Effects of a high-protein/low carbohydrate versus a standard hypocaloric diet on adipocytokine levels and insulin resistance in obese patients along 9 months

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Objective: Recent dietary trials and observational studies have focused on the effects of diet on health outcomes such as improvement in levels of surrogate biomarkers. The aim of our study was to examine the changes in weight, adipocytokines levels and insulin resistance after a high-protein/low carbohydrate hypocaloric diet vs. a standard hypocaloric diet during an intervention of 9 months.

Subjects and methods: 331 obese subjects were randomly allocated to one of two diets for a period of 9 months. Diet HP (n = 168) (high-protein hypocaloric diet) consisted in a diet of 1050 cal/day, 33% of carbohydrates, 33% of fats and 34% of proteins. Diet S (n = 163) (standard protein hypocaloric diet) consisted in a diet of 1093 cal/day, 53% carbohydrates, 27% fats, and 20% proteins.

Results: With the diets HP and S, BMI, weight, fat mass, waist circumference, waist-to-hip ratio, systolic blood pressure, total cholesterol, LDL-cholesterol, insulin and HOMA decreased. The decrease at 9 months of (BMI: −2.6 ± 1.3 kg/m² vs. −2.1 ± 1.2 kg/m²; p < 0.05), weight (−8.4 ± 4.2 kg vs. −5.0 ± 4.1 kg; p < 0.05), fat mass (−3.4 ± 4.2 kg; p < 0.05), systolic blood pressure (−5.1 ± 7.1 mmHg vs. −3.1 ± 2.1 mmHg; p < 0.05), insulin levels (−4.0 ± 4.8 UI/L vs. −2.2 ± 2.4 UI/L; p < 0.05) and HOMA (−0.8 ± 1.0 units vs. −0.3 ± 1.0 units; p < 0.05) was higher in diet HP than Diet S. With both diets, leptin levels decreased.

Conclusion: A high-protein/low carbohydrate hypocaloric diet shows a higher weight loss, insulin and HOMA-R decreased after 9 months than a standard hypocaloric diet. The improvement in adipokines levels was similar with both diets.

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1. Introduction

The prevalence of obesity is increasing worldwide, and there is a growing body of evidence that obesity-associated cardiovascular disease (CVD) morbidity and mortality are also increasing, mediated by increases in the risks for hypertension, type 2 diabetes, insulin resistance, and dyslipidemia (Aranceta, Perez Rodrigo, & Serra Majem, 1998). Recent studies have suggested no major differences between the effects of various dietary approaches, including low-carbohydrate and low-fat diets on body weight outcomes (Castaneda-Gonzalez, Bacardi Gascon, & Jimenez, 2011; Hu, Mills, Yao, et al., 2012; Nordmann, Nordmann, Briel, et al., 2006). However, other studies have reported that very low-carbohydrate ketogenic diets and the Mediterranean diet are superior to low-fat diets in reducing body weight (Bueno, de Melo, de Oliveira, et al., 2013; Nordmann, Suter-Zimmermann, Bucher, et al., 2011). In terms of the cardiometabolic outcomes, some dietary types have shown more beneficial effects than others. Compared with low-fat diets, low-carbohydrate diets have shown beneficial effects on lipid profile, such as triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels (Castaneda-Gonzalez et al., 2011; Hu, Mills, Yao, et al., 2012; Nordmann, Nordmann, Briel, et al., 2006).

Adipose tissue is considered an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation and immunity. Adipocytokines are proteins produced mainly by adipocytes (Matsuda, Shimomura, & Sata, 2002). These molecules have been shown to be involved in the pathogenesis of the metabolic syndrome and cardiovascular disease. Dietary patterns are associated with fluctuations in certain adipokine levels. In a recently published article, the Mediterranean, low-fat, and low carbohydrate diets were associated with decreased levels of leptin, retinol-binding protein 4, and vaspin, whereas adiponectin levels tended to increase throughout the intervention (Bluher, Rudich, Kloting, et al., 2012). Recent dietary trials and observational studies...
have focused on the effects of diet on health outcomes such as improvement in levels of surrogate biomarkers, obesity status, and reduction in the incidence of chronic diseases. Low-carbohydrate diet trials have also been shown to have favorable effects on weight control, cardiovascular parameters, and adipokine levels (Bradley, Spence, Courtney, et al., 2009; Ruth, Port, Shah, et al., 2013), similar to those of the Mediterranean diet (Esposito, Maiorino, Ciotola, et al., 2009; Esposito, Pontillo, Di Palo, et al., 2003; Fragopoulou, Panagiotakos, Pitsavos, et al., 2010; Hermsdorff, Zulet, Abete, et al., 2009; Mantzoros, Williams, Manson, et al., 2006), although the association has been less clear in studies of high-carbohydrate diets (Claessens, van Baak, Monsheimer, et al., 2009; Kasim-Karakas, Tsodikov, Singh, et al., 2006; Kitabchi, McDaniel, Wan, et al., 2013).

The aim of our study was to examine the changes in weight, adipokynes levels and insulin resistance after a high-protein/low carbohydrate hypocaloric diet vs. a standard hypocaloric diet during an intervention of 9 months.

2. Subjects and methods

2.1. Subjects and procedures

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving patients were approved by the ICUVA ethics committee. A sample of 331 obese non-diabetic outpatients was enrolled in a prospective way. These patients were recruited in a nutrition clinic unit. All participants provided informed consent to a protocol approved by the local ethical review boards. Inclusion criteria were body mass index > 30. Exclusion criteria included history of cardiovascular disease or stroke during the previous 24 months, total cholesterol > 200 mg/dl, triglycerides > 250 mg/dl, blood pressure > 140/90 mmHg, fasting plasma glucose > 110 mg/dl, as well as the use of metformin, sulphonylurea, thiazolidinedions, insulin, glucocorticoids, anitpeptidase agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors and psychoactive medications.

Basal fasting glucose, c-reactive protein (CRP), insulin, insulin resistance (HOMA-R), total cholesterol, LDL-cholesterol, HDL-cholesterol, plasma triglycerides concentration and adipokines (leptin, adiponectin, resistin and visfatin) levels were measured within the start of the trial and repeated after three months of both dietary intervention. Weight, height, a tetrapolar bioimpedance and blood pressure measures were realized within the start of the trial and repeated 9 months of intervention. These measures were realized at same time of the day (morning).

2.2. Procedure

331 obese subjects were randomly allocated to one of two diets for a period of nine months. Diet HP (n = 168) (high-protein hypocaloric diet) consisted in a diet of 1093 cal/day, 53% carbohydrates (144.3 g/day), 27% fats (32.6 g), and 20% proteins (55.6 g/day). The distribution of fats was: 23.5% of saturated fats, 63.8% of monounsaturated fats and 12.6% of polyunsaturated fats. Cholesterol was 215 mg/day, and dietary fats (32.6 g), and 20% proteins (55.6 g/day). The distribution of fats consisted in a diet of 1050 cal/day, 33% of carbohydrates (86.1 g/day), 33% of fats (39.0 g/day) and 34% of proteins (88.6 g/day). The distribution of fats was: 23.5% of saturated fats, 63.8% of monounsaturated fats and 12.6% of polyunsaturated fats. Cholesterol was 215 mg/day, and dietary fats was; 23.5% of saturated fats, 63.8% of monounsaturated fats and 12.6% of polyunsaturated fats.

2.3. Anthropometric measurements and blood pressure

Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged (Omrom, LA, CA). Body weight was measured to an accuracy of 0.1 Kg and body mass index computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to-hip ratio (WHR) were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 50 g (Lukaski & Johnson, 1985). The same investigator measured patients and controls. Precautions taken to insure valid BIA measurements were: no alcohol within 24 hours of taking the test, no exercise or food for four hours before taking the test.

3. Assays

CRP was measured by immunoturubimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0–7 mg/dl) and analytical sensitivity 0.5 mg/dl. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate–magnesium. LDL cholesterol was calculated using Friedewald formula. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5 mU/L (normal range 0.5–30 mU/L) (Durt, Arroyo, & Moreno, 2002), and the homeostasis model assessment for insulin resistance (HOMA) were calculated using these values (Mathews, Hosker, & Rudenski, 1985).

A single blood sample was obtained from each patient in tubes containing ethylenediaminetetraacetic acid (EDTA) in each visit of the study. Plasma samples were obtained after proper centrifugation. Samples were stored at −80 °C until hormone profiling. Plasma hormone levels were evaluated using the multiplex Biorad© 10 plex assay following manufacturer’s instructions (Bio-Rad®, Hercules, CA). This system allows for quantitative measurement of different hormones, while consuming a small amount of biological material; resistin, leptin, visfatin and adiponectin. Limits of detection were as follows (pg/ml): leptin (1.8), resistin (1.4), visfatin (1.5) and adiponectin (3.8).

4. Statistical analysis

Sample size was calculated to detect differences over 3 kg in body weight with 90% power and 5% significance (n = 160, in each diet group). The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed Student’s t-test. Non-parametric variables were analyzed with the Wilcoxon test. In order to retain a prescribed family wise error rate α in our analysis involving more than one comparison, Bonferroni correction method was used. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. A p-value <0.05 was considered significant.

5. Results

Three hundred and thirty one patients gave informed consent and were enrolled in the study. The mean age was 50.1 ± 13.2 years and the mean BMI 35.4 ± 5.3, with 25.7% males and 74.3% females. All patients completed the 9-month follow-up period without drop-outs in both branches (diet HP vs. diet S). Sex distribution was similar in groups, males (27.9% vs. 23.3%) and females (72.1% vs. 76.7%).
Age was similar in both groups (HP: 50.5 ± 13.1 years vs. S: 49.9 ± 12.0 years: ns).

The 168 subjects treated with diet HP basal, basal assessment of nutritional intake with a 3 days written food record showed a calorie intake of 2129.1 ± 677.6 kcal/day, a carbohydrate intake of 224.5 ± 67.9 g/day (40.3% of calories), a fat intake of 94.2 ± 32.3 g/day (41.5% of calories) and a protein intake of 87.6 ± 64.2 g/day (27.2% of calories). During the intervention, these subjects reached the recommended diet of; 1101.8 calories (34.1% of carbohydrates, 33.0% of lipids and 35.9% of proteins). The 163 subjects treated with diet S, basal assessment of nutritional intake with a 3 days written food record showed a calorie intake of 2037.8 ± 649.1 kcal/day, a carbohydrate intake of 205.3 ± 44.3 g/day (42.8% of calories), a fat intake of 91.2 ± 20.2 g/day (38.4% of calories) and a protein intake of 90.5 ± 18.1 g/day (38.4% of calories). During the intervention, these patients reached the recommendations of diet; 1193.8 calories (52.3% of carbohydrates, 28.9% of lipids and 18.8% of proteins).

Anthropometric characteristics of participants at baseline and at month 3 of intervention are shown in Table 1. With the diets HP and S, BMI, weight, fat mass, waist circumference, waist-to-hip ratio and systolic blood pressure decreased at 3, 6 and 9 months. The decrease at 9 months of (BMI: −2.6 ± 1.3 kg/m² vs. −2.1 ± 1.2 kg/m²; p < 0.05), weight (−8.4 ± 4.2 kg vs. −5.0 ± 4.1 kg; p < 0.05), fat mass (−5.1 ± 4.1 kg vs. −3.4 ± 4.2 kg; p < 0.05) and systolic blood pressure (−5.1 ± 7.1 mmHg vs. −3.1 ± 2.1 mmHg; p < 0.05) was higher in diet HP than Diet S. There were no significant differences between the effects on waist-to-hip ratio and diastolic blood pressure at 3, 6 and 9 months.

Table 2 shows the classic cardiovascular risk factors. With the diets HP and S, total cholesterol, LDL-cholesterol, insulin and HOMA decreased at 3, 6 and 9 months. Subjects with diet HP showed a significant decrease in glucose and triglyceride levels, too. The decrease at 9 months of (insulin levels −4.0 ± 4.8 UI/L vs. −2.2 ± 2.4 UI/L; p < 0.05) and HOMA (−0.8 ± 1.0 units vs. −0.3 ± 1.0 units; p < 0.05) was higher in diet HP than Diet S. There were no significant differences between the effects on total cholesterol and LDL cholesterol of the different diets at 3, 6 and 9 months. No differences were detected among basal and post-treatment values of the next biochemical variables (HDL-cholesterol and C reactive protein).

Table 3 shows levels of adipocytokines. With both diets, leptin levels decreased at 3, 6 and 9 months. With both diets the decrease of leptin levels (−18.2 ± 14.1 ng/ml vs. −17.1 ± 11.8 ng/ml) was similar. No differences were detected among basal and post-treatment values of the next biochemical variables (resistin, visfatin and adiponectin) at 3, 6 and 9 months.

6. Discussion

Because obesity are mandatory treated with weight reduction, we have performed a discriminative study comparing the effects of energy restriction with different dietary macronutrient composition on weight loss, cardiovascular risk factors and protein secretion from adipose tissue in obese humans. A high-protein/low carbohydrate hypocaloric diet shows a higher weight loss, insulin and HOMA-R decreased after 9 months than a standard hypocaloric diet. The improvement in adipokine levels was similar with both diets.

Meta-analyses of clinical trials with low-carbohydrate diets have shown that such diets have favorable effects on weight reduction and other major cardiovascular risk factors (Santos, Esteves, da Costa Pereira, et al., 2012). Meanwhile, high-carbohydrate diets are associated with greater insulin resistance and poorer lipid profiles, compared with low-carbohydrate diets, in type 2 diabetes patients (Kodama, Saito, Tanaka, et al., 2009). Compared with low-fat diets, low-carbohydrate diets have shown more favorable effects on TG and HDL-C levels; however, total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels were reduced to a greater extent by low-fat diets (Hu et al., 2012; Nordmann et al., 2006). Nevertheless, concerns over the long-term effects of low-carbohydrate diets still exist. A meta-analysis of observational studies has revealed that subjects who consume a low-carbohydrate diet may have increased risk for all-cause mortality (Noto, Goto, Tsujimoto, et al., 2013). Thus, further large trials are necessary. After 9 months, patients treated with a...
high-protein/low carbohydrate diet showed a higher improvement in weight loss, fat mass, HOMA-R and insulin levels, and these data are in agreement with previous studies.

In terms of adipokine changes, our results are in agreement with a number of studies in which leptin has been consistently shown to decrease in response to weight loss (Bastard, Jardel, & Bruckert, 2000; Hotta, Funahashi, & Arita, 2000; Okasaki, Himeono, Nani, Ogata, & Ikeda, 1999; Xenakis, Samojlik, Raghuwanshi, & Kirschner, 2001). The decrease in serum leptin in our patients was similar to reported previously. Some authors have reported a decrease of 22% in serum leptin (Ruth et al., 2013) or 45% (Bastard et al., 2000). In these studies the percentage of weight loss was similar than ours, for example 7% in first study (Xenakis et al., 2001) and 10% in second one (Bastard et al., 2000). Our study shows a 5 — 8% of weight loss with a decrease of leptin levels of 35 — 40%. Monzillo et al. (2003) detected a decrease of 14% in leptin levels in a weight loss of 7%. Perhaps, these differences in the literature may partially explain by differences in baseline BMI and leptin levels of participants.

Other authors (Xidakis et al., 2004), after 4–6 weeks of weight loss, detected marked improvement in glucose, insulin, leptin and triglycerides, whereas adiponectin and TNF-alpha concentrations did not change. Three studies (Brun, As, Verdich, Pedersen, & Toubo, 2003; Holta et al., 2000; Yang, Lee, Funahashi, Tanaka, & Matsuzawa, 2001) have demonstrated that a 10 — 20% weight reduction in obese individuals with very low calorie diets (VLCD) or bariatric surgery was associated with a 36 — 51% increase in adiponectin levels. Our data are consistent with the data from a 6 months weight loss study that achieved a 6% reduction in BMI with diet and exercise, without any significant change in adiponectin levels, too (Ryan, Nicklas, Berman, & Elahi, 2003). It is possible that a substantially greater and sustained weight reduction is necessary to correct altered adipocyte function, which could be determined by increased adiponectin and reduced levels of other adipocytokines (Esposito et al., 2003). Perhaps, the macronutrient composition, at least carbohydrate and fat contents, could influence in these differences. However, the results of Arvidsson, Viguier, Andersson, Verdich, & Langin, (2004) showed that the macronutrient composition of diets was not a major importance for the adipokine variations. Other comparative study of low- and highcarbohydrate diets designed for weight loss in middle-aged women has revealed that the diets are similarly effective in reducing fat mass and leptin levels (Miller, Volpe, Coleman-Kelly, et al., 2009). The effect of different type of fats on adiponectin levels has been evaluated in a systematic review, and this review has shown a potential positive association of saturated fatty acids with C-reactive protein but not with adiponectin levels (Santos, Olivese, & Lopes, 2013). Finally, depending on the protein sources quite large reductions in blood lipids can be seen on high-protein plant-based diets (Jenkins et al., 2014), and in cohort studies higher protein and fat from vegetable sources has been associated with lower incidence of heart disease and diabetes (Halton et al., 2006). In our study the protein source was 30% from vegetables and 7% from animals in both diets.

In conclusion, changes in serum adipokines levels due to a 9-month intervention with high-protein/low carbohydrate and standard hypocaloric diets were detected in our study, without changes in other adipokines. Weight and fat mass losses and improvement in HOMA-R and insulin levels were higher in subjects with high-protein/low carbohydrate diet than subjects treated with a standard hypocaloric diet during 9 months.

References


