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ORIGINAL ARTICLE

Effect of Mediterranean diet on lipid peroxidation marker TBARS in obese patients with OSAHS under CPAP treatment: a randomised trial

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Abstract
Purpose The aim of our study was to examine the possible effect of the Mediterranean diet on thiobarbituric acid reacting substances (TBARS) in obese patients with obstructive sleep apnoea/hypopnoea syndrome (OSAHS) who are under continuous positive airway pressure treatment.
Methods Nine hundred patients were evaluated during a 1-year period (November 2008–October 2009), and 21 obese patients who met the inclusion criteria, with moderate to severe OSAHS based on overnight attended polysomnography, were included in the study. After randomisation, 11 followed the Mediterranean diet and 10 a prudent diet for a 6-month period. TBARS were measured in serum.
Results TBARS levels decreased notably in both groups ($p<0.05$), but no difference was observed between them ($p>0.05$). There were significant differences in other characteristics. The Mediterranean diet group showed a greater reduction in weight (−10.8±3.8), body mass index (−3.9±1.6), waist circumference (−9.9±3.0) and percentage of body fat (−4.7±2.3) compared with the other group (−6.9±3.1, −2.5±1.0, −5.3±2.6 and −2.2±1.5, respectively; $p<0.05$).
Conclusions Our results showed that the Mediterranean diet did not reduce the TBARS more than the prudent diet.

Keywords OSAHS · Obesity · Lipid peroxidation · TBARS · Mediterranean diet

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Introduction

Patients with obstructive sleep apnoea–hypopnoea syndrome (OSAHS) experience cyclic alterations of arterial oxygen saturation that may represent a form of oxidative stress and lipid peroxidation [1], probably contributing to the wide spectrum of clinical features of OSAHS, including cardiovascular morbidity [2]. Oxidative stress has been independently associated with obesity in general [3] and central obesity in particular [4]. Obese patients suffering from OSAHS are likely to be at increased risk for cardiovascular diseases since the increased levels of oxidative stress stem from both obesity and from the syndrome itself could be expected to lead to endothelial dysfunction. Currently, the treatment of OSAHS is based on weight reduction and continuous positive airway pressure (CPAP); as first-line therapies, these benefit the patient by maintaining upper airway patency. There is evidence that weight loss can have a beneficial role in reducing lipid peroxidation [5] and its deleterious effects. Studies have also indicated that treatment with CPAP can attenuate lipid peroxidation in OSAHS [6–8]. Specific nutritional supplements have been proposed for the improvement of oxidative aspects of OSAHS, mostly with conflicting results [9]. To the best of our knowledge, the effect of a diet rich in antioxidants on lipid peroxidation has never been described in OSAHS patients under treatment with CPAP. Therefore, we aimed to investigate the effect of the Mediterranean diet compared to a prudent diet on lipid peroxidation, estimated using the thiobarbituric acid reacting substances (TBARS) method, in obese patients with OSAHS who were under CPAP treatment.

Material and methods

Participants

A series of consecutive patients, who were diagnosed with OSAHS by overnight attended polysomnography (PSG) in the Sleep Disorders Unit, Department of Thoracic Medicine, Medical School, University of Crete, during a 1-year period (November 2008–October 2009) were evaluated, and the study population was selected based on the following criteria. Inclusion criteria were as follows: (a) age 18–65 years, (b) body mass index (BMI) ≥30 kg/m^2 and (c) apnoea–hypopnoea index >15 events/h. Exclusion criteria were (a) diseases such as ischaemic heart disease, diabetes mellitus, thyroid disorders and malignancies; (b) upper airway surgery; (c) gestation; (d) alcoholism; (e) diet for weight reduction during the last 6 months; (f) eating habits close to the Mediterranean diet at the entry phase; (g) intake of antioxidant supplements; (h) medications affecting weight; (i) smoking; (j) therapy with sleeping pills; (k) use of anti-depressive medication and (l) BMI <30.0 kg/m^2. After the visit to the study physicians and the confirmation that the patients fulfilled the inclusion/exclusion criteria, the subjects were allocated randomly to two study groups using a computer-generated random number sequence. In view of the use of both diets, blinding of staff or patients was not considered feasible. The study flowchart is presented in Fig. 1. This study was approved by the ethical committee of the University of Crete, and all the participants provided their consent.

Intervention

Two groups of patients with moderate to severe OSAHS were formed. In both groups, the patients received CPAP therapy and lifestyle interventions. The lifestyle interventions included an initial weight reduction programme that involved increasing physical activity, mainly by walking for at least 30 min daily, and following a low-calorie diet (a

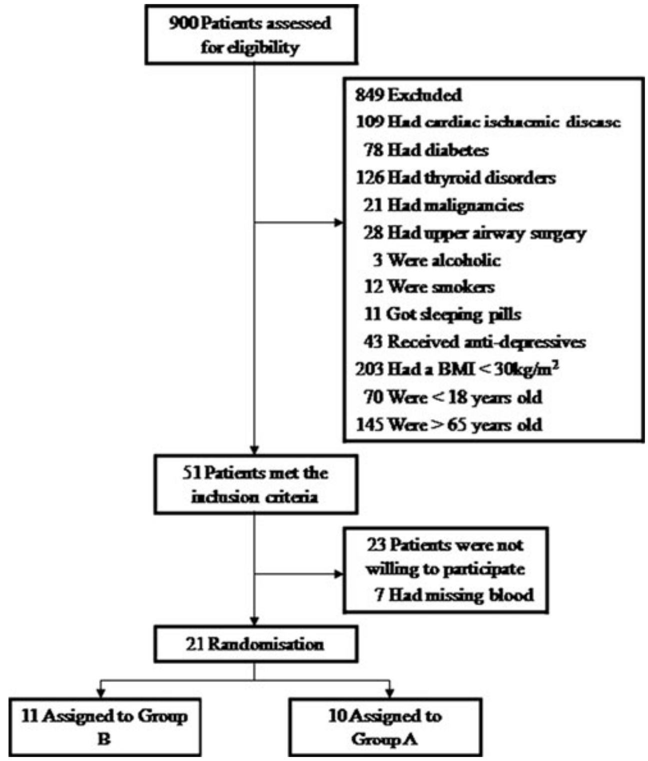

Fig. 1 Study flowchart. A total of 21 patients were enrolled: 11 were assigned to group B (following the Mediterranean diet) and 10 were assigned to group A (following a prudent diet)

prudent diet: group A or the Mediterranean diet: group B). Patients of both groups were offered a specific individualised programme and received education in reducing calorie intake. The energy intake restriction was limited to 1,500 kcal/day for women and 1,800 kcal/day for men. The general guidelines to the participants in group B were consumption of six servings per day of non-refined cereals, five or more servings per week of potatoes, five servings per day of various vegetables (two of them as salad), four servings per day of various fresh fruits, three or more servings per week of legumes, three servings per week of fish (at least one serving of fatty fish), one serving per day of nuts, three servings per week of poultry without skin, three servings per week of red meat and seven glasses each week of red wine. The recommended intake of fruits, vegetables, legumes, non-refined cereals and fish was three times higher in group B than in group A, whereas the red meat intake in group B was one third of that in group A. The moderate daily consumption of nuts and alcohol (red wine) was only recommended in group B. In both groups, the moderate consumption of olive oil was recommended because the people living on the island of Crete produce and consume this type of oil. Patients in both groups were also advised to eliminate or limit the consumption of cream, butter, margarine, carbonated and/or sugared beverages, commercial bakery products (e.g. sweet desserts, cakes, biscuits/cookies, puddings and custard), chips (French fries) or potato crisps (chips) and processed meats (i.e. burgers and sausages) and to consume two servings per day of low-fat dairy products (Table 1).

The intervention lasted for 6 months and consisted of seven visits with the study dietician. The study dietician was responsible for providing dietary and exercise counselling at each visit. Additionally compliance with CPAP therapy was monitored.

Procedures and measurements

PSG (Alice 5, Respironics) was performed in the Sleep Disorders Unit, University of Crete. Patients underwent a full diagnostic PSG study, according to standard techniques, with monitoring of the electroencephalogram (EEG) using frontal, central and occipital leads, electrooculogram, electromyogram, flow (by oronasal thermistor and nasal air pressure transducer), thoracic and abdominal respiratory effort by uncalibrated impedance plethysmography belts, oximetry and body position. Snoring was recorded by a microphone placed on the anterior neck. A single modified type II EKG lead was used for cardiac monitoring. Polysomnographic recordings were manually interpreted over 30-s periods, in accordance with the American Academy of Sleep Medicine (AASM) guidelines [10, 11]; the scorer was blinded to the PSG findings of the initial and final clinical assessment. The determination of sleep stages and arousals was performed according to the AASM 2007 criteria and by using EEG montages including frontal, central and occipital leads [11]. Hypopnoeas were scored with the "recommended" AASM hypopnoea rule that requires a ≥30% drop in the nasal pressure transducer signal and a desaturation ≥4% compared to the prior event baseline. The sleep recordings were conducted at baseline and at the end of the programme. Patients with apnoea–hypopnoea index of >15/h and Epworth sleepiness scale of >10 were assigned as having moderate to severe OSAHS and underwent a CPAP titration study.

Table 1 Recommended consumption of several food groups according to intervention groups

Food groups	Group A	Group B
Red meat (servings/week)	9	3
Poultry (servings/week)	3	3
Fish (servings/week)	1	3 (1 fatty fish)
Dairy products, low fat (servings/day)	2	2
Fruit (servings/day)	1–2	4
Vegetables (servings/day)	1.5	5
Legumes (servings/week)	1	≥3
Potatoes (servings/week)	≥5	≥5
Non-refined cereals (servings/day)	2	6
Red wine (glasses/day)	0	1–2
Daily use of olive oil	Moderate	Moderate

Group A patients receiving the prudent diet, Group B patients receiving the Mediterranean diet

Anthropometric measurements (weight, height, waist circumference (WC)) were carried out by an expert at baseline, and at each visit, a subject was defined as obese when BMI was ≥30.0 kg/m^2 [12]. Body fat was estimated by the four skinfold thickness method [13] at baseline and at the 6-month visit. Adherence to the diets was evaluated by a semi-quantitative food frequency questionnaire [14] at baseline, at the 3-month visit and at the 6-month visit. In addition, overall dietary habits were evaluated through a special diet score (MedDietScore, range 0–55) that assessed adherence to the Mediterranean dietary pattern [15]. Physical activity was assessed using the long version of the self-reported International Physical Activity Questionnaire (IPAQ) [16] at baseline, at the 3-month visit and at the 6-month visit. The continuous indicator was expressed as metabolic equivalent of the task (MET)-minutes per week derived from walking and vigorous- and moderate-intensity activities. Four millilitres of venous blood was also obtained from each patient in the morning (7 a.m.) after an overnight fast at baseline and at the 6-month visit. The concentration of TBARS, reflecting lipid peroxidation products, was determined following a previously published method with a slight modification [17]. Briefly, serum, spiked solutions or standard (400 μL) was mixed with 50 μL butylated hydroxytoluene in ethanol (concentrations ranging from 0 to 1 μmol/L) and 400 μL orthophosphoric acid (0.2 mol/L) in test tubes and stirred well. Fifty microlitres of thiobarbituric acid reagent (0.11 mol/L prepared by adding 800 mg of TBA in 50 mL NaOH 0.1 M) was added and again stirred well. The reaction mixture was incubated at 90°C for 45 min in a water bath. Then the test tubes were put on ice to stop further reaction. TBARS were extracted once with 1,000 μL *n*-butanol. To facilitate phase separation, 50-μL saturated NaCl solution was added, and the test tubes were centrifuged at 4,000 rpm for 5 min. Absorption of the upper butanol phase was read at 535 and 572 nm to correct baseline in a UV spectrometer (Shimadzu UV-1800). The range of the standard curve was 0–1.0 μmol/L. The within-day relative standard deviation (in percent) was determined by preparing and extracting five spiked blood samples at three concentration levels (0.1, 0.25 and 1 μmol/L) and measuring them during one working day. Within-day precision was estimated to be 19.5%, 6.6% and 2.2% at concentration levels of 0.1, 0.25 and 1 μmol/L, respectively. When the TBARS assay was performed, patients were not on CPAP. Participants were also questioned regarding their smoking status.

Statistical analysis

Mean values and standard deviations are used to describe the baseline characteristics of the two treatment groups. The Student's *t* test was used to evaluate differences between the treatment groups. Changes in sleep recordings and anthropometric measures during the follow-up were calculated by subtracting the baseline measurement from the 6-month follow-up measurement, and the mean difference in changes between the treatment groups was calculated. The statistical significance of between-group differences in changes was assessed with the Student's *t* test. A paired sample *t* test was performed to test the changes in the above measurements for each group independently, using the Statistical Package for Social Sciences version 18.0. A *p* value less than 0.05 was considered significant.

Results

Twenty-one patients were randomised to group A ($n=10$) or group B ($n=11$). All of them successfully completed the study. After CPAP initiation, patients were followed up by the Sleep Disorders Unit CPAP clinic. The hours per day and percentage of days that CPAP was used were monitored (IC card, Respironics, Inc., Murrysville, PA, USA) at the monthly clinical assessment. Patients exhibited good compliance, using CPAP >4 h/day and >5 days/week [18].

During the selection of the study population, 849 patients were excluded based on the above-mentioned exclusion criteria. Another 23 patients refused to participate in the intervention programme and seven had a missing blood sample (Fig. 1).

In general, the studied patients were middle aged (48.1± 12.4 years); the majority were men (80.96%) with moderate to severe OSAHS and a BMI of 36.6±3.7 kg/m^2. Table 2 depicts the changes in sleep and anthropometric parameters for each group after 6 months of intervention. Apart from the lowest SaO_2, weight, BMI, WC and percentage of body fat, which improved in both groups, group B also showed improvements in apnoea–hypopnoea index, desaturations per hour, mean oxygen saturation, MedDietScore and MET-minutes per week. Lipid peroxidation showed a decrease in both groups with a change of TBARS from 0.8±0.6 to 0.3 ±0.2 in group A ($p<0.05$) and 0.9±0.6 to 0.4±0.3 in group B ($p<0.05$). However, no significant difference was observed in TBARS between the two groups ($p>0.05$), as shown in Table 3. On the other hand, there were some notable differences with group B showing more improvement in other characteristics: weight, BMI, WC, percentage of body fat, MET-minutes per week ($p<0.05$) and MedDietScore ($p<0.001$; Table 2). More specifically, in group B, the change in weight was −10.8±3.8, the change in BMI was −3.9±1.6, the change in WC was −9.9±3.0 and the change in percentage of body fat was −4.7±2.3, compared with −6.9±3.1, −2.5±1.1, −5.3±2.6 and −2.2±1.5, respectively, in group A. In group B, the MedDietScore increased by 13.6±4.7 and the level of physical activity by 473.1±627.8, but in group A, both parameters decreased by −0.8±5.2 and −96.8±258.6, respectively. There was no significant difference in CPAP use for the 6 months between the treatment groups (hours per day group A, 6.03±0.9; group B, 6.4± 1.2, $p=0.47$) (days per week group A, 6.37±0.7; group B, 6.5±0.7, $p=0.48$).

Discussion

In this study, lipid peroxidation marker TBARS showed a substantial reduction in two groups of obese patients with moderate to severe OSAHS treated by CPAP and a weight reduction programme for a 6-month period. However, there was no significant difference in the change of TBARS concentrations between the two intervention groups. Numerous studies indicate that a reduced risk of various diseases is associated with a diet rich in plant foods that contain antioxidant compounds. Because the Mediterranean diet is characterised by an abundance of these foods, researchers have attributed a great deal of protective activities to the antioxidants included [19]. In the present study, patients in group B reported an increased adherence to the Mediterranean diet at the end of the intervention as indicated by the rise in the MedDietScore, whereas in group A, the same score did not change significantly since subjects were compliant to the prudent diet. Greater adherence to the Mediterranean diet (MedDietScore, 36–55) is associated with elevated total antioxidant capacity (TAC) levels [20]. Specifically, a 20% increase in the diet score, which signifies greater adherence to the Mediterranean dietary pattern, has been associated with an increase in TAC of about 6% and a 10% reduction in oxidized low-density lipoprotein-cholesterol concentrations [20]. In the present study, the increase in the MedDietScore was >20% in group B. In spite of this, no greater decrease in lipid peroxidation was noticed in group B than group A. The differences between the changes in weight, BMI, body fat as well as in abdominal fat in the two treatment groups could also lead to reduced lipid peroxidation in group B compared to group A, based on the fact that obesity, and especially central obesity, has been associated with increased levels of oxidative lipids [21]. The differences between the changes in the above anthropometric indices could be attributed to the greater adherence to dietary restrictions and the physical activity programme in the patients in group B. Potential mechanisms that could link adherence to the Mediterranean diet with better compliance

Table 2 Characteristics of intervention groups at entry to study and after 6 months

Characteristics	Group A (n=10)		Group B (n=11)	
	Baseline	After 6 months	Baseline	After 6 months
AHI (events/h)	45.8±31.5	37.2±29.2	46.6±33.9*	34.9±32.2
Desaturations/h	45.1±30.3	37.3±32.5	43.6±29.5*	32.8±26.9
$MSaO_2$	92.2±1.4	92.8±2.3	91.9±3.2*	93.6±2.2
$LSaO_2$	76.6±9.4*	79.9±9.3	77.3±7.0*	82.2±7.7
Weight (kg)	99.2±14.2**	92.3±11.9	105.2±12.0**	94.4±11.9
BMI (kg/m^2)	37.1±4.4 **	34.5±3.8	36.2±3.3**	32.4±3.2
WC (cm)	112.8±11.1 **	107.5±10.2	118.0±9.4**	108.1±9.2
BF (%)	33.5±5.8*	31.2±5.9	32.9±4.3**	28.3±4.3
MedDietScore	28.0±5.7	27.2±5.9	27.7±5.4**	41.4±2.9
MET (min/week)	2,064±1,381	1,943±1,436	1,848±1,728*	2,499±197
TBARS (μmol/L)	0.8±0.6 *	0.3±0.2	0.9±0.6*	0.4±0.3

Data are presented as mean±SD

Group A patients receiving the prudent diet, *Group B* patients receiving the Mediterranean diet, *AHI* apnoea–hypopnoea index, $MSaO_2$ mean oxygen saturation (haemoglobin), $LSaO_2$ lowest oxygen saturation (haemoglobin), *BMI* body mass index, *WC* waist circumference, *BF* body fat, *MedDietScore* Mediterranean diet score, *MET* metabolic equivalent of task, *TBARS* thiobarbituric acid reacting substances

*$p<0.05$; **$p<0.001$ [paired samples t test for within-group differences (6 months vs. baseline)]

with a weight loss programme, compared to the prudent diet used in this study, include its lower energy density [22] and also the relatively low glycaemic load [23], which along with its higher fibre [22] and water content [24] lead to increased satiation and lower calorie intakes. Moreover, the Mediterranean diet is highly palatable, which can increase its tolerance among consumers, and compliance with the Mediterranean diet has been found to be high [25]. Regarding physical activity, the higher conformance with the exercise programme by patients in the group B could be a fortuitous event, as the number of participants per group was small. In recent years, a variety of studies have shown that CPAP treatment reduces lipid peroxidation levels in OSAHS patients [6–8]. This particular type of therapy may constitute a very effective plan in combating lipid peroxidation in OSAHS patients. The net effect of Mediterranean diet on systemic oxidative stress induced by OSAHS syndrome could be investigated in a study where patients must be under no CPAP treatment. This approach, however, is definitely a malpractice as it excludes an established treatment from patients suffering a lethal disease. Perhaps patients having denied or not accepted CPAP therapy could be a candidate studied population, but this was not designed in this study.

Table 3 Comparison between intervention groups

Characteristics	Group A (n=10)	Group B (n=11)	p value
AHI (events/h)	−8.6±12	−11.7±9.9	NS
Desaturations/h	−7.8±14.8	−10.8±10.4	NS
$MSaO_2$	0.6±1.4	2.1±1.9	NS
$LSaO_2$	3.3±3.7	5.4±4.2	NS
Weight (kg)	−6.9±3.1	−10.8±3.8	*
BMI (kg/m^2)	−2.5±1.1	−3.9±1.6	*
WC (cm)	−5.3±2.6	−9.9±3.0	*
BF (%)	−2.2±1.5	−4.7±2.3	*
MedDietScore	−0.8±5.2	13.6±4.7	**
MET (min/week)	−96.8±258.6	473.1±627.8	*
TBARS (μmol/L)	−0.5±0.6	−0.6±0.4	NS

Data are presented as mean±SD

NS not significant as estimated by Student's t test, *Group A* patients receiving the prudent diet, *Group B* patients receiving the Mediterranean diet, *AHI* apnoea–hypopnoea index, $MSaO_2$ mean oxygen saturation (haemoglobin), $LSaO_2$ lowest oxygen saturation (haemoglobin), *BMI* body mass index, *WC* waist circumference, *BF* body fat, *MedDietScore* Mediterranean diet score, *MET* metabolic equivalent of task, *TBARS* thiobarbituric acid reacting substances

*$p<0.05$; **$p<0.001$ (Student's t test)

The present study has some limitations. The study is underpowered because of the small sample. The recruitment of a larger number of participants during the planned selection period was difficult in this study because of certain factors such as the characteristics of the population, the rigorous exclusion criteria and the lack of willingness of 23 patients to participate. We did not include patients with conditions that could affect the impact evaluation of the intervention. Another potential criticism is that TBARS have been claimed to be a nonspecific marker of lipid peroxidation [26]. However, it has been shown that under conditions of induced oxidative stress, which is presumably a characteristic of patients with OSA, there is a good correlation between TBARS and the levels of isoprostane, which is a more specific marker of lipid peroxidation [27]. Another concern is about the use of a subjective assessment of diet adherence and physical activity because questionnaires have shown low reliability. However, the semi-quantitative food frequency questionnaire used here has been used in the evaluation of interventions promoting healthy dietary patterns like the Mediterranean diet [28]. Moreover, even the subjective measurement of physical activity has several limitations; the long form of the IPAQ instrument has been validated against accelerometer and recommended in intervention studies [16]. Finally, the selection of all individuals with certain clinical characteristics and the exclusion of comorbidities decrease the external validity of our results. Then, the clinical application of the data should be considered with great caution as they may be valid only for patients with similar characteristics with the studied population.

In conclusion, in this randomised weight loss trial, the Mediterranean diet showed no difference in reducing the TBARS compared to a low-calorie diet based on the data presented. However, further studies with larger samples needed to confirm these observations.

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Conflict of interest The authors declare no conflict of interest.

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