Energy-restricted diets based on a distinct food selection affecting the glycemic index induce different weight loss and oxidative response

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Abbreviations:

GI: glycemic index; BMI: body mass index; REE: resting energy expenditure; MFA: monounsaturated fatty acid; PFA: polyunsaturated fatty acid; SFA: saturated fatty acids; FM: fat mass; MAA: muscle arm area; FFM: fat free mass; MO: mitochondrial oxidation.

Abstract

Background and aims: Low glycemic index (GI) based diets could influence the accompanying physiological adaptations to energy restriction in the treatment of obesity. It was aimed to investigate the effects of two energy-restricted diets with different food distribution and GI values on weight loss and energy metabolism in the nutritional treatment of obesity.

Subjects and Methods: Participants (n=32;BMI:32.5±4.3kg/m^2) were randomly assigned to follow two energy-restricted diets with higher-GI or lower-GI for 8 weeks. The energy restriction was -30% in relation to energy expenditure. Anthropometry, energy expenditure and mitochondrial oxidation were assessed at baseline and at the endpoint of the intervention. Body weight was also measured one year after the treatment. The work was approved by the ethical committees of the University of Navarra (54/2006).

Results: Volunteers consuming the lower-GI diet showed a significantly higher weight loss than their counterparts (-5.3±2.6% vs -7.5±2.9%;p=0.032), although the decrease in resting energy expenditure (REE) was similar between groups (p=0.783). Mitochondrial oxidation was significantly affected by the type of diet (p=0.001), being activated after the lower-GI treatment (p=0.022). Interestingly, one year after the nutritional intervention weight regain was only statistically significant in the higher-GI group (p=0.033).

Conclusions: Lower-GI energy-restricted diets achieved through a specific differential food selection can improve the energy adaptations during obesity treatment, favouring weight loss and probably weight maintenance compared with higher-GI hypocaloric diets.

Keywords: Obesity, glycemic index, weight loss, energy expenditure, mitochondrial oxidation, weight maintenance.

INTRODUCTION

The prevalence of obesity and related co-morbidities is substantially increasing worldwide (1). Although genetics plays a role in weight gain, it cannot independently explain the dramatic rise in obesity rates over the past several decades, which is mainly attributed to changes in dietary and physical activity patterns (2). In addition to obesity prevention strategies concerning physical activity promotion and nutritional advice, various pharmacological and surgical treatments have been proposed to treat the obese, but diet is still the basic therapeutic tool against obesity (3).

A positive energy balance due to overfeeding is often a causal factor of obesity (4). In this context, carbohydrate intake is being increased worldwide in the form of refined starchy foods and concentrated sugar beverages, which is associated with an elevated high glycemic index and a reduced fiber intake which has also been related with obesity rates (5). Physiological evidences indicate that the consumption of high-GI meals induce hormonal changes that not only influence the availability of metabolic fuels in the post-prandial period, but also may stimulate hunger and inhibit fat oxidation (6). Indeed, several trials have suggested that low-GI diets provide specific benefits for weight loss and cardiovascular risk reduction by regulating food intake (6, 7).

Based on the potential role of low-GI foods in weight control and obesity management, the tested hypothesis was that a hypocaloric diet with a selection of foods decreasing the GI can improve the energy response in the treatment of obesity. Thus, the aim of this study was to investigate the effects of two dietary energy-restricted approaches with similar macronutrient content, but different food distribution modifying the glycemic index on metabolic markers such as body weight, plasma biochemical indicators, energy expenditure and mitochondrial oxidation, which are expected to be affected during the hypocaloric treatment of obesity.

MATERIAL AND METHODS

Subjects

Thirty two obese subjects (BMI: 32.5±4.3 kg/m^2) were recruited to participate in the study (14 women and 18 men; 36±7 years old). Potential volunteers were contacted through internal and local advertisements. A physician performed the screening and the inclusion of volunteers by means of a medical history, physical examination and fasting blood profile to exclude subjects with evidence of diabetes, hypertension, liver, renal or haematological disease as well as other clinical disorders that could interfere with the weight loss process. Other exclusion criteria were weight change higher than ±3 kg within the three months before the start of the study, participation in another scientific study up to 90 days before, chronic pharmacological therapies, pregnancy, surgical or drug related obesity treatments, as well as alcohol or drug abuse. After a detailed explanation of the study protocol, all subjects gave written informed consent to participate in the trial, which was previously approved by the Ethics Committee of the University of Navarra (54/2006).

Study design

Subjects were enrolled in this prospective study and randomly assigned to one of the two dietary treatments: higher-GI and lower-GI energy-restricted approaches. Balanced diets were designed to provide the same distribution of macronutrients: 53% of energy as carbohydrates, 17% as proteins and 30% as fat (MFA: 18.8±1.7%, PFA: 3.7±0.3%, SFA: 5.3±0.6%). Participants were individually instructed to follow the prescribed dietary regime for eight consecutive weeks by a trained dietician within a strict dietary framework, which was repeated on a 3-day rotation basis (Table 1).

The glycemic index of lower-GI diet was reduced by counselling some modifications in the carbohydrate consumption patterns, which was achieved by advising a driven food selection, affecting also protein quality, fiber nutritional sources and cooking style.

Thus, most of the high-GI foods in the higher-GI diet were replaced by foods with low-GI carbohydrates in the lower-GI diet (Table 1). Foods considered in the GI adjustment threshold were potatoes, rice, bread, pasta and legumes as well as fish and meat products. This approach provided about 84% of total carbohydrates in the lower-GI diet from pasta and legumes and 84% of total carbohydrates in higher-GI diet from rice and potatoes. As mentioned earlier, the protein source was different in both intervention groups. Thus, the animal protein intake was decreased, while the plant protein (legumes/cereals) was accordingly increased in the lower-GI diet. The protein was mainly of animal origin in the other dietary group (Table 1). Diet records were assessed by using the Medisystem software adapted for Spanish foods (Sanocare, Spain) and the GI was calculated using a validated guide (8), which resulted in about 60-65 units in the higher-GI diet and about 40-45 units in the lower-GI diet.

The induced energy restriction of both hypocaloric diets was -30% with respect to the individually measured total energy expenditure specifically calculated from the REE assessed by indirect calorimetry (Deltatrac, Datex-Ohmeda, Finland) and corrected by the physical activity level of each participant (9). So, the mean energy provided by the intervention was 1495±245 kcal/day for the lower-GI group and 1568±225 kcal/day for the higher-GI group.

Volunteers were asked to maintain the same habitual physical activity habits during the intervention period, which was assessed through specific questions during the interviews. Weight loss was monitored weekly by a dietician and the intake was controlled by 3-day weighted food records (2 weekdays and 1 weekend day). Foods records were performed during the week before the beginning of the intervention (week -1) and during the week before the end of the nutritional trial (week +7). These data provided information about baseline intake and the adherence to the prescribed diets. Anthropometry, body composition, energy expenditure, blood and 12 h urine samples

were assessed at baseline (day 0) and at the endpoint (day 56) following standardized procedures (10-12).

Anthropometry and body composition

Body weight assessment was performed using a digital balance accurate to 0.1 kg (Seca 767, Vogel & Halke, Germany) and height accurate to 1cm, using a wall-mounted stadiometer (Seca 220, Vogel & Halke, Germany). Measurements were carried out in underwear after an overnight fast. The waist circumference was measured at the site of the smallest circumference between the rib cage and the iliac crest (10), and the hip circumference was measured on the maximum circumference over the buttocks (10) with the subject in standing position. Body composition was assessed by bioelectrical impedance (Quadscan 4000, Bodystat, UK), based on a previously validated procedure (11).

Blood pressure measurements and biochemical analysis in blood and urine.

Blood pressure was measured with a standard mercury sphygmomanometer (Minimus II, Riester, Germany) after the subject was quietly sitting for 5 min following OMS criteria.

Venous blood samples were drawn at fasting state (12 h) to measure basal circulating levels of selected biochemical markers. Plasma levels of glucose (ABX Diagnostics, Germany) and urine urea concentration (ABX Diagnostics, Germany) were assayed on a Cobas-Mira equipment (Roche, Switzerland). Plasma levels of insulin were assessed by a commercially available radioimmunoassay (DPC, USA) and insulin resistance was indirectly estimated by the homeostatic model assessment index (HOMA), as the product of fasting insulinemia (μU/ml) per glycemia (mM), which was divided per 22.5 (13, 14). Insulin resistance was considered if the HOMA index was higher than 3.5 (14). Serum leptin was measured by using a radioimmunoassay kit (DPC, USA).

The rate of urinary nitrogen excretion was calculated from urea urine concentration by using appropriate equations (15), since most of the urinary nitrogen (>80%) is in the form of urea (15, 16). Nitrogen balance was computed by the difference between dietary nitrogen (grams of protein intake/6.25) and nitrogen excretion (urine nitrogen + 3 g/day) (17).

Mitochondrial oxidation measurement

The 2-keto[1-^{13}C]isocaproate breath test was performed to study mitochondrial oxidation *in vivo* (18). After the indirect calorimetry was performed, the subjects received 6.5 μmol/kg 2-keto[1-^{13}C]isocaproate sodium salt (Euriso-top, France) together with 152.4 μmol/kg L-leucine USP (Sigma-Aldrich Chemicals, Spain) dissolved in 200 ml orange juice. This short chain keto acid is specifically metabolized by mitochondria, raising CO_2 after oxidative decarboxylation, which is eliminated through the lungs (14). The breath test estimates mitochondrial oxidation from the decarboxylation of the 2-ketoisocaproate labelled with ^{13}C in the carboxylic acid group, measuring the exhalation of $^{13}CO_2$ after the tracer ingestion (19). Thus, breath samples were recovered by exhaling through a straw into a tube (Labco, England) before and at 10-min intervals during the 2 h after ingestion of the 2-keto[1-^{13}C]isocaproate. Enrichment of $^{13}CO_2$ in breath was measured by isotope ratio mass spectrometry on a BreathMAT plus spectrometer (Finnigan, Germany) and the percent of 2-keto[1-^{13}C]isocaproate oxidized at 2 h after the test meal ingestion (% ^{13}C) was calculated (19). Volunteers repeated this protocol after the nutritional intervention trial once they lost weight.

Follow-up

After the caloric restriction was ended, a maintenance diet was given to each volunteer with specific nutritional recommendations to follow healthy dietary habits.

Participants were invited to come to the Metabolic Unit one year after the nutritional intervention to evaluate the body weight status after such period of time.

Statistical analysis

Sample size was established considering the weight loss as the main variable. Published values for the standard deviation (SD) of weight loss were applied and 2 kg was considered as the potential difference between means of the two interventions (20). The statistical power was set up at 80%. Therefore, and by applying a p-value <0.05, the sample size required was a minimum of 14 volunteers per group (21).

The Kolmogorov-Smirnov and the Shapiro-Wilk tests were used to determine the variable distribution and both within group and between group differences were analyzed. Thus, changes in weight loss were evaluated and compared by applying the paired parametric t-tests (baseline vs endpoint) and with the repeated measures ANOVA to evaluate the weight loss time-course (eight points). With respect to other variables, the Wilcoxon (non-parametric) and paired t-test (parametric) were applied to analyze within groups differences (baseline vs endpoint) as appropriate. The Student t-test (parametric) and the Mann-Withney U test (non-parametric) were used to analyze between groups differences (lower-GI vs higher-GI). The Pearson (parametric) or the Spearman (non-parametric) coefficients were used to set up the potential relationships among variables. A multivariate regression model with no more than three variables based on sample size was applied to describe the observed mitochondrial oxidation changes (dependent variable), considering diet, $leptin_{adjusted\text{-}FM}$ and REE as independent variables. Leptin blood levels were adjusted for fat mass, while REE was adjusted for fat free mass by using the residuals method. Results are reported as mean±SD, statistical significance was set up at $p<0.05$. All statistical analyses were performed using the SPSS 13.0 program (SPSS Inc., USA) for Windows XP (Microsoft, USA).

RESULTS

As designed, macronutrient distribution was similar between both experimental groups (Table 2), fulfilling the accepted recommendations for a healthy diet (carbohydrates: 53%, lipids: 30%, proteins: 17%), but with different daily glycemic index ranges (higher-GI diet: 60-65 vs lower-GI diet: 40-45 units)(8). Also, fiber content was statistically different between diets (Table 2).

At baseline, volunteers included in both experimental groups had similar characteristics with the exception of the total cholesterol. Therefore, within groups changes and the percentage of diet-related changes were compared (Table 3).

After the dietary intervention, the weight reduction was statistically different ($p<0.001$) in both experimental groups, being higher in participants that followed the lower-GI diet (Fig. 1). In fact, the weight loss directly correlated with fiber intake in this experimental group ($r=0.43$; $p=0.018$). Body composition (bioimpedance determinations and muscle arm area measurements) and other metabolic determinants such as insulin, leptin and REE changed in a similar way in both groups under dieting (Table 3).

As expected after a caloric restriction, nitrogen balance reached negative values after the intervention (higher-GI: -3.4±1.5 g/day; lower-GI: -2.7±0.9 g/day; $p=0.119$). The energy restriction produced approximately the same statistically decrease ($p=0.001$) in REE after both treatments (Table 3). This outcome was not affected when the variable was adjusted for fat free mass, even when the weight loss was different depending on diet (Fig. 2). Indeed, participants that followed the lower-GI diet showed an apparently smaller decrease than their counterparts when comparing the decrease of REE for each kilogram of FFM lost (-1.4 vs -4.0 kcal/day kg), although with no statistical impact ($p=0.360$).

Interestingly, the 2-keto[1-^{13}C]isocaproate oxidation statistically increased (p= 0.022) in the obese subjects ascribed to the lower-GI diet, while participants in higher-GI diet statistically decreased (p= 0.004) the amount of tracer oxidized after the trial. Therefore, mitochondrial oxidative response showed opposite trends (p=0.001) depending on the nutritional treatment (Fig. 2). In order to investigate factors related to the change in the percentage of tracer mitochondrially oxidized (% ^{13}C), a separate regression analysis for each variable was performed. This analysis showed that the type of diet accounted for 37% (p<0.001), the change in REE accounted for 25% (p=0.015) and the leptin$_{adjusted\text{-}FM}$ for 23% (p=0.009). Taking into account these variables as independent factors and the mitochondrial oxidation change as the dependent variable, the final model revealed that the lower-GI diet induced an increase in mitochondrial oxidation of 3.5-folds higher with respect to higher-GI diet (corrected r^2= 0.61; p<0.001).

One year after the end of the nutritional intervention, about 47% of volunteers came back to the Metabolic Unit to carry out the body weight follow-up assessment. Among these, volunteers treated by the higher-GI diet regained weight with statistical significance (n=8; +5.1±5.4 kg; p=0.003). This change was lower and not statistically significant for those participants that followed the lower-GI diet (n=7; +4.0±5.5 kg; p=0.101).

DISCUSSION

Short-term intervention studies in humans evaluating the effects of energy-restricted diets with different GI on body weight have produced controversial results (3, 22, 23). However, a recent prospective study showed that a low-GI diet may protect against increases in body weight (24). In this sense, our results have shown that a lower-GI diet (based on wholemeal cereals and legume consumption) can improve weight loss during an energy restriction period in comparison with a conventional hypocaloric diet (higher-GI and lower fiber content). Several authors have confirmed that some nutritional factors may influence the effectiveness of weight loss diets (25). Thus, legume and cereal components such as soluble fiber, low-GI carbohydrates, proteins and other bioactive substances can potentially improve weight loss by means of favourable effects on energy regulation (26). In fact, there are several studies indicating that increased legume consumption is inversely related with the body mass index (27, 28).

On the other hand, there is some evidence suggesting that high-GI diets may produce adverse effects, specifically favouring the catabolism of lean body mass (29). However, we did not observe this undesirable outcome, which could be due to the moderate GI value (8, 30) of the higher-GI diet. Fat free mass losses are expected during a weight loss period when physical activity is not accompanying the slimming strategy. Indeed, both dietary groups lost lean mass, which could be attributed to changes in truncal fat free mass and to the loss of body fluids (31).

This effect was confirmed by the change observed in the REE. Both experimental groups showed a similar decrease in REE despite differences in the weight loss. It is known that energy restriction reduces metabolic rate through loss of weight and metabolizing tissue mass (32). However, intervention studies indicated that the change in REE during an energy restriction period could be ameliorated by a low-GI diet (29, 33). Often, these studies combined low-GI and high-protein dietary content, which

should be considered when interpreting such data due to the thermogenic effect of protein (29). In the current work, the protein content was similar in both groups, so the metabolic response depended on the glycemic index, quality of proteins or other food components included in the diet.

The observed effect should be taken into account in the nutritional treatment of obesity, since the decrease in REE induced by hypocaloric diets has been related with the weight regain or rebound effect when the dietary intervention is finished (34). Indeed, weight regain was slightly lower in volunteers that followed the lower-GI diet one year after the end of the dietary treatment. Thus, resting energy expenditure seemed to be protected by the lower-GI intervention minimizing its decrease probably mediated by the activation of alternative metabolic pathways. Reinforcing this observation, we found a diet-related change in the mitochondrial oxidation of a keto acid closely related to the citric acid cycle (19). Activation of this mitochondrial pathway has been described after a successful nutritionally induced weight loss (14). The current research evidenced that the mitochondrial response seems to be modulated by the type of nutritional intervention, showing a specific oxidative activation mediated by the lower-GI diet.

Also, the measured biochemical markers followed the expected trends since the lipid and glycemic profile changes were more beneficial in the lower-GI group (35).

Thus, the important finding in this investigation was that both dietary regimens induced weight loss, but the impact of the lower-GI diet on energy metabolism (REE and MO), lipids (total cholesterol and LDL-cholesterol) and glycemic profiles (insulin and glucose) was improved beyond the expectations associated with the weight lowering, as compared with higher-GI diet. A lower-GI diet with a specific food selection (such as legumes or cereals) is able to differently affect weight losses and to modulate the energy adaptations to the caloric restriction. The fact that legumes were the selected foods to reduce the GI should be considered, since these grains may supply specific bioactive

compounds (antioxidants, starch blocking agents, fiber, etc.), and antinutritional factors with metabolic implications that could be involved in energy homeostasis.

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Figure 1

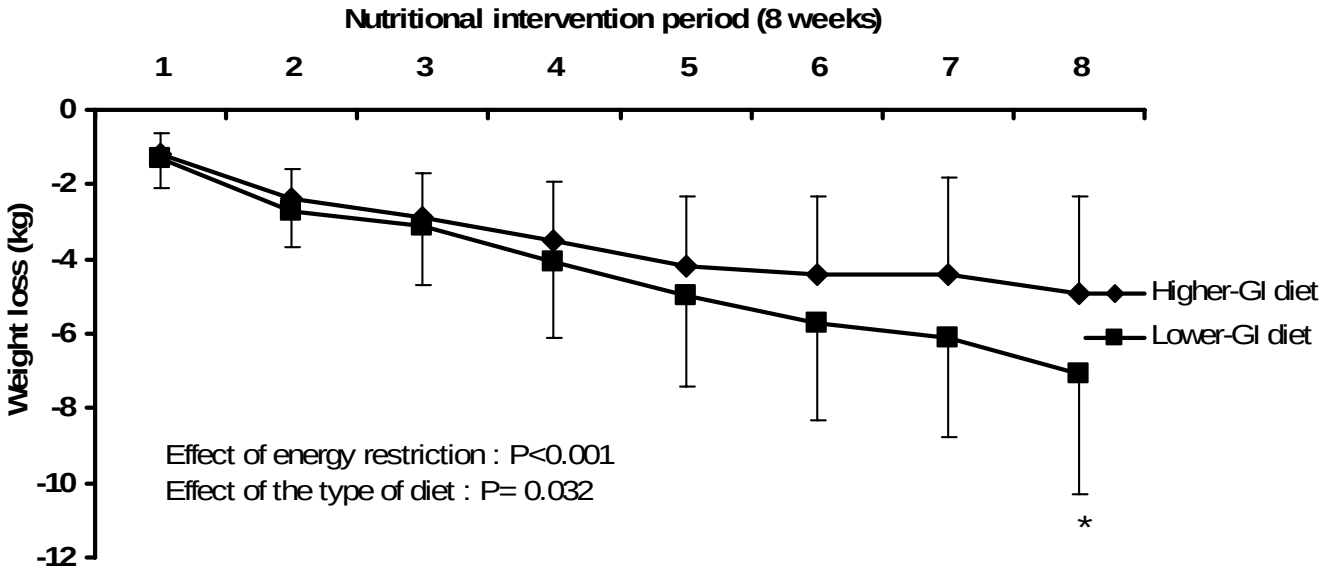

Figure 1: Weight loss in both dietary groups after the nutritional intervention. The asterisk indicates statistical differences between experimental groups. The statistical tests used were the paired parametric t-tests (baseline vs endpoint) and the repeated measures ANOVA.

Figure 2

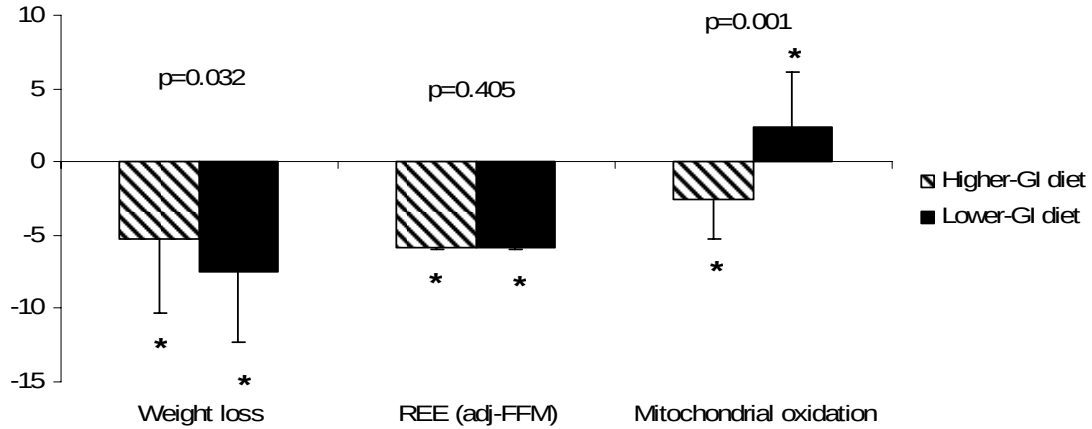

Figure 2: Changes (%) in body weight, resting energy expenditure and mitochondrial oxidation in the experimental groups after the nutritional intervention. The asterisk indicates statistical significance ($p<0.05$) between baseline and endpoint values within each dietary groups, while the p-values show differences of the change (%) between both dietary groups. The statistical tests used to analyze data were the paired parametric t-test and the t-test for independent variables.

Table 1: Examples of the menus of each dietary group for 3 days.

	1		2		3	
	Higher-GI	**Lower-GI**	**Higher-GI**	**Lower-GI**	**Higher-GI**	**Lower-GI**
Breakfast	Skimmed milk Bread	Skimmed milk Breakfast cereals (All-bran)	Skimmed milk Bread	Skimmed milk Breakfast cereals (All-bran)	Skimmed milk Bread	Skimmed milk Breakfast cereals (All-bran)
Snack	Low-fat yogurt fruit Cherries	Skimmed cheese Pear	Low-fat yogurt fruit Watermelon	Skimmed cheese Orange	Low-fat yogurt fruit Cantaloupe	Skimmed cheese Apple
Lunch	Vegetables Potatoes Lean meat Bread Low-fat yogurt fruit	Vegetables Pulses Bread Low-fat yogurt	Vegetables Rice Lean fish Bread Low-fat yogurt fruit	Vegetables Pasta Lean fish Bread Low-fat yogurt	Vegetables Potatoes Lean meat Bread Low-fat yogurt fruits	Vegetables Pulses Bread Low-fat yogurt
Afternoon snack	Watermelon	Peach	Pineapple	Apple	Banana	Pear
Dinner	Salad Bread Lean meat Cantaloupe	Salad Bread Lean meat Plums	Salad Bread Lean meat Kiwi	Salad Bread Lean meat Pear	Salad Bread Lean meat Pineapple	Salad Bread Lean meat Peach

The amounts of foods were individually adapted to produce the prescribed individual energy restriction and followed a 3-day rotation pattern.

Table 2: Nutritional composition of both experimental diets during the intervention period.

Variables	Higher-GI	Lower-GI	P-value
Carbohydrates (%)	47.8±6.8	50.2±1.8	0.214
Lipids (%)	32.6±4.3	31.5±1.6	0.370
Proteins (%)	19.6±5.6	18.3±1.6	0.384
Fiber (g/day)	18.5±5.1	24.9±5.1	0.002
Cholesterol (mg/day)	84.9±76.6	80.9±57.1	0.498

The statistical test used to analyze data was the t-test for independent variables (lower-GI vs higher-GI).

Table 3: Baseline characteristics and percentual change in the measured variables on the volunteers included in both nutritional intervention groups (higher vs lower-GI).

	Higher-GI diet (n=16)		Lower-GI diet (n=16)		Statistical significance between baseline points	Statistical significance between changes (%)
	Baseline	CHANGE (%)	Baseline	CHANGE (%)		
Women/men	6/10		8/8			
Weight (kg)	94.4±13.1	-5.3±2.6*	94.3±16.1	-7.5±2.9*	0.994	0.033
BMI (kg/m^2)	32.2±4.4	-5.4±2.5*	32.8±4.3	-7.6±3.0*	0.700	0.030
Waist circumference (cm)	102±10	-6.4±3.3*	99±10	-6.4±3.6*	0.414	0.988
Fat mass (kg)	32.0±11.7	-13.1±8.5*	32.9±11.1	-14.8±5.8*	0.830	0.552
Fat free mass (kg)	62.4±10.2	-1.3±3.9	61.4±13.6	-3.5±3.3*	0.822	0.126
Muscle arm area (cm^2)	2.3±0.2	-2.9±3.6*	2.3±0.3	-4.7±3.7*	0.926	0.189
Systolic blood pressure (mm Hg)	114±9	-3.7±5.3	115±11	-6.5±8.2	0.796	0.275
Diastolic blood pressure (mm Hg)	76±9	-5.7±8.6*	75±6	-7.5±7.5	0.906	0.551
Total cholesterol (mg/dl)	181±34	-3.5±10.6	215±37	-14.4±10.5	0.014	0.010
LDL-c (mg/dl)	112±29	-3.2±14.3	136±5	-15.9±16.6	0.124	0.037
HDL-c (mg/dl)	51±9	-5.5±14.9	50±12	-9.7±8.1	0.935	0.348
Triglycerides (mg/dl)	89±28	5.1±40.8	97±36	-2.4±18.0	0.513	0.531
Circulating glucose (mg/dl)	93±8	-1.9±6.3	95±7	-2.2±5.5	0.617	0.897
Circulating insulin (μUI/ml)	6.5±2.2	19.7±58.2	7.4±3.8	-15.7±44.5	0.333	0.085
HOMA index	1.5±0.8	20.6±65.8	1.6±0.8	-16.5±47.6	0.341	0.102
Circulating leptin$_{adjusted\text{-}FM}$ (ng/ml)	38.8±0.7	-21.1±1.8*	38.8±1.1	-22.4±2.2*	0.837	0.125
Resting energy expenditure (kcal/d)	1698±245	-6.7±5.0*	1621±287	-6.1±4.8*	0.423	0.783

The statistical tests used to analyze data were the t-test for independent variables (baseline vs baseline; change % in the higher-GI diet vs change % in the lower-GI diet) and the paired t-tests to analyze within groups differences (baseline vs endpoint). * When the change is significative within each dietary group (baseline vs end-point).