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ORIGINAL CONTRIBUTION

Adherence to Mediterranean diet and close dietetic supervision increase total dietary antioxidant intake and plasma antioxidant capacity in subjects with abdominal obesity

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Abstract
Purpose To determine the effect of Mediterranean-type diet and close dietetic supervision on dietary antioxidant intake and plasma total antioxidant capacity (TAC) in patients with abdominal obesity.
Methods Ninety subjects with abdominal obesity, 46 in intervention group, 44 in control group, participated in a 2-month, randomized, parallel dietary intervention. All participants were counseled on Greek Mediterranean diet. The intervention group was under close dietetic supervision, followed a specific relevant daily and weekly food plan consuming antioxidant-rich foods and food products. Total dietary antioxidant intake was calculated from the volunteers' food diaries, and plasma TAC using plasma ORAC assay and plasma ferric-reducing antioxidant power (FRAP) assay, both at baseline and at 2 months.
Results Following the 2-month period, total dietary antioxidant intake was increased in the intervention group compared to the control group ($P = 0.000$). In addition, increased intake of total fat, due to higher consumption of monounsaturated fatty acids, as well as increased intakes of dietary fiber, vitamin C and alcohol was also observed in the intervention group compared to the control group ($P < 0.05$). Plasma TAC was increased in the intervention group compared to the control group ($P = 0.039$) using the ORAC assay, while there was a trend toward a TAC increase ($P = 0.077$) using the FRAP assay.
Conclusion Adherence to a Mediterranean-type diet, with emphasis on an increase in foods rich in antioxidants and close dietetic supervision, can increase total dietary antioxidant intake and plasma TAC in patients with abdominal obesity.

Keywords Obesity · ORAC · FRAP · Antioxidants · Mediterranean diet

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Introduction

Obesity has become a major clinical and public health problem, affecting one-third of the population in industrialized world [1]. Abdominal obesity especially is an important predictor of cardiovascular disease (CVD) and diabetes mellitus type 2 [2] in addition to overall obesity. It is estimated that in Greece, using data from 2001 Census, about 2.3 million Greeks may have the metabolic syndrome, as defined by the American Heart Association and National Heart, Lung, and Blood Institute in 2004 [3], having abdominal obesity and arterial hypertension as the main abnormalities [4].

Abdominal obesity is associated with endothelial dysfunction, through different mechanisms, such as insulin resistance and production of adipokines and pro-inflammatory cytokines, which in turn induces oxidative stress

leading to reduced nitric oxide availability [5]. The generated free radicals in oxidative stress are scavenged by antioxidants that play a protective role against lipid peroxidation [6]. Thus, it seems that persons with abdominal obesity are at increased risk of CVD, and an increase in plasma antioxidants might have a positive effect in reducing this risk.

Mediterranean diet, which was firstly studied in the Seven-Countries study in 1950s to 1960s in the south of Europe [7], is identified as a dietary pattern that can modify the risk of developing CVD and metabolic syndrome [8]. Although the exact mechanisms responsible for the cardioprotective properties of the Mediterranean diet are still uncertain [9], one of the possible ones could be the reduction in oxidative stress. Vegetables, fruits, olive oil and red wine are basic food items in the Mediterranean diet and are rich in a variety of compounds with an antioxidant capacity, such as vitamin E, vitamin C, carotenoids and polyphenols [10]. However, only a limited number of observational and intervention studies tried to investigate the effect of the Mediterranean diet on plasma total antioxidant capacity (TAC). In particular, the ATTICA study, which is an observational study, showed that greater adherence to the Mediterranean diet is associated with elevated TAC in serum and that TAC was positively associated with the consumption of fruit, vegetables and olive oil [11]. Additionally, in an intervention study, the PREDIMED (Prevencion Dieta Mediterranea) trial, the results showed that the Mediterranean diet, especially rich in virgin olive oil, is also associated with higher levels of plasma antioxidant capacity [12].

On the other hand, in intervention studies, compliance is an issue. For example, in the PREDIMED study in subjects with body mass index (BMI) $\geq$25 kg/m^2, it was reported that nutritional intervention promoting the Mediterranean diet in group sessions, coupled with free provision of Mediterranean-related dietary products, was more effective in improving dietary habits of subjects than were verbal instructions and a leaflet recommending the third National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) dietary guidelines [13].

To the best of our knowledge, there are no studies investigating the effects of the Mediterranean diet in combination with close dietetic supervision on dietary total antioxidant intake and plasma TAC in patients with abdominal obesity.

Therefore, the aim of the present study was to advance further our study [2] and to assess the influence of a Mediterranean-type diet with the provision of foods relevant to Mediterranean diet, and in combination with close dietetic supervision, on total dietary antioxidant intake and plasma TAC.

Materials and methods

Subjects

Ninety subjects with abdominal obesity (waist circumference >102 cm for men and >88 cm for women) were recruited from the outpatient Cardiology Department of the "Attikon" University Hospital and Hygeias Melathron Infirmary, Athens Greece. They were picked out from 340 apparently healthy employees with abdominal obesity of the Hellenic National Bank, who had been referred for their annual checkup. The exclusion criteria used were presence of diabetes mellitus or CVD, age >70 year, use of multivitamins, alcohol drinking >500 g alcohol/week, presence of malignancy or other disease that might influence inflammatory markers such as autoimmune diseases. Furthermore, within 6 months preceding the study, participants should not have taken part in any nutritional intervention, weight-reducing program or extreme physical activity (>6 h of vigorous exercise/week).The study was approved by the ethics committees of Attikon Hospital, Hygeias Melathron Infirmary and the research committee of Agricultural University of Athens and was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration Helsinki. All subjects signed informed consent forms.

Experimental design

Upon entering the study, the participants were randomly divided into the intervention ($n = 46$) or the control ($n = 44$) group, with the use of a sequence of random binary numbers (i.e., 001110110 in which 0 represented the intervention group and 1 the control group). All participants were informed about the study at their first appointment with the dietician. They were asked to keep a 3-day food diary and to complete a basic questionnaire regarding their age, socioeconomic status, medical and family history, physical activity, smoking and alcohol consumption. All participants were provided with a copy of the Greek Mediterranean diet pyramid and were counseled on this eating pattern.

The intervention group received more counseling by a more detailed analysis on the food groups of the Greek Mediterranean diet pyramid, frequency and portion size of each food group to be consumed daily, weekly and monthly, and they were asked to follow a specific daily and weekly food plan. Within this food plan, the intervention group had to include the following food items, virgin olive oil, fruit juice, red wine, almonds, olive oil-based margarine, that were provided free of charge, in order to support the inclusion of important nutrients related to Mediterranean diet and also the increase in intake of antioxidants and

monounsaturated fatty acids (MUFA). The food plan included daily consumption of only whole wheat grains and products, 2–3 portions of only low-fat dairy products, 2 salads a day (one of which should contain ≥1 tomato) and ≥3 fruits daily together with a concentrated fruit juice made without preservatives (provided by ELAIS—Unilever Hellas SA), 5 mL (i.e., 1 tea spoon) olive oil-based margarine in order to increase MUFA intake (provided by ELAIS—Unilever Hellas SA), extra virgin olive oil as the main source of fat (provided by ELAIS—Unilever Hellas SA), 45 mL (i.e., 3 tablespoons) extra virgin olive oil with one of the two salads, 6 whole raw almonds, and 250 mL (1 glass) red wine (provided by Harlaftis Ltd, Greece) with their main meal. According to the food plan, the intervention group was required to consume weekly ≥1 portion of fish and at the most 1 portion of red meat.

The intervention group was closely supervised by a dietitian via weekly phone calls and weekly appointments. During these sessions, body weight was measured. In terms of compliance, participants of the intervention group at each of these appointments handed in a 3-day food diary (every second session) and completed a 24-h recall, by which the dietitian confirmed that they had consumed the required food items and they had followed a Mediterranean style diet. The intervention group was also asked to return the empty packets of the products they had received, showing that they had consumed the required food items in the exact portions and also returned a filled-in checklist of the foods they had consumed daily (i.e., whole wheat, low-fat dairy products, salads, fruits, fruit juice, olive oil and virgin olive oil, almonds, red wine) and weekly (i.e., meat and fish). The weekly telephone contacts and attendance to the arranged sessions with the dietitian confirmed the compliance of the intervention group.

Additionally, 3-day food diaries were used to measure dietary intake at both the beginning and the end of the study. Subjects in the control group met with the dietitian only at the beginning and at the end of the study.

The duration of dietary intervention was 2 months in both groups. Height (m), body weight (kg), waist circumference (cm) and blood pressure were measured in all participants at the beginning and at the end of the study. Body mass index (BMI) was calculated by dividing the participants' weight by their height squared (Kg/m^2). Body height was measured using a stadiometer. Body weight was measured using calibrated scales, after asking all subjects to remove their shoes and heavy outer garments. Abdominal obesity was measured by waist measurement using a non-stretchable tape over the lightly dressed abdomen, midway between the lowest rib and the iliac crest while the person was standing and having done a moderate expiration. Blood pressure refers to the arterial pressure and was measured at a subject's upper arm, on the inside of an elbow at the brachial artery, expressed in mmHg.

Energy, nutrient and food intake

The analysis of the food diaries was carried out with the use of Nutritionist V diet analysis software (version 2.1, 1999; First Databank, San Bruno, CA).

Total dietary antioxidant intake

Total dietary antioxidant intake was calculated from the food diaries using the USDA ORAC database of selected foods [14]. In particular, total dietary antioxidant intake was calculated for baseline and the end of the study as follows: for every subject from the 3-day food diary supplied for the 2 periods above, the average intake of each food item was calculated and subsequently multiplied with the respective total ORAC value of the database. The oxygen radical absorbance capacity values of total dietary antioxidant intake were expressed as μmol Trolox equivalents (TE)/day.

Blood sampling and laboratory methods

Venous blood samples were collected from the subjects at 08:00 h after a 12-h overnight fast at the beginning and the end of the study. Samples were centrifuged at 3,000 rpm for 10 min at 4 °C within 2 h from blood collection and were stored at −80 °C for further analysis.

Lipid measurements

Total cholesterol and triglycerides were measured by an enzymatic method in a Dimension of DATE BEHRING analyzer. High-density lipoprotein (HDL) cholesterol was assayed with the direct method of DATE BEHRING. LDL cholesterol was calculated according to Friedewald formula: LDL cholesterol = total cholesterol − (triglycerides/5 + HDL cholesterol).

The ORAC assay

Plasma TAC was determined using the ORAC assay [15]. Prior to the analysis, frozen plasma samples were brought to room temperature and subsequently diluted 200-fold with ice-cold phosphate-buffered saline. In brief, the assay was performed at 37 °C in fluorometer cuvettes. Phosphate buffer and the antioxidant compound Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid) were used instead of diluted plasma samples to make the blank and standard solutions, respectively. Each reaction was set up as follows: in each cuvette, 1,740 μL of phosphate buffer

(75 mM pH = 7) was added followed by 100 μL of sample (or phosphate buffer or Trolox (125 μM) in the case of blank and standard, respectively). Subsequently, 10 μL phycoerythrin (68 mg/L) was added. Each reaction was started with the addition of 150 μL of the peroxyl radical generator 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH, 160 mM), brief mixing of cuvette contents and immediate florescence measurement. Fluorescence was measured using a fluorescence spectrophotometer (emission 565 nm, excitation 540 nm) with readings being taken every 5 min until the fluorescence decreased to 5% of the initial value. ORAC values were calculated according to Cao and Prior [15] using 200 as sample dilution factor and 125 for Trolox concentration. ORAC values were expressed in μmol Trolox equivalents (TE)/L plasma (μM TE).

The FRAP assay

The FRAP assay was also used to measure plasma TAC, according to the method of Benzie and Strain [16] adapted to employ a plate reader. The FRAP reagent consisted of 25 mL acetate buffer (0.3 M, pH 3.6), mixed with 2.5 ml $FeCl_3 \cdot 6H_2O$, 20 mM, and 2.5 ml 2,4,6-tri(2-pirydyl)-s-triazine, 10 mM. Accordingly, 10 μL plasma was put in each well of a 96-well plate reader and diluted with 100 μL HCl, 40 mM, and the plate was immediately read at 595 nm, for the absorption at 0. Afterward, 10 μL of each sample was put in each well of a 96-well plate reader and diluted with 100 μL FRAP reagent, and the plate was read at 595 nm after 30 min. The plates were read in a multiwell absorbance plate reader (ELx808TM, BioTek Instruments, Inc., Vermont, USA) with temperature at 37 °C. Standards were prepared from $FeSO_4$ in HCl 0.01 N in concentrations ranging from 100 to 1,000 μM. FRAP values were expressed as μmol $FeSO_4$ per L.

Statistical analysis

Results are presented as mean ± SD for the normally distributed variables and frequencies (n) for the categorical ones. Normality was tested using the Shapiro–Wilk criterion, as well as graphical methods (i.e., Q–Q plots) for the continuous variables. The paired and the independent Student's t test were used to evaluate differences in mean values for the normally distributed variables. Pearson's chi-square test was used to evaluate the relationship between categorical variables, while Pearson's correlation coefficient was used to assess the relation between normally distributed continuous variables. Analysis of variance for repeated measures, with certain variables as the dependent outcome and the intervention as the main fixed effect covariate, was used to answer the research hypothesis. P-value < 0.05 was considered as statistically significant for two-sided tests. SPSS version 13 software was used for all calculations (SPSS Inc., Chicago, IL, USA). Sample size was considered adequate to evaluate differences at biomarker levels and anthropometric characteristics equal to 0.5 SDs at 5% significance level, with 70% achieved power.

Results

Baseline characteristics

Eighty-two of ninety recruited subjects completed the study (5 dropped out from the intervention group and 3 from the control group). The 2 groups were comparable in age, sex and smoking habits. Additionally, the 2 groups did not differ in lipid profile, weight and waist circumference (Table 1).

Anthropometry

There were no significant differences in body weight ($P = 0.975$) and waist circumference ($P = 0.537$) in the intervention group compared to the control group following between-group analysis, although a reduction in body weight and waist circumference was observed at the end of both 2-month periods (Table 2).

Energy and nutrient intake for the overall sample and by gender

Baseline nutrient intakes did not differ significantly between the 2 groups (Table 3). Baseline energy intakes decreased in both groups, but the degree of reduction did not differ significantly between the two groups.

Within-group analysis in the intervention group showed a significant decrease in saturated fatty acids (SFA), cholesterol, carbohydrate and protein intakes. On the other hand, total fat intake increased, because mainly of higher intakes of MUFA, and statistically significant increases were found in intakes of dietary fiber, vitamin C and alcohol. Within-group analysis in the control group showed a statistically significant reduction only in SFA intake. The intake of polyunsaturated fatty acids (PUFA) did not show any significant difference in neither group after the 2-month periods. Between-group analysis showed a significant increase in intakes of total fat, MUFA, fiber, vitamin C and alcohol and a significant decrease in intakes of protein and SFA, but no significant difference in the intake of PUFA in the intervention group compared with the control group.

According to the analysis by gender, women showed significant difference in almost all macronutrients and

Table 1 Descriptive characteristics of the two groups at baseline

	Intervention group ($n = 41$)	Control group ($n = 41$)	P^a-value
Age	50.2 ± 6.4	50.6 ± 8.1	0.777
Sex	23 men, 18 women	20 men, 21 women	0.658
Smoking habits	14	12	0.812
Body weight (kg)	94.1 ± 13.5[b]	94.0 ± 13.3	0.959
Body mass index (kg/m^2)	31.5 ± 3.8	32.8 ± 4.7	0.148
Waist circumference (cm)	106.1 ± 9.1	106.7 ± 9.5	0.691
Plasma glucose (mmol/L)	5.4 ± 0.9	5.4 ± 0.7	0.785
Total cholesterol (mmol/L)	5.5 ± 0.8	5.4 ± 0.9	0.575
Triglycerides (mmol/L)	1.7 ± 0.7	1.5 ± 0.7	0.122
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.3 ± 0.3	0.589
LDL cholesterol (mmol/L)	3.7 ± 0.6	3.5 ± 0.8	0.263
Systolic BP[c] (mmHg)	130.2 ± 14.4	129.6 ± 14.8	0.610
Diastolic BP (mmHg)	85.8 ± 7.6	86.3 ± 8	0.755

[a] *P*-values derived from unpaired *t* test; [b] $x \pm$ SD (all such values); [c] *BP* blood pressure

Table 2 Body weight and waist circumference before and after 2 months of dietary intervention

	Intervention group ($n = 41$)			Control group ($n = 41$)			Baseline comparisons P^b	Between-group comparison P^c
	Baseline	2 months after	P^a	Baseline	2 months after	P^a		
Body weight (Kg)	94.1 ± 13.5[d]	91.4 ± 13.6	<0.001	94.0 ± 13.3	91.9 ± 13.3	<0.001	0.959	0.975
BMI (Kg/m^2)	31.5 ± 3.8	30.6 ± 3.9	<0.001	32.8 ± 4.7	32.1 ± 4.6	<0.001	0.148	0.149
Waist circumference (cm)	106.1 ± 9.1	103.9 ± 9.3	<0.001	106.7 ± 9.5	105.7 ± 10.2	0.007	0.691	0.537

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA using the intervention as the main fixed covariate; data represent means ± SD; [d] $x \pm$ SD (all such values)

micronutrients apart from vitamin C, cholesterol and SFA in the intervention group compared with the control group, whereas men showed significant difference only for alcohol, alpha-tocopherol, SFA and protein intake (Table 4).

Food intake for the overall sample and by gender

At baseline, food intake did not differ significantly ($P > 0.05$) between the two groups (Table 5), apart from the consumption of red wine. Following the intervention periods, there was a significant change in the intakes of all food items except from nuts and legumes. Specifically, within-group analysis in the intervention group showed a significant increase ($P = 0.000$) in intake of olive oil, vegetables and whole grains. Moreover, a significant increase was shown also in intake of fruits ($P = 0.010$), fruit juice ($P = 0.027$), fish ($P = 0.001$) and red wine ($P = 0.001$). On the contrary, red meat consumption was significantly decreased ($P = 0.000$). Within-group analysis in the control group showed a significant increase in whole grain ($P = 0.046$) and a significant decrease in red meat ($P = 0.041$) intakes. Between-group analysis showed a significant increase in olive oil ($P = 0.001$), fruit juice ($P = 0.044$), vegetables ($P = 0.005$), fish ($P = 0.007$) and red wine ($P = 0.000$) and a strong trend toward a decrease in red meat ($P = 0.077$) in the intervention group compared with the control group. Accordingly, analysis between groups by gender also showed a significant increase in red wine for both women and men in the intervention group compared with the control ($P < 0.001$) (Table 6).

Total dietary antioxidant intake for the overall sample and by gender

At baseline, there were no significant differences between the two groups in total dietary antioxidant intake ($P = 0.433$).

Following the intervention, total dietary antioxidant intake increased significantly in the intervention group ($P = 0.000$), whereas it did not change in the control group ($P = 0.773$) (Table 7). Between-group analysis showed

Table 3 Distribution of energy and nutrient intakes before and after 2 months of dietary intervention for the overall sample [2]

	Intervention group ($n = 41$)			Control group ($n = 41$)			Baseline comparisons	Between-group comparison
	Baseline	2 months after	P[a]	Baseline	2 months after	P[a]	P[b]	P[c]
Total energy KJ/day (Kcal/day)	7,706 ± 1,193 (1,841 ± 285[d])	7,238 ± 1,273 (1,729 ± 304)	0.045	7,510 ± 2,152 (1,794 ± 514)	6,593 ± 2,055 (1,575 ± 491)	<0.001	0.617	0.228
Energy from:								
Carbohydrates (%)	41.9 ± 6.5	38.3 ± 5.3	0.009	42.4 ± 9.7	42.1 ± 7.6	0.839	0.780	0.107
Total fat (%)	40.7 ± 6.9	47.4 ± 6.4	<0.001	41.6 ± 9.4	40.3 ± 9.4	0.345	0.616	0.047
SFA[e] (%)	13.9 ± 2.6	9.5 ± 1.7	<0.001	14.5 ± 3.3	11.8 ± 2.5	<0.001	0.238	<0.001
MUFA[f] (%)	19.6 ± 4.2	26.4 ± 3.6	<0.001	20.5 ± 6.6	19.8 ± 7.7	0.534	0.572	0.007
PUFA[g] (%)	6.6 ± 4.1	6.2 ± 2.9	0.653	5.7 ± 2.2	5.7 ± 3.0	0.910	0.193	0.145
Protein (%)	16.1 ± 2.3	14.3 ± 2.5	0.002	16.6 ± 3.2	18.0 ± 4.8	0.079	0.418	<0.001
Fiber (g/day)	17.0 ± 6.3	21.4 ± 8	0.009	16.3 ± 6.6	16.6 ± 7.5	0.833	0.630	0.023
Alcohol (g/day)	5.9 ± 8.4	12.9 ± 5.3	<0.001	4.2 ± 8.6	4 ± 6.4	0.872	0.358	<0.001
Cholesterol (mg/day)	229.2 ± 71.3	142.1 ± 64.2	<0.001	235.9 ± 137	193.4 ± 120	0.074	0.783	0.119
Vitamin C (mg/day)	116.7 ± 80.9	167.8 ± 83.1	0.003	113.4 ± 68.8	117.1 ± 76.9	0.787	0.842	0.047
Alpha-tocopherol (mg/day)	4.5 ± 1.6	4.9 ± 4.1	0.494	5.1 ± 2.9	4.4 ± 3.1	0.160	0.327	0.978
Beta-carotene (μg/day)	550.6 ± 532	595.9 ± 489	0.704	456.2 ± 480	418.4 ± 384	0.587	0.401	0.091

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA using the intervention as the main fixed covariate; data represent means ± SD; [d] x ± SD (all such values); [e] *SFA* saturated fatty acids; [f] *MUFA* monounsaturated fatty acids; [g] *PUFA* polyunsaturated fatty acids

significant differences between the 2 intervention periods in total dietary antioxidant intake ($P = 0.000$). Such a result was also showed in between-group analysis for both women ($P < 0.001$) and men ($P = 0.001$) (Table 8).

Plasma total antioxidant capacity for the overall sample and by gender

Baseline plasma TAC was similar between the two groups using either the ORAC ($P = 0.527$) or the FRAP ($P = 0.516$) assays. However, within-group analysis, using the ORAC assay, revealed a statistically significant increase in plasma TAC in the intervention group following the 2-month period ($P = 0.036$), whereas no significant difference ($P = 0.844$) was observed in the control group. Most importantly, between-group analysis showed that plasma TAC significantly increased in the intervention compared to the control group ($P = 0.039$) following the 2-month intervention period. On the other hand, although no differences were observed within each group, using the FRAP assay, between-group analysis revealed a strong trend toward a statistical difference following the two 2-month interventions ($P = 0.077$), (Table 7). Between-group analysis for women showed that there was a strong trend toward an increase in plasma TAC using the FRAP assay ($P = 0.063$) and a significant increase in plasma ORAC ($P = 0.020$) for men (Table 8).

Finally, there was no correlation between the total dietary antioxidant intake and plasma TAC ($P > 0.05$).

Discussion

In the present study, we evaluated the effect of the adoption of Mediterranean-type diet with the provision of certain foods rich in antioxidants combined with close dietetic supervision in subjects with abdominal obesity and showed that there is an improvement in total dietary antioxidant intake as well as in plasma TAC.

Energy intake decreased in both groups following the intervention possibly due to substitution of high-energy meals by meals low in calories such as vegetable-based ones and to reduction in high-energy snacks and their replacement by fruits and whole-grain foods low in fat and sugar. Accordingly, on the basis of Mediterranean diet, both groups were advised to consume only Greek-type desserts, in the form of compote fruits only once a week, which further reduced the daily energy intake of both groups. The reduction in high-energy meals and snacks according to the Mediterranean diet and the consumption of legumes, vegetable-based foods, fruits and vegetables with reduced meat intake may explain the significant decrease in SFA, cholesterol, carbohydrate and protein intake as it is shown in the within-group analysis.

Table 4 Distribution of energy and nutrient intakes before and after 2 months of dietary intervention, by gender

	Intervention group			Control group			Baseline comparisons	Between-group comparison
	Baseline	2 months after	P^a	Baseline	2 months after	P^a	P^b	P^c
Females	($n = 18$)			($n = 21$)				
Total energy KJ/day (Kcal/day)	7,252 ± 1,235 (1,735 ± 295)[d]	6,596 ± 1,308 (1,578 ± 313)	0.087	6,449 ± 1,705 (1,543 ± 408)	5,563 ± 1,216 (1,331 ± 291)	0.009	0.124	0.030
Energy from:								
Carbohydrates (%)	41.7 ± 6.6	35.4 ± 5.6	0.009	45.9 ± 10.9	43.6 ± 6.9	0.295	0.185	0.007
Total fat (%)	44.2 ± 6.8	48.1 ± 7.2	0.030	38.7 ± 9.7	38.5 ± 8.4	0.929	0.060	0.003
SFA[e] (%)	13.5 ± 2.0	9.7 ± 1.9	<0.001	13.5 ± 3.3	11.5 ± 2.7	0.018	0.986	0.189
MUFA[f] (%)	22.0 ± 4.7	27.4 ± 3.4	0.001	18.6 ± 6.1	18.7 ± 5.9	0.944	0.072	<0.001
PUFA[g] (%)	8.9 ± 5.5	7.4 ± 4.5	0.457	5.6 ± 2.4	5.6 ± 3.7	0.977	0.022	0.008
Protein (%)	14.8 ± 1.7	15.0 ± 3.1	0.767	16.8 ± 2.9	18.5 ± 5	0.130	0.015	0.004
Fiber (g/day)	16.1 ± 6.4	18.4 ± 5.6	0.237	15.0 ± 6.0	13.3 ± 4.1	0.268	0.622	0.030
Alcohol (g/day)	4.2 ± 9.0	12.6 ± 6.5	0.008	2.8 ± 5.7	2.8 ± 5.4	0.979	0.558	0.001
Cholesterol (mg/day)	200.5 ± 60.8	143.7 ± 74.1	0.023	213.9 ± 147.6	159.4 ± 50.9	0.082	0.735	0.555
Vitamin C (mg/day)	118.0 ± 85.2	149.9 ± 74.2	0.099	93.6 ± 47.9	107.3 ± 62.0	0.426	0.277	0.077
Alpha-tocopherol (mg/day)	4.8 ± 2.1	5.9 ± 5.1	0.317	3.8 ± 1.7	3.4 ± 1.8	0.308	0.139	0.044
Beta-carotene (μg/day)	787.0 ± 674.5	544.8 ± 486.5	0.263	443.0 ± 302.4	404.6 ± 294.7	0.648	0.044	0.030
Males	($n = 23$)			($n = 20$)				
Total energy KJ/day (Kcal/day)	7,980 ± 1,095 (1,909 ± 262[d])	7,623 ± 1,091 (1,825 ± 261)	0.251	8,556 ± 2,057 (2,047 ± 492)	7,595 ± 2,236 (1,817 ± 535)	0.016	0.235	0.537
Energy from:								
Carbohydrates (%)	42.0 ± 6.6	40.1 ± 4.4	0.253	38.8 ± 6.9	40.6 ± 8.2	0.279	0.117	0.393
Total fat (%)	38.4 ± 6.0	47.0 ± 6.0	<0.001	44.7 ± 8.2	42.1 ± 10.3	0.213	0.005	0.739
SFA[e] (%)	13.9 ± 2.9	9.3 ± 1.6	<0.001	15.6 ± 3.0	12.2 ± 2.3	<0.001	0.068	<0.001
MUFA[f] (%)	18.5 ± 2.8	25.8 ± 3.7	<0.001	22.6 ± 6.5	21.1 ± 9.3	0.429	0.006	0.836
PUFA[g] (%)	5.8 ± 4.0	5.5 ± 4.9	0.67	5.7 ± 2.2	5.8 ± 2.1	0.736	0.857	0.899
Protein (%)	16.9 ± 2.3	13.8 ± 1.9	<0.001	16.3 ± 3.5	17.5 ± 4.8	0.349	0.493	0.050
Fiber (g/day)	17.6 ± 6.3	23.3 ± 8.9	0.022	17.6 ± 7.0	20.0 ± 8.7	0.169	0.983	0.365
Alcohol (g/day)	7.1 ± 7.9	13.1 ± 4.5	0.002	5.8 ± 10.8	5.3 ± 7.3	0.816	0.638	0.022
Cholesterol (mg/day)	247.6 ± 72.6	141.1 ± 58.7	<0.001	259.0 ± 125.5	229.2 ± 157.9	0.421	0.704	0.064
Vitamin C (mg/day)	115.8 ± 79.9	179.3 ± 87.9	0.015	134.1 ± 81.6	127.4 ± 90.5	0.766	0.454	0.386
Alpha-tocopherol (mg/day)	4.3 ± 1.1	4.3 ± 3.3	0.9	6.3 ± 3.5	5.5 ± 3.9	0.304	0.010	0.049
Beta-carotene (μg/day)	399.4 ± 356.0	628.6 ± 498.5	0.093	470.0 ± 622.8	432.9 ± 468.6	0.748	0.635	0.593

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA; data represent means ± SD; [d] x ± SD (all such values); [e] *SFA* saturated fatty acids; [f] *MUFA* monounsaturated fatty acids; [g] *PUFA* polyunsaturated fatty acids

Table 5 Food consumption before and after 2 months of dietary intervention for the overall sample

	Intervention group ($n = 41$)			Control group ($n = 41$)			Baseline comparisons	Between-group comparison
	Baseline	2 months after	P^a	Baseline	2 months after	P^a	P^b	P^c
Olive oil (g/day)	17.5 ± 12.4[d]	51.0 ± 9.7	0.000	22.4 ± 22.2	25.0 ± 25.4	0.595	0.240	0.001
Fruits (g/day)	161.5 ± 171.7	237.2 ± 146.0	0.010	209.9 ± 215.7	192.3 ± 158.5	0.674	0.280	0.954
Fruit juice (ml/day)	85.3 ± 167.3	143.8 ± 101.5	0.027	46.2 ± 109.9	82.4 ± 127.2	0.078	0.228	0.044
Vegetables (cups/day)	1.7 ± 1.2	2.7 ± 1.4	0.000	1.6 ± 1.0	1.7 ± 1.0	0.663	0.681	0.005
Whole grain (g/day)	23.1 ± 37.6	63.5 ± 47.9	0.000	21.8 ± 32.2	39.1 ± 42.3	0.046	0.872	0.089
Nuts (g/day)	4.3 ± 11.8	6.8 ± 5.9	0.189	4.4 ± 12.4	2.3 ± 6.0	0.367	0.980	0.164
Legumes (g/day)	20.5 ± 42.5	13.7 ± 34.2	0.489	33.4 ± 45.4	20.0 ± 51.3	0.656	0.772	0.230
Red meat (g/day)	65.1 ± 46.1	21.9 ± 31.5	0.000	69.0 ± 53.1	49.0 ± 52.1	0.041	0.731	0.077
Fish (g/day)	23.7 ± 31.2	51.3 ± 44.7	0.001	23.3 ± 28.7	17.5 ± 22.1	0.250	0.957	0.007
Red wine (g/day)	39.8 ± 64.9	121.7 ± 25.4	0.001	13.2 ± 30.5	16.9 ± 28.4	0.588	0.023	0.000

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA using the intervention as the main fixed covariate; data represent means ± SD; [d] x ± SD (all such values)

Intakes of total fat and MUFA increased in the intervention group through the increased consumption of olive oil, by the daily addition of virgin olive oil in their salads and of olive oil-enriched margarine. Increased fiber intake resulted from the consumption of 2 salads, >3 fruit, concentrated fruit juice, and whole-grain products on a daily basis. Increase in MUFA and fiber is in accordance with the increased intake of olive oil and fiber-rich food items, such as vegetables and whole grains.

Increased total dietary antioxidant intake in the intervention group, as was also observed in the analysis by gender, resulted from the increase in the consumption of food items rich in antioxidants, that is, olive oil, fruits, fruit juice, vegetables, whole grains and red wine. Vitamin C was also increased in the intervention group. The increase in alcohol intake could be the result of the increased red wine intake, since this was the main alcoholic beverage consumed during the intervention period. Such an increase was also shown from the between-group analysis by gender. Red wine is rich in polyphenols [17] and may affect the total antioxidant intake values. In contrast, the reduction in SFA intake could be the result from the instructions for less consumption of red meat, full-fat dairy products, and butter in both groups. The lack of change in PUFA intake was probably due to the lack of special advice in neither group regarding the consumption of other vegetable oils, apart from olive oil, and nuts. Furthermore, it seems that the increase in almond consumption in the intervention group did not have an overall effect on PUFA intake. According to the results by gender analysis for nutrient intake and food consumption, women seem to have better compliance than men, but this does not reflect major differences in plasma TAC, which may be due to inadequate sample size for performing subgroup analysis.

The improvement in the overall dietary intake and the increase in total dietary antioxidant intake in the intervention group could have been enhanced from the close dietetic supervision that increases compliance of the subjects. Similar results, regarding the positive results of individual motivational interventions together with free provision of Mediterranean diet foods, were observed in the PREDIMED study [13]. Similarly, an improvement in the dietary intake after close dietetic supervision was also observed in a nutritional intervention, promoting the Mediterranean food pattern in healthy women from the Quebec City Metropolitan area within the context of MARGARIN (Mediterranean Alpha Linolenic enRiched Groninger dietary Intervention) project [18, 19]. In particular, a posted leaflet available to participants was not as effective as intensive dietary counseling in group sessions regarding changing dietary behavior [18]. Furthermore, in another nutritional intervention for promoting the Mediterranean diet, close dietetic supervision was associated with a decrease in oxidized LDL concentration in healthy French Canadian women [19]. Additionally, in a nutrition intervention based on a hypocaloric diet with guidelines for adherence to a Mediterranean dietary pattern, the role of the dietitian in explaining the detailed meal plan for 8 weeks and by asking for weekly visits that included reinforcement messages to ensure compliance resulted in improved dietary intake of the subjects, as well as improved pro-inflammatory markers and some metabolic syndrome features–related measurements [20].

Our study also showed that, following a 2-month intervention period, plasma TAC significantly increased, using the ORAC assay, and there was a trend toward also an increase, using the FRAP assay, in the intervention group compared to the control group. An increase in

Table 6 Food consumption before and after 2 months of dietary intervention by gender

	Intervention group			Control group			Baseline comparisons	Between-group comparison
	Baseline	2 months after	P^a	Baseline	2 months after	P^a	P^b	P^c
Females	($n = 18$)			($n = 21$)				
Olive oil (g/day)	20.8 ± 14.6[d]	50.2 ± 7.4	<0.001	16.3 ± 14.5[d]	20.3 ± 20.0	0.456	0.379	<0.001
Fruits (g/day)	175.3 ± 199.2	242.0 ± 183.5	0.284	153.8 ± 135.2	177.3 ± 134.1	0.620	0.710	0.307
Fruit juice (ml/day)	59.1 ± 128.2	117.6 ± 63.1	0.142	30.7 ± 53.8	66.1 ± 95.5	0.196	0.380	0.067
Vegetables (cups/day)	1.7 ± 1.1	2.2 ± 1.2	0.151	1.65 ± 1.0	1.7 ± 1.0	0.834	0.912	0.370
Whole grain (g/day)	12.3 ± 21.8	36.7 ± 33.1	0.012	17.3 ± 19.9	31.1 ± 29.7	0.163	0.495	0.966
Nuts (g/day)	6.7 ± 15.1	5.3 ± 0.7	0.738	0.2 ± 0.8	2.6 ± 6.0	0.099	0.063	0.020
Legumes (g/day)	19.6 ± 36.4	8.8 ± 36.4	0.430	23.4 ± 44.5	16.6 ± 43.4	0.664	0.789	0.545
Red meat (g/day)	43.5 ± 38.0	8.2 ± 14.5	0.003	52.7 ± 39.7	43.1 ± 51.8	0.436	0.501	0.057
Fish (g/day)	22.8 ± 31.9	50.1 ± 38.6	0.058	28.8 ± 28.4	18.4 ± 18.6	0.130	0.563	0.100
Red wine (g/day)	8.6 ± 22.6	126.0 ± 22.7	<0.001	5.9 ± 19.2	15.9 ± 29.9	0.257	0.704	0.000
Males	($n = 23$)			($n = 20$)				
Olive oil (g/day)	15.5 ± 10.9	51.5 ± 10.9	<0.001	28.7 ± 27.1	30.0 ± 29.9	0.885	0.035	0.368
Fruits (g/day)	153.4 ± 157.5	234.3 ± 123.5	0.009	268.9 ± 267.9	208.1 ± 183.1	0.395	0.085	0.327
Fruit juice (ml/day)	100.6 ± 187.2	159.1 ± 116.8	0.101	62.6 ± 147.9	99.6 ± 154.6	0.245	0.473	0.240
Vegetables (cups/day)	1.7 ± 1.2	3.0 ± 1.4	0.001	1.5 ± 0.9	1.6 ± 1.0	0.687	0.614	0.007
Whole grain (g/day)	29.4 ± 43.6	79.1 ± 48.7	0.000	26.5 ± 41.5	47.4 ± 51.9	0.158	0.830	0.151
Nuts (g/day)	2.9 ± 9.4	7.6 ± 7.3	0.010	8.7 ± 16.9	1.9 ± 6.0	0.130	0.159	0.988
Legumes (g/day)	21.3 ± 48	17.8 ± 32	0.339	23.5 ± 47.0	36.9 ± 56	0.339	0.880	0.318
Red meat (g/day)	77.7 ± 46.4	29.8 ± 36.0	0.000	86.2 ± 60.7	55.1 ± 53.2	0.049	0.607	0.170
Fish (g/day)	24.3 ± 31.4	51.9 ± 48.6	0.007	17.5 ± 28.9	17.6 ± 25.7	0.998	0.475	0.031
Red wine (g/day)	57.9 ± 74.6	119.2 ± 27.1	0.002	20.8 ± 38.1	18.1 ± 27.4	0.809	0.055	0.000

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA using the intervention as the main fixed covariate; data represent means ± SD; [d] x ± SD (all such values)

plasma TAC was also observed in another study [21], with healthy volunteers on a low phenolic diet, after drinking red wine for a week in comparison with the week they were drinking water. Therefore, the increase in red wine consumption observed in our study, as mentioned previously, could partly explain the increased plasma TAC observed, along with the increase in the concentrated fruit juice, vegetable and olive oil consumption. Unfortunately, we did not observe a correlation between total dietary antioxidant intake and plasma TAC. This could be possibly and at least partly explained by the fact that the role of diet rich in antioxidants still remains unclear in the regulation of antioxidants in plasma [22]. In addition, since the USDA ORAC database is not complete, this could be a case of possibly underestimating the total dietary antioxidant intake calculated in our study.

Until today, there are many observational and clinical studies investigating the influence of individual food items, rich in antioxidants, such as fruits and vegetables on the plasma/serum concentration of antioxidants or TAC values in healthy subjects. These studies have indicated an increase in plasma antioxidants after the consumption of such foods [23–27]. Studies on Mediterranean diet though, as a whole, regarding its influence on plasma antioxidants, are limited. In the study of Leighton et al. [28], in healthy volunteers, the Mediterranean diet supplemented with red wine (during the second month) was compared with a high-fat diet supplemented with red wine, and after 3 months, the Mediterranean diet resulted in increased plasma TAC. In the PREDIMED trial, a randomized dietary trial that was carried out in high-cardiovascular-risk patients, a Mediterranean diet, especially rich in virgin olive oil, was found to be associated with higher plasma antioxidant capacity [12]. The results of the above study seem to be reinforced from the results of another study [29] that showed a relation between dietary TAC and a number of metabolic syndrome variables, such as systolic blood pressure, serum glucose and free fatty acids.

Regarding ORAC and FRAP assays used in our study, ORAC assay showed stronger results compared with FRAP assay. Similarly, Rautiainen et al. [30], using these methods for the validation of an FFQ-based TAC estimates with one measurement on plasma TAC, showed that there was no correlation between any of the FFQ-based TAC and the

Table 7 Total dietary antioxidant intake and plasma total antioxidant capacity, before and after 2 months of dietary intervention, for the overall sample

	Intervention group ($n = 41$)			Control group ($n = 41$)			Baseline comparisons	Between-group comparison
	Baseline	2-months after	P^a-value	Baseline	2-months after	P^a-value	P^b	P^c
ORAC (μmol TE/day)	6,752 ± 5,049[d]	14,753 ± 3,799	<0.001	5,987 ± 4,586	6,166 ± 3,281	0.773	0.433	<0.001
Plasma ORAC μmol TE/L	12,615 ± 2,073	13,774 ± 2,358	0.036	12,220 ± 2,808	12,330 ± 3,178	0.844	0.527	0.039
Plasma FRAP μmol $FeSO_4$/L	495 ± 234	508 ± 116	0.732	459 ± 277	423 ± 132	0.432	0.516	0.077

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA using the intervention as the main fixed covariate; [d] data represent means ± SD

Table 8 Total dietary antioxidant intake and plasma total antioxidant capacity, before and after 2 months of dietary intervention, by gender

	Intervention group			Control group			Baseline comparisons	Between-group comparison
	Baseline	2-months after	P^a-value	Baseline	2-months after	P^a value	P^b	P^c
Females	($n = 18$)			($n = 21$)				
ORAC (μmol TE/day)	6,066 ± 4,112[d]	13,892 ± 2,799	<0.001	4,839 ± 2,378	5,631 ± 3,480	0.028	0.262	<0.001
Plasma ORAC μmol TE/L	11,973 ± 1,907	13,696 ± 2,816	0.099	12,304 ± 2,754	12,439 ± 3,497	0.840	0.683	0.545
Plasma FRAP μmol $FeSO_4$/L	460 ± 200	501 ± 81	0.488	390 ± 197	409 ± 146	0.668	0.296	0.063
Males	($n = 23$)			($n = 20$)				
ORAC (μmol TE/day)	7,192 ± 4,488	15,304 ± 4,284	<0.001	7,220 ± 5,942	6,728 ± 3,044	0.632	0.985	0.001
Plasma ORAC μmol TE/L	13,026 ± 2,107	13,824 ± 2,075	0.211	12,131 ± 2,933	12,214 ± 2,892	0.928	0.240	0.020
Plasma FRAP μmol $FeSO_4$/L	518 ± 255	513 ± 135	0.917	531 ± 331	438 ± 118	0.255	0.883	0.547

[a] *P*-values derived from Student's paired *t* test; [b] *P*-values derived from unpaired *t* test; [c] *P*-values derived from repeated-measures ANOVA using the intervention as the main fixed covariate; [d] data represent means ± SD

plasma TAC using FRAP assay, whereas plasma TAC using ORAC assay correlated positively modestly with FFQ-based TAC. The mechanism by which plasma antioxidant capacity is influenced by a Mediterranean-type diet, rich in antioxidant, has, to our knowledge, not been studied before. However, it is plausible that more than one mechanism as well as synergistic effects may lead to the observed increase in plasma TAC. In particular, our study showed an increase, both in total dietary antioxidant intake and in plasma TAC, mainly through an increase in using ORAC assay. This difference between results obtained from the two assays may suggest that the observed increase in TAC may occur through different mechanisms. For example, FRAP assay measures compounds that exert antioxidant effects through electron transfer reactions, thus it measures compounds that have reducing properties, such as ascorbic acid or some phenolic compounds although it does not measure low-molecular-weight SH-group–containing antioxidants [31]. On the other hand, the ORAC assay is suitable for measuring water- and lipid-soluble antioxidants, such as β-carotene, phenolic acids, flavonoids and vitamin C [32]. These antioxidants are found in fruits and vegetables, nuts, wine and virgin olive oil that are the main characteristic foods of a Mediterranean diet [33]. Furthermore, such a diet, which is rich in antioxidants, might provide an adequate amount of antioxidants to cover the even higher needs of patients with abdominal obesity where inflammation process exists [10, 34].

Some underreporting may be a weakness of our study [35]. However, we did not observe very low energy intake,

and consequently none of the subjects recruited were excluded from the study.

In conclusion, we demonstrated that consumption of a Mediterranean-type diet according to a specific food plan, together with the distribution of free products relevant to Mediterranean diet and dietetic supervision for 2 months, increases total dietary antioxidant intake and plasma TAC. This study provides further evidence to recommend Mediterranean diet as a useful tool against CVD and metabolic syndrome, through its high content of antioxidants.

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Conflict of interest The authors declare that they have no conflict of interest.

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