions using a single allocation ratio. One person, not involved in data handling and analysis, was responsible for preload allocation. The crossover design was selected to limit the inter-subject variability between the interventions [11]. Before the initiation of the study days, all subjects completed a 3-day food diary in order to evaluate their habitual energy and macronutrient intakes. Volunteers consumed in random order the two snacks (40g of carob snack and 40g chocolate cookie) with one-week wash-out period. During each test day, subjects reported after a 10-14h fast, without having performed strenuous exercise during the last 24h and without having consumed alcohol for 24h prior to the breakfast meals.

At 9:00 AM participants were offered a standardized breakfast (2 slices of bread and honey), consumed over a 10 min period, which provided approximately 350 kcal. Immediately after, at 11:00 AM, subjects were provided with the preload, consumed over a 10-15min period. The preloads of the two different study days had similar energy contents and macronutrients (Table 2; Proteins were assessed by ISO 1871:2009 and AOAC 201.11; total fat by ISO 6492:1999; saturated fat by AOAC 996.06; available carbohydrates by AOAC 991.43; sugars by AOAC 977.20 and dietary fibers by AOAC 985.29). Three hours after, at 14:00 PM, subjects were given *ad libitum* access to a meal (lunch and dessert), consumed over a 20-30min period. The meal consisted of rice and roasted chicken breast and chocolate cake and subjects were encouraged to consume as much or as little as they wished. All offered foods were weighed at the time of serving and any leftovers were weighed again after meal to determine the amount of food consumed. Subjects had free access to water during the experimental periods. At the end of the study days, all subjects were asked to keep detailed food and beverages intake records for the next 24h using household measures.

Fingertip capillary blood glucose samples were taken using an automatic lancet (FORA Comfort lux GD50, ATCARE ltd, Greece; same as in study 1), at baseline (before breakfast); 2h after breakfast, which was also the time just before preload consumption); 2h post-preload consumption; 3h post-preload consumption, which was also the time just before the *ad libitum* meal (lunch and dessert) consumption; 1h post-meal consumption and 2h post-meal consumption. Blood glucose was measured with Glucose Dehydrogenase – FAD test strips. The CV (%) was less than 5% both in intermediate precision and repeatability. The blood glucose value recorded was the mean of three measurements.

Besides monitoring and weighing the actual amount of food consumed during the study days, subjects rated their hunger, desire to eat, preoccupation with food, perceived fullness, motivation to eat and thirst, on 100mm line visual analogue scales (VAS) which were given in the form of a booklet, one scale per page [12]. VAS ratings were obtained before and every 45min post-preload consumption (0, 45, 90, 135 and 180min), and immediately after completing the meal. Shortly after the consumption of the preloads and meal, subjects rated on VAS the experienced pleasure.

Anthropometric measurements

Height, body weight, waist and hip circumferences were measured. Body mass index (BMI) was calculated. Fat mass and fat-free mass were assessed by single frequency bioimpedance (BIA, Tanita Total Body Composition Analyzer, TBF-300 500, Tanita Europe, Amsterdam, The Netherlands). Percent body fat was calculated by using the manufacturer's software.

Statistical Analysis

Data are means± SEM, unless otherwise stated. For GI determination, glycemic response and VAS scores, the incremental area under the curve (iAUC) values were calculated [10]. To calculate the iAUC, the raw data of the blood glucose and VAS scores were used and were determined for each subject as well as for both test and reference products. To calculate glucose concentrations and VAS scores at the various time points, the raw data were zero-value corrected. To calculate the iAUCs for blood glucose, a period of time from zero to two hours was taken into account. The GI of the carob snack and chocolate cookie was then calculated from the iAUCs that were recorded for glucose. For this a ratio was created using the mean value of all individual iAUCs of the test snacks and the reference iAUC. To calculate the iAUCs for VAS scores, a period of time from zero to three hours was likewise taken into account. Statistical analysis was performed on the basis of the data's distribution type (Kolmogorov–Smirnov adjustment test). In the case of normal distribution, analysis was performed using single-factor analysis of variance (ANOVA); in the case of abnormally distributed data, the Mann–Whitney U test was used. Differences in glucose concentrations and VAS ratings between and among the test snack preloads at the different time points were tested using repeated measures ANOVA with two within-subjects factors (snack type and time). In the absence of normality, variables were ranked and then the Friedman non-parametric statistical test was used. The study had 80% power ($\alpha = 0.05$) to detect differences between dietary groups of 15 ± 2 mg/dl in postprandial plasma glucose. Statistical significance was determined to be $p < 0.05$ and marked using an asterisk: *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$. Data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, Il, USA).

Results

Determination of glycemic index

Volunteers' baseline characteristics are presented in Table 1. Table 2 shows the nutritional composition of the preload snacks. GI results and the preloads' glycemic response are summarized in Table 3 and Figure 2A. At baseline there were no significant differences in glucose concentrations between carob snack, chocolate cookie, white bread and glucose (P=0.14). There was a statistically significant difference in blood glucose ($F_{(18,77)}=17.63$, $p<0.001$) and an interaction between blood glucose and snack ($F_{(18,77)}=0.48$, $p=0.003$). The carob snack showed markedly less effect on blood glucose concentration compared with the chocolate cookie snack and the reference products at 15' (p=0.03), 30' (p=0.02), 45' (p<0.001), 60' (p=0.007) and

120’ (p=0.007; Figure 2A). The iAUC of carob snack was likewise lower compared with the cookie snack and reference products (p=0.01; Table 3, Figure 2B).

Effects of consuming a preload including the carob snack on blood glucose responses and subjective appetite ratings

Mean age of the subjects was 25±6years and mean BMI was 23±3 kg/m² (Table 1). There was no significant effect of gender in blood glucose responses or assessed VAS ratings at any time point and, therefore, our results are presented for the whole sample.

Table 4 presents the energy and nutrient intake during the meal and 24 h after the completion of both study days. There were no differences in self-reported energy intake between the two preload study days (Mean energy intake 24hr before the intervention, day of the intervention, 24hr after the intervention for carob snack: 2034±135 kcal, 2233±103 kcal, 2029±143 kcal, respectively; 24hr before the intervention, day of the intervention, 24hr after the intervention for the chocolate cookie snack: 2399±486 kcal, 2216±100 kcal, 1995±118 kcal, respectively). The total energy intake at the meal (energy intake from lunch and dessert) was significantly lower when subjects consumed the carob preload snack compared to chocolate cookie ($F_{(1,49)}$=4.685, p=0.035; Table 4). The total g of consumed food (meal and dessert) was significantly lower when subjects consumed the carob preload snack compared to chocolate cookie ($F_{(1,49)}$=11.546, p=0.001; Table 4). Protein consumption at meal (lunch and dessert) was significantly lower when subjects consumed the carob preload compared to chocolate cookie ($F_{(1,49)}$=4.612, p=0.037; Table 4). Carbohydrate consumption at meal (lunch and dessert) was lower when subjects consumed the preload that included the carob preload snack compared to chocolate cookie ($F_{(1,49)}$=4.131, p=0.048; Table 4). Rice consumption was significantly lower when subjects consumed the carob preload snack compared to chocolate cookie ($F_{(1,49)}$=11.528, p=0.001; Table 4). Chicken consumption tended to be lower after consumption of the carob preload compared to chocolate cookie, but it was not statistically significant (p=0.08; Table 4). Dessert consumption did not differ between the two preload snacks (Table 4). Energy intake 24h following the consumption of lunch did not differ between the two study days (Table 4).

Blood glucose was not significantly different between subjects on both preload study days at baseline. Figure 3 presents capillary blood glucose concentrations before and after breakfast, after preload snack consumption and after *ad libitum* meal (lunch and dessert) consumption. There was a significant blood glucose x time interaction ($F_{(5,45)}$=52.730, p<0.001) and a significant blood glucose x preload snack interaction ($F_{(1,49)}$=10.272, p=0.002). There was a significant main effect of time on blood glucose ($F_{(3,129)}$=124.512, p<0.001), a significant main effect of preload snack on blood glucose ($F_{(1,49)}$=10.272, p=0.002) and a significant main effect of time x preload snack interaction ($F_{(3,153)}$=3.523, p=0.015). Blood glucose concentrations were similar 2h and 3h after consumption of the two preload snacks (Figure 3). There was a significant main effect of time on blood glucose for meal (lunch and dessert) consumption ($F_{(2,98)}$=162.678, p<0.001), a significant main effect of preload snack ($F_{(1,49)}$=8.228, p=0.006) and a significant main effect of time x preload snack interaction ($F_{(2,98)}$=4.404, p=0.015). After meal (lunch and dessert) consumption, blood glucose was significantly lower at 60 min and 120 min when subjects consumed the carob preload snack (133.4±4.3 mg/dl and 128.0±3.9 mg/dl, respectively) compared to chocolate cookie preload (145.2±4.3 mg/dl and 142.4±3.6 mg/dl, respectively; $F_{(1,49)}$=6.219, p=0.016 for 60min and $F_{(1,49)}$=7.745, p=0.008 for 120min, respectively; Figure 3). Overall, mean blood glucose was lower after meal consumption when subjects consumed the carob preload snack by 8.4±2.9 mg/dl (p=0.006). The iAUC for glucose for the period of meal (lunch and dessert) consumption was significantly lower when subjects consumed the carob preload snack (4011±338 mg*min/dl) compared to chocolate cookie (5220±374 mg*min/dl; $F_{(1,49)}$=7.863, p=0.007; Figure 3).

No significant differences were observed in the VAS ratings before the consumption of the preload (baseline) between the two different test days (p>0.05). Figure 3 presents the subjective VAS scores. There a significant hunger x time interaction ($F_{(5,45)}$=198.904, p<0.001), a significant hunger x preload snack interaction ($F_{(1,49)}$=5.445, p=0.024) and a significant hunger x time x preload snack interaction ($F_{(5,45)}$=4.571, p=0.002). There was a significant main effect of preload snack ($F_{(1,49)}$=5.445, p=0.024) and a significant main effect of hunger x time x preload snack ($F_{(4,188)}$=3.523, p=0.009). Hunger was significantly lower at 45min ($F_{(1,49)}$=15.739, p<0.001), and tended to be lower at 135min (p=0.06) and 180min (p=0.07), when subjects consumed the carob preload snack (Figure 4). Overall, mean perceived hunger was significant lower when subjects consumed the carob preload (3.8±0.2 mm) compared to chocolate cookie (4.3±0.3 mm; p=0.02). The iAUC for perceived hunger was significantly lower after the carob preload (100±46 mm*min) compared to chocolate cookie (214±37; $F_{(1,49)}$=4.094, p=0.048) and tended to be lower even after the meal (lunch and dessert) consumption when subjects consumed the carob preload snack (p=0.06). There was a significant food desire x time interaction ($F_{(5,45)}$=219.276, p<0.001), a significant food desire x preload snack interaction ($F_{(1,49)}$=6.621, p=0.013) and a significant food desire x time x preload snack interaction ($F_{(5,45)}$=2.637, p=0.036). Food desire was significantly lower at 45min ($F_{(1,49)}$=17.041, p<0.001) and 135min ($F_{(1,49)}$=4.137, p=0.047) compared to chocolate cookie (Figure 4). Overall mean food desire was lower when subjects consumed the carob preload by (3.88±0.21 mm) compared to chocolate cookie (4.56±0.27 mm; $p=0.013$). There was a significant preoccupation with thoughts of food x time ($F_{(5,45)}=118.689$, $p<0.001$) and a significant preoccupation with thoughts of food x snack interaction ($F_{(1,49)}=4.085$, $p=0.049$). There was a significant main effect of time on preoccupation with food ($F_{(4,196)}=146.485$, $p<0.001$) and main effect of snack on preoccupation with food ($F_{(1,49)}=4.085$, $p=0.049$). Preoccupation with food was significantly lower at 45min ($F_{(1,49)}=6.686$, $p=0.013$) and tended to be lower at 135min ($p=0.051$) when subjects consumed the carob preload (Figure 4). Overall mean preoccupation with food was lower when subjects consumed the carob preload (3.75±0.20 mm) compared to chocolate cookie (4.27±0.28 mm; $p=0.049$). The iAUC for preoccupation with food was significantly lower after the carob preload (194.58±68.02 mm*min) compared to chocolate cookie (211.41±40.26 mm*min) ($F_{(1,49)}=6.434$, $p=0.014$). There was a significant perceived fullness x time interaction ($F_{(5,45)}=158.336$, $p<0.001$) and a main effect of time on perceived fullness ($F_{(4,198)}=186.589$, $p<0.001$), without preload snack interaction or main effects. Subjects consuming the carob preload has higher perceived fullness at 45min compared to chocolate cookie ($F_{(1,49)}=4.316$, $p=0.04$; Figure 4). The iAUC for perceived fullness was significantly higher after meal consumption when subjects consumed the carob preload (150.97±58.09 mm*min) compared to chocolate cookie (145.66±43.90; $F_{(1,49)}=9.062$, $p=0.004$). There was a significant motivation to eat x time interaction ($F_{(5,45)}=211.744$, $p<0.001$) and a significant motivation to eat x preload snack interaction ($F_{(1,49)}=4.365$, $p=0.042$). Motivation to eat tended to be lower at 90min when subjects consumed the carob preload compared to chocolate cookie ($p=0.076$; Figure 4). Overall mean motivation to eat was lower when subjects consumed the carob preload (4.18±0.21 mm) compared to chocolate cookie (4.53±0.23; p=0.042). There was a significant thirst x time x preload snack interaction ($F_{(5,45)}$=2.636, p=0.036) and a main effect of thirst x time x preload snack interaction ($F_{(4,201)}$=2.739, p=0.029). Perceived thirst was significantly lower at 90min when subjects consumed the carob preload compared to chocolate cookie ($F_{(1,49)}$=8.212, p=0.006; Figure 4). Prospective food consumption tended to be lower when subjects consumed the carob preload snack compared to chocolate cookie, although it did not reach statistical significance (p=0.051). There was a significant pleasure from foods x preload snack interaction ($F_{(1,43)}$=74.877, p<0.001). Overall mean perceived pleasure from foods was significantly lower when subjects consumed the carob preload snack (5.47±0.24 mm) compared to chocolate cookie (8.13±0.18 mm; p<0.001).

Discussion

The main findings of these studies were that 1: the carob snack was a low GI food (≤55 on the glucose scale) and 2. results support the hypothesis that a preload including the carob snack, in comparison to a similar energy and macronutrient chocolate cookie snack, created for the needs of this study, would be associated with a stronger satiety effect and subsequently reduce meal time energy intake (energy coming from lunch and dessert) and total amount (grams) of meal consumed. Furthermore, the reduced energy intake from the meal after the consumption of the carob snack was partly supported by the results of subjective measures of hunger, preoccupation with thoughts of food, desire to eat and perceived fullness as assessed with the use of VAS. Moreover, after the carob snack preload, rice consumption at the following meal was significantly lower and chicken consumption tended to be lower leading to significant reduction in the glycemic response. This translated in significantly lower intake of carbohydrates and dietary protein at meal (lunch and dessert) when subjects consumed the carob preload snack compared to the chocolate cookie preload snack.

The carob snack's GI value obtained is in agreement with others [4]. The GI value difference observed based on the reference food has been thoroughly discussed [10]. The carob snack decreased significantly the glycemic response to a following meal (lunch and dessert) compared to chocolate cookie snack, which may be due to its low GI value and higher dietary fiber content. Furthermore, the low GI of our carob snack may be due to its hazelnut content, which made up almost a quarter of the product. One study that compared the effects of three different types of hazelnut enriched breads compared to a control bread without hazelnuts, on gastrointestinal tolerance, postprandial glucose values and satiety in healthy, normal-weight, adults showed that consumption of hazelnut-enriched bread reduced the postprandial glycemic response, without significantly affecting satiety or short-term energy intake [13]. It has been shown that a low GI may be sufficient to achieve a lower glycemic response from one meal to the next [14-17]. Following the carob preload snack, rice consumption was significantly lower (by 34±10 g) and chicken consumption tended to be lower (by 12±7 g) at lunch. Whether a carob snack leads to lower desire for overall food intake, particularly starchy foods, such as rice, and subsequently lower consumption at the following meal needs further investigation.

One study showed that when carob flour is used in traditional Yemenite bread (malawach), it leads to improved glycemic control in people with diabetes[18]. However, other short-term trials have produced contradictory findings with one showing that consumption of polyphenol-rich insoluble fiber preparation from carob pulp did not affect postprandial plasma glucose or serum insulin responses in healthy adults and this was dose dependent [19] and another showing that carob fiber-enriched foods (white bread, spice cake, soup, and milk drink) increased glucose response in healthy adults [20].

Since the macronutrient composition and the energy density of the two different preloads were similar, the increased satiety feeling following the consumption of the carob snack preload as well as the reduced energy and total grams of meal consumed may be due to its low GI value and higher fiber content. Others, have also shown that slow absorption and digestion of starch from a meal improved glucose tolerance at the second meal (lunch) [21]. Moreover, it has been shown that a low GI may reduce subsequent energy intake and that the postprandial glycemic response has an important effect on short-term appetite sensations [22].

Our results may be partly explained by another study's findings showing that consumption of polyphenol-rich insoluble fiber preparation from carob pulp decreased postprandial acylated ghrelin, an orexigenic hormone, leading to increased satiety [19]. In contrast, another short-term study showed that consumption of carob fiber-enriched foods (white bread, spice cake, soup, and milk drink) increased total and acylated plasma ghrelin in healthy adults, leading to decreased satiety [20]. There are no studies available examining the effects of carob flour on biological markers of satiety and energy homeostasis and thus, we are unable to draw conclusions from the few available and contradicting studies regarding short- and long-term effects of carob flour consumption on satiety and energy intake regulation.

In our study, a preload including a carob snack led to an approximately 270 kJ reduction in energy intake at the following meal. Furthermore, self-reported 24hr energy intake was not greater following the carob snack compared with the chocolate cookie snack indicating that volunteers did not compensate for the lower energy consumed during the carob snack intervention; indicating that products made with carob may be offer some advantages to weight control. Whether the induction of more carob flour-including snacks during the day would reduce the daily energy intake and exclude or reduce consumption of energy dense foods needs to be investigated.

The strengths of our studies include the randomized, crossover design. Moreover, we tested the GI of a novel snack made with carob and compared it to a chocolate cookie snack. Among the limitations of the study one could mention that one serving of a preload snack may not suffice to obtain clinically meaningful glycemic response data. Also, we did not measure biochemical indices of satiety and appetite, such as ghrelin and leptin. Moreover, our second study offered only acute observations and we do not know which may be the long-term effects of carob snack consumption on blood glucose, subjective appetite and energy intake. Although we tried to control for several factors known to affect blood glucose levels (i.e. stress, exercise, illness) and the crossover design of the study also contributed to that; remaining confounding factors cannot be ruled out. Despite these limitations, this study adds to a growing body of evidence supporting that carob flour may be a dietary alternative to body weight and glycemic control due to its natural sweetness and low GI properties.

In conclusion, our results showed that the carob snack was a low GI food which increased satiety, led to lower energy intake at the following meal and to lower glycemic response; indicating that it may be a dietary alternative for both weight and glycemic control.

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Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this paper. The authors have nothing to disclose.

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Figure Legends:

Figure 1: Schematic showing the outline of the two studies, including the timings of capillary blood samples and visual analogue scale ratings (VAS)

Figure 2: Glycemic response (A) and incremental area under the curve (iAUC) (B) for glucose after consumption of test snacks (carob snack and chocolate cookie) and reference foods (white bread and glucose), (n=10).

Data are means ± SEM. Data were compared by post hoc analysis of repeated measures ANOVA. To convert mg/dl to mmol/l, values need to be divided by the number 18.

Figure 3. Glycemic response and incremental area under the curve (iAUC) after consuming *ad libitum* meal (lunch and dessert) following a preload snack which includes carob and chocolate cookie (n=50)

Data are means ± SEM. * $p < 0.05$ carob preload snack vs. chocolate cookie preload snack by post hoc analysis of repeated measures ANOVA. Subjects had significantly lower glucose concentrations at 60min ($p = 0.02$) and 120min ($p = 0.008$) after meal (lunch and dessert) consumption when they consumed the carob preload snack compared to the chocolate cookie snack. To convert mg/dl to mmol/l, values need to be divided by the number 18.

Figure 4. **Subjective appetite ratings from visual analogue scales (VAS), (n=50)**

Data are means ± SEM. *$p < 0.05$, **$p < 0.005$ carob preload snack vs. chocolate cookie preload snack by post hoc analysis of repeated measures ANOVA.

Table 1. Anthropometric characteristics of participants at baseline (n=50)

Age (years)	25 ± 6
Sex (n – male / female)	22 / 28
Height (cm)	172 ± 8
Body weight (kg)	69 ± 13
Waist circumference (cm)	79 ± 11
Hip circumference (cm)	100 ± 14
Body mass index (kg/m^2)	23 ± 3
Total Body Fat (%)	22 ± 8
Fat mass (kg)	15 ± 6
Fat-free mass (kg)	52 ± 13
Basal Metabolic Rate (BMR, kcal)	1603 ± 334

All values are means ± SD.

Table 2. Average nutrition facts of the carob snack and chocolate cookie as retrieved by laboratory analysis

Per 100 g serving	Carob snack	Chocolate cookie snack
Energy content (kcal)	405.5 (1697 kJ)	403.8 (1690 kJ)
Carbohydrates (total) (g)	53.00	53.50
Available carbohydrate (g)	38.50	58.14
Sugars (g)	24.00	36.40
Starch (g)	10.08	10.80
Dietary fiber (g)	14.50	4.90
Soluble fiber (g)	14.00	1.09
Protein (g)	4.50	4.25
Fat (g)	19.50	19.30
Saturated fat (g)	2.50	12.70
Polyunsaturated fat (g)	2.40	1.68
Monounsaturated fat (g)	13.60	4.45
Trans fatty acids (g)	<0.10	0.60
Salt (g)	0.06	0.82
Energy density (kcal/g)	4.14	4.04
Main ingredients for producing 100 g	35 g of carob powder (roasted carob flour), 32 g of carob syrup, 23 g ground hazelnuts, 10 g of soft vegetable fat (homogenizing agent).	18 g butter 13 g white all-purpose flour 13 g coconut milk 12 g cocoa 44 g honey

The energy and macronutrient content of the preloads were analyzed at the laboratory of food quality control and hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens. The nutritional value of 100g of carob flour is the following: 222 kcal (929 kJ), 89 g total carbohydrates, 49 g sugars, 40 g dietary fiber, 5 g proteins, 1 g fat, 0 g saturated fat, 0 g trans fat, 0 mg cholesterol, 35 mg sodium, 35% calcium, 16% iron.

Table 3. Determination of the incremental area under the curve (iAUC) and glycemic index (GI) of the preload snacks

Food	iAUC ($mg*120min*dl^{-1}$)	GI (glucose as reference food)	GI (white bread as reference food)
Glucose	1766 ± 370	–	–
White bread	1622 ± 299		
Carob snack	699 ± 173	39.58 ± 4.77	43.09 ± 5.78
Chocolate cookie snack	1373 ± 199	77.75 ± 5.38	84.65 ± 6.66

Data are means ± SEM. Each value represents the mean of ten replicates. To convert mg/dl to mmol/l, values need to be divided by the number 18.

Table 4. Post preload consumption

	Carob snack day	Chocolate cookie day	P-value
Energy intake form meal (including lunch and the dessert) (kcal/kJ)[a]	950 ± 36 kcal/ 3975 ± 151 kJ	1014 ± 41/ 4243 ± 172 kJ	0.035
Lunch consumption (g)[b]	453 ± 16	499 ± 19	0.001
Dessert consumption after lunch (g)	104 ± 8	111 ± 8	0.525
Protein (g) at lunch$_c$	43 ± 2	47 ± 2	0.037
Carbohydrates (g) at lunch[d]	133 ± 5	143 ± 6	0.048
Fat (g) at lunch	27 ± 1	28 ± 1	0.544
Chicken (g)	183 ± 7	196 ± 8	0.079
Rice (g)[e]	270 ± 10	304 ± 13	0.001
Energy intake 24h after study days (kcal)	2029± 143	1995 ± 118	0.797

Data are Means ± SEM. Means were compared by repeated measures ANOVA.
[a, b, c, d, e] Significant main effect of preload snack.

Figure 1

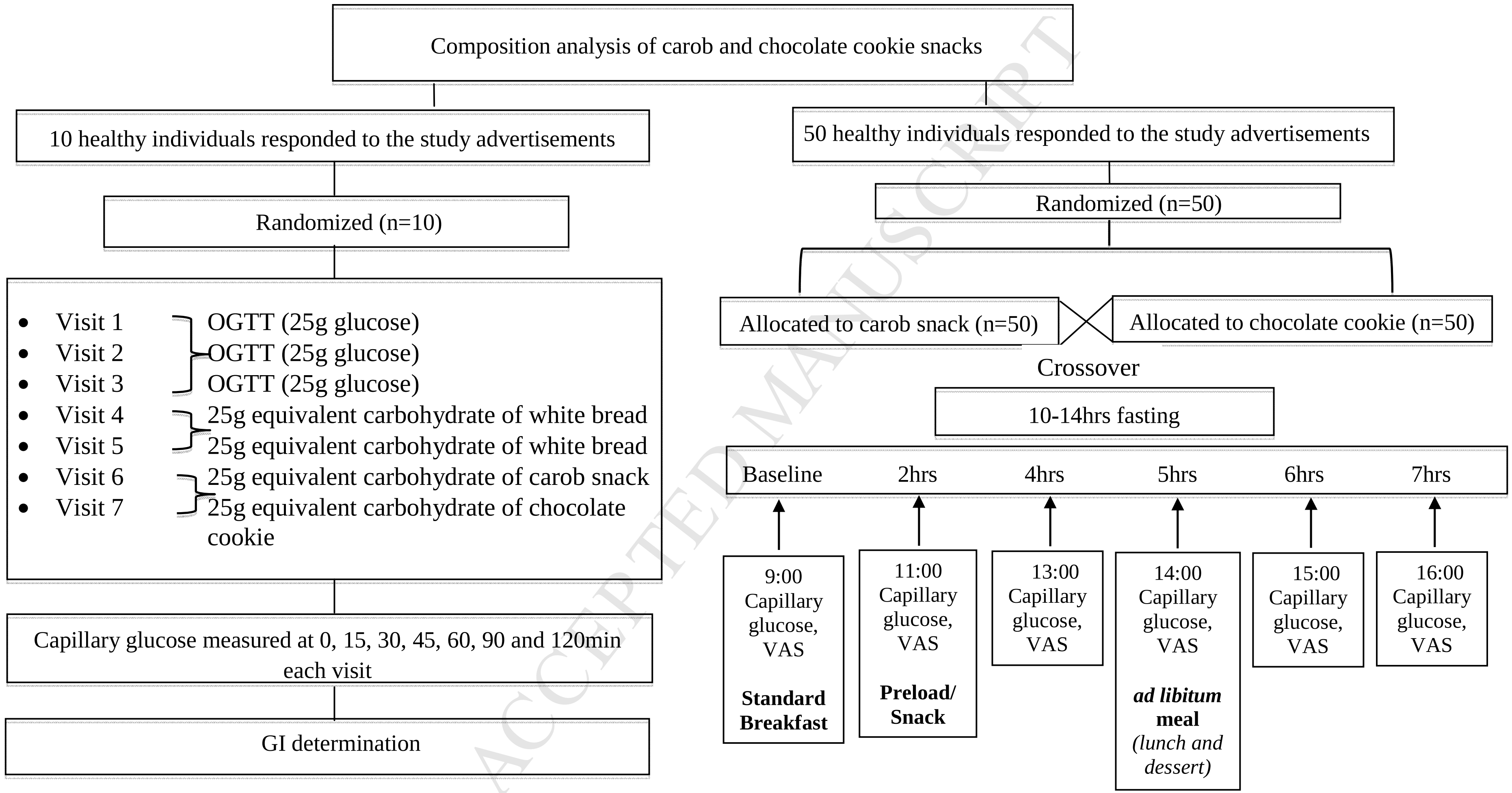
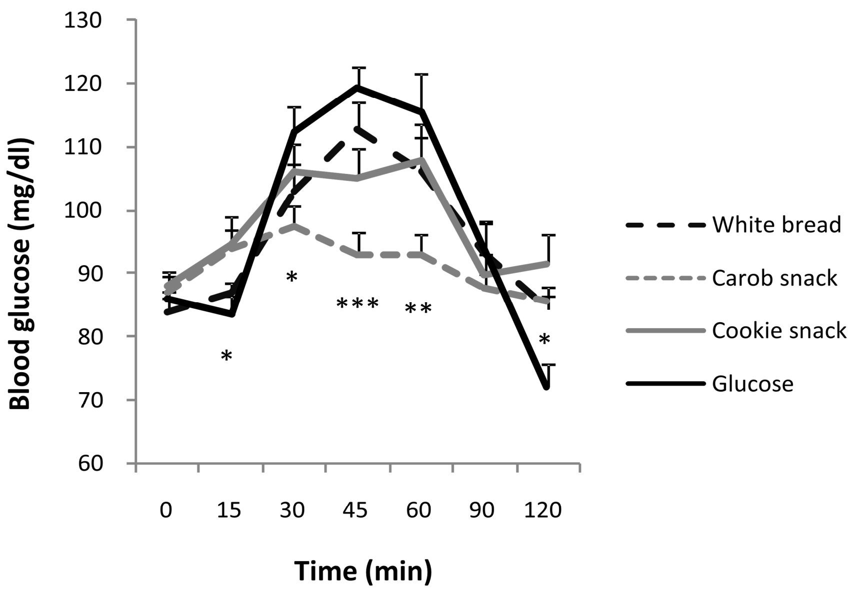

Composition analysis of carob and chocolate cookie snacks
10 healthy individuals responded to the study advertisements
Randomized (n=10)
Visit 1 OGTT (25g glucose)
Visit 2 OGTT (25g glucose)
Visit 3 OGTT (25g glucose)
Visit 4 25g equivalent carbohydrate of white bread
Visit 5 25g equivalent carbohydrate of white bread
Visit 6 25g equivalent carbohydrate of carob snack
Visit 7 25g equivalent carbohydrate of chocolate cookie
Capillary glucose measured at 0, 15, 30, 45, 60, 90 and 120min each visit
GI determination
50 healthy individuals responded to the study advertisements
Randomized (n=50)
Allocated to carob snack (n=50)
Allocated to chocolate cookie (n=50)
Crossover
10-14hrs fasting
Baseline
2hrs
4hrs
5hrs
6hrs
7hrs
9:00 Capillary glucose, VAS
Standard Breakfast
11:00 Capillary glucose, VAS
Preload/ Snack
13:00 Capillary glucose, VAS
14:00 Capillary glucose, VAS
ad libitum meal (lunch and dessert)
15:00 Capillary glucose, VAS
16:00 Capillary glucose, VAS

Figure 2.

A


Blood glucose (mg/dl)
130
120
110
100
90
80
70
60
0
15
30
45
60
90
120
Time (min)
White bread
Carob snack
Cookie snack
Glucose
*
*
***
**
*

B

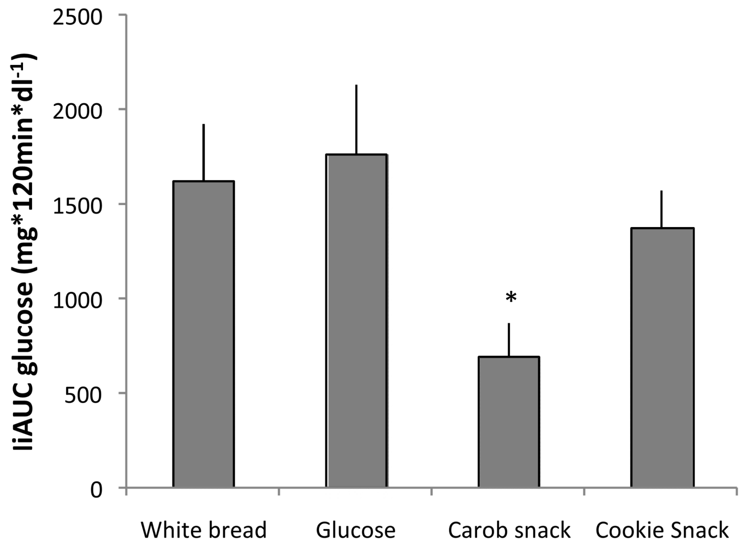
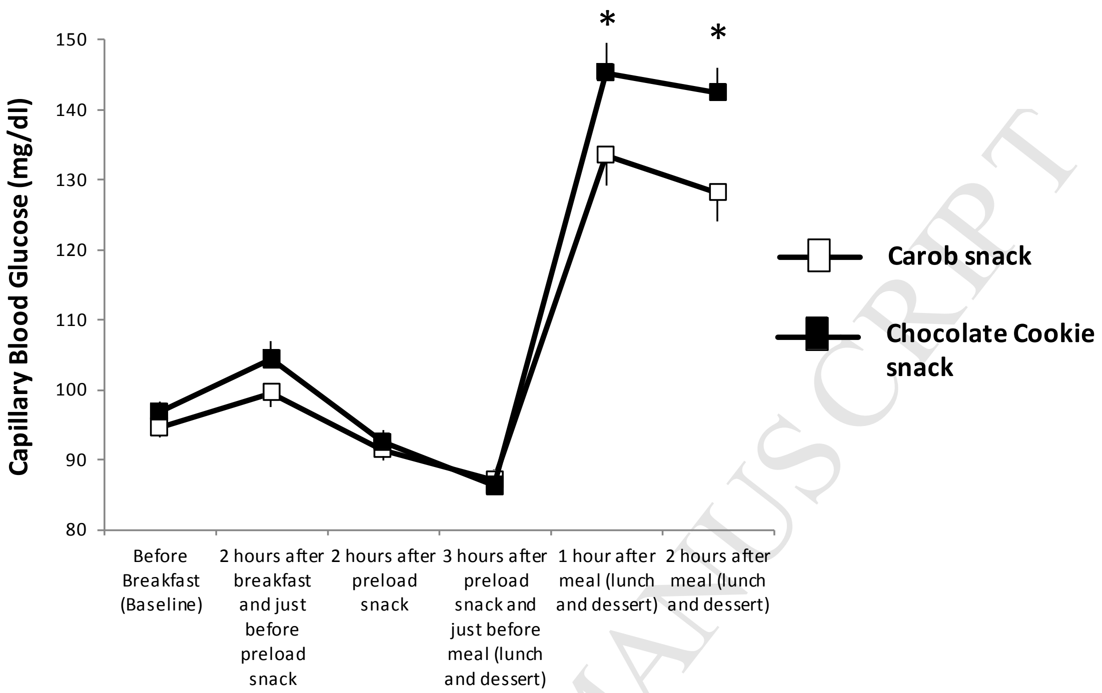
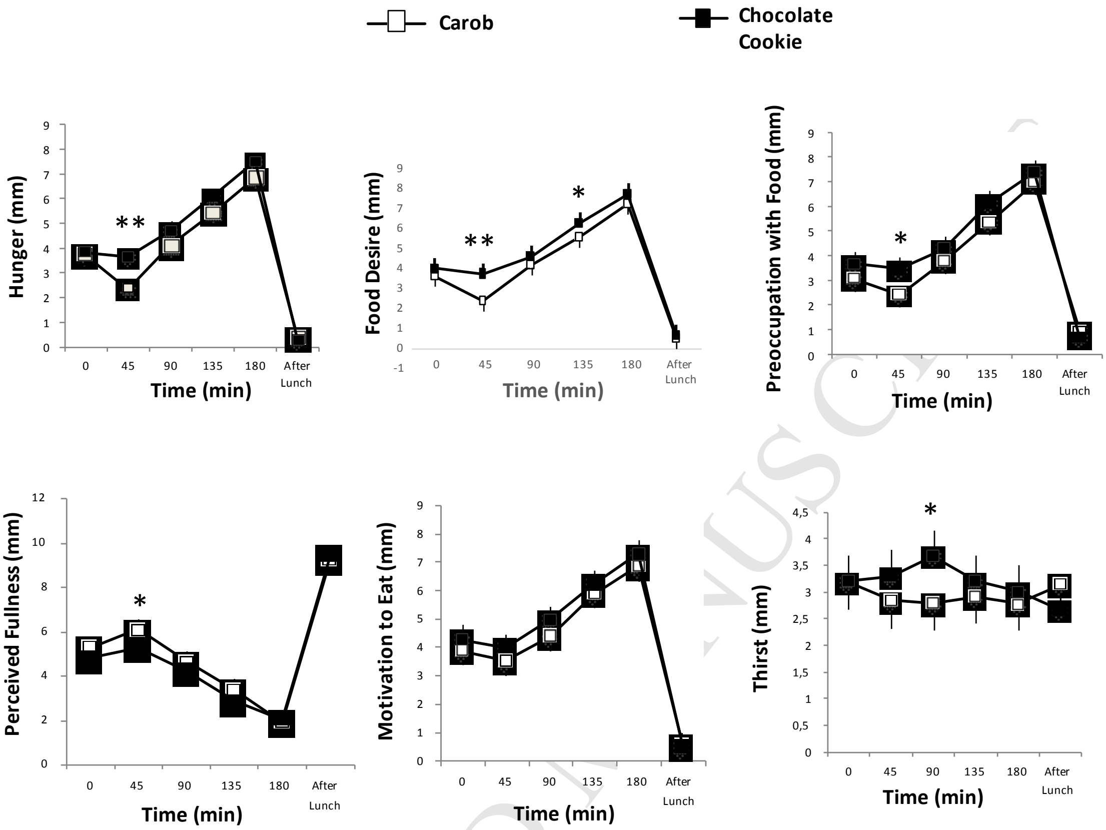

IiAUC glucose (mg*120min*dl-1)
2500
2000
1500
1000
500
0
White bread
Glucose
Carob snack
Cookie Snack
*

Figure 3.



Capillary Blood Glucose (mg/dl)
150
140
130
120
110
100
90
80
Before Breakfast (Baseline)
2 hours after breakfast and just before preload snack
2 hours after preload snack
3 hours after preload snack and just before meal (lunch and dessert)
1 hour after meal (lunch and dessert)
2 hours after meal (lunch and dessert)
*
*
Carob snack
Chocolate Cookie snack

Figure 4.


Carob
Chocolate Cookie
Hunger (mm)
Time (min)
Food Desire (mm)
Time (min)
Preoccupation with Food (mm)
Time (min)
Perceived Fullness (mm)
Time (min)
Motivation to Eat (mm)
Time (min)
Thirst (mm)
Time (min)
0
45
90
135
180
After Lunch

Highlights

- A carob based snack was found to be a low glycemic index (GI) food.
- The carob snack led to increased satiety and lower energy intake at *ad-libitum* meal.
- The carob preload snack decreased significantly post-meal glycemic response.
- Snacks formulated with carob flour may offer advantages to body weight and glycemic control.