Effects of different protein content and glycemic index of *ad libitum* diets on risk factors for diabetes in overweight adults after weight loss: the DIOGenes multicentre, randomised, dietary intervention trial

Estibaliz Goyenechea, Claus Holst, Wim H.M. Saris, Susan A. Jebb, Anthony Kafatos, Andreas Pfeiffer, Svetoslav Handjiev, Petr Hlavaty, Steen Stender, Thomas M. Larsen, Arne Astrup, J. Alfredo Martinez, on behalf of DIOGenes

Dpt. Physiology and Nutrition, University of Navarra, Pamplona, Spain (EG, JAM); Institute of Preventive Medicine, Centre for Health and Society, Copenhagen, Denmark (CH), Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands (WS); MRC Human Nutrition Research, Cambridge, UK (SAJ); Department of Social Medicine, Preventive Medicine & Nutrition Clinic, University of Crete, Heraklion, Greece (AK); Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuhetal, Germany (AP); National Multiprofile Transport Hospital, Sofia, Bulgaria (SH); Obesity Management Centre, Institute of Endocrinology, Prague, Czech Republic (PH); Department of Clinical Biochemistry, Gentofte University Hospital, Denmark (SS); Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark (TL, AA).

Running head: Diabetes risk factors in the DIOGenes study

Correspondence to:

Prof. J. Alfredo Martínez

Dpt. of Physiology and Nutrition

University of Navarra

Irunlarrea 1. 31008 Pamplona, Spain

E-mail: jalfmtz@unav.es

Telephone: +34948425600

Fax: +34948425649

Abstract

Aims/hypothesis: Diets which differ in protein and glycemic index (GI) may distinctively contribute to body weight control and metabolic consequences, especially those related to insulin sensitivity.

Methods: In DIOGenes, overweight/obese adults in 8 European countries who lost ≥ 8% of initial body-weight following a low calorie diet (LCD) were subsequently randomly assigned into five *ad libitum* interventional groups during 6 months: Low Protein [LP]/Low GI [LGI]; LP/High GI [HGI]; High Protein [HP]/LGI; HP/HGI and a healthy conventional control diet. Body weight, fasting insulin as well as an oral glucose tolerance test (OGTT), HOMA-IR, adiponectin and fructosamine levels were determined at the three time points.

Results: The LCD period was initiated by 932 adults, 773 were randomised to the 5 diets and 548 completed the intervention. The HP and LGI diets were related to weight-loss management and to a lower drop-out rate. The LP/HGI diet induced a greater increase in HOMA-IR during the 6 month period. The HP and LGI diets decreased HOMA-IR ($p<0.001$) in those patients with the highest weight-loss ($\geq$10% of initial body-weight). The LGI diets also lead to a decrease in fructosamine levels ($p<0.05$), and plasma insulin response was lower in the HP/LGI after 60 and 90 min of the OGTT at the end of the 6-months intervention ($p<0.05$) as compared to others.

Conclusion/Interpretation: An increase in dietary protein and a reduction in GI content over a 6-month *ad libitum* dietary intervention produced better compliance and favorable effects on glycaemic control and insulin sensitivity in overweight/obese subjects after an initial body-weight loss.

The study was registered with ClinicalTrials.gov, number NCT00390637.

Introduction

The worldwide pandemic of overweight/obesity is a major public health concern and is strongly linked to the rising prevalence of diabetes and cardiovascular disease [1, 2]. While an excess in caloric intake and positive energy balances are clearly associated with the development of these diseases and their consequences [3], dietary composition may also be important [4].

A diet-based weight loss program is an essential treatment for obesity and related diseases [4, 5]. However, it is well known that most individuals do not succeed in long-term weight-loss maintenance [6] and weight cycling and relapses are common features after weight lowering. Moreover, current strategies to avoid weight regain have been unconvincing [7]. Thus, today's challenge is to find an approach to maintain body weight-loss and to prevent subsequent relapse of characteristic metabolic alterations, such as insulin resistance.

Different dietary approaches concerning macronutrient composition of the diet has been suggested to promote long term weight loss. For weight-loss maintenance, there is currently a vigorous debate regarding the optimal dietary macronutrient composition to achieve such a goal [8-11]. A number of low-carbohydrate and high-protein content diets have been described to be effective in controlling blood glucose and insulin levels [12-14]. However, there are still conflicting results concerning this issue [8], while the effects on weight cycling have been poorly investigated [15]. Other clinical interventional programs have been focused on analysing different nutritional strategies to improve weight management and metabolic related disturbances, not only varying in macronutrient composition [9, 16], but also with different approaches, such as

manipulating the dietary glycemic index (GI) values [10, 17], or increasing the intake of calcium, or antioxidant or fruit [17-20]. Studies with low GI diets have shown a number of favourable effects such as rapid weight loss, better management of blood glucose and insulin levels, triglycerides and blood pressure reduction, among others [21, 22]. However, other investigations have reported no differences depending on the GI values [23].

On the other hand, *ad libitum* nutritional programs with different macronutrient composition, have been described to improve the adherence to the diet and weight loss [10, 24]. *Ad libitum* low-fat, low-carbohydrate and high-protein diets have all been proposed as a means to facilitate weight lowering and stability thereafter [25-27]. Also, several studies have shown that an increase in dietary protein content in the context of an *ad libitum* diet results in more pronounced weight loss in overweight individuals [12, 13] and contributes to better weight-loss maintenance [28, 29]. A meta-regression analysis suggested that a reduction in dietary GI under *ad libitum* or limited restriction conditions is associated with a modest body weight reduction [30]. So far, there is little evidence for the conjoint role of dietary protein content and GI in the context of *ad libitum* in body weight control and related metabolic disorders management.

Thus, the main aim of the study was to evaluate the role of five dietary groups varying in protein and GI when provided *ad libitum* in the prevention of 6-month weight regain and in diabetes related risk factors after an energy-restricted body weight loss period of at least 8% in overweight and obese adults.

The study is part of the European integrated project on Diet, obesity and genes (DIOGenes; www.diogenes-eu.org), which focuses on dietary means for weight (re)gain prevention [31, 32], and was performed in 8 European centres.

Methods

Subjects

Volunteer families were enrolled in eight European centres (The Netherlands, Denmark, United Kingdom, Greece, Spain, Germany, Bulgaria and the Czech Republic) between November 2005 and April 2007, using a number of recruitment strategies [31]. Initial screening evaluations included a medical history, physical examination, fasting blood profile and urine sample collection, to exclude subjects with evidence of diabetes, hypertension, liver, renal or haematological disease, or other clinical disorders that could interfere with the weight-loss process [31].

Families (two-parent or single-parent) were eligible for participation if they were generally healthy and (1) at least one parent was overweight (BMI > 27 kg/m^2) and aged < 65 y; (2) at least one child was between 8 and 15 y of age [32]. Exclusion criteria for adults were: BMI > 45 kg/m^2; diagnosed psychiatric diseases, eating disorders, infectious or inflammatory diseases, untreated hypo- or hyperthyroidism, gastro-intestinal, liver or kidney diseases, cardiac diseases, type 1 or type 2 diabetes mellitus, cancer within the last 10 years; food allergies; blood pressure ≥ 160/100 mm Hg; triglycerides > 3 mM; total cholesterol > 7 mM; fasting blood glucose ≥ 6.1 mM; urinary protein, glucose, pH, ketones and hemoglobin outside accepted reference ranges; use of prescription medication that might influence the outcome of the study; alcohol consumption > 21

alcohol units/week (males), > 14 units/week (females); planned major changes in physical activity during the study period; blood donation within the past 2 months; weight change >3 kg within the 3 months before the start of the study, participation in another scientific study up to 3 months before, drug treatment, pregnancy or lactation, surgically or drug-treated obesity, drug abuse; inability/unwillingness to engage in 8-week low calorie diet or 6-month randomised diet; special diet; inability to give informed consent [31]. If one parent of a family was eligible and the other not, the family was included as a single-parent family.

After a detailed explanation of the study, a written informed acceptance was obtained from all participants before the beginning of the trial, in agreement with the Helsinki Declaration. The study protocol and informed consent document were approved by the Medical Ethical Committees of each enrolled European centres.

Study design

A schematic overview of the study protocol and trial is shown in Figure 1. The full study protocol has been described in detail [31, 32] in the papers by Larsen et al (2009) and Moore, (2009). At baseline point (preLCD test day), all adults completed a 3-day weighed dietary record (2 weekdays and 1 weekend day). Subjects were asked to eat normally and to refrain from excessive alcohol consumption or exercise on the day preceding the test day, avoiding extreme exercise. On the test day subjects came in the morning after an overnight fast of at least 10 hours. Anthropometrical and body composition variables and blood pressure were measured. A cannula was inserted and

fasting blood samples were collected. Subsequently, a 2-h 75-g oral glucose tolerance test (OGTT) was performed.

After baseline measurements subjects started a 8-week low-calorie diet (LCD) providing 800-880 kcal/d through a commercially available diet: Modifast® (Nutrition et Santé, France). Subjects used 4 sachets per day and could choose from drinks, creams and soups with different tastes. Additional intake of tomatoes (200 g/d), cucumber (125 g/d) and lettuce (50 g/d) was allowed. During the first 3 weeks of the LCD, weight loss and adverse events were assessed weekly and 2-weekly during the remaining time of the diet period. If at least one of the parents of a two-parent family or the parent in a single-parent family had attained a weight loss of ≥ 8% of initial body weight, the family was randomised into one of five dietary groups, varying in protein content and glycemic index, for the 6-month dietary intervention period. Before the start of the randomised phase (post LCD), subjects came for the second test day, which was similar to the pre LCD test day, but with no weighed food records. A dietician gave instructions on the *ad libitum* randomised diet during the test day [31].

Two centres were enrolled in a shop system [26, 33] and six centres followed the dietary instruction procedure [31, 32]. During the first 6 weeks of the randomised phase, subjects came to the research centre on a 2-weekly basis and thereafter on a monthly basis to meet with a dietician. At all visits body weight, adherence to the diet and adverse events were monitored and dietary counselling was provided [31]. After 6 months subjects returned for the third test day (post intervention), and were evaluated as for preLCD test day [32]. The study was registered with ClinicalTrials.gov, number NCT00390637.

Diet groups and dietary instruction

Subjects were randomised into 5 dietary groups: (1) low protein/low glycemic index (LP/LGI); (2) low protein, high glycemic index (LP/HGI); (3) high protein, low glycemic index (HP/LGI); (4) high protein, high glycemic index (HP/HGI); and (5) standard recommended healthy conventional control diet: rich in fruits and vegetables every day; eat fish several times per week; eat potatoes, rice or pasta and whole-grain bread every day; limit sugar intake, especially from liquids, candy and cakes; eat less fat, especially from dairy and meat; eat a varied diet and keep weight stable. All diets were low in fat (25-30% of energy from fat) and were offered *ad libitum*, i.e. no energy restriction was imposed. The aim was to obtain a protein consumption of 10-15% of total energy intake in the low protein groups and of 23-28% in the high protein groups. With respect to GI, a distinction was made between high and low GI foods within each food group. The assignment of GI values to foods was performed as described by Aston et al. [34]. Subjects in the low GI groups were advised to consume the low GI foods within a food group, those in the high GI groups the high GI foods. The aim was to attain a 15-point difference in glycemic index between the high and low GI groups [31].

All enrolled subjects were advised on nutritional content of foods, recipes, cooking and aspects of behaviour modification. More detailed information on the diets and dietary counselling is provided in the paper by Moore et al. [31].

Measurements

Anthropometric measurements

Body weight was measured on a calibrated digital balance and height using a wall-mounted stadiometer. BMI was calculated as the body-weight/height^2 (kg/m^2). Waist and hip circumferences and sagital diameter were measured twice following standardized procedures (www.diogenes-eu.org). Body composition was determined by dual-energy X-ray absorption (DEXA) (Lunar Radiation, Madison, WI, USA) or by bioelectric impedance analysis (BIA) (QuadScan 4000; Bodystat, Douglas, Isle of Man, British Isles).

Blood analysis

Venous blood samples were drawn after an overnight fast of 12 h and plasma and serum were stored at -80 ºC until analysis. Fasting and OGTT serum glucose and insulin concentrations were measured by a colorimetric assay (Ortho-Clinical Diagnostics, Johnson & Johnson, Birkerød, Denmark). Fasting serum levels of fructosamine were assessed by a colorimetric assay (Roche Diagnostics). Plasma adiponectin was analysed by a human adiponectin ELISA kit (BioVendor GmbH, Heidelberg, Germany).

Oral glucose tolerance test (OGTT)

Subjects drank a solution containing 75 g of glucose, and blood samples were colleted every 30 min (before and 30, 60, 90 and 120 min) for the measurements of plasma glucose and insulin concentrations. Insulin sensitivity and β-cell function indexes were calculated at fasting using the HOMA-IR and HOMA-B (Homeostasis Model

Assessment: insulin resistance and ß-cell function) and QUICKI (quantitative insulin sensitivity check index) [35] and during the OGTT by using the ISIcomp, MCRest, OGIS and IGI mathematical models as previously described [35-38]. Incremental areas under the curve (ΔUCs) of plasma glucose and insulin concentrations during the OGTT were calculated according to the trapezoid rule. The insulinogenic index or incremental ΔUC of insulin (ΔI [AUC]) dividing by the incremental AUC of glucose (ΔG [AUC]) was calculated during the 0- to 30-min (early) and to 0- and 120-min (total) time period of the OGTT [39].

Statistical analysis

The Kolmovorov-Smirnov and the Shapiro-Wilk tests were used to determine variable distribution. Normally distributed variables are presented as mean ± standard deviation (SD) and non-normally distributed variables are expressed as median together with the interquartilic range (IQR). Changes in clinical variables are expressed as means together with the 95% confidence interval (CI). Changes in the weight loss and interventional periods were evaluated and compared between groups with ANOVA and paired student's t-test, when variable distribution was parametric. The Kruskal–Wallis and the Wilcoxon for matched pairs were applied to analyse non-parametric data. Differences in the dietary groups and potential interaction between GI and protein content were analysed by factorial ANCOVA. A multiple linear regression analysis was used to evaluate the effect of the dietary components on HOMA-IR changes considering the 10% of body weight-loss during the initial LCD phase as cut off. Weight-changes during the intervention, baseline values, centre and drop-put rate were included as covariates in the analyses. The

weight loss cut-off criterion was considered based on previously published works [40, 41]. A two way ANOVA was applied to evaluate possible interactions between dietary patterns and variables changes considering the weight-loss response.

Statistical analyses were carried out using the SPSS 16.0 program for Windows XP (Microsoft, USA). A two-tailed p-value less than 0.05 was chosen as the level of statistical significance.

Results

The schematic overview of the study protocol (Figure 1) shows that a total of 1209 adults (mean age 41 years, mean BMI 34kg/m^2) were screened. Out of 932 adults (male: n=312; female: n=620) initiated the LCD period, and 773 adults from 634 families were randomised to the 5 diets, 263 of them (2 centers) were provided with all foods for free using a shop system, and 510 (6 centers) were provided dietary instruction only. The number of adults that remained at the end of the intervention period was 548 (71%). The drop out rate was highest (37%) in the LP/HGI diet group (p=0.039), and assignment to both HP (27%; p=0.046) and LGI (25%; p=0.021) diets was associated with lower drop-out rates (Figure 1).

Changes in anthropometrical and clinical variables

Data on anthopometrical and clinical variables measured on the test days (Table 1) showed that the mean body weight loss was -11.0 ± 3.5 kg (p<0.001). Except for fructosamine (p=0.223) and adiponectin (p=0.860), all parameters decreased and the 2h glucose concentrations increased significantly (all p < 0.001) in the whole studied cohort

after the LCD. A repeated measures ANOVA showed statistically significant changes for all clinical features among the three test days (Table 1).

The postLCD changes in weight, body composition and other clinical factors were analysed by an ANOVA test on the studied 5 randomised diets (Table 2. Only a marginal difference in body weight changes between groups (p=0.081) were found. The HP and LGI diets when conjointly considered were related to better body-weight control during the intervention as compared to the LP/HGI diet (p<0.05). No overall statistically significant differences between groups were found for changes in the remaining antropometrical and clinical measurements (Table 2).

At the end of the 6-month weight maintenance period body weight and other anthropometrical and clinical variables remained significantly lower (all P < 0.001) than at baseline preLCD (Table 1).

Changes in diabetes risk factors during the intervention

Before randomisation to the *ad libitum* diets, plasma concentrations of glucose and insulin at fasting and after 2h OGTT, the incremental glucose and insulin AUC and the rest of insulin sensitivity and β-cell function indexes did not differ significantly between dietary groups (p>0.05). Neither were any differences observed in the plasma concentrations of adiponectin and fructosamine concentration between groups (data not shown).

The change in body weight during the randomised phase contributed significantly (p<0.05) to the improvements in fasting and 2h glucose, HOMA-IR and adiponectin concentrations.

Changes in HOMA-IR during the 6 months intervention period showed no differences between the diets (Figure 2). However, in the LP/HGI diet the return of HOMA-IR towards baseline was higher (p<0.05) during the 6 months randomised dietary period as compared to the control healthy recommended diet (Figure 2) and to the HP diets (p=0.046). Moreover, when a factorial ANCOVA was used to test the effect of the protein and GI content of the diet on HOMA-IR changes, a significant main effect of the LP/HGI was observed during the intervention period (p=0.038). This finding was attributed to the increased insulin concentrations during the diet intervention in this dietary group (Table 2) and factorial ANCOVA showed a significant effect of the LP/HGI diet on insulin changes during the intervention (p=0.022). After adjusting for the 6 months weight change and other main covariables (centre and drop-out) a marginal significance were observed for HOMA-IR changes (p=0.072) tough insulin concentrations remained significantly different (p=0.040). Also, a trend was observed when analysing the effect of this diet on glucose concentrations changes among the intervention period (p=0.053). To further analyse the effect of dietary components on the change in clinical variables during the intervention period, we considered 10% of the initial body weight-loss as the cut off (Table 3). Thus, the individual effect of HP dietary intervention on insulin and HOMA-IR changes as compared to the LP/HGI was more evident when considering those patients with the higher weight-loss (weight loss $\geq 10\%$ of the initial body weight). The same trend was observed for the LGI diets, but did not reach statistical significance (Table 3). When HOMA-IR changes were compared by means of a multiple lineal regression after adjustment for weight change, baseline value, centre and drop-out (Figure 3), the HP (r^2=0.14; p<0.001) and LGI (r^2=0.17; p<0.001)

14

dietary patterns were related to lower HOMA-IR changes as compared to LP/HGI diet in those patients with higher (55.9%) body weight loss (Figure 3). This observation was also observed (r^2=0.11; p=0.020) in those patients presenting a weight-loss lower than the 10% who were following a HP diet (Figure 3).

With respect to the fructosamine changes, significant differences were observed in the HP dietary groups depending on the GI content (Table 2). The factorial ANCOVA with fructosamine changes as the dependent variable and adjusting for relevant covariables (weight change, drop-out and centre) revealed a significant main effect of the dietary GI content (p=0.012) on fructosamine changes. Changes in adiponectin concentration did not significantly differ between dietary groups (Table 2).

At the end of the 6 month period fasting glucose and insulin concentrations, along with 2h glucose levels were lower (p <0.001) than at baseline (preLCD) point (Table 1). Plasma levels of fructosamine and adiponectin remained higher (P < 0.001) after the interventional period when compared to the baseline point (Table 1). All these variables showed no differences between dietary groups at the end of the randomised period (data not shown).

Several approaches were used to assess the impact of the dietary intervention in insulin sensitivity and β-cell function at fasting and after the OGTT (see methods). Fasting plasma glucose, insulin, HOMA-IR and other fasting indexes did not differ between the studied groups 6-months after the intervention (p>0.05). The application of the ANOVA test for repeated measures showed no differences between the dietary groups among the OGTT or the 2h plasma glucose and the incremental glucose AUC during the OGTT were not different between groups (Figure 4). However, the LP/LGI diet was

accompanied by a higher glucose concentration after 30 minutes and a tendency to be different 60 minutes from the beginning when comparing to the recommended diet (Figure 4). Although the incremental insulin AUC was not different between groups, the time course of insulin response was different among the diets (Figure 4). In fact, the plasma insulin response was lower in the HP/LGI diet as compared to the LP/LGI after 60 min and to the three other dietary composition groups after 90min of the beginning of the OGTT (Figure 4). The insulin secretion during the 120-min OGTT, measured by ΔI (AUC)/ ΔG (AUC) and the early insulin response (ΔI_{0-30}/ ΔG_{0-30}) did not significantly differ between groups ($p > 0.05$). Insulin sensitivity, as expressed by ISIcomp, MCRest, IGI and OGIS indexes, did neither differ between diets at the end of the intervention (data not shown).

Discussion

The persistence of an epidemic of obesity and type 2 diabetes suggests that new nutritional strategies are needed to overcome them [42]. Many short-term dietary and behavioural weight loss programmes have proved to be successful, but long-term weight maintenance is still a major problem [7, 43, 44].

The group of patients within the high-protein and low-GI diet continued to lose weight during the 6 months intervention period, despite the fact that the diets were offered *ad libitum*. These findings are in accordance to previous investigations that described a better weight-loss maintenance with an *ad libitum* dietary pattern containing a higher protein content [28, 29, 45, 46] or lower in GI [17, 47, 48]. The reason for these effects could be due to the satiating and thermogenic effects of protein and to the improvement

in appetite regulation and subsequent decrease in energy intake that could be produced by a low GI diet [17, 49, 50].

The LP/HGI diet was accompanied with the highest drop-out rate during the 6 months interventional period as compared to the HP and LGI dietary patterns [32]. This effect could be due to the satiating effect produced by the HP and LGI diets, which could induce higher adherence to the nutritional intervention. So, as previously published [9, 50, 51], diets high in protein content and low in GI were more acceptable to be followed for overweight and obese patients.

A number of nutritional approaches and dietary patterns with different proportions of macronutrients, micronutrients and specific dietary components are being investigated to overcome obesity and diabetes [11, 18, 51, 52]. However, the conjoint long-term effect of protein and GI content on insulin resistance and glucose metabolism has been poorly investigated. Our study demonstrated that the LP/HGI diet is accompanied with an increasing risk of insulin resistance during the 6-month weight-loss maintenance period, as expressed by the increasing values in insulin concentration and HOMA-IR changes during this period. When adjustment for weight changes, similar figures were observed for insulin concentration changes, although HOMA-IR was no longer statistically significant, suggesting the mayor benefits though the maintenance of weigh-loss. When most successful weight-loss subjects were considered (weight-loss $\geq$ 10% of the initial body weight), based on previously published reports [41, 42], the HP and LGI diets were accompanied by a enhanced insulin sensitivity action during the 6 months intervention as expressed by decreasing values of insulin and HOMA-IR changes. Moreover, the role of these dietary traits was more evident in more successful subjects, showing lower HOMA-

IR changes values independently of the body weight changes and other main variables. This outcome reinforces previously published observations describing the favourable role of both LGI and HP diets on the weight control methods [22, 50, 53-56]. Moreover, these observations clearly support a specific role for both HP and LGI diets in terms of long term interventional controlled and randomised trial, in the insulin sensitivity action on weight management strategies of obesity. It is not definitely established how HP and LGI diets exert there effects on insulin sensitivity, but it could be hypothesized apart from the previously mentioned mechanisms on appetite regulation and thermogenic regulation [50, 51], that it is through the lowering effect on glucose uptake, which produces a lower insulin secretion which could derivate in an insulin sensitivity action [50, 53, 54].

In addition, the LGI diets were accompanied with reduced levels of fructosamine changes during the 6-month dietary intervention. Fructosamine is a measure of non-enzymatically glycated proteins in the blood and a marker of glycaemia in the previous three weeks [57]. Other authors have previously used the fructosamine levels as a marker of glycaemic control in humans [58], but our study demonstrated for the first time that lower changes in fructosamine concentrations following LGI diets could indicate a more appropriate glycaemia control in obese subjects, and therefore reinforces the beneficial effects of LGI diets concerning insulin sensitivity management, suggesting a possible mechanism resulting from this action.

The use of the OGTT is extended for the evaluation of glucose metabolism and insulin sensitivity [36-38]. In the present study, we observed that plasma insulin concentrations during the OGTT were lower after the 6 months dietary intervention in the HP/LGI at 60 and 90 minutes after the beginning of the test, which suggest a better insulin sensitivity

during the intervention period. This diagnosis criteria has been briefly studied for insulin sensitivity and ß-cell function [59-61], and their relationships with the different dietary components on insulin sensitivity [62, 63]. However, we failed to find differences between dietary groups in the insulin sensitivity and ß-cell function indexes, at fasting or after the OGTT 6 months after the interventional diet.

The strength points of this research are the number of patients following every dietary pattern, the study of the weight-management state after following a low-calorie diet, the involvement of patients coming from 8 European countries and the fact that diets where offered *ad libitum*. The *ad libitum* dietary approach could induce an improvement in the adherence to the nutritional therapy and achieve a better weight-loss management. In fact, several authors have demonstrated more satisfactory results in weight-loss and maintenance of the weight with *ad libitum* programs varying in macronutrient content [10, 24-27] with respect to energy-restricted diets. We further support a role of this nutritional design on insulin sensitivity action related to the different protein content and glycemic index patterns, despite that controversy in this area is still warranted [64].

In conclusion, an increase in dietary protein content and a reduction in glycemic index values over a 6-month dietary intervention within an *ad libitum* approach are related to a lower drop-out rate, better glycaemia control and insulin sensitivity action in overweight or obese subjects after an initial body weight loss as well as to improved weight-loss maintenance in this large randomised study including 8 different European centres.

Figure 1. Schematic overview of study protocol and trial profile.
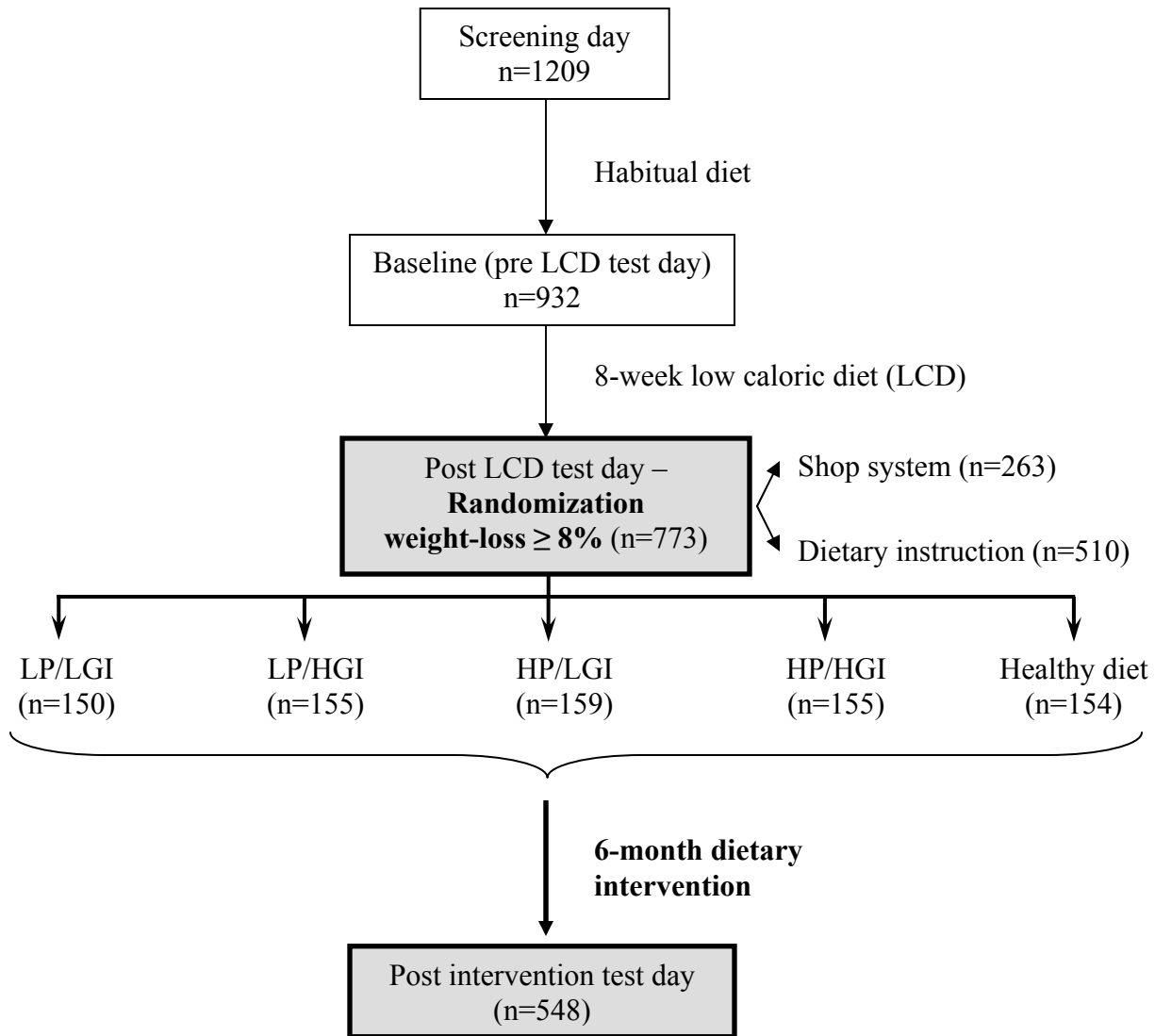
Abbreviations: LP/LGI, low protein/low glycemic index; LP/HGI, low protein/high glycemic index; HP/LGI, high protein/low glycemic index; HP/HGI, high protein/high glycemic index.

Table 1. Anthropometrical and clinical characteristics at baseline (preLCD), after the 8-week low-caloric diet (postLCD) and after the 6 months randomised diet period (post intervention).

	preLCD	postLCD	6 month post intervention	*p* value *	*p* value #
Weight (kg)	100.0 ± 17.5 *n=773*	89.0 ± 15.7 *n=773*	88.7 ± 16.0 *n=548*	<0.001	0.018
BMI (kg/m^2)	34.4 ± 4.9 *n=773*	30.7 ± 4.5 *n=773*	30.6 ± 4.6 *n=548*	<0.001	0.029
WC (cm)	107.7 ± 13.0 *n=763*	97.9 ± 12.4 *n=760*	97.4 ± 12.1 *n=547*	<0.001	0.093
HC (cm) §	112.6 (13.6) *n=763*	106.4 (14.1) *n=760*	105.3 (14.6) *n=547*	<0.001	0.240
Sagital diameter (cm)	25.2 ± 3.8 *n=747*	22.1 ± 3.5 *n=752*	22.1 ± 5.1 *n=538*	<0.001	0.557
FFM (kg)	59.6 ± 12.8 *n=679*	56.4 ± 10.4 *n=665*	57.2 ± 12.0 *n=530*	<0.001	0.047
FM (kg)	40.1 ± 11.2 *n=679*	32.4 ± 10.5 *n=665*	31.1 ± 10.2 *n=530*	<0.001	0.011
Glucose (mM)	5.11 ± 0.69 *n=747*	4.83 ± 0.62 *n=755*	4.94 ± 0.51 *n=542*	<0.001	<0.001
2h glucose (mM)	6.66 ± 2.14 *n=736*	6.80 ± 2.04 *n=735*	5.80 ± 1.67 *n=524*	<0.001	<0.001
Insulin (mU/L)	11.7 ± 10.7 *n=732*	8.13 ± 7.8 *n=672*	9.46 ± 9.53 *n=495*	<0.001	<0.001
AUC glucose (mM/180min)	20.1 ± 3.6 *n=719*	19.4 ± 3.1 *n=718*	18.6 ± 3.2 *n=517*	<0.001	<0.001
Fructosamine (µM)	208.4 ± 24.4 *n=762*	209.3 ± 22.9 *n=758*	218.1 ± 19.2 *n=545*	<0.001	<0.001
Adiponetin (mg/L) §	6.59 (4.17) *n=759*	7.19 (4.76) *n=763*	8.69 (6.03) *n=540*	<0.001	<0.001

Abbreviations: AUC, area under the curve; BMI, body mass index; FFM, fat free mass; FM, fat mass; HC, hip circumference; WC, waist circumference.

Normally distributed variables are presented as mean ± standard deviation (SD), non-normally distributed variables (§) are expressed as median (interquartile range - IQR).

* Repeated measures analysis of variance (ANOVA) taking into account the whole studied period (preLCD, post LCD and 6 months post intervention test days).

Comparisons between postLCD and the 6 month post intervention test days by using paired student t test or Wilcoxon for matched pairs.

Table 2. Changes (Δ) in anthropometrical and clinical variables over the 6 months randomised diet period (between Post intervention and Post LCD test day) represented as means together with the 95% confidence interval.

	All dietary groups (n=548)	LP/LGI (n=106)	LP/HGI (n=97)	HP/LGI (n=124)	HP/HGI (n=107)	Control diet (n=114)	p va
Weight (Δ, kg)	0.56 (0.04, 1.08)	0.33 (-0.74, 1.40)	1.67 (0.48, 2.87) †	-0.38 (-1.70, 0.93) †	0.57 (-0.65, 1.78)	0.84 (-0.17, 1.86)	0.
FFM (Δ, kg)	0.95 (0.47, 1.43)	0.68 (-0.19, 1.55)	0.86 (-0.56, 2.28)	0.02 (-0.89, 0.93)	1.83 (0.46, 3.20)	1.51 (0.65, 2.37)	0.
FM (Δ, kg)	-0.56 (-1.16, 0.03)	-0.69 (-1.98, 0.59)	-0.16 (-1.39, 1.08)	-0.92 (-2.22, 0.38)	-0.18 (-1.96, 1.60)	-0.73 (-1.79, 0.33)	0.
WC (Δ, cm)	0.52 (-0.17, 1.25)	0.64 (-0.99, 2.27)	0.86 (-0.94, 2.65)	-0.08 (-1.53, 1.38)	0.41 (-1.20, 2.02)	0.85 ±(-0.52, 2.23)	0.
HC (Δ, cm)	-0.31 (-0.90, 0.28)	-0.53 (-1.94, 0.88)	0.15 (-1.32, 1.63)	-0.52 (-1.80, 0.76)	-0.07 (-1.49, 1.34)	-0.49 (-1.66, 0.68)	0.
SD (Δ, cm)	0.05 (-0.16, 0.27)	0.09 (-0.28, 0.46)	0.20 (-0.29, 0.69)	-0.17 (-0.59, 0.26)	-0.08 (-0.65, 0.49)	0.26 (-0.24, 0.77)	0.
Adiponectin (Δ, mg/L)	1.50 (1.14, 1.86)	1.71 (0.84, 2.20)	1.76 (1.15, 2.89)	1.59 (0.59, 2.78)	1.29 (0.49, 1.89)	1.26 (0.40, 1.91)	0.
Fructosamine (Δ, μM)	9.9 (7.7, 12.0)	11.3 (7.1, 15.1)	6.7 (2.5, 15.2)	11.3 (7.9, 16.5)	5.4 (2.2, 11.8)	9.6 (4.8, 14.8)	0.
Glucose (Δ, mM)	0.12 (0.06, 0.17)	0.14 (0.02, 0.23)	0.21 (0.07, 0.29)	0.08 (-0.39, 0.22)	0.06 (-0.09, 0.22)	0.10 (0.02, 0.24)	0.4
2h glucose (Δ, mM)	-1.0 (-1.2; -0.8)	-1.2 (-1.6; -0.9)	-1.0 (-1.3; -0.6)	-1.0 (-1.3; -0.6)	-1.0 (-1.3; -0.6)	-0.8 (-1.1; -0.5)	0.
AUC glucose (Δ, mM/180min)	-0.8 (-1.1; -0.6)	-1.0 (-1.6; -0.4)	-0.6 (-1.2; 0.1)	-0.8 (-1.2; -0.3)	-0.8 (-1.4; -0.3)	-0.9 (-1.4; -0.4)	0.
Insulin (Δ, mU/L)	1.52 (0.92, 2.11)	0.98 (-0.95, 2.56)	2.97 (0.96, 5.32)	1.51 (0.68, 2.24)	1.24 (0.15, 2.34)	1.15 (0.45, 2.02)	0.
AUCglucose (Δ, mM)	-0.8 (-0.9; -0.4)	-0.8 (-1.4; -0.1)	-0.6 (-1.3; 0.1)	-0.6 (-1.1; -0.1)	-0.6 (-1.2; -0.1)	-0.7 (-1.2; -0.1)	0.

Abbreviations: AUC, area under the curve; C, cholesterol; CRP, C reactive protein; FM, fat free mass; FM, fat mass; HC, hip circumference; HDL, high density lipoproteins; HP/LGI, high protein/low glycemic index; HP/HGI, high protein/high glycemic index; LDL, low density lipoproteins; LP/LGI, low protein/low glycemic index; LP/HGI, low protein/high glycemic index; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, sagital diameter; TG, triacylglycerides; WC, waist circumference.

* p value for comparison among the dietary groups using ANOVA.

† Tukey post hoc test showed statistical differences between dietary groups (p<0.05).

Table 3. Changes (Δ) in clinical markers related to diabetes risk over the 6 months randomised diet period (between Post intervention and Post LCD test day) considering the 10% of weight-loss criteria as cut off and comparing the LPHGI diet vs. HP and LGI patterns.

|		**LPHGI** (n=7441)	**HP** (n=18187)	**LGI** (n=17880)	*p* value HP vs. LPHGI	*p* value LGI vs. LPHGI
Glucose (Δ, mM)	WL < 10%	0.18 (0.01; 0.31)	0.07 (-0.07; 0.21)	0.01 (-0.21; 0.21)	0.249	0.343
	≥ 10%	0.29 (0.03; 0.37)	0.13 (0.04; 0.27)	0.13 (0.04; 0.22)	0.107	0.228
2h glucose (Δ, mM)	WL < 10%	-1.2 (-1.8; -0.5)	-0.7 (-1.2; -0.3)	-0.8 (-1.3; -0.4)	0.175	0.450
	≥ 10%	-0.8 (-1.3; -0.4)	-1.1 (-1.4; -0.9)	-1.1 (-1.4; -0.9)	0.218	0.477
AUC glucose (Δ, mM/180min)	WL < 10%	-0.6 (-1.7; 0.4)	-1.0 (-1.7; -0.4)	-1.1 (-1.7; -0.6)	0.540	0.362
	≥ 10%	-0.5 (-1.3; 0.2)	-1.1 (-1.1; -0.3)	-0.8 (-1.2; -0.3)	0.617	0.613
Insulin (Δ, mU/L)	WL < 10%	1.62 (0.14; 3.44)	1.36 (0.50; 2.23)	1.12 (0.06; 2.14)	0.574	0.576
	≥ 10%	4.19 (0.44; 8.19)	1.04 (-0.57; 2.51)	1.52 (0.64; 2.40)	0.046	0.090
HOMA (Δ)	WL < 10%	0.47 (0.05; 0.89)	0.34 (0.09; 0.59)	0.26 (-0.04; 0.57)	0.429	0.752
	≥ 10%	1.01 (0.23; 1.80)	0.28 (-0.20; 0.73)	0.40 (0.15; 0.64)	0.155	0.096
Adiponectin (Δ, mg/L)	WL < 10%	1.27 (0.05; 2.49)	1.56 (0.46; 2.27)	1.32 (0.01; 2.27)	0.636	0.439
	≥ 10%	2.67 (1.43; 3.92)	1.71 (0.85; 2.41)	1.64 (0.86; 2.42)	0.046	0.236
Fructosamine (Δ, mU/L)	WL < 10%	6.5 (-0.6; 13.9)	9.6 (5.7; 13.5)	7.5 (2.7; 11.5)	0.820	0.360
	≥ 10%	10.8 (0.3; 21.2)	2.2 (9.1; 17.8)	11.3 (6.8; 15.9)	0.562	0.161

Abbreviations: AUC, area under the curve; HOMA, homeostasis model of assessment for insulin resistance; LGI, Low Glycemic index diets; LP/HGI, Low Protein-High Glycemic index; WL, weight-loss.

Data is represented as means together with the 95% confidence interval.

A two way ANOVA showed no interaction between the dietary patterns and weight-loss response among the 6 month intervention changes (p>0.05).

p value for comparison among the dietary groups using student t test.

Figure 2. Change (Δ) in HOMA-IR over the 6 months randomised diet period (between Post intervention and Pot LCD test day), represented as mean together with the 95% confidence interval. (ANOVA p=0.357). Statistically relevant differences are indicated in the figure, the remaining comparisons did not reach the p<0.10 statistical value.
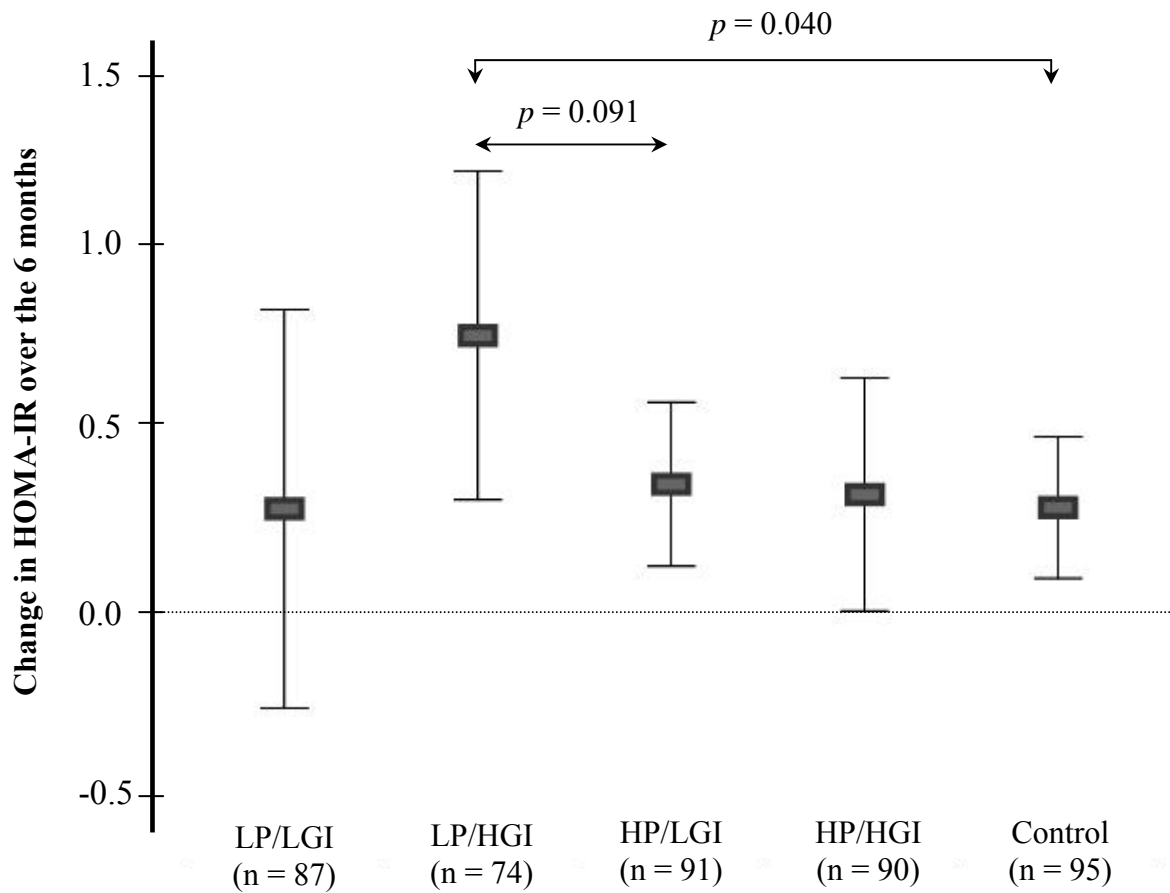
Abbreviations: LP/LGI, low protein/low glycemic index; LP/HGI, low protein/high glycemic index; HP/LGI, high protein/low glycemic index; HP/HGI, high protein/high glycemic index.

Figure 3. Changes (Δ) in HOMA-IR over the 6 months randomised diet period (between post intervention and post LCD test days) concerning the 10% of weight-loss criteria and after adjusted for weight-change, baseline value, centre and drop-out.
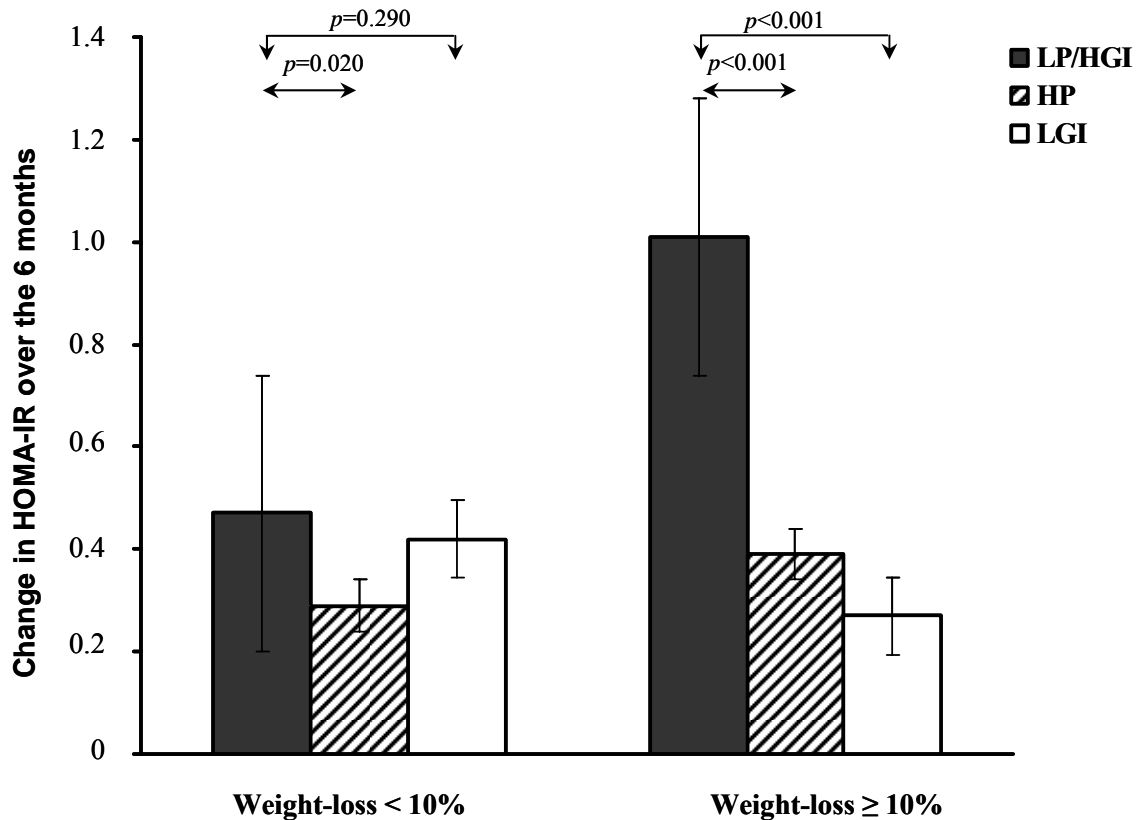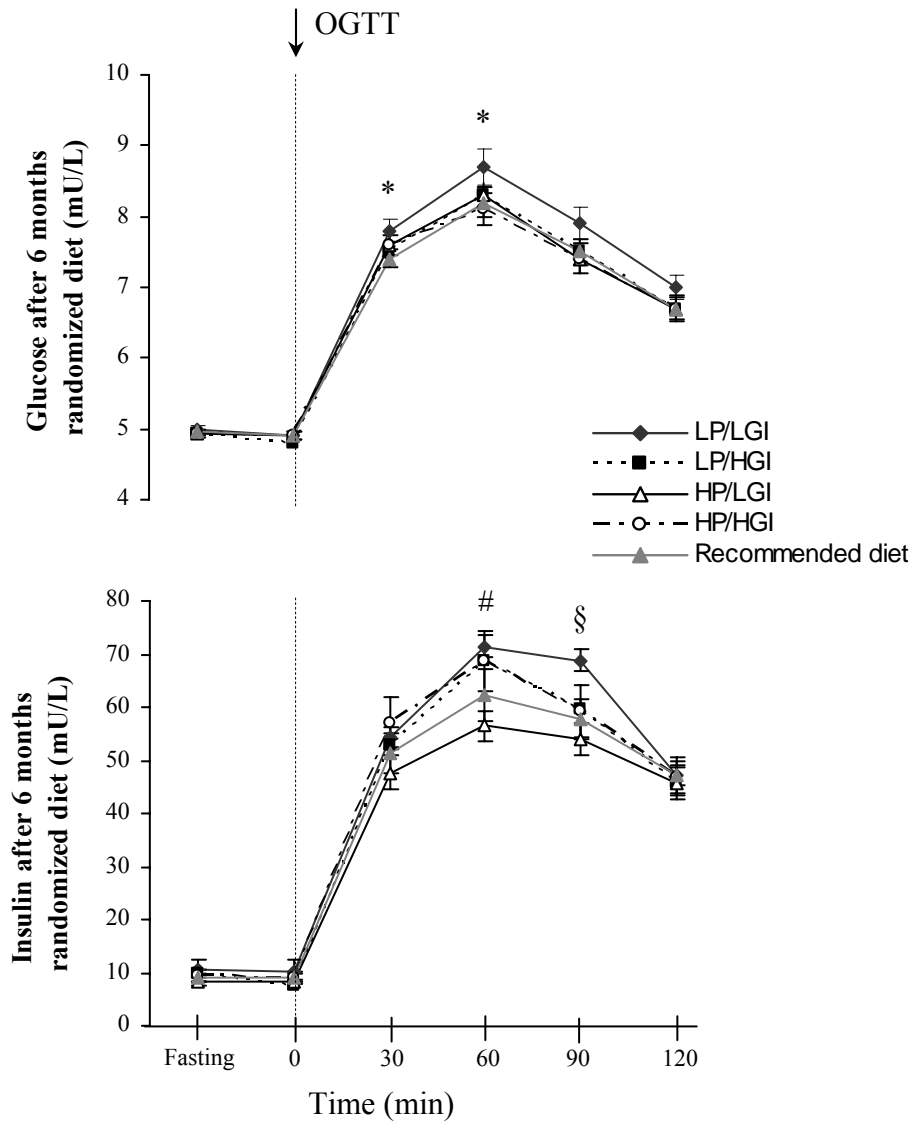

Abbreviations: LGI, Low Glycemic index diets; LP/HGI, Low Protein-High Glycemic index; HOMA-IR, homeostasis model of assessment for insulin resistance; HP, High Protein diets.

p values for comparisons between LP/HGI and HP or LGI dietary patterns are obtained thought a multiple lineal regression analysis.

Data is represented as means together with the 95% confidence interval after adjusted for potential covariables and obtained though an ANCOVA test.

Figure 4. Plasma glucose and insulin concentrations during an oral glucose tolerance test (OGTT) 6 months after the randomised diet.

Abbreviations: LP/LGI, Low Protein-Low Glycemic index diet; LP/HGI, Low Protein-High Glycemic index; HP/LGI, High Protein-Low Glycemic index diet; HP/HGI, High Protein-High Glycemic index diet.

* Higher glucose concentrations in LP/LGI diet as compared to recommend diet at minute 30 (p=0.019) and a tendency at minute 60 (p=0.055).

\# Lower insulin concentrations in HP/LGI as compared to LP/LGI diet (p=0.022).

§ Lower insulin concentrations in HP/LGI diet as compared to LP/LGI, LP/HGI and HP/HGI (p<0.05).

References

1. Klein S, Sheard NF, Pi-Sunyer X, et al. Weight management through lifestyle modification for the prevention and management of type 2 diabetes: rationale and strategies: a statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. Diabetes Care 2004; 27: 2067-2073.

2. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006; 113: 898-918.

3. Martinez JA, Moreno MJ, Marques-Lopes I, Marti A. Causes of obesity. An Sist Sanit Navar 2002; 25 Suppl 1: 17-27.

4. Abete I, Parra MD, Zulet MA, Martinez JA. Different dietary strategies for weight loss in obesity: role of energy and macronutrient content. Nutr Res Rev 2006; 19: 5-17.

5. Astrup A. Dietary management of obesity. JPEN J Parenter Enteral Nutr 2008; 32: 575-577.

6. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 2001; 74: 579-584.

7. Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr 2005; 82: 222S-225S.

8. Succurro E, Marini MA, Grembiale A, Lugarà M, Andreozzi F, Sciacqua A, Hribal ML, Lauro R, Perticone F, Sesti G. Differences in cardiovascular risk profile based on relationship between post-load plasma glucose and fasting plasma levels. Diabetes Metab Res Rev 2009; 25: 351-356.

9. Brehm BJ, D'Alessio DA. Weight loss and metabolic benefits with diets of varying fat and carbohydrate content: separating the wheat from the chaff. Nat Clin Pract Endocrinol Metab 2008; 4: 140-146.

10. Kushner RF, Doerfler B. Low-carbohydrate, high-protein diets revisited. Curr Opin Gastroenterol 2008; 24: 198-203.

11. Bravata DM, Sanders L, Huang J, et al. Efficacy and safety of low-carbohydrate diets: a systematic review. JAMA 2003; 289: 1837-1850.

12. Due A, Toubro S, Skov AR, Astrup A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. Int J Obes Relat Metab Disord 2004; 28: 1283-1290.

13. Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. Int J Obes Relat Metab Disord 1999; 23: 528-536.

14. Volek JS, Phinney SD, Forsythe CE, et al. Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. Lipids 2009; 44: 297-309.

15. Grieb P, Klapcinska B, Smol E, et al. Long-term consumption of a carbohydrate-restricted diet does not induce deleterious metabolic effects. Nutr Res 2008; 28: 825-833.

16. Brand-Miller J, McMillan-Price J, Steinbeck K, Caterson I. Carbohydrates--the good, the bad and the whole grain. Asia Pac J Clin Nutr 2008; 17 Suppl 1: 16-19.

17. Abete I, Parra D, Martinez JA. Energy-restricted diets based on a distinct food selection affecting the glycemic index induce different weight loss and oxidative response. Clin Nutr 2008; 27: 545-551.

18. Rodriguez MC, Parra MD, Marques-Lopes I, De Morentin BE, Gonzalez A, Martinez JA. Effects of two energy-restricted diets containing different fruit amounts on body weight loss and macronutrient oxidation. Plant Foods Hum Nutr 2005; 60: 219-224.

19. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. Int J Obes 2005; 29: 391-397.

20. Zulet MA, Puchau B, Hermsdorff HH, Navarro C, Martinez JA. Dietary selenium intake is negatively associated with serum sialic acid and metabolic syndrome features in healthy young adults. Nutr Res 2009; 29: 41-48.

21. Pereira MA, Swain J, Goldfine AB, Rifai N, Ludwig DS. Effects of a low-glycemic load diet on resting energy expenditure and heart disease risk factors during weight loss. JAMA 2004; 292: 2482-2490.

22. Radulian G, Rusu E, Dragomir A, Posea M. Metabolic effects of low glycaemic index diets. Nutr J 2008; 8: 5.

23. Sloth B, Krog-Mikkelsen I, Flint A, et al. No difference in body weight decrease between a low-glycemic-index and a high-glycemic-index diet but reduced LDL

cholesterol after 10-wk ad libitum intake of the low-glycemic-index diet. Am J Clin Nutr 2004; 80: 337-347.

24. Raben A, Astrup A, Vasilaras TH, et al. The CARMEN trial: increased intake of carbohydrates--simple or complex--and unchanged blood lipids in overweight subjects. Ugeskr Laeger 2002; 164: 627-631.

25. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003; 348: 2082-2090.

26. Saris WH, Astrup A, Prentice AM, et al. Randomized controlled trial of changes in dietary carbohydrate/fat ratio and simple vs complex carbohydrates on body weight and blood lipids: the CARMEN study. The Carbohydrate Ratio Management in European National diets. Int J Obes Relat Metab Disord 2000; 24: 1310-1318.

27. Stern L, Iqbal N, Seshadri P, et al. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. Ann Intern Med 2004; 140: 778-785.

28. Claessens M, van Baak MA, Monsheimer S, Saris WH. The effect of a low-fat, high-protein or high-carbohydrate ad libitum diet on weight loss maintenance and metabolic risk factors. Int J Obes 2009; 33: 296-304.

29. Westerterp-Plantenga MS, Lejeune MP, Nijs I, van Ooijen M, Kovacs EM. High protein intake sustains weight maintenance after body weight loss in humans. Int J Obes Relat Metab Disord 2004; 28: 57-64.

30. Livesey G, Taylor R, Hulshof T, Howlett J. Glycemic response and health--a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. Am J Clin Nutr 2008; 87: 258S-268S.

31. Moore CS, Lindroos AK, Kreutzer M, et al. Dietary strategy to manipulate ad libitum macronutrient intake, and glycaemic index, across eight European countries in the Diogenes Study. Obes Rev 2009; DOI:10.1111/j.1467-789X.2009.00602.x

32. Larsen TM, Dalskov S, van Baak M, et al. The Diet, Obesity and Genes (Diogenes) Dietary Study in eight European countries - a comprehensive design for long-term intervention. Obes Rev 2009; DOI:10.1111/j.1467-789X.2009.00603.x

33. Skov AR, Toubro S, Raben A, Astrup A. A method to achieve control of dietary macronutrient composition in ad libitum diets consumed by free-living subjects. Eur J Clin Nutr 1997; 51: 667-672

34. Aston LM, Gambell JM, Lee DM, Bryant SP, Jebb SA. Determination of the glycaemic index of various staple carbohydrate-rich foods in the UK diet. Eur J Clin Nutr 2008; 62: 279-285

35. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. Best Pract Res Clin Endocrinol Metab 2003; 17: 305-322.

36. Pei D, Hsiao CF, Hung YJ, et al. The insulin sensitivity, glucose sensitivity, and acute insulin response to glucose load in adolescent type 2 diabetes in Taiwanese. Diabetes Metab Res Rev 2006; 22: 26-33.

37. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.

38. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 2000; 23: 295-301.

39. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes 2006; 55: 1430-1435.

40. Forsythe LK, Wallace JM, Livingstone MB. Obesity and inflammation: the effects of weight loss. Nutr Res Rev 2008; 21: 117-133

41. Madsen EL, Rissanen A, Bruun JM, et al. Weight loss larger than 10% is needed for general improvement of levels of circulating adiponectin and markers of inflammation in obese subjects: a 3-year weight loss study. Eur J Endocrinol 2008; 158: 179-187.

42. Tsismenakis AJ, Christophi CA, Burress JW, Kinney AM, Kim M, Kales SN. The obesity epidemic and future emergency responders. Obesity 2009; 17: 1648-1650

43. Goyenechea E, Dolores Parra M, Alfredo Martinez J. Weight regain after slimming induced by an energy-restricted diet depends on interleukin-6 and peroxisome-proliferator-activated-receptor-gamma2 gene polymorphisms. Br J Nutr 2006; 96: 965-972.

44. Weinsier RL, Hunter GR, Desmond RA, Byrne NM, Zuckerman PA, Darnell BE. Free-living activity energy expenditure in women successful and unsuccessful at maintaining a normal body weight. Am J Clin Nutr 2002; 75: 499-504.

45. Lejeune MP, Kovacs EM, Westerterp-Plantenga MS. Additional protein intake limits weight regain after weight loss in humans. Br J Nutr 2005; 93: 281-289.

46. Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. Am J Clin Nutr 2000; 72: 1135-1141.

47. Agus MS, Swain JF, Larson CL, Eckert EA, Ludwig DS. Dietary composition and physiologic adaptations to energy restriction. Am J Clin Nutr 2000; 71: 901-907.

48. Roberts SB. High-glycemic index foods, hunger, and obesity: is there a connection? Nutr Rev 2000; 58: 163-169.

49. Thomas DE, Elliott EJ, Baur L. Low glycaemic index or low glycaemic load diets for overweight and obesity. Cochrane Database Syst Rev 2007: CD005105.

50. Layman DK, Clifton P, Gannon MC, Krauss RM, Nuttall FQ. Protein in optimal health: heart disease and type 2 diabetes. Am J Clin Nutr 2008; 87: 1571S-1575S.

51. McAuley KA, Hopkins CM, Smith KJ, et al. Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. Diabetologia 2005; 48: 8-16.

52. Plodkowski RA, St Jeor ST. Medical nutrition therapy for the treatment of obesity. Endocrinol Metab Clin North Am 2003; 32: 935-965.

53. Brand-Miller J, Marsh K. The low glycemic index diet: new way of eating for all? Pol Arch Med Wewn 2008; 118: 332-334.

54. Dewan S, Gillett A, Mugarza JA, Dovey TM, Halford JC, Wilding JP. Effects of insulin-induced hypoglycaemia on energy intake and food choice at a subsequent test meal. Diabetes Metab Res Rev 2004; 20: 405-410.

55. Holub I, Gostner A, Hessdorfer S, et al. Improved Metabolic Control After 12-Week Dietary Intervention with Low Glycaemic Isomalt in Patients with Type 2 Diabetes Mellitus. Horm Metab Res 2009; 41: 886-92.

56. Warren JM, Henry CJ, Simonite V. Low glycemic index breakfasts and reduced food intake in preadolescent children. Pediatrics 2003; 112: e414

57. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 1995; 18: 440-447.

58. Sievenpiper JL, Kendall CW, Esfahani A, et al. Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. Diabetologia 2009; 52: 1479-1495.

59. Mari A, Gastaldelli A, Foley JE, Pratley RE, Ferrannini E. Beta-cell function in mild type 2 diabetic patients: effects of 6-month glucose lowering with nateglinide. Diabetes Care 2005; 28: 1132-1138.

60. Ferrannini E, Massari M, Nannipieri M, Natali A, Ridaura RL, Gonzales-Villalpando C. Plasma glucose levels as predictors of diabetes: the Mexico City diabetes study. Diabetologia 2009; 52: 818-824.

61. Rabbone I, Bobbio A, Rabbia F, et al. Early cardiovascular autonomic dysfunction, beta cell function and insulin resistance in obese adolescents. Acta Biomed 2009; 80: 29-35.

62. Wolever TM, Mehling C, Chiasson JL, et al. Low glycaemic index diet and disposition index in type 2 diabetes (the Canadian trial of carbohydrates in diabetes): a randomised controlled trial. Diabetologia 2008; 51: 1607-1615.

63. Shikany JM, Phadke RP, Redden DT, Gower BA. Effects of low- and high-glycemic index/glycemic load diets on coronary heart disease risk factors in overweight/obese men. Metabolism 2009; 58: 1793-1801.

64. Delbridge EA, Prendergast LA, Pritchard JE, Proietto J. One-year weight maintenance after significant weight loss in healthy overweight and obese subjects: does diet composition matter? Am J Clin Nutr 2009; 90: 1203-1214.