

Effects of two energy-restricted diets differing in the carbohydrate/protein ratio on weight loss and oxidative changes of obese men

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Abstract

Introduction Low-carbohydrate, high-protein diets are a current challenge in the nutritional treatment of obesity.

Objective To compare the effect of a low-carbohydrate high-protein diet with a traditional hypocaloric diet on weight loss and mitochondrial oxidative metabolism.

Subjects and methods Nineteen obese men (age 36 ± 6 years; body mass index 34 ± 2 kg/m²) were randomized to follow one of the two diets—control diet (15% protein; 30% lipids; 55% carbohydrates) or high-protein diet (30% protein; 30% lipids; 40% carbohydrates)—over an 8-week period. Anthropometry, biochemical variables, resting energy expenditure and mitochondrial oxidation were measured at the start and at the end of the intervention.

Results The high-protein diet produced a greater weight loss ($-8.3 \pm 1.2\%$ versus $-5.5 \pm 2.5\%$, $P=0.012$) than the control diet. Interestingly, an activation in the mitochondrial oxidation was found in the high-protein-fed group. This stimulation was positively correlated with the final resting energy expenditure and negatively associated with the final fat mass content.

Conclusion Low-carbohydrate high-protein diets could involve specific changes in mitochondrial oxidation that could be related to a higher weight loss.

Keywords: Energy restriction, high-protein diets, mitochondrial oxidation, resting energy expenditure, weight loss

Introduction

Low-carbohydrate diets are generally considered those that contain less than 100 g carbohydrate per day with a typical energy distribution of 50–60% from fat, less than 30% from carbohydrate and 20–30% from protein (Bilborough and Crowe 2003; Martínez and Parra 2006). Previous investigations have reported that low-carbohydrate diets promote a greater degree of weight loss in the short term than the high-carbohydrate low-fat diets (Hu 2005; Yancy et al. 2005; Abete et al. 2006).

Additionally, the current available scientific evidence reveals that such diets acutely induce a number of favourable effects, such as a rapid weight loss, decrease in fasting

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glucose and insulin levels, reduction of circulating triglyceride levels and improvement of blood pressure (Brehm et al. 2003; Yancy et al. 2004; McAuley et al. 2005). However, some less desirable effects such as ketosis (Adam-Perrot et al. 2006), enhanced lean body mass loss, renal overload, increased urinary calcium loss and increased low-density lipoprotein (LDL) cholesterol have been also reported (Astrup 2005). Furthermore, the effects of the high saturated fat and the high dietary cholesterol intake commonly found in such diets still remain unclear. Data obtained from nutritional intervention studies suggest that some of the harmful effects of a low-carbohydrate diet may be counteracted by a higher protein intake (Halton and Hu 2004). In this context, high-protein low-fat diets may have favourable effects on appetite and hunger, lean body mass preservation and effective fat mass reduction as well as beneficially impacting on insulin sensitivity and the blood lipid profile (Johnstone et al. 2008). In addition, it has been reported that diets with a fat content fixed at 30% of calories produce more weight loss when high in protein (25% of energy) than when normal (12% of energy) in protein (Weigle et al. 2005). Thus, the objective of the present study was to compare the effects of two energy-restricted diets with different carbohydrate/protein ratios, but similar amounts of fat on weight loss and related metabolic changes, with emphasis on mitochondrial oxidation.

Subjects and methods

Subjects

Nineteen obese men (36 ± 6 years old; body mass index 34 ± 2 kg/m²) were recruited to participate in this nutritional intervention study. Initial screening evaluations included medical histories, physical examinations and fasting blood profiles, to exclude subjects with clinical evidence of diabetes, hypertension, liver, renal, haematological disease, or other clinical disorders. Other exclusion criteria included weight changes of over 3 kg within the 3 months before the start of the study, participation in other scientific studies within the previous 90-day period, pharmacological therapy for chronic diseases, surgical or drug obesity treatments and alcohol or drugs abuse. All subjects gave written informed consent, which had previously been approved by the Ethics Committee of University of Navarra (54/2006).

Study design

Participants were alternatively distributed to one of the two dietary treatments: control diet (C-diet) with 15% of energy from protein, 55% from carbohydrates and 30% from fat; or to the high-protein diet (HP-diet), which contained 30% of energy from protein, 40% from carbohydrates and 30% from fat. The C-diet was adjusted to the recommendations for a healthy diet, while the HP-diet had an increase in the protein content, reducing the carbohydrate proportion. The protein source was mainly of animal origin. The total fat intake was similar in both dietary groups. Each subject was instructed by the dietician over the 8-week trial period to follow the designed meal plan (Table I). The energy restriction was -30% from the total energy expenditure, which was calculated from resting energy expenditure measured by indirect calorimetry (Deltatrac; Datex-Ohmeda, Helsinki, Finland) and corrected by physical activity levels (OMS 2000; Labayen et al. 2004). Volunteers were instructed to maintain their habitual physical activity patterns during the trial. Specific questions

Table I. Example of a 1-day meal plan for each experimental diet.

	C-diet	HP-diet
Breakfast	250 ml skimmed milk 60 g white bread	250 ml skimmed milk 30 g white bread 30 g boiled ham 60 g fresh cheese
Mid-morning snack	60 g white bread 30 g cured ham 250 g apple	1 skimmed yoghurt
Lunch	200 g Greens 80 g potatoes 60 g roast beef 60 g white bread 30 g olive oil 1 skimmed yoghurt	200 g Greens 80 g potatoes 210 g roast beef 90 g white bread 25 g olive oil 1 skimmed yoghurt
Afternoon snack	125 ml skimmed milk 175 g naranja	350 g manzana 30 g boiled ham
Dinner	100 g salad 90 g white bread 80 g skimmed cheese 25 g olive oil 125 g kiwi	200 g salad 60 g white bread 90 g cured ham 90 g skimmed cheese 20 g olive oil 200 g kiwi

about lifestyle habits were asked weekly by the dietician. Each participant received a detailed meal plan to be followed for 8 weeks, which was directly explained by the dietician, including portion size. This task was facilitated by giving a calibrated scale and a menu plan for a whole week to each participant.

The intake was controlled by 3-day weighed food records (two weekdays and one weekend day), which 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 compliance to the prescribed diets (Xinying et al. 2004). Weight loss was monitored weekly by the dietician. Body composition, energy expenditure and blood samples were assessed at baseline and at the end, following standardized procedures (Gibson 2005; Pérez et al. 2005). Dietary records were analysed and quantified using the Medisystem program (Sanocare, Alcobendas, Spain).

Anthropometry

Body weight measurements were performed using a digital balance accurate to 0.1 kg (Seca 767; Vogel & Halke, Hamburg, Germany) and height measurements using a wall-mounted stadiometer (Seca 220; Vogel & Halke). 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 (Gibson 2005).

Body composition

Body composition was measured with a bioelectric impedance equipment (Quadscan 4000; Bodystat, Douglas, UK), based on a previously described standardized

procedure (Pérez et al. 2005). Testing conditions were controlled to avoid acute changes in hydration status. Thus, subjects were instructed to avoid vigorous exercise for at least 12 h and to abstain from alcohol and caffeine consumption for 48 h before assessment. In the fasting state, with minimum clothing, the subject lay in supine position with arms and legs abducted and not touching the body.

Indirect calorimetry

The indirect calorimetry measurements were carried out in a canopy-hood system. On the day of the test, patients arrived by car or bus at the department at 8:00 h in a fasting condition from 20:00 h on the day before. Subjects were instructed not to perform any strenuous physical activity during the 24 h before the measurement. Respiratory exchange was measured continuously for 30 min on two occasions during the study period (baseline and end-point). Respiratory exchange measurements were determined by means of an open-circuit computerized indirect calorimeter (Deltatrac; Datex-Ohmeda) after the daily calibration with room air, and then against a standard gas mixture consisting of 95% oxygen and 5% carbon dioxide in nitrogen (Datex-Ohmeda), as described elsewhere (Para et al. 2005).

Blood pressure measurement and blood sample analyses

Blood pressure was measured with a standard mercury sphygmomanometer after the subject had been sitting quietly for 5 min (Minimus II; Riester, Juingem, Germany), following standard World Health Organization criteria. Venous blood samples were drawn in a fasting state (12 h) to measure basal circulating levels of selected biochemical markers. Ethylenediamine tetraacetic acid plasma and serum were separated from whole blood by centrifugation ($1,400 \times g$, 15 min, 5°C) and stored at -80°C until assay. Plasma levels of glucose, triacylglycerol, total cholesterol and high-density lipoprotein (HDL) were assayed on Cobas-Mira equipment (Roche, Basel, Switzerland). The reported LDL cholesterol was calculated by the Friedewald equation (Friedewald et al. 1972). Plasma levels of insulin were assessed using commercially available radioimmunoassay kits (DPC, Los Angeles, CA, USA). Insulin resistance was estimated by the homeostatic model assessment index (HOMA), as the product of fasting insulinaemia ($\mu\text{U/ml}$) per glycaemia (mM), which was divided by 22.5 (Matthews et al. 1985). Insulin resistance was considered if the HOMA index was higher than 3.5 (Parra et al. 2005).

Mitochondrial oxidation measurement

The 2-keto[1- ^{13}C]isocaproate breath test was performed to study mitochondrial oxidation *in vivo* (Parra and Martinez 2006). After the indirect calorimetry was performed, the subjects received $6.5 \mu\text{mol/kg}$ 2-keto[1- ^{13}C]isocaproate sodium salt (Euriso-top, Saint-Aubin, France) together with $152.4 \mu\text{mol/kg}$ l-leucine USP (Sigma-Aldrich Chemical, Madrid, 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 (Parra et al. 2005). 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 $^{13}\text{CO}_2$ after the tracer ingestion (Parra et al. 2003). Thus, breath

samples were recovered by exhaling through a straw into a tube (Labco, High Wycombe, UK) before and at 10-min intervals during the 2 h after ingestion of the 2-keto[1-¹³C]isocaproate. Enrichment of ¹³CO₂ in breath was measured by isotope ratio mass spectrometry on a BreathMAT plus spectrometer (Finnigan, Bremen, Germany) and the percent of 2-keto[1-¹³C]isocaproate oxidized at 2 h after the test meal ingestion (% ¹³C) was calculated (Parra et al. 2003). Volunteers repeated this protocol after the nutritional intervention trial.

Statistical analysis

The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine variable distribution. Changes in weight loss were evaluated and compared applying paired parametric *t*-tests (baseline and endpoint) and with the repeated-measures analysis of variance to evaluate the weight loss during 8 weeks. Wilcoxon's test was applied to analyse within-group differences. Two-tailed Mann–Whitney U-tests were used to analyse differences between groups as appropriate. The Spearman coefficient was used to set up the potential relationships among variables (resting energy expenditure, fat mass and mitochondrial oxidation). Results are reported as the mean ± standard deviation. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the SPSS 13.0 program (SPSS Inc., Chicago, IL, USA) for Windows XP (Microsoft, Redmond, WC, USA).

Results

Both experimental diets supplied the same energy content per kilogram of body weight (about 18 kcal/kg), while they were significantly different in the carbohydrate/protein distribution (Table II). The C-diet was prepared in accordance with the recommendations for a healthy hypocaloric diet, while the energy-restricted HP-diet was higher in protein and lower in carbohydrate content. The total fat content was similar in both dietary groups; however, the HP-diet group showed a higher saturated fatty acid intake in comparison with their control counterparts (Table II). The fibre consumption was not statistically different between groups; however, as expected, the dietary cholesterol content was significantly higher in the HP-diet group ($P < 0.001$).

Baseline characteristics of the participants were not markedly different ($P > 0.05$) between the two dietary groups (Table III), unless for the HDL-cholesterol values, which were unexpectedly higher in the C-diet group than in the HP-diet group.

Table II. Nutritional characteristics and composition of both experimental diets during the 8-week intervention period.

Variable	C-diet	HP-diet	<i>P</i> value
Energy (kcal)/body weight (kg)	17.5 ± 1.7	18.7 ± 1.5	0.113
Proteins (% energy)	17.5 ± 0.5	30.7 ± 0.8	<0.001
Carbohydrates (% energy)	55.9 ± 2.1	43.2 ± 2.0	<0.001
Lipids (% energy)	30.1 ± 1.9	28.5 ± 2.8	0.133
Saturated fatty acids (% energy)	5.8 ± 2.0	10.0 ± 3.8	0.020
Fibre (g/day)	20.2 ± 5.4	15.8 ± 3.0	0.147
Cholesterol (mg/day)	81.2 ± 67.7	338.1 ± 26.4	0.001

Table III. Changes related to both nutritional interventions (control and high-protein diets) after 8 weeks of controlled energy-restriction

Variable	Control-diet group			High-protein-diet group			P value between changes
	Baseline	End-point	Change (%)	Baseline	End-point	Change (%)	
Body mass index (kg/m ²)	31.4±3.4	29.8±3.4	-4.9±3.0*	33.1±1.9	30.1±1.9	-9.3±1.6*	0.001
Waist circumference (cm)	104.4±7.7	98.0±6.5	-6.1±2.9*	108.9±6.7	97.6±6.6	-9.8±2.4*	0.034
Fat mass (kg)	28.0±5.8	24.5±5.9	-12.7±7.2*	31.1±5.1	25.3±4.9	-18.4±3.4*	0.079
Systolic blood pressure (mmHg)	117.5±8.5	114.5±8.6	-2.4±4.8	128.3±14.3	115.0±3.1	-9.4±10.2	0.181
Diastolic blood pressure (mmHg)	78.0±9.1	72.5±8.5	-6.6±9.3*	83.3±8.1	78.3±7.5	-5.2±12.1	0.875
Resting energy expenditure (kcal/day)	1,813.0±187.2	1,676.0±144.7	-7.2±5.7*	1,907.7±155.6	1,741.3±149.3	-8.6±3.7*	0.661
Total cholesterol (mg/dl)	185.5±31.3	172.9±26.3	-6.0±9.3	182.5±27.6	166.6±39.0	-5.3±12.1	0.875
LDL-cholesterol (mg/dl)	116.1±28.8	107.8±23.5	-5.6±12.8	124.5±25.8	115.2±34.5	-8.2±12.9	0.796
HDL-cholesterol (mg/dl)	49.2±7.6	45.5±10.8	-7.5±15.9	40.1±4.8	34.5±5.7	-13.4±12.6*	0.546
Triglycerides (mg/dl)	101.1±28.3	98.1±49.8	2.3±47.0	89.6±45.9	85.1±42.6	3.1±45.7	0.999
Total cholesterol/HDL-cholesterol ratio	3.8±0.8	3.9±0.9	3.7±18.7	4.6±0.8	4.8±0.7	5.4±10.1	0.605
Glucose (mg/dl)	93.8±8.3	90.7±5.9	-2.9±6.5	91.3±6.5	87.0±4.8	-4.2±9.7	0.863
Insulin (μIU/ml)	9.5±6.8	8.7±4.9	-11.3±70.5	13.6±7.9	7.7±4.1	-37.3±24.7*	0.050
HOMA-IR	2.17±1.59	1.82±1.15	-32.3±79.2	3.12±1.99	1.65±0.91	-109.2±142.4*	0.114

P values indicate the statistical comparison of the nutritionally induced changes by both hypocaloric diets (non-parametric: Mann-Whitney U-test). *Statistical difference between baseline and end-point (non-parametric: Wilcoxon *t*-test).

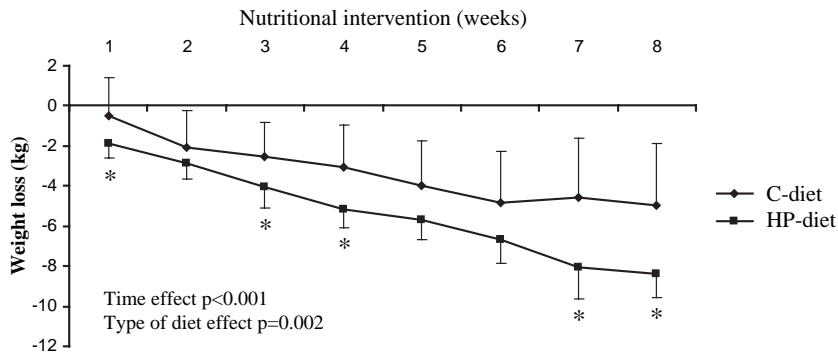


Figure 1. Weight loss in both dietary groups after the nutritional intervention. *Statistical differences between experimental groups. The statistical tests used were the paired parametric *t*-tests (baseline versus endpoint) and the repeated-measures analysis of variance.

After the dietary intervention, the body mass index and waist circumference were significantly reduced in both dietary groups. Moreover, the change in both variables was significantly higher in the HP-diet group (Table III). Similarly, the change in body weight was significantly higher ($-8.3 \pm 1.2\%$ versus $-5.5 \pm 2.5\%$, $P=0.012$) in the HP-diet group as compared with the change in the C-diet group (Figure 1).

Body composition and other metabolic determinations such as systolic and diastolic blood pressure, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride levels, total-cholesterol/HDL-cholesterol ratio and glucose concentration changed in a similar way in both groups under dieting (Table III).

On the other hand, insulin levels were significantly reduced only in the HP-diet group, also reaching statistical differences between both groups ($P=0.05$). Moreover, this change was reflected in the HOMA-IR index, which resulted significantly improved ($P=0.015$) after the HP diet (Table III).

As previously mentioned, body composition measurements (bio-impedance determinations and body water percentage) followed a similar trends in both dietary groups. Body fat significantly decreased in both groups (Table III). The change in the percentage of this variable was higher in the HP-diet group, but did not reach statistical significance (C-diet, $-12.7 \pm 7.2\%$; HP-diet, $-18.4 \pm 3.4\%$; $P=0.079$). As expected after a caloric restriction, the fat-free mass content also decreased in both dietary groups. Thus, from the total weight loss, the fat-free mass loss was about 42.0% in the C-diet group and was 43.0% in the HP-diet group with no statistical differences between groups ($P=0.423$). However, the change in total body water content was significantly higher in those patients included in the HP-diet group (C-diet, $-1.3 \pm 0.6\%$; HP-diet, $-2.1 \pm 0.2\%$; $P=0.008$).

Resting energy expenditure significantly decreased after the caloric restriction period with no statistical differences between groups (Table III). Interestingly, when the variable was adjusted for fat-free mass ($REE_{adj-FFM}$), the change in $REE_{adj-FFM}$ was similar in both groups even when the weight loss was different depending on diet (C-diet, $-7.7 \pm 0.04\%$; HP-diet, $-7.7 \pm 0.02\%$; $P=0.999$). Indeed, participants that followed the HP-diet showed an apparently smaller decrease than their counterparts when comparing the decrease of resting energy expenditure for each kilogram of fat-free mass lost (-47.5 ± 27.1 kcal/day_xkg versus -91.1 ± 89.7 kcal/day_xkg), although with no statistical impact ($P=0.243$).

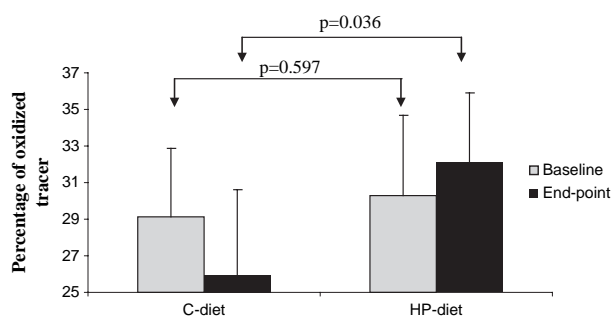


Figure 2. Percentage of the mitochondrial oxidation of each dietary group at the end of the nutritional intervention period.

On the other hand, the mitochondrial oxidation percentage was similar in both dietary groups at the beginning of the nutritional intervention (C-diet, $29.1 \pm 3.8\%$; HP-diet, $30.3 \pm 4.4\%$; $P=0.597$). Interestingly, obese men included in the HP-diet group tended to increase the percentage of oxidized tracer after the dietary intervention period, while patients included in the C-diet group tended to decrease the percentage of this variable (Figure 2). Thus, the percentage of oxidized tracer was statistically different between dietary groups at the end of the nutritional treatment (C-diet, $26.0 \pm 4.6\%$; HP-diet, $32.1 \pm 3.8\%$; $P=0.036$). Moreover, the change in mitochondrial oxidation was related to the final $REE_{\text{adj-FFM}}$ ($r=0.684$; $P=0.007$) (Figure 3a). Also, the final percentage of oxidized tracer tended to be inversely related to the final body weight ($r=-0.502$; $P=0.068$). In addition, the final percentage of oxidized tracer was inversely related ($r=-0.615$; $P=0.015$) to the final body fat content (Figure 3b).

Discussion

Low-carbohydrate-based dietary approaches may represent a way to reduce caloric intake and promote weight loss (Noakes et al. 2006; Cassady et al. 2007). The present study examined the effects of two diets with different macronutrient composition by comparing a traditional hypocaloric diet with a moderate low-carbohydrate high-protein diet on weight loss as well as on associated metabolic and oxidative changes. The prescribed high-protein diet contained higher saturated fat than the control diet, but apparently not enough high to produce detrimental effects on cardiovascular risk factors (Woodside and Kromhout 2005). The absolute amount of protein consumption in the HP-diet group was about 150 g/day (<2 g/kg/day), which is not a harmful challenge for the kidney in normal conditions (Skov et al. 1999a). Overall, the weight loss achieved with the HP-diet was considerably higher than the weight loss registered in the other dietary group. Some authors indicate that the high-protein content of these diets may contribute to their success in inducing weight lowering (Hu 2005; Cassady et al. 2007; McClernon et al. 2007). In this context, it has been reported that relative low-carbohydrate diets have a better efficacy for weight-loss promotion than low-fat diets during a short-term period (Nordmann et al. 2006). Also, a nutritional intervention study showed that an increase in dietary protein from 15% to 30% of energy and a reduction in fat from 35% to 20%, at a constant carbohydrate intake, produced a sustained decrease in *ad libitum* caloric intake and results in significant

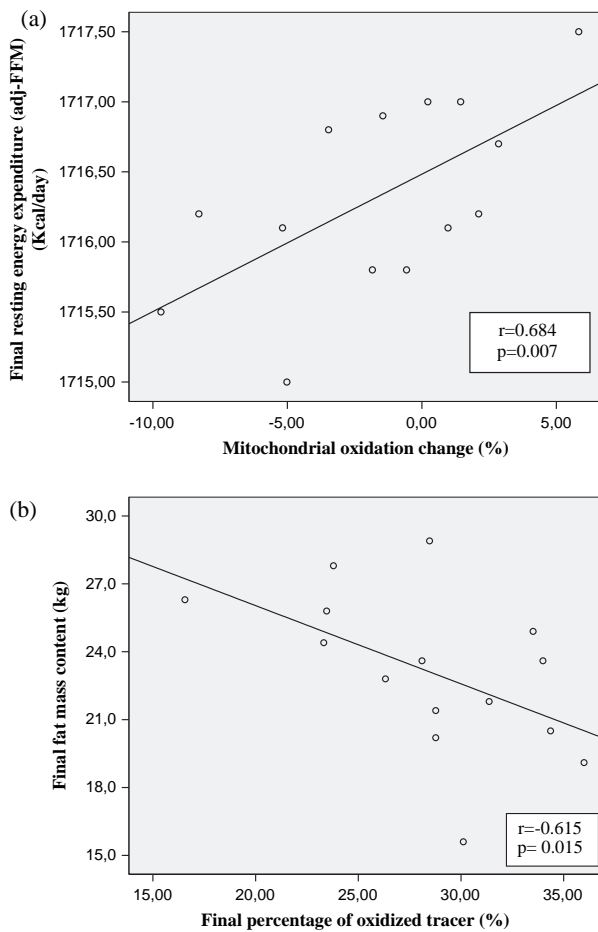


Figure 3. (a) Final resting energy expenditure rate related to the change in the mitochondrial oxidation after the nutritional intervention period. (b) Final oxidized tracer percentage inversely related to the final fat mass content.

weight loss (Weigle et al. 2005). In this sense, it has been confirmed that protein consumption reduced food intake, being more satiating than carbohydrate (Due et al. 2004). Thus, in accordance with our results, diets with a fat content fixed at 30% of calories produce more weight loss when high in protein (25% of energy) than when protein intake is lower (12% of energy). However, both experimental diets were energy-restricted diets, but not *ad libitum* diets, so, patients did not show a 'free' decrease in their total energy intake.

High-protein and high-fat diets, such as the Atkins diet, may also suppress food intake by producing ketosis, accompanying glycogen store depletion as induced by a severe restriction of carbohydrates (Astrup 2005). The HP-diet included in the trial was not associated with a severe carbohydrate restriction, in order to avoid ketosis and its related negative effects. In addition, it was also suggested that high-protein diets resulted in a significant loss of visceral fat independent of the loss of total body fat (Due et al. 2004). Despite the loss of total body fat not being significantly different, participants included in the HP-diet group registered a higher waist circumference

decrease in comparison with their counterparts in the other dietary group, which could be related to a higher visceral fat decrease and be involved in the improvement in the metabolic markers. In this sense, the measured lipid markers tended to decrease in both dietary groups, but with no statistical significance between them.

Experimental studies have suggested that exchanging animal protein for carbohydrates in diets significantly reduced LDL-cholesterol and triacylglycerol levels and increased HDL-cholesterol levels (Wolfe 1995); however, we did not observe this specific effect on lipid metabolism. Thus, total cholesterol and LDL-cholesterol slightly decreased in both dietary groups, while the HDL-cholesterol was decreased in the HP-diet group. This finding did not support the contention that high-protein diets increase the HDL-cholesterol levels showed by other nutritional intervention studies (Keller and Rastalsky 2005; Noakes et al. 2006; Westman et al. 2006). However, experimental data have shown marked increases in LDL-cholesterol in subjects following a low-carbohydrate diet and especially in those who were following a ketogenic diet (Adam-Perrot et al. 2006; Johnston et al. 2006), suggesting that blood lipid concentrations should be always monitored. On the other hand, scientific evidences suggest that higher protein content diets importantly decrease triglyceride levels compared with lower protein diets (Skov et al. 1999b; Farnsworth et al. 2003), but, in agreement with other studies, patients in the HP-diet group showed no significant change in this variable (Alford et al. 1990). On the other hand, a decrease in insulin concentration and an improvement in the HOMA index have been reported after the consumption of HP-diets (Bisschop et al. 2001; Volek et al. 2004). Thus, it has been suggested that a high-protein low-carbohydrate diet should be an important option for those patients with hyperinsulinaemia or insulin resistance, at least in the early phase of weight loss (Johnstone et al. 2008).

There is limited evidence in the literature that a higher protein intake during weight loss is a significant factor for long-term success. However, in a follow-up to an intensive 6-month weight-loss trial, subjects assigned to a high-protein diet reported greater abdominal fat loss (Due et al. 2004). In another 12-month study, McAuley et al. (2006) reported improved weight-loss maintenance with a higher-protein diet than with a high-carbohydrate diet or a high-fat diet. Similarly, Clifton et al. (2008) concluded that a higher protein intake appears to confer some weight-loss benefit after 64 weeks. Moreover, unlike the results of other studies, they also found that the total body bone mineral density was unchanged, which indicated that bone mass was preserved despite weight loss (Clifton et al. 2008).

Several studies mention the hypothesis that high-protein diets spare the lean body mass of those on energy-restricted diets (Halton and Hu 2004). In this context, Layman et al. (2003) compared two iso-energetic diets, differing only in the carbohydrate to protein ratio. Although they found no differences in body weight reduction between the two dietary groups, they did determine that the higher protein diet was more effective in improving body composition. Thus, the high-protein group lost more body fat and preserved more lean tissue compared with the high-carbohydrate group (Layman et al. 2003). According with this research, patients included in the HP-diet group tended to decrease more fat mass content, while the change in the fat-free mass was not different (42% versus 43%) after the dietary treatment. Interestingly, the fat-free mass change in the HP-diet group was not apparently related to the muscle mass loss, since the decrease in resting energy expenditure was lower than it could be expected in comparison with the other group.

The change in fat-free mass would be also due to changes in truncal fat free mass (i.e. size of splanchnic organs) and total body water loss. However, we did not perform specific approaches to study these changes. Thus, patients included in the HP-diet group showed a better energy restriction tolerance, since the decrease in resting energy expenditure was smaller than the decrease registered in the C-diet group, regarding total weight lost. In this context, several authors support the concept that calorie-reduced diets high in protein facilitate weight loss, in part, by preserving the metabolic rate (Baba et al. 1999; Mikkelsen et al. 2000; Brehm et al. 2005; Schoeller and Buchholz 2005; Meckling and Sherfey 2007). Also, it has been suggested that high-protein diets enhance metabolic rate due to an increased dietary thermogenesis (Halton and Hu 2004; Westman et al. 2007). Overall, resting energy expenditure reduction seemed to be protected by the HP-diet intervention minimizing its decrease, which suggested the metabolic activation of alternative oxidation pathways.

Previous nutritional studies have evidenced that the 2-keto[1-¹³C]isocaproate breath test reflected the adaptive modifications in mitochondrial oxidation in response to caloric restriction. Thus, the activation of this mitochondrial pathway was described after a successful nutritionally induced weight loss (Parra et al. 2003). However, in the current work, the mitochondrial response seems to be also modulated by the type of diet, showing a specific oxidative activation mediated by the HP-diet. In addition, this change in the mitochondrial oxidation was involved in the resting energy expenditure protection when an energy restriction is followed as well as in the greater weight loss achieved by the HP-diet. Thus, an important finding in this investigation was that the HP-diet regimen induced a higher weight loss as well as a greater fat mass loss, while the impact on energy metabolism (resting energy expenditure and mitochondrial oxidation) was improved as compared with a traditional hypocaloric diet, which could be hypothesized as trying to protect the resting energy expenditure decrease associated to the caloric restriction.

Thus, moderate HP-diets appear as a valid strategy to improve weight loss and fat mass loss during an energy restriction period, potentially avoiding the resting energy expenditure reduction by the activation of mitochondrial oxidation pathway, which may be also associated with a better body weight regulation after the nutritional intervention period.

Acknowledgements

Thanks are due to the Government of Navarra that supported this work included in the *Linea Especial* of the University of Navarra (LE/97) and IBERCAJA as well as to volunteers who participated in the study. The authors also thank their nurse Salomé Pérez and their technician Ana Lorente for excellent technical assistance.

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