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ORIGINAL ARTICLE

A 3 years follow-up of a Mediterranean diet rich in virgin olive oil is associated with high plasma antioxidant capacity and reduced body weight gain

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Background/Objectives: The aim of this study was to analyze the influence of a Mediterranean dietary pattern on plasma total antioxidant capacity (TAC) after 3 years of intervention and the associations with adiposity indexes in a randomized dietary trial (PREDIMED trial) with high cardiovascular risk patients.
Subjects/Methods: 187 subjects were randomly selected from the PREDIMED-UNAV center after they completed 3-year intervention program. Participants were following a Mediterranean-style diet with high intake of virgin olive oil or high intake of nuts, or a conventional low-fat diet. Adiposity indexes were measured at baseline and at year 3. Plasma TAC was evaluated using a commercially available colorimetric assay kit.
Results: Plasma TAC in the control, olive oil and nuts groups was 2.01 ± 0.15, 3.51 ± 0.14 and 3.02 ± 0.14 mM Trolox, respectively after adjusting for age and sex. The differences between the Mediterranean diet and control groups were statistically significant ($P < 0.001$). Moreover higher levels of TAC were significantly associated with a reduction in body weight after 3 years of intervention among subjects allocated to the virgin olive oil group (B = −1.306; 95% CI = −2.439 to −0.173; $P = 0.025$, after adjusting for age, sex and baseline body mass index).
Conclusions: Mediterranean diet, especially rich in virgin olive oil, is associated with higher levels of plasma antioxidant capacity. Plasma TAC is related to a reduction in body weight after 3 years of intervention in a high cardiovascular risk population with a Mediterranean-style diet rich in virgin olive oil.
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Introduction

An imbalance between tissue-free radicals, reactive oxygen species (ROS) and antioxidants causing oxidative damage might be a major mechanism underlying obesity-related comorbidities (Higdon and Frei, 2003). Numerous studies have found elevated oxidative stress biomarkers in obesity (Keaney *et al*., 2003), and have suggested that oxidative stress may be the linking mechanism in the pathway leading from obesity to obesity-related diseases (Higdon and Frei, 2003; Morrow, 2003). In this sense, a number of pathways capable of generating injury-inducing ROS are known to be present in obesity, including lipoprotein oxidation, increased production of cytokines, upregulation of nicotinamide adenine dinucleotide phosphate oxidase(s) and other oxidative enzymes present in vascular tissue. In addition, if obesity is a condition of increased oxidative stress, obese individuals may benefit from a better antioxidant status (Morrow, 2003). Therefore an antioxidant treatment should delay or prevent obesity phenotypes and obesity-related diseases.

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The Mediterranean dietary pattern has been associated with a lower risk of obesity or weight gain (Schroder *et al*., 2004; Mendez *et al*., 2006; Sanchez-Villegas *et al*., 2006) and also with lower cardiovascular morbidity and mortality (Trichopoulou *et al*., 2003; Knoops *et al*., 2004; Martinez-Gonzalez and Sanchez-Villegas, 2004; Sanchez-Tainta *et al*., 2008; Sofi *et al*., 2008). Current studies have suggested that this protective effect may be related to a decrease in oxidative stress mediated by the antioxidant capacity of the diet (Visioli and Galli, 2001; Pitsavos *et al*., 2005; Fito *et al*., 2007; Dai *et al*., 2008). Some authors have underlined the idea that is preferable to analyze the whole dietary pattern rather than single components thought to be antioxidants (Martinez-Gonzalez and Sanchez-Villegas, 2004). In this context, there are studies that analyzed the adherence to a Mediterranean diet and its effects on plasma antioxidant capacity, however, to the best of our knowledge, there are no randomized controlled intervention studies assessing the effect of this dietary pattern on plasma total antioxidant capacity (TAC) and adiposity indexes.

The assessment of TAC considers the cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated approach rather than the simple sum of measurable antioxidants. With this approach the capacity of both known and unknown antioxidants and their synergistic interaction is, therefore, included, and provides a better insight into the delicate *in vivo* balance between cellular oxidants and antioxidants (Serafini and Del Rio, 2004).

In the frame of a randomized dietary trial assessing the effect of a Mediterranean-style diet for primary cardiovascular prevention among high cardiovascular risk patients (PREDIMED), this substudy was aimed to analyze the effect of this dietary pattern on plasma TAC after 3 years of intervention and the association between the dietary pattern and its antioxidant capacity with adiposity indexes.

Subjects and methods

Study design

The PREDIMED study is a large, parallel-group, multicenter, randomized controlled, 4-year clinical trial aimed to assess the effects of the traditional Mediterranean diet (TMD) on the primary prevention of cardiovascular disease. The methods of this trial have been described in detail elsewhere (Estruch *et al*., 2006; Zazpe *et al*., 2008).

The inclusion criteria were either diabetes mellitus type II or at least three of the following risk factors: current smoking, hypertension, hyperlipidemia, high-density lipoprotein cholesterol <1.034 mmol/l, overweight/obesity or family history of premature coronary heart disease.

1055 subjects, at high cardiovascular risk, were recruited in the AP-UNAV center of the PREDIMED trial, and were randomly assigned to three intervention groups: TMD + free provision of extra virgin olive oil (VOO); TMD + free provision of nuts; and a low-fat diet. All participants provided informed consent and the protocol was approved by the institutional review boards according to the Declaration of Helsinki Principles.

Participants

This study assesses the effects of the intervention after 3 years of recruitment. The population sample consisted on 187 subjects (59 control, 65 TMD + VOO and 63 TMD + Nuts subjects) randomly selected within those who had been 3 years in the intervention program.

Dietary assessment

The dietary habits of the participants, both at baseline and after follow-up for 36 months, were assessed using a semiquantitative 137-item Food Frequency Questionnaire previously validated in Spain (Martin-Moreno *et al*., 1993).

After the screening visit, and based on a baseline short (14-item) questionnaire specifically targeted to assess adherence to the Mediterranean diet (Martinez-Gonzalez *et al*., 2004; Zazpe *et al*., 2008; Razquin *et al*., 2009), each participant was given personalized dietary advice by the dietician during a 30-min session. Participants allocated to a low-fat diet were advised to reduce all types of fat and were given written recommendations according to American Heart Association guidelines (Krauss *et al*., 2000). The TMD participants received instructions directed to upscale the TMD 14-item score, including (1) the use of olive oil for cooking and dressing; (2) increased consumption of vegetables, nuts, and fish products; (3) consumption of white meat instead of red or processed meat; (4) preparation of home-made sauce by simmering tomato, garlic, onion and aromatic herbs with olive oil to dress vegetables, pasta, rice and other dishes; and (5) for alcohol drinkers, to follow a moderate pattern of red wine consumption. No energy restrictions were suggested for the TMD groups. Participants in the TMD groups were given free VOO (15 liter for 3 months) or sachets of walnuts, hazelnuts and almonds (1350 g of walnuts (15 g per day), 675 g of hazelnuts (7.5 g per day) and 675 g of almonds (7.5 g per day), for 3 months). To improve compliance and account for family needs, participants in the corresponding TMD groups were given excess VOO or additional packs of nuts. One week after a participant's inclusion, 1-h group session (up to 20 participants) for each TMD group was held by the dietician. Each session consisted of an informative talk and written material with elaborated descriptions of typical Mediterranean foods, seasonal shopping lists, meal plans and recipes. All participants had free and continuous access to their dietician throughout the study (Fito *et al*., 2007; Zazpe *et al*., 2008; Corella *et al*., 2009).

Outcome measures

Anthropometric data were obtained by standardized methods (Estruch *et al*., 2006).

The samples were obtained from overnight fasting peripheral blood. Plasma TAC was measured with a colorimetric test (Cayman Chemical Corporation, Ann Arbor, MI, USA) on plasma samples. It is based on the determination of antioxidant capacity (measured as the ability of inhibiting the oxidation of ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) by metmyoglobin) of both aqueous and lipid-soluble antioxidants by comparison with that of Trolox, a water-soluble tocopherol analogue (TEAC).

Statistical analysis

The Kolmogorov–Smirnov test was used to determine variable distribution. Descriptive analyses of variables between the three interventional groups were performed using parametric tests (Student's *t*-tests, analysis of variance followed by Bonferroni's *post hoc* tests). Assuming two-tailed α error of 0.05 and a minimum difference between group of 0.8 mM Trolox, with two equally sized groups ($n=50$) the statistically power would be 1.0 (100%).

Means of TAC were also compared among the three randomized groups using general linear models adjusting for age and sex.

Multiple linear regression models were used to analyze the effects of diet on plasma TAC levels and the effects of these levels on changes on adiposity indexes after 3 years of nutritional intervention.

Results

Characteristics of participants according to the nutritional group at baseline and after 3 years of intervention are presented in Table 1. Although the baseline body weight and waist circumference were significantly different in these subsamples between control and VOO groups ($P=0.015$ and 0.005, respectively), when they were adjusted for height (body mass index (BMI) and waist to height), no statistically significant differences were found.

The measurement of plasma TAC after 36 months of intervention showed that both Mediterranean diet groups presented significantly higher levels of this antioxidant capacity parameter compared to control subjects ($P<0.001$). Moreover participants in the TMD + VOO tended to exhibit a slightly higher TAC than those in the TMD + Nuts group ($P=0.066$). When these values were adjusted for age and sex in a general linear model, the differences between control and Mediterranean diet groups remained statistically significant, and the TMD + VOO presented significantly higher levels of TAC when compared to TMD + Nuts group ($P=0.048$).

To analyze the effects of the diet on TAC levels, we investigated the effectiveness of the nutritional intervention. For this purpose the macronutrient distribution and the intake of some specific food items of the TMD (VOO and nuts) were analyzed and were compared with the control group (advised to follow a low-fat diet) (Table 2). We observed that the distribution of macronutrient intake was significantly different among the three groups (Table 2). The control group had the highest protein and carbohydrate intake, whereas TMD subjects had the highest intake of mono- and polyunsaturated fat but not of saturated fat. Moreover, we confirmed that the highest intake of VOO was present in the TMD + VOO group ($P<0.001$), and that the Nuts group had also significantly higher intake

Table 1 Characteristics of the population according to the nutritional intervention group

	Control (n = *59*)	*Virgen olive oil* (n = *65*)	*Nuts* (n = *63*)
Baseline			
Age	69.00 ± 5.94	67.48 ± 5.82	68.40 ± 5.82
Sex (% females)	54	52	46
Weight (kg)	71.98 ± 11.59	78.46 ± 12.11[a]	74.10 ± 9.80
BMI (kg/cm^2)	28.55 ± 3.36	29.96 ± 2.96	28.95 ± 2.93
Waist circumference (cm)	93.79 ± 9.78	98.83 ± 10.14[b]	96.67 ± 9.30
Waist to height	0.59 ± 0.05	0.61 ± 0.05	0.60 ± 0.05
3 years			
3-year weight change (kg)	0.36 ± 3.49	0.10 ± 5.11	−0.02 ± 3.18
3-year waist change (cm)	0.11 ± 4.56	−0.63 ± 4.76	−0.23 ± 3.60
Plasma TAC (mM Trolox)[c]	2.05 ± 0.97[d]	3.49 ± 1.08[e]	3.03 ± 0.90
Plasma TAC (mM Trolox)[f]	2.01 ± 0.15[d]	3.51 ± 0.14[g]	3.02 ± 0.14

Abbreviations: BMI, body mass index; TAC, total antioxidant capacity.
The data are presented as mean ± standard deviation, except when indicated.
[a]The differences between control and virgin olive oil groups were statistically significant ($P=0.015$).
[b]($P=0.005$).
[c]Unadjusted means.
[d]The differences between control and virgin olive oil or nuts group were statistically significant ($P<0.001$).
[e]The differences between virgin olive oil and nuts groups tended to be statistically significant ($P=0.066$).
[f]Means adjusted for age and sex.
[g]The differences between virgin olive oil and nuts groups were statistically significant ($P=0.048$).

Table 2 Distribution of macronutrients and Mediterranean diet specific nutrients after 3 years of nutritional intervention according to the nutritional group

	Control (n = 59)[a]	*TMD + VOO (n = 65)*[a]	*TMD + Tree nuts (n = 63)*[a]	P-*values for the between-group differences*		
				TMD + VOO vs control	*TMD + Nuts vs control*	*TMD + Nuts vs TMD + VOO*
Total energy intake (kcal/day)	2266.4 ± 657.1	2565.6 ± 562.2	2607.3 ± 648.1	**0.025**	**0.009**	1.000
Carbohydrates (% total energy intake)	43.69 ± 7.0	40.26 ± 5.8	37.82 ± 5.4	**0.006**	**<0.001**	0.075
Proteins (% total energy intake)	16.51 ± 2.8	15.30 ± 2.3	15.36 ± 2.2	**0.019**	**0.030**	1.000
Total fat (% total energy intake)	38.19 ± 5.9	41.84 ± 5.1	43.67 ± 5.1	**0.001**	**<0.001**	0.169
Saturated fat (% total energy intake)	9.20 ± 2.5	9.19 ± 1.7	9.64 ± 1.7	1.000	0.692	0.616
MUFA (% total energy intake)	19.60 ± 3.9	22.71 ± 3.2	23.23 ± 3.5	**<0.001**	**<0.001**	1.000
PUFA (% total energy intake)	5.77 ± 1.8	6.52 ± 1.6	7.48 ± 1.09	**0.023**	**<0.001**	**0.002**
VOO (10 g) (servings/day)	3.32 ± 2.3	6.39 ± 1.2	4.54 ± 2.8	**<0.001**	**0.008**	**<0.001**
Nuts (25 g) (servings/day)	0.15 ± 0.3	0.33 ± 0.3	0.53 ± 0.2	**0.001**	**<0.001**	**<0.001**

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; TMD, traditional Mediterranean diet; VOO, virgin olive oil.
[a]Mean ± standard deviation.
P-values in bold indicate that they are statistically significant.

Table 3 Multiple regression model to predict the plasma TAC according to age, sex and the nutritional intervention

		B *(95% CI)*[a]	P-*value*
Age	(× 1 additional year)	0.016 (−0.838 to 3.158)	0.253
Sex	Males	0 (ref.)	
	Females	−0.160 (−0.490 to 0.171)	0.341
Nutritional intervention	Control	0 (ref.)	
	Virgin olive oil	1.497 (1.095–1.900)	**<0.001**
	Nuts	1.011 (0.605–1.416)	**<0.001**

Abbreviation: B, coefficient of the multiple linear regression model.
[a]Dependent variable: total plasma antioxidant capacity (mM Trolox).
P-values in bold indicate that they are statistically significant.

Table 4 Multiple regression model assessing the association between plasma TAC and body weight changes after 3 years of nutritional intervention with a Mediterranean diet rich in VOO ($n = 65$)

		B *(95% CI)*[a]	P-*value*
Age	(× 1 additional year)	0.119 (−0.097 to 0.335)	0.274
Sex	Males	0 (ref.)	
	Females	0.735 (−1.725 to 3.195)	0.551
BMI		−0.768 (−1.170 to −0.366)	**<0.001**
TAC (mM Trolox)		−1.306 (−2.439 to −0.173)	**0.025**

Abbreviations: B, coefficient of the multiple linear regression model; BMI, body mass index; TAC, total antioxidant capacity.
[a]Dependent variable: body weight changes after 3 years of nutritional intervention (body weight at year 3-baseline body weight).
P-values in bold indicate that they are statistically significant.

of VOO than the control group ($P = 0.008$). Likewise, the highest intake of nuts was observed in the TMD + Nuts group ($P < 0.001$), having the TMD + VOO group significantly higher intake of nuts compared to the control group ($P = 0.001$).

Taking into account these data, we performed a multiple regression model to predict the plasma TAC levels at year 3 according to the nutritional group and adjusting for age and sex (Table 3). Both TMD + VOO ($B = 1.497$) and TMD + Nuts ($B = 1.011$) interventions predicted significantly higher levels of plasma TAC ($P < 0.001$) independently of sex and age.

Accordingly to our objective, we investigated whether the plasma TAC levels modified body weight or adiposity (waist circumference) after 3 years of intervention. First of all, changes in body weight were analyzed. Therefore, a multiple regression model, adjusted for sex, age and baseline BMI, for each intervention group was fitted. In Table 4, we observed that, within the TMD + VOO group, the 3-year plasma TAC was significantly associated with a reduction in

body weight change ($B=-1.306$; $P=0.025$). Moreover this model showed that the highest baseline BMI, the highest body weight change reduction ($B=-0.768$; $P<0.001$). No statistically significant association was observed within the control or TMD + Nuts group (data not shown).

When waist circumference change at year 3 was analyzed, the same decreasing tendency within VOO group was observed, although the results were not statistically significant (data not shown).

To clarify the potential explanation for the significantly higher levels of plasma TAC found in the TMD + VOO group that seemed to be leading to a reduction in weight change, we analyzed the association between VOO intake and plasma TAC levels. A statistically significant partial correlation, adjusted for age and sex, was observed between the two parameters ($r=+0.302$; $P<0.001$), showing that the higher the VOO intake, the higher plasma TAC levels.

Discussion

We have found a robust association between a nutritional intervention with a Mediterranean dietary pattern and the plasma TAC in subjects at high cardiovascular risk. This relationship was higher in subjects with higher intake of VOO. Interestingly, plasma TAC levels were associated with a reduction in weight changes after 3 years of intervention.

To the best of our knowledge, this is the first study analyzing the effects of a Mediterranean diet on plasma TAC in the context of a randomized nutritional intervention. There are short-term interventional studies analyzing the effects of the Mediterranean diet on circulating oxidative stress biomarkers, but not measuring the TAC (Hagfors *et al*., 2003; Ambring *et al*., 2004; Zulet *et al*., 2008; Puchau *et al*., 2009). Moreover, this study goes further and analyzes the influence of this antioxidant capacity of the three nutritional interventions on adiposity indexes.

The Mediterranean diet has been related to lower rates of obesity (Schroder *et al*., 2004) and cardiovascular disease (Trichopoulou *et al*., 2003; Knoops *et al*., 2004; Martinez-Gonzalez and Sanchez-Villegas, 2004; Sanchez-Tainta *et al*., 2008; Sofi *et al*., 2008). Our dietary pattern is not restrictive on the quantity of fat as other hypocaloric dietary programs directed to decrease body weight. The main characteristic of the TMD is the change in the distribution of fat (high intake of mono- and polyunsaturated fat) being the main source of fat, olive oil and especially VOO. Thus, the mechanisms that link the Mediterranean diet with lowering the risk of obesity need to be further clarified.

There are studies showing that obese subjects present high levels of oxidative stress biomarkers (Keaney *et al*., 2003), suggesting that potential therapies may act in two ways: decreasing body weight that would be accompanied by lower oxidative stress or decreasing oxidative stress what may result in lower body weight. Thus, an antioxidant treatment could be a satisfactory therapy for obesity.

There are some cross-sectional studies analyzing the antioxidant capacity and the adherence to a Mediterranean diet and reporting an association between this dietary pattern and high levels of antioxidant capacity (Lapointe *et al*., 2005; Pitsavos *et al*., 2005; Dai *et al*., 2008). There are also short-term interventional studies analyzing the Mediterranean diet and its potential association with other oxidative stress biomarkers (Hagfors *et al*., 2003; Ambring *et al*., 2004), but there are not long-term interventional studies about this issue. Our study is a 3-year randomized trial that supports the idea that a greater adherence to Mediterranean diet is associated with higher plasma antioxidant capacity (Pitsavos *et al*., 2005; Dai *et al*., 2008). It shows that both Mediterranean diet interventions, with high intake of VOO as well as high intake of nuts, presented significantly higher levels of plasma TAC compared to a control group following a low-fat diet.

Another important finding is the significant correlation found between the intake of VOO and plasma TAC in our population. Related to this result, some authors highlighted the idea that VOO is important in the antioxidant capacity of Mediterranean diet (Perez-Jimenez *et al*., 2005; Mataix *et al*., 2006), explained by the fact that VOO is rich in monounsaturated fatty acids and polyphenols that have high antioxidant activity.

On the other hand, we observed that the TMD + Nuts group presented higher plasma TAC compared to control group levels as reported in previous studies (Torabian *et al*., 2009). However, the supplementation with nuts is not equally effective to increase antioxidant capacity as compared to VOO. It seems that a high intake of VOO in a Mediterranean-style diet is essential to obtain higher dietary antioxidant contribution.

Moreover, a potential connection between plasma antioxidant capacity and lower body weight is observed. In the TMD + VOO group, higher plasma TAC predicted higher reduction in body weight change. This result agrees with the potential link between oxidative stress and obesity and also with the hypothesis that decreasing oxidative stress could improve obese phenotypes. It has been suggested that inadequacy of antioxidant defenses observed in obese subjects probably begins with a low dietary intake of antioxidants and phytochemicals that possess antioxidant capacity. In fact, several studies showed that obese individuals have a lower intake of phytochemical-rich foods (fruits, vegetables, whole grains, legumes, wine, olive oil, seeds and nuts) compared with nonobese persons. In addition, phytochemical intake is inversely correlated with waist circumference, BMI and plasma lipid peroxidation in several populations (Wallstrom *et al*., 2001; Reitman *et al*., 2002; Vincent *et al*., 2007).Therefore, the intake of a Mediterranean dietary pattern rich in VOO appeared to be a good strategy to increase antioxidant capacity of the organism and thus allowing to decrease oxidative stress and adiposity.

This study has some limitations because the TAC was measured rather than a specific antioxidant biomarker

that could be more precise. Despite this, TAC was measured because it considers the single antioxidant activity as well as the synergistic interactions of the redox molecules present in complex matrixes, giving an integrated insight into the assessment of the nonenzymatic antioxidant network (Serafini *et al*., 2006). On the other hand, we did not measure baseline TAC but we could assume that there were little differences between groups due to the randomization. This study used 187 subjects at high cardiovascular risk who were a random subsample of the 1055 subjects enrolled in the PREDIMED trial in our center (AP-UNAV). Although further studies with larger sample size are necessary to corroborate these findings, we have enough power to detect statistically significant differences between groups. Participants who died during this follow-up period ($n = 40$) or failed to comply with the 3-year measurement protocol in our center ($n = 99$) were not eligible for this substudy, and, thus, the randomization advantage was not completely preserved in the subset of participants here included. However, the multivariable adjustment of estimates that we have used is very likely to correct the small potential between-group imbalances.

On the other hand, this study has several strengths. First of all, its design allows us to find results in real-life conditions such as with home-prepared foods (Fito *et al*., 2007). Moreover, the period of intervention is longer than any previous study carried out with the Mediterranean diet. Thus, the results obtained may be more accurate and valid.

In conclusion, Mediterranean diet, especially rich in VOO, is associated with higher levels of plasma antioxidant capacity. Interestingly, the antioxidant capacity is related to a reduction in body weight in a high cardiovascular risk population after 3 years of intervention with a Mediterranean-style diet rich in VOO.

Conflict of interest

The authors declare no conflict of interest.

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