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A hypocaloric diet enriched in legumes specifically mitigates lipid peroxidation in obese subjects

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Abstract

Legume intake could specifically protect against lipid peroxidation in addition to the effects associated to weight loss when included in hypocaloric diets. Thus, 30 obese subjects (age: 36 ± 8 years and BMI: 32.0 ± 5.3 kg/m^2) were nutritionally treated by a 8-week energy restriction (− 30% energy expenditure) with a legume enriched diet (4 days/week servings, $n = 15$) or without legumes (control diet (CD), $n = 15$). Body weight, circulating cholesterol, oxidized LDL (ox-LDL), malondialdehyde (MDA) and urinary 8-isoprostane $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) were measured at baseline and at endpoint. After the nutritional intervention, all obese subjects lost weight, specially those individuals who followed the legumes-enriched diet as compared to the CD (− 7.7 ± 3 vs. − 5.3± 2.7%; $p = 0.023$), which was accompanied by marked decreases in total cholesterol levels ($p < 0.001$) and statistically significant diet-related reductions on plasma ox-LDL, plasma MDA and urinary 8-iso-$PGF_{2\alpha}$ output.

Therefore, a balanced diet with moderate caloric restriction including 4 day/week legume servings empowered the oxidative stress improvement related to weight loss through a reduction in lipid peroxidation as compared to a control hypocaloric diet.

Keywords: *Obesity, oxidative stress, caloric restriction, lipid peroxidation, antioxidant status, dietary TAC*

Abbreviations: *TAC, total antioxidant capacity; MDA, malondialdehyde; ox-LDL, oxidized LDL; 8-iso-$PGF_{2\alpha}$, 8-isoprostane $F_{2\alpha}$; AOP, total plasma antioxidant power*

Introduction

Oxidative stress is produced by an imbalance between the levels of free radicals and the antioxidant mechanisms, which has been repeatedly recognized as a cause of cell degeneration [1–3]. In this situation, reactive oxygen species promote lipid peroxidation, resulting in chain reactions that generate a sustained increase in reactive compounds able to oxidize cellular macromolecules such as lipids, proteins and DNA [4].

There is an increasing interest about the role of nutrition on this process. Thus, food intake has been related to oxidative stress induction [5–8], while energy restriction in the obese and fasting conditions in the lean subjects can decrease oxidative stress mediators [9]. Specifically, it has been described a relationship between excess in body fat and inflammation, in which oxidative stress could participate [10,11]. In this context, obesity is a complex multifactorial disease in which environmental and genetic factors are involved [12,13]. Among those, inadequate eating habits are important contributors to obesity development as well as being linked to a number of metabolic impairments associated to oxidative stress [10,11,14–16].

On the other hand, cells contain a variety of chain-breaking antioxidants, such as uric acid, bilirubin, ascorbic acid, vitamin E or protein thiols, that provide

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protection against oxidative stress [1,2]. Taking into account diet as the major antioxidants source and weight loss as a powerful oxidative stress lowering factor [16,17], calorie-restricted diets including antioxidants-enriched foods could be a doubly effective strategy to inhibit oxidative injuries [18]. Indeed, we previously described that the inclusion of fruit-derived antioxidant capacity within a hypocaloric diet is a useful nutritional design to improve the oxidative balance related to obesity [19].

Nowadays, legumes are nutritionally recognized for their high protein content as well as for their soluble-fiber and other bioactive compounds [20]. While the effects of dietary fiber on serum lipoproteins have received much attention, it is very likely that other bio-functional components could be involved in the cardio-protective effects attributed to legumes [21]. In fact, beneficial effects on blood lipids of dietary soy protein are well documented and several studies have suggested that soy intake aids against oxidative stress by means of isoflavons, saponins and other compounds with antioxidant capacity [22,23]. However, in European countries the consumption of soybean is low, whereas lentils, chickpeas, peas and faba beans consumption is more common [24].

Based on these facts, the current nutritional intervention study was designed to determine whether a hypocaloric diet enriched in non-soybean legumes could decrease oxidative stress in obese subjects in addition to the recognized effects associated to weight loss, with special emphasis to lipid peroxidation.

Subjects and methods

Subjects

Thirty obese subjects (17 men and 13 women) with mean age of 36 ± 8 years and mean body mass index (BMI) of 32.0 ± 5.3 kg/m^2, were recruited through local advertisements. All participants were in apparent good health as determined by medical history, physical examination, and routine laboratory tests. They reported to have not used supplemental vitamin or minerals nor regular prescription of medications during the previous 3 months, and body weight was ± 3 kg during this time. A written informed consent to participate in the trial was obtained before the start of the study. This consent, as well as the study protocol, was previously approved by the Ethical Committee of the University Clinic of Navarra.

Study design

Volunteers were randomly divided into two groups, which were assigned to two different energy-restricted dietary treatments for 8 weeks by receiving a hypocaloric diet with 4 days/week non-soybean legumes: lentils, chickpeas, peas and faba beans servings (LD; $n = 15$) or without legumes consumption: control diet (CD; $n = 15$) during this period. The hypocaloric diets were designed to produce a similar caloric restriction of −30% E with respect to the subject's energy expenditure, which was individually measured by indirect calorimetry at baseline (Deltratac, Datex Ohmeda, Finland), using conventional protocols [25] and adjusted for personal physical activity level [26]. The macronutrient content was designed to supply 20% energy as proteins, 50% energy as carbohydrates and 30% energy as lipids in both diets. The energy and nutrient intake compliance was assessed by 3-day weighted food records (2 week days and 1 weekend day), calculating the nutrient composition through the Medisystem Nutritional Database (Sanocare Human Systems L.S, Spain). Total antioxidant capacity (TAC) values of the prescribed diet was calculated using the total antioxidant concentration of each food as previously reported, which was expressed as TAC in μmol/100 g of fresh food and reported as μmol/kcal [27,28].

Before and after the nutritional intervention, body weight was measured and fasting blood and 8-hour urine samples were obtained. The EDTA-plasma and serum samples were separated from whole blood by centrifugation, which were frozen immediately at −80°C until assay as well as the urine samples.

Total cholesterol and oxidative stress markers analyses

Total cholesterol, HDL-c, triglycerides, glucose, uric acid and total bilirubin were measured by specific colorimetric assays (Horiba ABX Diagnostics, Montpellier, France) using an automatized system (COBAS MIRA, Roche, Switzerland). The reported plasma LDL-cholesterol data were calculated by the Friedewald equation [29]. Serum malondialdehyde (MDA) and total plasma antioxidant power (AOP) were evaluated by means of commercially available colorimetric assay kits (OXIS International, Portland, OR, USA). Insulin concentrations were assessed by using a radioimmunoassay method (DPC, Los Angeles, CA, USA).

Plasma level of circulating oxidized LDL (ox-LDL) and urinary excretion of 8-isoprostane $F_{2\alpha}$ concentration (8-iso-$PGF_{2\alpha}$) were measured by enzyme-linked immunosorbent assay kits (Mercodia, AB, Uppsala, Sweden and OXIS International, Portland, OR, USA, respectively). The colorimetric reactions were read at corresponding wavelengths (Multiskan Spectrum, Thermo Electron Corporation, Finland). The 8-iso-$PGF_{2\alpha}$ values were expressed per milligram creatinine [30]. Creatinine concentration was calorimetrically measured (Horiba ABX Diagnostics) by an automatized system (COBAS MIRA).

Statistical analysis

The sample size in the dietary groups ($n = 15$) was set up according to Mera et al. [31], taking into account published values of the standard deviation for MDA [32]. The normal distribution was explored through the Kolmogorov-Smirnov and Shapiro Wilk tests. The student *t*-test was applied to detect differences before and after weight loss as well as differences between both hypocaloric diets response. Percentage changes in variables were calculated based upon the comparison between endpoint and baseline measurements for both control and legume-based hypocaloric diets. The differences between both diets were tested through the Mann Whitney-*U*-test. Statistical analysis of ox-LDL and 8-iso-$PGF_{2\alpha}$ data were carried out with the log transformation on the raw data [30]. The Pearson coefficient was used to evaluate the potential association between weight loss and changes in total cholesterol and oxidative stress markers, while the Spearman test was used to explore associations between biochemical variables and dietary TAC and fiber content. Multivariate linear regression models were fitted to explain differences observed in oxidative stress biomarkers, considering diet or TAC adjusted for energy intake [33], weight loss and total cholesterol changes as independent variables. Regression models were not adjusted for potential confounding factors (gender, BMI and age), since no statistical differences between dietary groups were observed ($p > 0.10$) in accordance with the design. Untransformed data are reported as the mean ± standard deviation and confidence intervals (95%CI) are used to show statistical trends in changes and to describe linear regression coefficient (*B*) values.

Statistical analysis was performed by SPSS 12.0 software (SPSS Inc, USA) for Windows XP (Microsoft, USA) and *p*-value <0.05 was considered as statistically significant.

Results

Dietary assessment

The mean caloric intake at baseline was 2479 ± 1832 kcal/d and the registered values at the endpoint were 1462 ± 354 kcal/d ($p = 0.001$). As designed, macronutrient distribution was similar in both diets, and no statistical differences were found concerning the energy provided by carbohydrates (50.2 ± 6.0 vs. 50.7 ± 1.6%; $p = 0.741$), lipids (33.4 ± 4.4 vs. 30.8 ± 5.6%; $p = 0.179$) and proteins (18.9 ± 3.4 vs. 18.9 ± 1.6%; $p = 0.991$) between both dietary experimental groups. With respect to cholesterol content, no statistical differences ($p = 0.641$) were observed between the CD (93 ± 80 mg/d) and the LD (81 ± 57 mg/d) experimental interventions. The TAC of the prescribed non-soybean legumes diet was 2.8% higher than CD ($p < 0.001$) and, as expected, fibre content in the LD was statistically higher than in the CD (25 ± 6 vs. 18 ± 5 g/d; $p = 0.005$).

Changes in body weight and oxidative stress biomarkers

After the nutritional intervention, body weight statistically decreased in relation to baseline in both dietary groups (Table I), showing higher weight loss in those volunteers that followed the LD as compared to CD (−7.7 ± 3 vs. −5.3 ± 2.7%; $p = 0.023$). In fact, the decrease in body weight correlated with the dietary fiber content ($r = 0.46$; $p = 0.014$).

The intervention resulted in reductions in total plasma cholesterol concentrations (Table I), being significantly different between both diets (−14.4 ± 10.6 vs. −3.9 ± 10.7%; $p < 0.001$). The decrease in total cholesterol was directly correlated with body weight loss ($r = 0.50$; $p = 0.006$) and with the increase in fiber intake ($r = 0.44$; $p = 0.022$). Glucose and insulin values at baseline and at the

Table I. Changes in anthropometric variables, plasma total cholesterol, glucose and insulin, and oxidative stress markers in response to the calorie-restricted intervention.

	Intervention-related changes ($n = 30$)		
	Baseline	Endpoint	*p*-value
Weight (kg)	93.4 ± 14.5	87.3 ± 13.7	<0.001
BMI (kg/m^2)	32.5 ± 4.5	30.4 ± 4.3	<0.001
Total cholesterol (mg/dl)	199 ± 39	178 ± 29	<0.001
Glucose (mg/dl)	95 ± 8	92 ± 6	0.847
Insulin (μU/ml)	9.42 ± 7.90	7.36 ± 4.1	0.290
Oxidized LDL (U/l)	115 ± 67	110 ± 58	0.569
MDA (μM)	2.15 ± 0.72	1.92 ± 0.79	0.004
8-Isoprostane $F_{2\alpha}$ (ng/mg creatinine)	0.68 ± 0.41	0.44 ± 0.39	0.005
Total bilirrubin (mg/dl)	0.78 ± 0.35	0.86 ± 0.47	0.246
Uric acid (mg/dl)	5.64 ± 1.07	4.98 ± 0.91	<0.001
AOP (mM)	0.51 ± 0.11	0.50 ± 0.09	0.398

endpoint were not statistically different (Table I) after following both diets.

Taken together both dietary intervention groups (Table I), MDA and 8-iso-$PGF_{2\alpha}$ concentrations significantly decreased, and also the circulating levels of ox-LDL, without reaching statistical significance. However, the ox-LDL reduction was related to body weight loss ($r = 0.42$; $p = 0.020$).

Considering both groups together, a trend was observed in the relationship between fiber intake and ox-LDL levels ($r = -0.38$; $p = 0.121$). The correlation was statistically significant for LD ($r = -0.62$; $p = 0.013$), but was not statistically relevant for CD ($r = 0.21$; $p = 0.482$).

Changes in these oxidative stress biomarkers differed between both dietary groups. Thus, the LD tended to decrease ($p = 0.091$; 95%CI: -6; 51) plasma ox-LDL levels (Table II) with respect to baseline and considering the two groups, this change was statistically higher ($p = 0.039$) after following the LD than after CD. Furthermore, the LD induced a statistically significant reduction in MDA levels ($p = 0.008$) and 8-iso-$PGF_{2\alpha}$ ($p = 0.035$) as compared with baseline (Table II), while the decreases were no statistically significant in the CD. However, when comparing both groups, no statistical differences were observed.

Reductions in total cholesterol concentrations that occurred after the intervention were positively associated with decreases in MDA ($r = 0.50$; $p = 0.006$) and with ox-LDL ($r = 0.58$; $p = 0.001$). These statistical relationships were only maintained in the legume-enriched intervention group (Figure 1A,B) when intervention groups were separately analyzed.

The urinary 8-iso-$PGF_{2\alpha}$ decrease was diet-related associated ($r = 0.54$; $p = 0.037$) with TAC of diet, while no relationships were found ($p > 0.05$) between the 8-iso-$PGF_{2\alpha}$ and the other biomarkers involved in free radical production.

With respect to antioxidant status, total plasma antioxidant power (AOP) correlated with uric acid ($r = 0.45$; $p = 0.013$) and marginally tended to be associated with total bilirubin ($r = 0.36$; $p = 0.055$). No statistical changes were found between both intervention groups with respect to this parameter after dietary intervention ($p = 0.404$), as well as for bilirubin. Uric acid significantly decreased after following both energy restricted-diets (Table I), but the diet-related effect had no apparent clinical relevance since all values were found within the healthy normal range.

Different models were examined by using multiple regression analysis in order to explore specific dietary effects on oxidative stress markers (Table III). Thus, the 26% of the variability in total cholesterol changes were conjointly explained by the body weight loss and the type of diet.

The regression model predicting changes in ox-LDL concentrations (Table III) showed a 25% of decrease mainly depending on total cholesterol changes and also in relation with dietary TAC and body weight loss. The 37% MDA decrease was explained by changes in total cholesterol concentrations when the regression model was adjusted for circulating MDA baseline (Table III). Interestingly, linear regression analyses to explain the differences in 8-iso-$PGF_{2\alpha}$ concentrations showed the participation of the estimated-dietary TAC in decreasing the levels of this oxidative biomarker (Table III).

Discussion

A number of epidemiological studies have shown that Asian people consuming soy in their staple diet have much lower mortality and morbidity from cardiovascular disease than their counterparts in Western countries [34]. These beneficial effects have been partially attributed to the antioxidant potential of soy, possibly due to the occurrence of isoflavones, specific amino acids, the fiber composition and other bioactive compounds [20,35,36]. In the European countries, the more frequently consumed pulses are non-soybean legumes, such as lentils, chickpeas, peas and faba beans [24], which present a comparable nutritional composition to soy, but whose potential antioxidant benefits have been under-studied. Based on this, modification of lipid peroxidation markers related to non-soybean legumes consumption were studied in

Table II. Changes in body weight, plasma total cholesterol and oxidative markers in response to calorie-restricted intervention by hypocaloric diets with and without legumes content.

	Control diet ($n = 15$)			Legume-based diet ($n = 15$)		
	Baseline	Endpoint	*p*-value	Baseline	Endpoint	*p*-value
Body weight (kg)	92.5 ± 13.2	87.7 ± 13.0	<0.001	94.4 ± 16.1	87.0 ± 14.7	<0.001
Total cholesterol (mg/dl)	181 ± 35	173 ± 32	0.140	215 ± 37	182 ± 27	<0.001
Oxidized LDL (U/l)	109 ± 56	121 ± 67	0.260	121 ± 78	99 ± 46	0.091
MDA (μM)	1.83 ± 0.76	1.70 ± 0.89	0.148	2.46 ± 0.54	2.14 ± 0.64	0.015
8-Isoprostane $F_{2\alpha}$ (ng/mg creatinine)	0.58 ± 0.31	0.41 ± 0.34	0.073	0.76 ± 0.48	0.47 ± 0.44	0.035
AOP (mM)	0.53 ± 0.10	0.50 ± 0.09	0.226	0.50 ± 0.11	0.50 ± 0.08	0.993

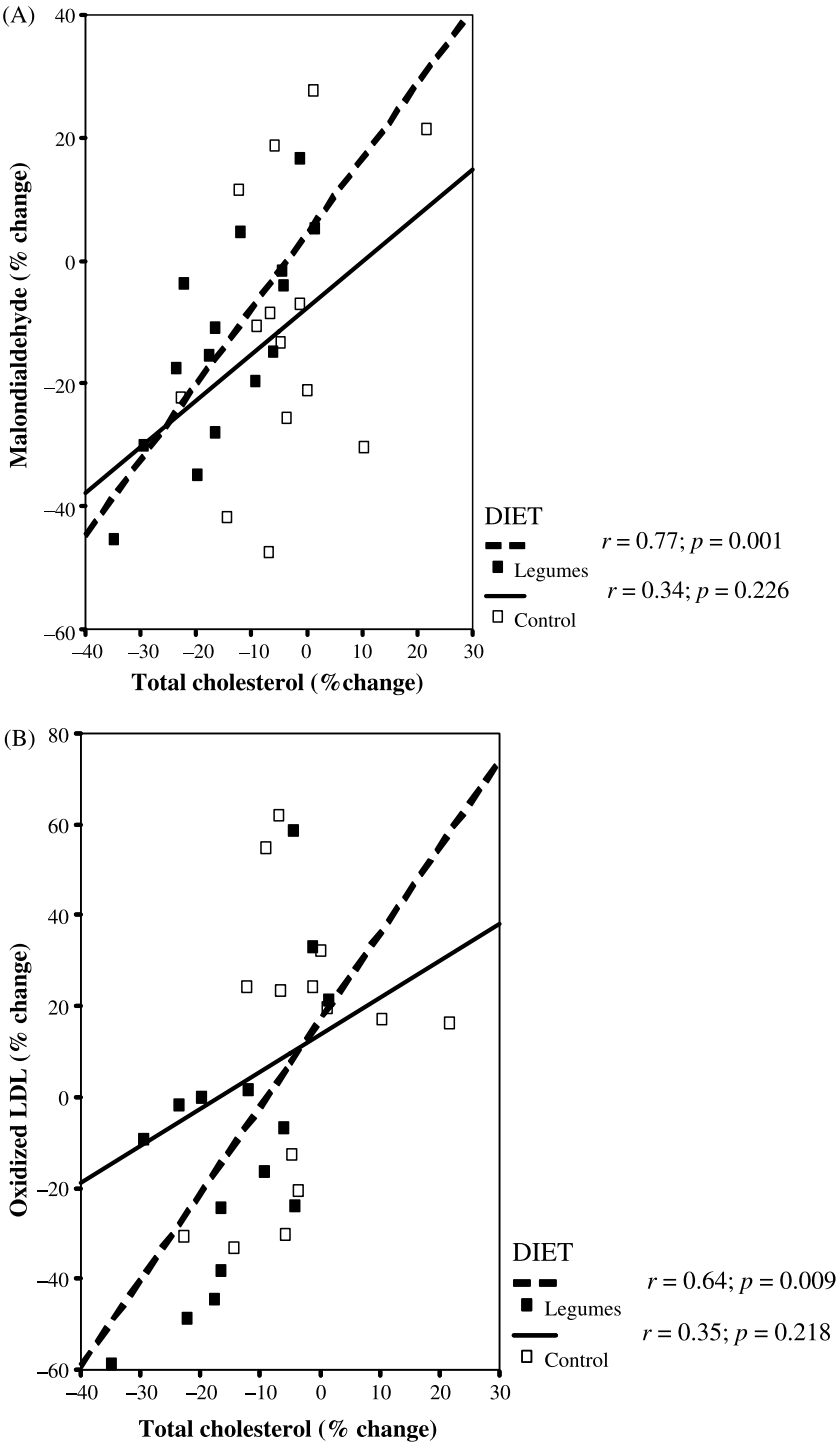

Figure 1. Association between total cholesterol and MDA (A) and ox-LDL (B). Data shows that reductions in total cholesterol concentrations were associated with decreases in MDA ($p = 0.001$) and ox-LDL ($p = 0.009$) mainly in LD group.

the current work when included as components of a hypocaloric diet designed to lose weight.

Legumes are an excellent source of dietary fiber since one serving provides 2–4 g of a mixture of soluble and insoluble fiber [21]. In this context, it has been suggested that fiber acts as a physiological obstacle to energy intake, by displacing available calories and nutrients from the diet, by increasing satiety and by decreasing the absorption efficiency [37]. In fact, populations reporting high fiber intake have low obesity rates [38]. In agreement with this observation, our results showed that a legumes diet induced higher body weight loss than CD probably due among other compounds to legumes–fiber content.

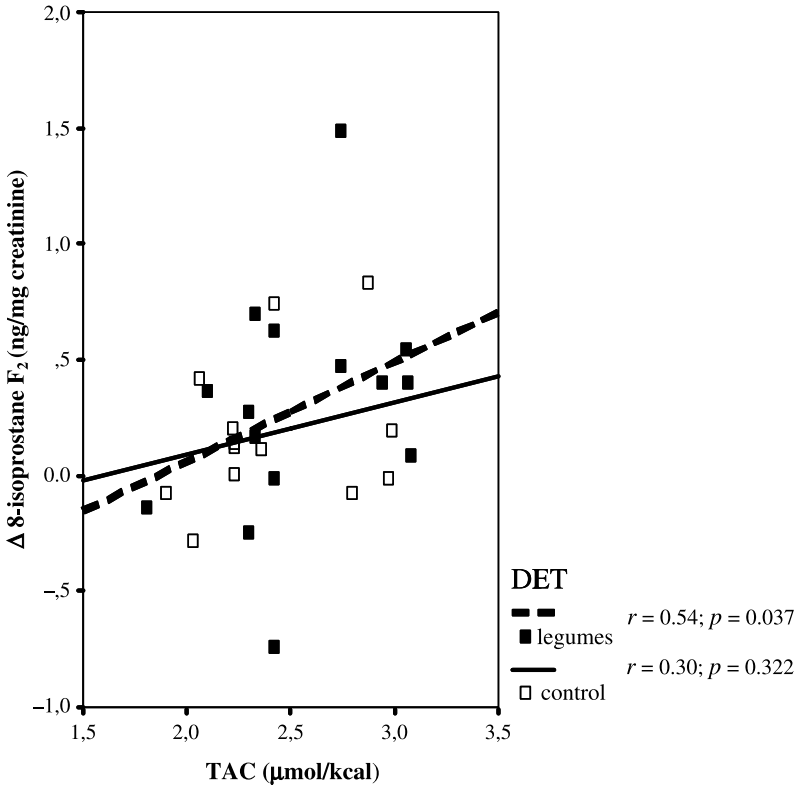

Figure 2. Association between 8-isoprostane $F_{2\alpha}$ and TAC of diet. Data evidence that 8-isoprostane $F_{2\alpha}$ decrease was associated with increase in TAC mainly in LD group ($p = 0.037$).

Table III. Independent effects of dietary intervention markers* on changes in total cholesterol (%), ox-LDL (%), MDA (μM) and 8-isoprostane $F_{2\alpha}$ (ng/mg creatinine) Data show that weight loss and the type of diet explained the variability in total cholesterol while decreases in ox-LDL and MDA mainly depend on total cholesterol changes. Variability of 8-isoprostane $F_{2\alpha}$ (global oxidative stress) adjusted for baseline values was mainly explained by antioxidant capacity of diet.

	B (95% CI)	*P*-value
Model for changes in cholesterol		
Diet	−7 (−15;1)	0.095
Body weight loss	1.4 (0.1;2.8)	0.040
Corrected R^2 = 0.28		*0.006*
Model for changes in oxidized LDL		
Dietary TAC	−16 (−46;14)	0.274
Body weight loss	0.7 (−1.7;6.3)	0.738
Change cholesterol	1.5 (0.4;2.6)	0.010
Corrected R^2 = 0.25		*0.017*
Model for ΔMDA		
Change cholesterol	0.02 (0.01;0.04)	<0.001
MDA baseline	0.03 (−0.16;0.21)	0.786
Corrected R^2 = 0.37		*0.001*
Model for Δ8-isoprostane $F_{2\alpha}$		
8-isoprostane $F_{2\alpha}$ baseline	−0.6 (−0.9; − 0.2)	0.001
Dietary TAC	−0.35 (−0.71;0.01)	0.054
Corrected R^2 = 0.36		*0.001*

* Type of diet (0: control, 1: legumes) and TAC of diet (μmol/kcal), body weight loss (%), changes in total cholesterol (%), MDA (μM) and 8-isoprostane $F_{2\alpha}$ (ng/mg creatinine) at baseline as independent variables.

Several studies have attributed antioxidants properties to fiber-enriched diets, since this compound enhances the capacity to detoxify free radicals [39]. However, although the non-soybean legumes diet improved lipid peroxidation as compared to the CD, this beneficial effect was not reflected in the assessed plasma antioxidant capacity. A possible explanation is that the method applied to detect plasma antioxidant capacity measured water-soluble antioxidants, such as uric acid and bilirubin [40,41], which apparently changed, but without clinical relevance. Indeed, the values were within the normal laboratory range. Therefore, the antioxidant mechanisms of legumes could be probably mediated by amphipathic substances, such as isoflavones or through additive and synergistic effects of fiber and others substances with the antioxidant capacity provided by legumes. In agreement with this assumption, we found an association between fiber intake and reduction in plasma total cholesterol and ox-LDL.

Several decades ago, different authors suggested that non-soybean pulses contribute to decrease serum cholesterol level, improving cardiovascular risk [21,42]. Accordingly, we observed this lowering effect in obese volunteers that followed the non-soybean legumes diet. As previously mentioned, the experimental intervention was rich in fiber, which is known to influence fat metabolism by reducing elevated LDL

cholesterol without changing HDL fraction [43]. This hypocholesterolemic effect could be related with soluble fiber, since it binds bile acids and cholesterol during intraluminal formation of micelles, reducing hepatocyte cholesterol content [44]. Other proposed mechanism is the inhibition of fatty acid synthesis in liver mediated by fiber fermentation products, such as acetate, butyrate and propionate [45].

With respect to oxidative stress, circulating MDA is widely used as lipid peroxidation marker [46–49], while ox-LDL, a product of the oxidative conversion of LDL cholesterol, indicates lipid susceptibility to free radical attack [50] being, considered to be a key event in the initiation and acceleration of the development of the early atherosclerotic lesion [51,52]. In the current study, legume-based dietary intake induced a decrease in ox-LDL levels with respect to CD, in agreement with previous studies that reported protection against ox-LDL by soy intake [22]. A decrease in MDA, one of the end products of this oxidative process [53], also evidenced the improvement in the oxidative stress mediated by the legumes diet. Thus, the cardioprotective effects of the non-soybean legumes could be explained, at least to some extent, by decreasing lipid peroxidation.

The observed dietary effects on ox-LDL and MDA were associated with changes in total cholesterol. Therefore, the decrease in lipid peroxidation biomarkers was directly influenced by the hypocholesterolemic diet-related effect. Moreover, we found an association between changes in ox-LDL levels and weight loss. Consequently, the major protective mechanism by which non-soybean legumes diet improved oxidative stress was apparently mediated by weight loss and decrease in circulating cholesterol.

To further assess the effect of the dietary intervention on oxidative stress, urinary excretion of 8-isoprostane $F_{2\alpha}$ was measured since it is a widely-accepted indicator of overall lipid peroxidation [46,54,55]. This marker decreased after the intervention, specifically in response to the enriched legumes diet. These changes were associated with the dietary TAC which reinforcing the initial hypothesis. So, the effect of the diet on 8-iso-$PGF_{2\alpha}$ was mainly associated to the dietary antioxidant content rather than body weight loss.

In conclusion, a nutritional intervention based upon a hypocaloric balanced diet enriched in non-soybean legumes showed *in vivo* effects by mitigating oxidative stress through the hypocholesterolemic effect of fiber and the participation of other antioxidant components. This outcome appeared in addition to the recognized beneficial effect associated to weight loss. Therefore, the inclusion of non-soybean legumes 4 days/week in the nutritional treatment of obesity by moderate calorie-restricted diets is able to specifically ameliorate oxidative stress associated to lipids.

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