Eur J Nutr (2011) 50:61–69
DOI 10.1007/s00394-010-0115-x

ORIGINAL CONTRIBUTION

A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects

Helen Hermana M. Hermsdorff · M. Ángeles Zulet · Itziar Abete · J. Alfredo Martínez

Received: 29 January 2010 / Accepted: 10 May 2010 / Published online: 25 May 2010

Abstract
Background The nutritional composition of the dietary intake could produce specific effects on metabolic variables and inflammatory marker concentrations. This study assessed the effects of two hypocaloric diets (legume-restricted- vs. legume-based diet) on metabolic and inflammatory changes, accompanying weight loss.
Methods Thirty obese subjects (17 M/13F; BMI: 32.5 ± 4.5 kg/m^2; 36 ± 8 years) were randomly assigned to one of the following hypocaloric treatments (8 weeks): Calorie-restricted legume-free diet (Control: C-diet) or calorie-restricted legume-based diet (L-diet), prescribing 4 weekly different cooked-servings (160–235 g) of lentils, chick-peas, peas or beans. Body composition, blood pressure (BP), blood biochemical and inflammatory marker concentrations as well as dietary intake were measured at baseline and after the nutritional intervention.
Results The L-diet achieved a greater body weight loss, when compared to the C-diet (−7.8 ± 2.9% vs. −5.3 ± 2.7%; $p = 0.024$). Total and LDL cholesterol levels and systolic BP were improved only when consuming the L-diet ($p < 0.05$). L-diet also resulted in a significant higher reduction in C-reactive protein (CRP) and complement C3 (C3) concentrations ($p < 0.05$), compared to baseline and C-diet values. Interestingly, the reduction in the concentrations of CRP and C3 remained significantly higher to L-diet group, after adjusting by weight loss ($p < 0.05$). In addition, the reduction (%) in CRP concentrations was positively associated with decreases (%) in systolic BP and total cholesterol concentration specifically in the L-diet group, independent from weight loss ($p < 0.05$).
Conclusion The consumption of legumes (4 servings/week) within a hypocaloric diet resulted in a specific reduction in proinflammatory markers, such as CRP and C3 and a clinically significant improvement of some metabolic features (lipid profile and BP) in overweight/ obese subjects, which were in some cases independent from weight loss.

Keywords Legumes · Hypocaloric diet · Weight loss · Inflammation · C-reactive protein · Complement C3

Introduction

Excessive body fat is associated with increased concentrations of several proinflammatory and proatherogenic markers such as C-reactive protein (CRP), complement C3 (C3), interleukin-6 (IL6), tumor necrosis factor-alpha (TNFα) and homocysteine [1–4]. Furthermore, the occurrence of a low-grade chronic inflammation in obese subjects has been implicated in the development of insulin resistance, diabetes, hypertension, dyslipidemia and atherosclerosis [3, 5, 6].

In this context, in addition to the loss of body fat and the subsequent improvement in common metabolic syndrome features produced by hypocaloric diets, some nutritional intervention trials have as a target the improvement in obesity-related proinflammatory status [7, 8]. In turn, legumes are foods containing important nutritional and functional factors that may play a crucial role in health maintenance and disease treatment, such as vegetable protein, fiber, oligosaccharides, phytochemicals, minerals

H. H. M. Hermsdorff · M. Á. Zulet · I. Abete · J. A. Martínez (✉)
Department of Nutrition, Food Science, Physiology and Toxicology, University of Navarra, C/ Irunlarrea, 1, 31008 Pamplona, Spain
e-mail: jalfmtz@unav.es

(e.g. potassium) and other bioactive compounds, such as saponins and polyphenols [9–11].

The consumption of non-soy legumes, such as lentils, beans, peas and chickpeas, has been associated with diet quality—lower fat, higher bioactive compounds intake and higher vegetable protein supply—as well as with lower waist circumference and blood pressure values, according to results from the National Health and Nutrition Examination Survey [12, 13]. Moreover, the consumption of this staple food-group has consistently revealed important hypocholesterolemic effects [9, 14], in addition to the potential benefits concerning the glycemic control [15]. In fact, specific beneficial effects of a hypocaloric diet enriched with legumes have been reported as changes in metabolic syndrome features and mitochondrial oxidation in obese subjects [16, 17]. Also, the consumption of a legume-based hypocaloric diet has mitigated lipid peroxidation associated with a higher weight loss [18].

Overall, this study aimed at assessing the effects accompanying weight loss of two hypocaloric diets with different food patterns (a control-restricted- vs. a legume-based diet) on metabolic and inflammatory changes.

Subjects and methods

Subjects

Thirty obese subjects, 17 men/13 women (BMI: 32.5 ± 4.5 kg/m^2; Age: 36 ± 8 years), were enrolled in the study. A physician performed the screening and the inclusion of volunteers by medical history, physical examination and fasting blood profile to exclude subjects with evidence of diabetes, hypertension, liver, renal or haematological disease and other clinical disorders. Other exclusion criteria were weight change of ±3 kg in the last months, participation in another scientific study up to 90 days before, chronic pharmacological therapies, surgical or drug related-obesity treatments, as well as alcohol or drugs abuse. In addition, those subjects who declared not to consume any of the proposed non-soy legumes (chickpeas, peas, beans or lentils) were not enrolled in the study to avoid different consumption patterns among these legumes. After a detailed explanation of the study protocol, all subjects gave written informed consent to participate in the trial, which was previously approved by the Ethics Committee of the University of Navarra (ref. no 54/2006).

Study design

The hypocaloric diets were designed to produce a calorie restriction of −30% of total energy requirements in relation to the individual daily resting energy expenditure, assessed by indirect calorimetry (Deltatrac, Datex-Ohmeda, Finland) and corrected by the declared physical activity level (1.3–1.6) of each participant following the WHO criteria [19].

The average consumption of legumes in Spain is approximately 2 servings/week [20], while the maximal recommended amount is 4 servings/week, which was prescribed in this trial [21]. Thus, volunteers were randomly distributed into two groups which were assigned to two different calorie-restricted dietary treatments for 8 weeks: a legume-restricted diet as a control diet (C-diet; $n = 15$) or a legume-based diet (L-diet; $n = 15$) with 4 servings/week of traditional Spanish non-soybeans legumes (lentils, chickpeas, peas and beans). The macronutrient-balanced diets (control and legume-based dietary approaches) were designed to provide a similar macronutrients distribution: 53% of energy as carbohydrates, 17% as proteins and 30% as fat.

The participants were individually instructed by a registered dietician to follow the prescribed dietary regime for 8 consecutive weeks. In this context, the volunteers received a 7-day menu in relation to the assigned diet, with the food-groups they should consume each day and an exchange-food list with amounts in grams of each food, expressed in raw and in cooked terms, to facilitate the adherence to the dietary regime. Regarding legume consumption, the participants in the L-diet group received a 7-day menu in which they should weekly consume all of four different types of legumes. The advised amounts in grams for each legume were between 60 and 90 g (raw), or 160 and 235 g (cooked), according to daily energy intake of each participant. The participants received a food-weighing scale (Odag 5025, Roque Monasterio, Spain) to rigorously follow the prescribed dietary regimes.

Furthermore, diet compliance was weekly assessed by a trained dietician who asked routine questions and gave advices to improve the monitoring of the prescribed diets. In addition, dietary intake was controlled by 3-day weighed food records (2 weekdays and 1 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) as previously described [18, 22]. Diet records were assessed by using the Medisystem software adapted for Spanish foods (Sanocare, Alcobed, Spain). These data also provided information about baseline intake and the compliance to the prescribed diets.

Volunteers were weekly asked to maintain the same habitual physical activity habits during the intervention period, which was reinforced through specific questions during the interviews [23]. Weight loss was monitored by the dietician on a weekly basis.

Data about anthropometry, body composition, energy expenditure and blood markers were collected at baseline (day 0) and at the end-point (day 56) following standardized procedures [24].

Anthropometry and body composition

The body weight assessment was performed using a digital weight-scale with accurate to 0.1 kg (Seca 767, Vogel & Halke, Germany) and height, which was accurate to 0.1 cm, using a wall-mounted stadiometer (Seca 220, Vogel & Halke, Germany), carried out in underwear after an overnight fast [25]. The waist circumference was measured at the site of the smallest circumference between the rib cage and the iliac crest, and the hip circumference was measured on the maximum circumference over the buttocks with the subject in standing position [26]. Body composition was assessed by bioelectrical impedance (Quadscan 4000, Bodystat, UK), based on a previously validated procedure [25].

Blood pressure measurement and blood sample analyses

Blood pressure (BP) was measured following WHO criteria [19]. Venous blood samples were drawn in a fasting state (12 h). EDTA plasma and serum were separated from whole blood by centrifugation (3,500 rpm, 15 min, 5 °C) and stored at −20 °C until assay. Plasma concentrations of triglycerides, total cholesterol and HDL-c, glucose (Horiba ABX Diagnostics, Montpellier, France) and homocysteine (Demitec Diagnostic GmbH, Kiel-Wellsee, Germany) were measured by specific colorimetric assays, while complement C3 was assessed by turbidimetric assay (Horiba ABX Diagnostics, Montpellier, France), by using an automated analyzer system (COBAS MIRA, Roche, Basel, Switzerland). The reported LDL-c was calculated by the Friedewald equation [27]. Plasma insulin concentration was assessed by using commercially available radioimmunoassay kits (DPC, Texas, USA). Insulin resistance was calculated by the HOMA-IR index [28], which was estimated as follows: HOMA-IR = [fasting glucose (mmol/l) × fasting insulin (μIU/l)]/22.5. Plasma concentrations of CRP, high-sensitive IL6 (hs-IL6) and TNFα (hs-TNFα) were assessed by enzyme-immunoassay kits using an automated analyzer system (Triturus, Grifols, Barcelona, Spain). CRP was measured by using an Immundiagnostik AG kit, (Bensheim, Germany) and the cytokines by Quantikine immunoassay kits (hs-IL-6 and hs-TNFα) from R&D Systems (Minneapolis, USA). In our laboratory, all the inter- and intra-assay variability for all determinations were <10%.

Statistical analysis

Results are reported as mean ± SD (95% confidence interval—95% CI). Sample size was calculated considering: (1) a change in plasma CRP as main outcome; (2) published SD values (±0.9) for plasma CRP concentration [29]; (3) an statistical power of 80% and (4) p-value < 0.05 as statistically significant. For all these criteria, the sample size required was a minimum of 12 volunteers per group [30].

The Shapiro–Wilk tests were used to determine variable distribution. Changes in weight loss were evaluated by the repeated measures ANOVA (weight loss time-course: 8 points), and diet was used as a between-subject factor to evaluate the interaction time × diet and effect of diet separately. Changes in study variables within each group were evaluated by applying the Wilcoxon signed rank test to analyze non-parametric data (triglycerides, hs-IL6 and hs-TNFα variables) and the paired t-test to analyze the remaining data. To analyze the changes in variables between dietary groups, the Mann–Whitney U-test was applied to analyze non-parametric data (triglycerides, hs-IL6 and hs-TNFα variables) and the Student's t-test to analyze the remaining data. In order to evaluate the independent effect of dietary group with respect to weight loss, proinflammatory marker concentrations were also adjusted for weight loss (kg) by the residuals method prior to comparison analyses [31]. Pearson coefficients were used to evaluate the potential associations between changes in metabolic and inflammatory variables. All statistical analyses were performed using the SPSS 15.0 program (SPSS Inc., USA) for Windows XP (Microsoft, USA), and statistical significance was set up at a p-value < 0.05.

Results

Data from 3-day weighed food records showed that dietary intake before the study was not different between both experimental groups. The recorded mean value of caloric intake at baseline was 2,479 ± 1,832 (95% CI: 1,798; 2,523) kcal/d and at the endpoint was 1,462 ± 354 (95% CI: 1,337; 1,515) kcal/d ($p < 0.001$). As designed, macronutrient distribution was similar in both diets, and statistical differences were not found concerning the calories provided by carbohydrates [50.2 ± 6.0 (95% CI: 46.0; 53.4) vs. 50.7 ± 1.6 (95% CI: 50.0; 52.1) %; $p = 0.741$], lipids [33.4 ± 4.4 (95% CI: 30.0; 34.0) vs. 30.8 ± 5.6 (95% CI: 29.6; 31.4) %; $p = 0.179$] and proteins (18.9 ± 3.4 (95% CI: 16.1; 23.2) vs. 18.9 ± 1.6 (95% CI: 18.1; 19.6)%; $p = 0.991$).

In turn, the consumption of legumes by the participants at baseline was 1 ± 1 servings/week, with no statistical difference between groups ($p > 0.05$). As designed in the nutritional intervention, the subjects consumed 0–1 serving/week in the C-diet group and 4 servings/week in the L-diet group ($p < 0.05$), according to the weekly reports and to the 3-day weighed food records (week +7). Among

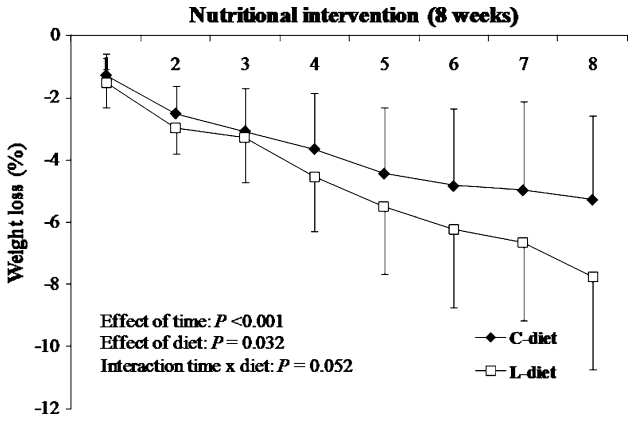

Fig. 1 Time-course in the weight loss change (%) during the 8-week nutritional intervention induced by control and legume-based diets

the non-soy legumes prescribed in the dietary regime, the consumption of lentils, chickpeas, peas and beans was the same during the 8-week intervention, as encouraged by dietician. Furthermore, fiber content was higher [26.0 ± 6.0 (95% CI: 22.4; 28.6) vs. 18.0 ± 5.0 (95% CI: 16.1; 21.7) g/d; $p = 0.005$], and saturated fatty acid was lower in the L-diet versus C-diet [5.0 ± 0.3 (95% CI: 4.8; 5.2) vs. 5.4 ± 0.9 (95% CI: 4.9; 5.9) %; $p < 0.001$]. The cholesterol intake was significantly ($p < 0.001$) lower during the intervention period [C-diet: −301.0 ± 58.1 (95% CI: −447; −267); L-diet: −323.0 ± 51.5 (95% CI: −508; −220.6) mg/d], when compared to baseline values, with no difference between dietary groups ($p > 0.05$). Interestingly, only individuals fed on the L-diet had significantly higher intake of potassium, compared to baseline values [3,775 ± 267 (95% CI: 3,275; 3,459) vs. 2,965 ± 117.2 (95% CI: 2,291; 3,137) mg/d; $p = 0.022$], and the increase in the magnesium intake was significantly higher within the L-diet group, when compared to the C-diet group [+160 ± 33 (95% CI: +103; +221) vs. +65 ± 33 (95% CI: +10; +117) mg/d; $p = 0.019$]. Volunteers reported that they kept their physical activity pattern during the trial.

The calorie restriction produced a statistically significant weight loss in both dietary group, but L-diet achieved the greater body weight loss [−7.8 ± 2.9% (95% CI: −9.4; −6.1) vs. −5.3 ± 2.7 (95% CI: −6.8; −3.8) %; $p = 0.024$], compared to the C-diet (Fig. 1). Also, the body fat (%) and waist circumference were statistically decreased in the both dietary groups by the nutritional intervention, with no statistical differences between both experimental groups (Table 1).

Regarding to clinical and metabolic variables, the L-diet was the only dietary approach inducing a significant reduction in systolic BP (SBP) measurements, total cholesterol, LDL-c and HDL-c concentrations (Table 1). Also, diastolic BP measurement was significantly reduced by both dietary groups, with no statistical differences between them (Table 1).

Concerning to changes in inflammatory marker concentrations, plasma concentrations of CRP, complement C3 and hs-TNFα significantly decreased in the L-diet group, while in the C-diet group there were significant reductions in plasma complement C3 and hs-TNFα concentrations (Table 2). In both dietary groups, plasma IL6 and homocysteine concentrations showed no statistically significant changes (Table 2). Interestingly, the participants who followed L-diet had a higher reduction (% change from baseline values) in plasma CRP and C3 concentrations, when compared to C-diet (Fig. 2). As the weight loss was significantly higher in the L-diet group, we also adjusted the change in proinflammatory markers concentrations for this variable, using the residuals method. In such analysis, the L-diet group showed a marked reduction in the CRP [−1.2 ± 3.2 (95% CI: −3.0; +0.2) vs. +0.4 ± 2.8 (95% CI: −1.2; +1.6) mg/dl, $p = 0.044$] and C3 [−0.8 ± 0.7 (95% CI: −2.0; +0.2) vs. +0.4 ± 0.4 (95% CI: 0.2; 0.5) g/l, $p = 0.044$] concentrations, when compared with C-diet group.

In order to evaluate the potential associations between metabolic and inflammatory changes independent from change in body weight, Pearson correlation coefficients were performed with the variables adjusted for weight loss. Interestingly, only in the L-diet group, the reduction (%) in plasma CRP concentrations was positively associated with the decreases (%) in systolic blood pressure (L-diet: $r = 0.78$, $p = 0.001$; C-diet: $r = -0.11$, $p = 0.721$) as well as with total cholesterol concentration (L-diet: $r = 0.81$, $p = 0.001$; C-diet: $r = -0.47$, $p = 0.750$).

Discussion

A novel finding of this study was that the L-diet resulted in a significant higher reduction in CRP and C3 concentrations, compared to baseline values and to C-diet group, accompanying a higher weight loss and significant improvement in some metabolic features.

Previous studies have also demonstrated the effect of prescribing hypocaloric diets on the reduction in some inflammatory markers, related to weight loss [7, 32–35]. However, reductions in CRP and C3 levels remained significantly higher in the L-diet group, after adjusting for weight loss. In this sense, a legume-enriched (250 g/d), low glycemic index (GI 38) diet, following during 4 weeks under weight-stable conditions, has also resulted in a significant reduction of CRP (−20.2 %) concentrations in men at risk for colorectal cancer [36]. Furthermore, feeding a hypocaloric diet enriched with legumes has resulted in a statistical reduction in oxidative stress markers, such as

Table 1 Change in anthropometrical, clinical, and metabolic variables after 8 weeks following calorie-restricted diets (C-diet vs. L-diet)

Characteristics	Control diet ($n = 15$)		Legume diet ($n = 15$)		p-value between baselines[c]	p-value between endpoints[c]	p-value between changes[c]
	Baseline	Endpoint	Baseline	Endpoint			
Men/Women (n)	9/6		8/7				
BMI (kg/m^2)	31.3 ± 4.0 (29.1; 33.8)	29.4 ± 4.1[a] (27.3; 32.1)	33.7 ± 4.7 (30.5;35.5)	31.7 ± 3.9[a] (28.1;32.7)	0.634	0.942	*0.014*
WC (cm)	100.5 ± 9.9 (95.5; 107)	92.9 ± 10.1[a] (89; 100)	100.7 ± 9.9 (95;106)	95.0 ± 9.4[a] (88;99.2)	0.641	0.649	0.942
Body fat (%)	30.4 ± 8.2 (25.7; 36.0)	28.5 ± 6.6 (25.4; 34.0)	35.2 ± 9.4 (29.5;40.3)	33.5 ± 10 (28.2;38.7)	0.556	0.553	0.864
Systolic BP (mmHg)	115 ± 9 (110;121)	111 ± 12 (105; 119)	115 ± 13 (110;122)	106 ± 10[a] (101;111)	0.635	0.193	*0.043*
Diastolic BP (mmHg)	76 ± 9 (71; 82)	72 ± 10[b] (67; 77)	76 ± 6 (72;79)	70 ± 6[a] (66;73)	0.999	0.444	0.377
Glucose (mg/dl)	92.4 ± 9.6 (88.2; 99.9)	89.7 ± 6.7 (87.5; 94.4)	93.2 ± 5.7 (91.1;99.8)	92.4 ± 5.3 (89.9;96.3)	0.617	0.298	0.727
Insulin (μIU/ml)	10.5 ± 10 (4.9; 18.3)	8.2 ± 4.3 (6.0; 11.3)	7.5 ± 3.8 (5.4;9.6)	5.9 ± 4.0 (3.7;8.2)	0.312	0.163	0.752
HOMA-IR	2.1 ± 1.7 (1.0–4.6)	1.6 ± 1.0 (1.3–2.5)	1.8 ± 0.9 (1.2–2.3)	1.6 ± 0.9 (0.8–1.9)	0.341	0.200	0.643
TC (mg/dl)	181 ± 35 (161–205)	173 ± 30 (152–192)	215 ± 27 (195–236)	182 ± 27[a] (167–197)	0.088	0.408	*0.005*
HDL-c (mg/dl)	58 ± 10 (45–56)	49 ± 12 (40–54)	49 ± 10 (44–58)	44 ± 7[b] (40–50)	0.093	0.417	0.244
LDL-c (mg/dl)	128 ± 23 (95–133)	120 ± 20 (89–122)	142.2 ± 41 (125–165)	121 ± 28[a] (104–132)	0.441	0.445	*0.010*
Triglycerides (mg/dl)	104 ± 28 (80–111)	104 ± 51 (70–123)	99 ± 38 (78–118)	97 ± 37 (74–114)	0.513	0.795	0.756

Data are mean ± standard deviation (95% confidence interval). Values in italics are statistically significant

[a] $p < 0.01$; [b] $p < 0.05$ from paired t-test or for Wilcoxon signed rank test (triglycerides), as statistical significance within group differences (baseline vs. endpoint)

[c] p-value from Student's t-test or Mann–Whitney U-test (triglycerides), as statistical significance between diet differences (C-diet vs. L-diet)

BP blood pressure; *BMI* body mass index; *HDL-c* high-density lipoprotein; *HOMA-IR* homeostatic model of assessment of insulin resistance; *LDL-c* low-density lipoprotein; *TC* total cholesterol; *WC* waist circumference

Table 2 Change in inflammatory marker concentrations after 8 weeks following calorie-restricted diets (C-diet vs. L-diet)

Characteristics	Control diet ($n = 15$)		Legume diet ($n = 15$)		*p*-value between baselines[c]	*p*-value between endpoints[c]	*p*-value between changes[c]
	Baseline	Endpoint	Baseline	Endpoint			
Men/Women (*n*)	9/6		8/7				
CRP (mg/l)	2.0 ± 1.0 (0.9–2.4)	1.9 ± 0.8 (0.6–2.3)	2.7 ± 2.4 (1.8–4.0)	1.6 ± 0.9[a] (1.2–3.0)	0.104	0.092	*0.043*
Complement C3 (g/l)	1.6 ± 0.2 (1.4–1.6)	1.4 ± 0.5[a] (1.3–1.5)	1.5 ± 0.2 (1.4–1.7)	1.1 ± 0.2[b] (0.8–1.4)	0.501	0.150	*0.039*
IL6 (pg/ml)	2.2 ± 1.2 (1.5–2.8)	2.2 ± 1.4 (1.4–2.5)	2.6 ± 1.8 (1.4–2.8)	2.3 ± 1.7 (1.1–2.7)	0.516	0.937	0.272
TNFα (pg/ml)	6.0 ± 2.8 (3.0–7.1)	3.0 ± 1.8[a] (0.9–4.1)	4.7 ± 3.2 (3.1–7.5)	2.2 ± 1.2[a] (1.2–2.5)	0.980	0.447	0.691
Homocysteine (μmol/l)	9.8 ± 3.8 (6.7–12.4)	10.0 ± 4.2 (9.4–13.6)	10.5 ± 3.6 (8.1–12.2)	10.6 ± 2.6 (8.7–12.2)	0.567	0.684	0.331

Data are mean ± standard deviation (95% confidence interval). Values in italics are statistically significant

[a] $p < 0.01$; [b] $p < 0.05$ from paired *t*-test or for Wilcoxon signed rank test (IL-6 and TNFα), as statistical significance within group differences (baseline vs. endpoint)

[c] *p*-value from Student's *t*-test or Mann–Whitney *U*-test (IL-6 and TNFα), as statistical significance between diet differences (C-diet vs. L-diet)

CRP C-reactive protein; *IL-6* interleukin-6; *TNF-α* tumor necrosis factor-alpha

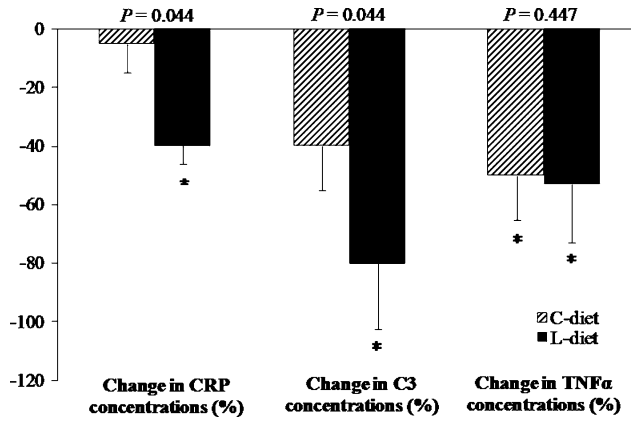

Fig. 2 Change (% from baseline values) in inflammatory markers concentrations, according to assigned calorie-restricted diet (C-diet vs. L-diet). * $p < 0.05$, for paired *t*-test: baseline versus endpoint values within each dietary group. *p*-values, for Student's *t*-test or Mann–Whitney *U*-test (non-normally variable: TNFα): differences in the change (%) between both dietary groups. CRP, C-reactive protein; C3, complement C3; TNFα, tumor necrosis factor-alpha

plasma malondialdehyde and urinary 8-isoprostane-$F_{2\alpha}$ [18]. Thus, the effect of high legume consumption on these inflammatory markers could be associated with the nutritional composition of these foods.

Legumes are foods containing important nutrients and bioactive factors, such as vegetable protein, fiber, oligosaccharides, phytochemicals, minerals (potassium and magnesium), saponins and other compounds as well as providing a low fat content [10, 37]. In this context, it has observed an inverse association between the intake of total dietary fiber (soluble and insoluble fiber) and CRP concentrations in cross-sectional and longitudinal analyses [38]. In healthy subjects, a fiber intake of about 30 g/d from a diet naturally rich in fiber or from a supplement, during 3 weeks, has reduced the levels of CRP [39], being hypothesized as a possible mechanism, a slowing of absorption of glucose and a modulation of anti-inflammatory cytokines, associated with a favorable gut flora outcome responding to fiber [38, 39]. Also, a high magnesium intake could decrease CRP values, being related to modulating microvascular function of magnesium, which stimulates endothelial growth and inhibits the synthesis of nitric oxide and some inflammatory markers [40]. In observational studies, individuals with a higher magnesium intake have presented a lower prevalence of diabetes, metabolic syndrome features and lower plasma CRP and E-selectin concentrations [40, 41]. Although some effects of bioactive compounds in legumes on pro-inflammatory markers have been reported, there is little information concerning the influence of high consumption of these foods on inflammatory and oxidative stress status [18, 36]. In this sense, current trial provides additional and relevant data about the effect of a calorie-restricted diet with high

content in legumes on proinflammatory markers, although the involved mechanisms need further investigations.

In this study, the TNFα concentrations were significantly reduced in both dietary groups, with no difference between groups. This outcome is in some contradictory with data obtained in a previous study, in which a favorable effect of a Mediterranean dietary pattern on TNFα concentrations was found independent from weight loss [7], which may be attributed to other nutrients/ food ingredients included in this dietary pattern.

Moreover, the legume-based hypocaloric diet was the only dietary approach inducing a significant reduction in SBP measurement, total cholesterol and LDL-c concentrations in the current study, which is in accordance with published reports [9, 13, 17, 18]. In this context, legume consumption has been related with high fiber and low glycemic index meals, which have been related to higher satiety, favouring dietary compliance [17, 42]. In addition, the consumption of whole grains and legumes-based diet, with a low glycemic index, resulted in higher mitochondrial oxidation, compared to diet with a high glycemic index, suggesting other potential mechanism of foods with high content of fiber and low glycemic index, such as legumes, in weight loss and control [16, 17].

Furthermore, legumes are low in sodium, being excellent sources of other minerals, including calcium, copper, iron, magnesium, phosphorus, potassium and zinc [43], which may contribute in reducing the risk of hypertension. In fact, young adults (20–40 years) who consumed variety beans consumption had a risk of elevated SBP reduced in a 47% [13]. In this sense, the L-diet achieved higher intake of magnesium and potassium than C-diet, which could explain, in part, the reduction in SBP only in this dietary group. Although a promising outcome, repeated measurements of BP within 24-h interval could provide information of interest.

In turn, the hypocholesterolemic effect of legume-based hypocaloric diet is corroborated by a previous meta-analysis of randomized controlled trials [9], which has reported consistent effect of legume-based diets in decreasing total cholesterol and LDL-c, compared to control diets. Soluble fiber and resistant starch content of legumes has been related, since it binds bile acids and cholesterol during intraluminal formation of micelles, reducing hepatocyte cholesterol content and inhibits fatty acid synthesis in liver mediated acetate, butyrate and propionate production [10, 37, 44]. The saponins, which are hydrolyzed by intestinal bacterial to diosgenin, may also have a positive effect in decreasing cholesterol absorption [37]. In addition, the legume-based hypocaloric diet resulted in a reduction in oxidized LDL-c, which was positively associated with higher content of fiber of this dietary group [18].

Furthermore, the reduction (%) in plasma CRP concentrations was significantly associated with the decreases (%) in SBP and total cholesterol values, but only in the L-diet group, independently of weight loss. Since that proinflammatory status has been considered a potential link between obesity and metabolic disorders, including insulin resistance, atherogenic lipid profile and hypertension [3, 4, 45], these results provide additional evidence that a reduction/ improvement in the proinflammatory status can be a potential mechanism to ameliorate metabolic features through a calorie-restricted diet with high content of legumes in overweight and obese subjects. In fact, the change in total cholesterol levels by consuming a hypocaloric diet enriched with legumes has been associated with a reduction in oxidative stress markers [18].

In the current study, we did not find any statistical change due to the experimental diets in glucose and insulin levels and HOMA-IR, although a slight reduction in the values of these variables induced by both dietary regimes was noted. The consumption of diets with low glycemic index, including fruits, vegetables, whole cereals and legumes has resulted in an improvement glycemic control in diabetic patients [15], which has been associated with the content of resistant starch and fiber content on these foods. One reason for this result may be the normal glucose profile presented by our population at the baseline, which contrasts with other trials involving subject with insulin resistance or diabetes features [15, 42].

Furthermore, we did not find any change in IL6 and homocysteine concentrations. To explain this case, two arguments may be taken into account: first, the sample size was not able to achieve statistical difference, since CRP concentrations were used as the main variable; the second, the achieved weight loss in this study was not enough to produce changes in the concentrations of these markers. Given the subsequent improvements in metabolic and inflammatory status, the last possibility may be more reasonable. In fact, a similar calorie-restriction intervention with a mean weight loss of -4.4 ± 2.5 kg (% weight loss: -6.2 ± 2.9) has not induced statistical changes in these proinflammatory markers, in 41 overweight and obese subjects [7].

In summary, specific consumption of legumes within a hypocaloric diet by overweight and obese subjects could result in a consistent reduction in some proinflammatory markers, such as CRP and C3 and a significant improvement in clinical and metabolic features, independent from weight loss. This finding reinforces the recommendation of a regular consumption of legumes of about 4 portions/week versus habitual consumption of 2 servings/week to achieve specific benefits in the treatment of obesity and related-metabolic disorders and inflammatory status. Indeed, this

dietary pattern provided additional healthy outcomes than that normally accompanying weight loss alone.

Acknowledgments We wish to thank our physician (Blanca E. Martínez de Morentin), our nurse (Salomé Pérez) and our technicians (Ana Lorente and Verónica Ciaurriz), for excellent technical assistance. This work was supported by *Línea Especial* about Nutrition, Obesity and Health (University of Navarra LE/97) and by Health Department of the Government of Navarra (22/2007). The Capes Foundation-Ministry of Education of Brazil also provided research grant to H. H. M. Hermsdorff (no 3756/05-0).

Conflict of interest statement The authors declared no conflict of interest.

References

1. Puchau B, Zulet MA, González de Echavarri A, Navarro-Blasco I, Martínez JA (2009) Selenium intake reduces serum C3, an early marker of metabolic syndrome manifestations, in healthy young adults. Eur J Clin Nutr 63:858–864
2. Wald DS, Wald NJ, Morris JK, Law M (2006) Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. BMJ 333:1114–1117
3. Zulet MA, Puchau B, Navarro C, Marti A, Martínez JA (2007) Inflammatory biomarkers: the link between obesity and associated pathologies. Nutr Hosp 22:511–527
4. Hermsdorff HHM, Monteiro JB (2004) Visceral, subcutaneous or intramuscular fat: where is the problem? Arq Bras Endocrinol Metabol 48:803–811
5. Rondinone CM (2006) Adipocyte-derived hormones, cytokines, and mediators. Endocrine 29:81–90
6. Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444:875–880
7. Hermsdorff HHM, Zulet MA, Abete I, Martínez JA (2009) Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects. Endocrine 36:445–451
8. Hermsdorff HHM, Zulet MA, Bressan J, Martínez-González MA, Martínez JA (2009) Impact of caloric restriction and Mediterranean food-based diets on inflammation accompanying obesity and metabolic syndrome features. Obe Metab 5:69–77
9. Bazzano LA, Thompson AM, Tees MT, Nguyen CH, Winham DM (2009) Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis. doi:10.1016/j.numecd.2009.08.012
10. Trinidad TP, Mallillin AC, Loyola AS, Sagum RS, Encabo RR (2010) The potential health benefits of legumes as a good source of dietary fibre. Br J Nutr 103:569–574
11. Zulet MA, Martínez JA (2001) Mediterranean diet: legumes and cholesterolemia. Rev Chil Nutr 28:312–320
12. Mitchell DC, Lawrence FR, Hartman TJ, Curran JM (2009) Consumption of dry beans, peas, and lentils could improve diet quality in the US population. J Am Diet Assoc 109:909–913
13. Papanikolaou Y, Fulgoni VL 3rd (2008) Bean consumption is associated with greater nutrient intake, reduced systolic blood pressure, lower body weight, and a smaller waist circumference in adults: results from the national health and nutrition examination survey 1999–2002. J Am Coll Nutr 27:569–576
14. Pittaway JK, Robertson IK, Ball MJ (2008) Chickpeas may influence fatty acid and fiber intake in an ad libitum diet, leading to small improvements in serum lipid profile and glycemic control. J Am Diet Assoc 108:1009–1013
15. Venn BJ, Mann JI (2004) Cereal grains, legumes and diabetes. Eur J Clin Nutr 58:1443–1461
16. Abete I, Parra D, Martinez JA (2009) Legume-, fish-, or high-protein-based hypocaloric diets: effects on weight loss and mitochondrial oxidation in obese men. J Med Food 12:100–108
17. Abete I, Parra D, Martinez JA (2008) Energy-restricted diets based on a distinct food selection affecting the glycemic index induce different weight loss and oxidative response. Clin Nutr 27:545–551
18. Crujeiras AB, Parra D, Abete I, Martínez JA (2007) A hypocaloric diet enriched in legumes specifically mitigates lipid peroxidation in obese subjects. Free Radic Res 41:498–506
19. WHO (2000) World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep 894:1–253
20. Aranceta J (2001) Spanish food patterns. Public Health Nutr 4:1399–1402
21. Dapcich V, Salvador-Castell G, Ribas-Barba L, Pérez-Rodrigo C, Aranceta J, Serra-Majem L (2004) Guía de la alimentación saludable. SENC. Sociedad Española de Nutrición Comunitaria, Madrid. [Guide for a health diet]
22. Abete I, Parra D, De Morentin BM, Alfredo Martinez J (2009) Effects of two energy-restricted diets differing in the carbohydrate/protein ratio on weight loss and oxidative changes of obese men. Int J Food Sci Nutr 60(Suppl 3):1–13
23. Martínez-González MA, López-Fontana C, Varo JJ, Sánchez-Villegas A, Martínez JA (2005) Validation of the Spanish version of the physical activity questionnaire used in the nurses' health study and the health professionals' follow-up study. Public Health Nutr 8:920–927
24. Pérez S, Parra MD, Martínez de Morentin B, Rodríguez MC, Martínez JA (2005) Evaluación de la variabilidad intraindividual de la medida de composición corporal mediante bioimpedancia en voluntarias sanas y su relación con el índice de masa corporal y el pliegue tricipital. Enferm Clin 15:307–314
25. Labayen I, Diez N, Parra MD, González A, Martínez JA (2004) Time-course changes in macronutrient metabolism induced by a nutritionally balanced low-calorie diet in obese women. Int J Food Sci Nutr 55:27–35
26. Gibson R (2005) Principles of nutritional assessment. Oxford University Press, New York
27. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
29. Sharman MJ, Volek JS (2004) Weight loss leads to reductions in inflammatory biomarkers after a very-low-carbohydrate diet and a low-fat diet in overweight men. Clin Sci (Lond) 107:365–369
30. Dean AG, Sullivan KM, Soe MM (2008) OpenEpi source epidemiology statistics for public health, version 2.3. www.OpenEpi.com, update 2008/20/05
31. Willett WC (1998) Nutritional epidemiology. Oxford University Press, New York
32. Abete I, Parra D, Crujeiras AB, Goyenechea E, Martinez JA (2008) Specific insulin sensitivity and leptin responses to a nutritional treatment of obesity via a combination of energy restriction and fatty fish intake. J Hum Nutr Diet 21:591–600
33. Crujeiras AB, Parra D, Milagro FI et al (2008) Differential expression of oxidative stress and inflammation related genes in

peripheral blood mononuclear cells in response to a low-calorie diet: a nutrigenomics study. Omics 12:251–261
34. Goyenechea E, Parra D, Crujeiras AB, Abete I, Martinez JA (2009) A nutrigenomic inflammation-related PBMC-based approach to predict the weight-loss regain in obese subjects. Ann Nutr Metab 54:43–51
35. Shai I, Schwarzfuchs D, Henkin Y et al (2008) Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. N Engl J Med 359:229–241
36. Hartman TJ, Albert PS, Zhang Z et al (2010) Consumption of a legume-enriched, low-glycemic index diet is associated with biomarkers of insulin resistance and inflammation among men at risk for colorectal cancer. J Nutr 140:60–67
37. Rochfort S, Panozzo J (2007) Phytochemicals for health, the role of pulses. J Agric Food Chem 55:7981–7994
38. Ma Y, Griffith JA, Chasan-Taber L et al (2006) Association between dietary fiber and serum C-reactive protein. Am J Clin Nutr 83:760–766
39. King DE, Egan BM, Woolson RF, Mainous AG 3rd, Al-Solaiman Y, Jesri A (2007) Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. Arch Intern Med 167:502–506
40. Bo S, Durazzo M, Guidi S et al (2006) Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. Am J Clin Nutr 84:1062–1069
41. Song Y, Li TY, van Dam RM, Manson JE, Hu FB (2007) Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women. Am J Clin Nutr 85:1068–1074
42. Rizkalla SW, Bellisle F, Slama G (2002) Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. Br J Nutr 88(Suppl 3):S255–S262
43. Bazzano LA, He J, Ogden LG et al (2001) Legume consumption and risk of coronary heart disease in US men and women: NHANES I Epidemiologic Follow-up Study. Arch Intern Med 161:2573–2578
44. Mallillin AC, Trinidad TP, Raterta R, Dagbay K, Loyola AS (2008) Dietary fibre and fermentability characteristics of root crops and legumes. Br J Nutr 100:485–488
45. Martínez JA, Margareto J, Marti A, Milagro FI, Larrarte E, Moreno Aliaga MJ (2001) Resistin overexpression is induced by a beta3 adrenergic agonist in diet-related overweightness. J Physiol Biochem 57:287–288