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ORIGINAL ARTICLE

Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects

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

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Abstract Personalized nutritional strategies to treat obesity may specifically influence inflammatory markers, in addition to reduce body weight. In the present study, we evaluated the effect of a hypocaloric diet based on a Mediterranean dietary pattern (MDP) on nutritional status as well as on plasma concentrations of retinol binding protein-4 (RBP4) and other proinflammatory markers. Fourty-one subjects (24F/17M; age: 37 ± 7 years; BMI: 32.2 ± 3.9 kg/m^2) were assigned to follow a MDP within a caloric-restricted diet over an 8-week period. Anthropometrical, clinical, and biochemical variables were measured at baseline and endpoint after the nutritional program. Dietary intervention resulted in a mean weight loss of −4.4 ± 2.5 kg ($P < 0.001$) and marked reductions ($P < 0.05$) in plasma concentrations of RBP4, leptin, C-reactive protein, complement C3, and tumor necrosis factor-alpha (TNFα). Individuals with a higher adherence to the MDP during the nutritional intervention presented differentially higher reductions ($P < 0.05$) in plasma RBP4, IL6, and TNFα. In addition, the increase in the Mediterranean diet score from baseline was a significant and independent predictor factor for the decrease in plasma RBP4 concentration ($P < 0.05$). In conclusion, our findings suggest that following a hypocaloric diet accompanying a high adherence to a MDP resulted in specific reductions on proinflammatory markers, in addition to a significant improvement in some metabolic syndrome features induced by weight loss, which could be a good combined strategy to treat obesity as well as related metabolic and inflammatory disorders.

Keywords Caloric restriction · Mediterranean diet · Weight loss · Inflammation · Retinol binding protein-4 · Complement C3

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

Introduction

Excessive fat accumulation in the adipose tissue is associated with increased concentrations of several proinflammatory and proatherogenic markers, such as retinol binding protein-4 (RBP4), C-reactive protein (CRP), complement C3, interleukin-6 (IL6), and tumor necrosis factor-alpha (TNFα) and homocysteine [1–4]. In turn, these biomarkers have been implicated in the etiology and development of insulin resistance, type II diabetes, and atherosclerosis [3, 5, 6]. In this context, in addition to the loss of body fat and the subsequent improvement of metabolic syndrome features (hypertension, insulin resistance, and dyslipidemias), some nutritionally oriented intervention trials have as a target the specific improvement of obesity-related proinflammatory status [7, 8]. Information from nutritional studies has indicated that caloric restriction can reduce circulating inflammatory markers [9–13]. In addition, the traditional Mediterranean diet, rich in olive oil, nuts, vegetables, legumes, fruits and fish, low in meat and processed foods, and moderate in dairy products, may be a good source of mono- and polyunsaturated fatty acids, dietary fiber, antioxidants, and other phytochemicals with specific healthy benefits [14]. All these food components have been reported as affecting inflammatory markers [15–18]. Furthermore, some studies have suggested that the healthy effect of following a Mediterranean dietary pattern (MDP) could be explained in part by modulating some inflammatory markers [19–21]. The effects of this dietary style on C3 and RBP4 concentrations have not been reported. Overall, the aim of this study was to evaluate the effects of following a hypocaloric diet with an high adherence to MDP on the nutritional status and on plasma concentrations of RBP4 and selected proinflammatory markers (CRP, complement C3, IL6, TNFα, and homocysteine) and metabolic syndrome manifestation in obese subjects.

Results

Diet composition and adherence to MDP during nutritional intervention

As a consequence of the nutritional intervention period, energy intake was significantly lower ($P < 0.001$) compared to baseline (Table 1). Regarding macronutrients intake, carbohydrate proportion was significantly higher, while protein and lipid distribution was lower in comparison with the habitual diet of the volunteers, as assessed at the beginning of the study (Table 1). The consumption of fiber was significantly higher, while saturated fatty acid (SFA) and cholesterol intakes were significantly lower during the intervention period (Table 1). Also, following the intervention diet resulted in a significant increase in the intake of vegetables, fruits and in the monounsaturated fatty acid (MUFA)/SFA ratio as well as a decrease in the consumption of meats, leading to statistically significant increase in the Mediterranean diet score (MDS) during 8 weeks of implementation (Table 1).

Table 1 Change of daily intake and adherence to the Mediterranean dietary pattern during the nutritional intervention

Dietary characteristics	Baseline diet	Intervention diet	*P* value[a]
Energy intake (kcal/day)	2067 ± 721	1529 ± 247	<0.001
Carbohydrate (% EI)	37.9 ± 7.8	53.0 ± 1.8	0.009
Fiber (g)	16.8 ± 7.8	20.8 ± 5.8	0.012
Protein (% EI)	20.1 ± 4.9	17.0 ± 0.7	<0.001
Lipid (% EI)	42.0 ± 7.1	30.0 ± 0.8	<0.001
MUFA (% EI)	19.8 ± 4.1	19.1 ± 1.9	0.305
PUFA (% EI)	6.6 ± 4.7	4.0 ± 0.05	<0.001
SFA (% EI)	11.0 ± 2.9	5.4 ± 0.07	<0.001
MUFA/SFA ratio	1.8 ± 0.4	3.5 ± 0.4	<0.001
Cholesterol (mg)	405.8 ± 199.5	55.8 ± 22.9	<0.001
Cereals (g)	199.2 ± 109.4	219.9 ± 84.4	0.231
Vegetables (g)	177.2 ± 98.0	301.7 ± 93.8	<0.001
Fruits (g)	198.3 ± 177.5	303.4 ± 132.6	0.006
Fish and related products (g)	82.7 ± 68	64.0 ± 41.0	0.254
Legumes (g)	69.9 ± 75	88.5 ± 90.3	0.330
Dairy products (g)	266.5 ± 195.2	324.1 ± 121.5	0.069
Meat (g)	201.2 ± 102	71.3 ± 47.9	<0.001
Mediterranean diet score	2.9 ± 1.3	6.5 ± 0.5	<0.001

EI energy intake, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid

Data are mean ± SD. Diet composition in habitual and intervention period were from three weighed food records

[a] *P* value from paired *t*-test

Clinical, metabolic, and inflammatory response after nutritional intervention

Caloric restriction resulted in a mean weight loss of −4.4 ± 2.5 kg (% weight loss: −6.2 ± 2.9) (Fig. 1). The body mass index, body fat (kg), waist circumference, and blood pressure values were significantly reduced over the 8-week trial period as well as plasma concentrations of glucose, total cholesterol, HDL-c, LDL-c, and HOMA-IR index (Table 2). Final non-esterified fatty acid (NEFA) concentrations were significantly higher than baseline values. Finally, resting energy expenditure was significantly reduced by the nutritional treatment (Table 2). Plasma RBP4 concentration showed a statistically significant reduction (−43 ± 29% from baseline) after the dietary intervention. Moreover, plasma concentrations of leptin, CRP, complement C3, and hs-TNFα were significantly decreased, while circulating hs-IL6 and homocysteine values remained unchanged (Table 2).

Effect of weight loss and MDP in metabolic and inflammatory response

Interestingly, the reduction (%) in plasma RBP4, hs-IL6, and hs-TNFα concentrations was significantly higher in

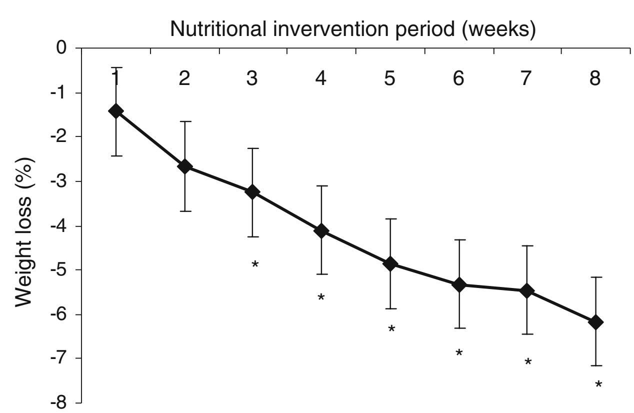

Fig. 1 Percent of weight loss (mean ± SD) during the 8-weeks nutritional intervention by caloric restriction with high adherence to the Mediterranean diet. Effect of caloric restriction, $P < 0.001$, from ANOVA repeated measures test. * Significant difference compared to weight loss (%) in the first week, $P < 0.001$, from Dunnet's post-hoc tests

Table 2 Changes in anthropometrical, clinical, metabolic and inflammatory variables after 8 weeks following a hypocaloric diet based on Mediterranean dietary pattern

Variables	Baseline	Endpoint	*P* value[a]
BMI (kg/m^2)	32.2 ± 3.9	30.3 ± 3.7	0.010
WC (cm)	100.3 ± 9.7	94.4 ± 9.5	0.010
Body fat (kg)	31.8 ± 10.0	28.4 ± 9.8	0.010
SBP (mmHg)	115 ± 10	109 ± 10	0.010
DBP (mmHg)	75 ± 8	70 ± 7	0.010
RMR (kcal/day)	1657 ± 275	1546 ± 226	0.010
Glucose (mg/dl)	95.1 ± 7.6	91.7 ± 6.6	0.012
Insulin (μUI/ml)	10.2 ± 7.6	7.5 ± 4.6	0.030
HOMA-IR	2.4 ± 2.0	1.7 ± 1.0	0.030
Total cholesterol (mg/dl)	200 ± 36	180 ± 31	0.010
HDL-c (mg/dl)	48.5 ± 10.7	45.5 ± 10.3	0.030
LDL-c (mg/dl)	131.9 ± 35	115 ± 28	0.001
TC/HDL-C ratio	4.4 ± 1.4	4.1 ± 1.1	0.001
Triglycerides (mg/dl)	102.6 ± 42	93.5 ± 39	0.151
NEFA (mmol/l)	0.30 ± 0.1	0.43 ± 0.2	0.034
Leptin (ng/ml)	27.8 ± 4.1	23.9 ± 3.6	0.004
C-reactive protein (mg/l)	2.6 ± 1.6	1.7 ± 1.2	0.012
Complement C3 (g/l)[b]	1.5 ± 0.2	1.4 ± 0.4	0.045
IL6 (pg/ml)	2.2 ± 1.5	2.3 ± 1.5	0.930
TNFα (pg/ml)	5.1 ± 3.2	2.5 ± 1.7	0.004
Homocysteine (μmol/l)	10.3 ± 3.7	10.4 ± 3.4	0.953
RBP4 (μg/ml)	33.9 ± 25.4	19.3 ± 7.4	0.004

BMI body mass index, *DBP* diastolic blood pressure, *HDL-c* high density lipoprotein, *HOMA-IR* homeostatic model of assessment of insulin resistance, *IL6* interleukin-6, *LDL-c* low density lipoprotein, *NEFA* non-esterified fatty acid, *RBP4* retinol binding protein-4, *RMR* resting metabolic rate, *SBP* systolic blood pressure, *TC* total cholesterol, *TNFα* tumor necrosis factor-alpha, *WC* waist circumference

Datas are mean ± SD

[a] *P* value from paired *t*-test or from Wilcoxon signed-rank test (non-normally distributed variables: triglycerides, C3, RBP4, and IL-6), after *P* value adjustment for Hochberg's step-up method for each domain (anthropometrical, metabolic and inflammatory variables)

[b] $n = 38$. All variables, $n = 41$

individuals with a higher adherence to MDP (point-increment in MDS > 3) than individuals with lower adherence ($P < 0.05$), independent of the caloric restriction (kcal/day). The reduction in C3 concentration showed a tendency ($P = 0.09$) to significance (Table 3). On the other hand, the values of metabolic features such as HOMA-IR, blood pressure, and total cholesterol/HDL-c were significantly lower ($P < 0.05$) in individuals with higher weight loss (% weight loss > 6.5%), compared with individuals with lower weight loss, while the reductions in inflammatory marker concentrations were not statistically different, except to change in leptin concentration ($P < 0.05$) and to reduction in CRP concentration with a tendency to significance (Table 3). Interestingly, the reduction of the plasma RBP4 concentration was positively and significantly associated ($r = 0.412$; $P = 0.006$) with the increment of MDS value (Fig. 2). In multivariate regression models, which was adjusted for gender and age, the MDS change was a predictive factor ($P < 0.05$) of the RBP4 decrease, independent of caloric restriction or weight loss (Table 4).

Discussion

The main aim of this study was to evaluate and discriminate the conjoint impact of following a hypocaloric diet with a high adherence to MDP on plasma concentrations of RBP4 and other proinflammatory markers (CRP, C3, hs-IL6, hs-TNFα, and homocysteine) and on metabolic syndrome features. In fact, nutritional intervention resulted in a statistically significant reduction of plasma RBP4, leptin, CRP, C3, and hs-TNFα concentrations. Several studies have demonstrated the effect of a hypocaloric diet on the reduction of some inflammatory markers such as leptin, CRP, IL6, and TNFα, related to weight loss [9–13, 22]. Regarding to the dietary effects on circulating RBP4 and C3 values, the current knowledge is still limited [13, 23–26]. In this context, we hypothesized whether an adherence to MDP would have additional beneficial effects on modulating these inflammatory markers with respect to weight loss. In observational studies, point-increments in the MDS have been given a protective role on the incidence of type 2 diabetes, overall mortality, and coronary disease-related to mortality [27, 28]. At the same time, higher MDS values have been associated with lower concentrations of inflammatory markers such as IL6, fibrinogen, homocysteine, CRP, vascular and intercellular adhesion molecules-1, and TNFα [19, 21, 29], which could, in part, explain the effects on mortality rate. In addition, clinical studies based on the MDP screening also have obtained a reduction on inflammatory and endothelial dysfunction markers such as IL6, IL17, IL18 [20] as well as vascular and intercellular adhesion molecules-1 [30, 31]. In this study, the reduction of RBP4 and hs-TNFα was significantly higher in individuals who presented a higher adherence to MDP, independent of the caloric restriction, while the reduction of C3 had tendency to statistical significance. The reduction of hs-IL6 concentrations, which was not significant when all participants were evaluated, also presented significant difference between individuals with higher and lower adherence. Interestingly, the change in MDS was a significant predictive factor of the reduction of plasma RBP4 concentrations, independent of age, gender, and caloric restriction. Thus, our results also showed an additional beneficial effect of a MDP on inflammation-related to obesity, independent of weight loss. The effect of MDP on plasma RBP4 and C3 concentrations is apparently reported for first time.

Table 3 Change (%) in metabolic features and inflammatory markers from baseline, according to cut-off median of weight loss[a] and cut-off median of adherence to Mediterranean dietary pattern[b]

Change (%)	Lower weight loss ($n = 20$)	Higher weight loss ($n = 21$)	P value[c]	Lower adherence ($n = 20$)	Higher adherence ($n = 21$)	P value[c]
Change in weight	−5.3 ± 2.4	−10.0 ± 1.5	<0.001	−5.6 ± 2.7	−6.5 ± 3.2	0.361
Change in MDS	+3.4 ± 1.3	+4.0 ± 0.7	0.299	+ 2.6 ± 0.8	+4.5 ± 0.9	<0.001
Change in HOMA-IR	−7.0 ± 54.2	−39.2 ± 30.5	0.034	−5.8 ± 48.0	−15.2 ± 27.0	0.558
Change in SBP	−3.2 ± 7.6	−10.3 ± 4.8	0.034	−2.9 ± 7.6	−6.0 ± 7.8	0.212
Change in DBP	−5.1 ± 7.6	−11.3 ± 5.5	0.034	−5.2 ± 8.9	−6.9 ± 6.8	0.309
Change in TC/HDL-c ratio	−0.1 ± 18.2	−15.6 ± 6.0	0.034	−3.9 ± 21.0	−3.4 ± 15.0	0.734
Change in leptin	−5.8 ± 126.0	−57.0 ± 11.8	0.020	−26.8 ± 27.1	−39.4 ± 165.0	0.224
Change in CRP	−5.4 ± 110.0	−23.4 ± 46.5	0.079	4.2 ± 109.0	−15.9 ± 9.2	0.139
Change in C3[d]	−9.3 ± 23.2	−8.5 ± 4.3	0.505	−4.9 ± 22.7	−14.5 ± 20.4	0.085
Change in IL6	+24.4 ± 132	+3.3 ± 34.8	0.824	+27.4 ± 172.0	−12.0 ± 34.2	0.044
Change in TNFα	+6.7 ± 141.0	−29.6 ± 106.4	0.552	+36.3 ± 171.2	−46.3 ± 45.0	0.044
Change in homocysteine	+41.9 ± 243.0	+15.1 ± 39.8	0.759	+3.6 ± 27.9	+4.6 ± 31.5	0.218
Change in RBP4	−27.4 ± 41.7	−18.3 ± 51.9	0.798	−11.7 ± 45.1	−41.2 ± 30.7	0.044

CRP C-reactive protein, *HDL-C* high density lipoprotein, *IL6* interleukin-6, *MDS* Mediterranean dietary score, *RBP4* retinol binding protein-4, *SPB* systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TNFα* tumor necrosis factor-alpha

Data are mean ± SD

[a] Sample was segregated in lower and higher weight loss, according cut-off median % weight loss from baseline (weight loss <6.5 vs. >6.5%)

[b] Sample was segregated in lower or higher adherence to Mediterranean dietary pattern, according to median point-increment in Mediterranean diet points-score from baseline to endpoint as cut-off (<3 points-increment vs. ≥3 points-increment). Change in Mediterranean diet points-score was ajdusted for energy intake

[c] P value from paired t-test or from Wilcoxon signed-rank test (non-normally distributed variables: triglycerides, C3, RBP4, and IL-6), after P value adjustment for Hochberg's step-up method for each domain (metabolic and inflammatory variables)

[d] $n = 38$. Remaining variables, $n = 41$

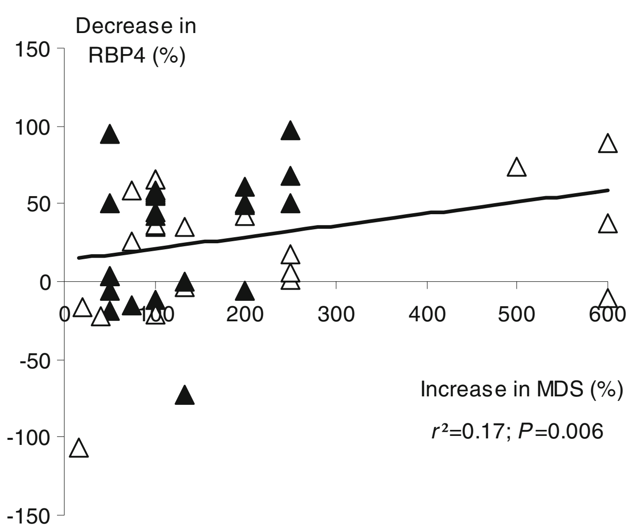

Fig. 2 Association of decrease in plasma RBP4 concentration with the increase in MDS (%). (*Filled triangle*) Men; (*open triangle*) Women

Table 4 Regression models results using to decrease (%) in plasma RBP4 concentration (dependent variable) as the outcome

Multivariate models[a]	B coefficient (95% CI)	P value
Model 1		
Corrected $R^2 = 0.19$		*0.020*
Change in MDS (%)	0.10 (0.02; 0.19)	0.020
Change in energy intake (%)	0.57 (0.10; 1.13)	0.050
Model 2		
Corrected $R^2 = 0.15$		*0.040*
Change in MDS (%)	0.11 (0.03; 0.20)	0.012
Change in weight (%)	3.21 (−0.70; 5.97)	0.173

MDS Mediterranean diet score

[a] Adjusted for gender (M/F) and age (year)

Furthermore, we also found significant improvements in clinical and metabolic characteristics, particulary in the glycemic and lipid profile. This effect was expected, since a weight loss related to a hypocaloric diet has been recommended for an improvement in these variables [32]. Specifically, our nutritional intervention resulted in a reduction of total cholesterol/HDL-c ratio comparable with previous studies, which applied a caloric-restricted Mediterranean diet [20, 33], while a higher reduction in HOMA-IR values was found. The improvements in these metabolic features were significantly higher in those individuals that presented a weight loss higher than 6.5%, but were not different between subjects with higher and lower adherence to MDP ($P > 0.05$). Additionally, the differences among studies may be due to differences in caloric restriction and in the baseline metabolic conditions of the studied participants (non-diabetics versus diabetics versus subjects with metabolic syndrome). Our study has some limitations, since it was designed without a specific control group, but we were able to discriminate the effects of weight loss and MDP adherence. Indeed, our data are in agreement to previous reported findings [19–21, 29], concerning the beneficial effect of Mediterranean food based dietary pattern on inflammatory modulation. In addition, the dietary intervention presented a higher consumption of dairy products, what could decrease the MDS, according the used score. However, we found a high consumption of whole dairy foods in the habitual diet, while the participants only consumed skimmed dairy foods during dietary intervention. This change in the quality of dairy foods is likely related to decrease in SFA and cholesterol intakes [34]. Also, we did not assess serum retinol concentration due to the lack of sample, but a reduction in the levels of retinol could be related to changes in RBP4 concentrations. In summary, following a nutritional intervention based on a high adherence to MDP within a hypocaloric diet resulted in a specific and differentiated reduction of proinflammatory markers RBP4, C3, CRP, IL6, and TNFα, besides to a significant improvement in the some metabolic syndrome features related-measurements. Thus, a high adherence to the MDP associated within a hypocaloric program could be a good strategy to treat obesity accompanying inflammatory related factors, in which both caloric-restriction and MDP adherence seem to play a specific role.

Subjects and methods

Subjects

Forty-one subjects (24 women and 17 men; 37 ± 7 years old) with excessive body weight-for height (BMI: 32.2 ± 3.9 kg/m^2; range 28.0–43.0 kg/m^2) were recruited via local advertisements. Exclusion criteria were metabolic, cardiovascular, or systemic diseases or any drug treatment as well as weight changes of over 3 kg within the 3 months before the start of the study, irregular menstruation, pregnancy, participation in other scientific studies within the previous 90-day period and alcohol/drugs abuse. All recruited subjects were not taking any medication, vitamins or supplements and declared to have sedentary habits (<2 h of physical activity per week). After receiving clear explanations, all subjects signed a written informed consent to participate in the study, which had previously been approved by the Ethics Committee of the University of Navarra (ref. 57/2006).

Study design

Each subject was instructed by the same trained dietitian over the 8-week trial period to follow a −30% total energy expenditure caloric restriction, accompanied by guidelines to assure a tight compliance in the adherence to a MDP as previously performed [35, 36]. Total energy expenditure was calculated from individual resting energy expenditure measured by indirect calorimetry (Deltatrac, Datex-Ohmeda, Helsinki, Finland) and corrected by physical activity levels as previously described [11, 37]. Volunteers were instructed to maintain their habitual physical activity patterns during the trial, being the physical activity pattern monitored and evaluated weekly by individual interviews during the course of 8 weeks study. Each participant received a detailed meal plan for 8 weeks, which was 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 as described elsewhere [38]. The 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). These data provided information about baseline intake and adherence to the prescribed diets [36]. In the weekly visits, reinforcement messages were also made to ensure compliance. Weight loss also was monitored weekly by the dietitian. Anthropometry, body composition, energy expenditure, and blood samples were assessed at baseline (day 0) and at the end (day 56), following standardized procedures [39, 40]. Diet records were analyzed and quantified using the Medisystem program (Sanocare, Madrid, Spain) based on Spanish food composition tables.

Nutritional intervention

In addition to generate a 30% caloric deficit over the total energy expenditure, the experimental diet was designed to provide about 30, 53, and 17% of the total energy intake from lipids, carbohydrates, and proteins, respectively, and, to increase the adherence to the MDP. In this context, the adherence to the Mediterranean diet was assessed according to a score created by Trichopoulou et al. [14, 41]. This index included nine components to define the Mediterranean diet: high ratio of MUFA/SFA, moderate intake of alcohol, high intake of grains, vegetables, fruit and nuts, legumes and fish, low intake of meat and meat products, and moderate intake of milk and dairy products. Briefly, the MDS assigns a score of 0 or 1 according to the daily intake of each of the nine components. For each of the six protective components (MUFA/SFA acid ratio, legumes, grains, fruits, vegetables, or fish) and two non-protective components (dairy products or meat), participants received one point if their intakes were over and below (specific per gender) the sample median, respectively. For alcohol, one point was scored if consumption was 10–50 g/day for men or 5–25 g/day for women. A highest score is nine points, reflecting maximum adherence [14, 41].

Anthropometry and body composition

Body weight measurements were performed using a digital balance accurate to 0.1 kg (Seca 767, Vogel & Halke, Hamburg, Germany) and height using a wall-mounted stadiometer (Seca 220, Vogel & Halke, Germany). 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, and the hip circumference was measured on the maximum circumference over the buttocks [39] with the subject in a standing position. Body composition was measured by a bioelectric impedance equipment (Quadscan 4000, Bodystat, Isle of Man, UK), following previously described procedure [39].

Blood pressure measurement and blood sample analyses

Blood pressure was measured following WHO criteria [37]. Venous blood samples were drawn in a fasting state (12 h). EDTA plasma and serum were separated from whole blood by centrifugation (3500 rpm, 15 min, 5°C) and stored at −20°C until assay. Plasma concentrations of triglycerides, total cholesterol and HDL-c, NEFA, glucose (Horiba ABX Diagnostics, Montpellier, France), and homocysteine (Demitec Diagnostic GmbH, Kiel-Wellsee, Germany) were measured by specific colorimetric assays, while complement C3 (Horiba ABX Diagnostics, Montpellier, France) was assessed by turbidimetric assay, by using an automated analyzer system (COBAS MIRA, Roche, Basel, Switzerland). The reported LDL-c was calculated by the Friedewald equation [42]. Plasma concentrations of insulin and leptin were assessed by using commercially available radioimmunoassay kits (DPC, Texas, USA), being the inter-assay variability about 6 and 5%, while the intra-assay variability was about 4 and 5%, respectively. Insulin resistance was estimated by the HOMA-IR index [43], which was estimated as follows: HOMA-IR = [fasting glucose (mmol/l) × fasting insulin (μIU/l)]/22.5. Plasma concentrations of RBP4, CRP, high-sensitive IL6 (hs-IL6), and TNFα (hs-TNFα) were measured by commercially available enzyme immunoassay kits using an automated analyzer system (Triturus, Grifols, Barcelona, Spain). RBP4 and CRP were measured by using Immundiagnostik AG kits, (Bensheim, Germany) and the cytokines by Quantikine immunoassay kits (hs-IL-6 and hsTNFα) from R&D Systems (Minneapolis, USA). The inter- and intra-assay variability for CRP, RBP4, hs-IL-6, and hs-TNFα were 10 and 4%, 8 and 6%, 12 and 6%, 10 and 5%, respectively.

Statistical analysis

Results are reported as mean ± SD. Sample size was calculated considering: (1) a reduction of 20% in plasma RBP4 concentration as an appropriate outcome; (2) published SD values (±7.9) for plasma RBP4 concentration [13]; (3) an statistical power of 80%; and (4) P value <0.05 as statistically significant. The calculation power was performed by the two-sided paired t-test in the OpenEpi version 2.3 [44]. For all these criteria, the sample size required was a minimum of 37 volunteers. The Shapiro–Wilk tests were used to determine variable distribution. Changes in weight loss were evaluated by paired t-test (baseline and endpoint) and by the repeated measures ANOVA (weight loss time-course: 8 points) following by the Dunnet's post-hoc test. In order to analyze changes in metabolic factors and proinflammatory marker concentrations with respect to caloric-restriction-related weight loss, the change in weight loss (%) was taken as suitable variable considering its median as cutoff value (6.5% weight loss) and categorizing the subsequent population group in "lower" and "higher" weight loss according to this value. To analyze change in metabolic and inflammatory variables related to adherence to MDP, the points-increment in MDS [14, 41] was taken as suitable variable considering its median cutoff value (three points-increment) and categorizing the subsequent population group in "lower" and "higher" adherence to the MDP according to this value. The median cutoff criteria have been previously applied [45–47] and is based on a valid and reliable method to assign two groups of risk in epidemiological studies [48]. The Wilcoxon signed-rank test was applied to analyse non-parametric data (triglycerides, complement C3, hs-IL6, and RBP4 variables) and the paired t-test to analyse the remaining data. After the application of these tests, P values were adjusted by Hochberg's step-up method to each domain of variables (anthropometrical, metabolic, and inflammatory variables) to avoid type 1 error. A multivariate regression model was finally applied to describe the observed change in plasma RBP4 concentration (dependent variable), considering age and gender as well as the change in MDS, caloric restriction, and in weight loss as independent variables. Statistical analyses were performed using the SPSS 15.0 program (SPSS Inc., USA) for Windows XP (Microsoft, USA).

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