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Postprandial antioxidant effect of the Mediterranean diet supplemented with coenzyme Q_{10} in elderly men and women

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Abstract Postprandial oxidative stress is characterized by an increased susceptibility of the organism towards oxidative damage after consumption of a meal rich in lipids and/or carbohydrates. We have investigated whether the quality of dietary fat alters postprandial cellular oxidative stress and whether the supplementation with coenzyme Q_{10} (CoQ) lowers postprandial oxidative stress in an elderly population. In this randomized crossover study, 20 participants were assigned to receive three isocaloric diets for periods of 4 week each: (1) Mediterranean diet supplemented with CoQ (Med+CoQ diet), (2) Mediterranean diet (Med diet), and (3) saturated fatty acid-rich diet (SFA diet). After a 12-h fast, the volunteers consumed a breakfast with a fat composition similar to that consumed in each of the diets. CoQ, lipid peroxides

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(LPO), oxidized low-density lipoprotein (oxLDL), protein carbonyl (PC), total nitrite, nitrotyrosine plasma levels, catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities and ischemic reactive hyperaemia (IRH) were determined. Med diet produced a lower postprandial GPx activity and a lower decrease in total nitrite level compared to the SFA diet. Med and Med+CoQ diets induced a higher postprandial increase in IRH and a lower postprandial LPO, oxLDL, and nitrotyrosine plasma levels than the SFA diet. Moreover, the Med+CoQ diet produced a lower postprandial decrease in total nitrite and a greater decrease in PC levels compared to the other two diets and lower SOD, CAT, and GPx activities than the SFA diet.

In conclusion, Med diet reduces postprandial oxidative stress by reducing processes of cellular oxidation and increases the action of the antioxidant system in elderly persons and the administration of CoQ further improves this redox balance.

Keywords Aging · Mediterranean diet · Coenzyme Q10 · Oxidative stress · Postprandial phase

Abbreviations

Apo	Apolipoprotein
BMI	Body mass index
CAT	Catalase
CoQ	Coenzyme Q10
GPx	Gluthatione peroxidase
H_2O_2	Hydrogen peroxide
HDL-C	HDL cholesterol
IRH	Ischemic reactive hyperaemia
LDL-C	LDL cholesterol
LPO	Lipid peroxidation products
Med diet	Mediterranean diet
Med+CoQ diet	Mediterranean supplemented with CoQ
MUFA	Monounsaturated fatty acid
NO	Nitric oxide
oxLDL	LDL oxidized
PC	Protein carbonyl
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SFA-diet	Saturated fatty acid-rich diet
SOD	Superoxide dismutase
TC	Total cholesterol
TG	Triacylglycerol

Introduction

Aging is a complex process that is manifested within an organism at genetic, molecular, cellular, organ, and system levels. Current knowledge points towards reactive oxygen species (ROS) and oxidative stress as the primary determinants of aging. Mediterranean Diet (Med diet) has been associated with a lower prevalence of coronary heart disease, metabolic disorders, and other aging-related diseases (Trichopoulou et al. 2003). The leading hypothesis on the mechanism of this association is a decrease of oxidative stress due to the antioxidant capacity of this diet (Visioli and Galli 2001).

When evaluating the influence of diet on the oxidative stress, classical studies aimed to analyze the different factors in the fasting state after different dietary models. However, fasting is not the typical physiological state of the modern human being, which spends most of the time in the postprandial state. Furthermore, there are data supporting that many of the oxidative stress variations may be expressed mainly in the postprandial state and that is, precisely in the postprandial state, where dietary interventions may show its maximal influence on oxidative status.

In line with the above argument, we have recently demonstrated that the high-monounsaturated fatty acids diet limits the postprandial oxidative stress compared with low-fat, high complex carbohydrate, and high saturated fatty acid diets in patients with metabolic syndrome (MetS) (Perez-Martinez et al. 2010). Nevertheless, there is still a lack of data in this field, and the influence of dietary interventions on postprandial oxidative status remains an active field of research, especially relating to age and age-associated diseases.

As exposed above, Med diet has been proposed as a healthy dietary model in which the main underlying mechanism for its favorable effects is the modulation of the oxidative stress. Although there are data suggesting so, there is no established body of evidence in the influence of the Med diet in the postprandial oxidative status, and clarifying this question was the main rationale for the present study.

Having in mind the above data, our hypothesis was that Med diet may provoke a substantial part of its favorable effects on the oxidative stress compared with the high saturated fatty acid diets during the postprandial state and that this hypothetical improvement could be boosted by supplementation with a natural antioxidant, like coenzyme Q_{10} (CoQ, 2,3-dimethoxy-5-methy-6-decaprenyl-1,4-benzoquinone), a natural antioxidant agent (Turunen et al. 2004).

Experimental procedures

Participants and recruitment

Volunteers were recruited using various methods, including the use of general practitioner databases, and poster and newspaper advertisements. A total of 63 persons were contacted among those willing to enter the study. All participants underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrolment and gave their informed consent before joining the study. Inclusion and exclusion criteria were fulfilled by 20 patients (age ≥65 years; 10 men and 10 women). Clinical inclusion criteria were age ≥65 years, body mass index (BMI) 20–40 kg/m^2, total cholesterol concentration equal to or <8.0 mmol/L, and non-smokers. The clinical exclusion criteria were age <65 years, diabetes or other endocrine disorders, chronic inflammatory conditions, kidney or liver dysfunction, iron deficiency anemia (hemoglobin <12 g/dL men, <11 g/dL women), prescribed hypolipidemic and anti-inflammatory medication, fatty acid supplements including fish oil, consumers of high doses of antioxidant vitamins (A, C, E, β-carotene), highly trained or endurance athletes or those who participate in more than three periods of intense exercise per week, weight change equal or >3 kg within the last 3 months, smokers, alcohol or drug abuse (based on clinical judgment). The study protocol was approved by the Human Investigation Review Committee of the Reina Sofia University Hospital, according to institutional and Good Clinical Practice guidelines.

Study design

Participants were randomly assigned to receive, in a crossover design, three isocaloric diets for a 4-week period each (Supporting Fig. S1). The three diets were as follows: (1) Mediterranean diet supplemented with coenzyme Q (Med+CoQ diet) (200 mg/day in capsules), containing 15% of energy as protein, 47% of energy as carbohydrate and 38% of total energy as fat [24% monounsaturated fatty acid (MUFA, provided by virgin olive oil), 10% saturated fatty acid (SFA), 4% polyunsaturated fatty acid (PUFA)]. (2) Mediterranean diet not supplemented with CoQ (Med diet), with the same composition of the first diet, but supplemented by placebo capsules, and (3) Western diet rich in saturated fat (SFA diet), with 15% of energy as protein, 47% of energy as carbohydrate, and 38% of total energy as fat (12% MUFA, 22% SFA, 4% PUFA).

The cholesterol intake was kept constant (<300 mg/day) during the three periods. Both the CoQ and the placebo capsules were specially produced by the same company (Kaneka Corporation, Osaka, Japan) and were identical in weight and external aspect. Patients taking capsules were unaware whether they were in the Med+CoQ or Med dietary period (Table 1). The composition of the experimental diets was calculated by using the US Department of Agriculture (1987) food tables and Spanish food composition tables for local foodstuffs (Varela 1980).

Before the start of the intervention period, volunteers completed a 3-day weighed food diary and an extensive Food Frequency Questionnaire, which allowed identification of foods to be modified. At the start of the intervention period, each patient was provided with a handbook for the diet to which they had been randomized. Advice was given on foods to choose and those to avoid if eating outside home. They were also instructed to write down in the diary about any menu eaten out of the home and to call the monitoring study nurse reporting such an event. At baseline, volunteers were provided with a supply of study foods to last for 2 weeks. They collected additional study foods every fortnight or when required. At these times, a 24-h recall of the previous day's food intake and a short food-use questionnaire based on the study foods were completed to monitor and motivate volunteers to adhere to the dietary advice. A points system was used to assess the number of food exchanges achieved in the 24-h recall and additional advice was given if either the 24-h recall or food-use questionnaire showed

Table 1 Composition of diet at end of intervention period, alongside dietary targets

	Diet		
	Med	Med+CoQ	SFA
Target (MJ/day)			
%E from fat	38	38	38
%E from SFA	<10	<10	22
%E from MUFA	24	24	12
%E from PUFA	4	4	4
End of intervention			
Number	20	20	20
Energy (MJ/day)	8.22±0.35	8.16±0.32	8.21±0.31
%E from fat	39.11±2.02	39.73±2.65	40.38±2.03
%E from SFA	8.78±0.61a	9.04±0.71a	20.75±0.87b
%E from MUFA	24.24±0.92a	24.36±1.47a	13.46±0.71b
%E from PUFA	4.15±0.23a	4.28±0.29a	3.92±0.25b
%E from CHO	44.64±1.86	44.31±2.29	43.64±1.73
%E from protein	16.33±0.52	16.07±0.64	16.16±0.53
Total α-tocopherol (mg/day)	18.91±0.73a	18.95±0.84a	6.97±0.76b
Ascorbic acid (mg/day)	187.54±5.83	181.17±6.12	195.62±6.86
β-carotene (mg/day)	3.11±0.92	3.13±0.66	3.21±0.23
Total fibre (g/day)	27.93±2.22	27.17±2.38	27.42±2.34
Cholesterol (mg/day)	345.71±35.93	345.86±36.92	378.18±59.29

Values are means±SE, n=20. Means in a row with lowercase letters without a common letter differ, $p<0.05$

CHO carbohydrate

inadequate intake of food exchange options. Volunteers were asked to complete 3-day weighed food diaries at baseline, week 2 and week 4. Weighed food intake over two weekdays and one weekend day was obtained using scales provided by the investigators. Fat foods were administered by dietitians in the intervention study. The dietary analysis software Dietsource version 2.0 was used. (Novartis S.A., Barcelona, Spain).

At the end of the dietary intervention period, the subjects were given a fatty breakfast with the same fat composition as consumed in each of the diets. Patients presented at the clinical centers at 8-h following a 12-h fast (time 0), abstained from alcohol intake during the preceding 7 days. After cannulation of a blood vessel, a fasting blood sample was taken before the test meal, which was then ingested within 20 min under supervision. The test meal reflected the fatty acid composition of each subject after the chronic dietary intervention. Subsequent blood samples were drawn at 2 and 4 h. Test meals provided an equal amount of fat (0.7 g/kg body weight), cholesterol (5 mg/kg of body weight), and vitamin A (60,000 IU/m^2 body surface area). The test meal provided 65% of energy as fat, 10% as protein and 25% as carbohydrates. The composition of the breakfasts was as follow: Med with CoQ (400 mg in capsules) breakfast (12% SFA, 43% MUFA, 10% PUFA); Med with placebo capsule breakfast (12% SFA, 43% MUFA, 10% PUFA); and SFA-rich breakfast (38% SFA, 21% MUFA, 6% PUFA).

Biochemical determinations

Plasma samples

Samples were collected in tubes containing 1 g EDTA/L or 3.8% citrate and were stored in containers with ice and kept in the dark. Particular care was taken to avoid exposure to air, light, and ambient temperature. Plasma was separated from whole blood by low-speed centrifugation at 1,500×g for 15 min at 4°C within 1 h of extraction.

Lipid analysis

The lipid variables were analyzed with a modular autoanalyzer (DDPPII Hitachi; Roche, Basel, Swit-

zerland) with the use of Boehringer-Mannheim reagents. Total cholesterol (TC) and triglycerides (TG) in plasma and lipoprotein fractions were assayed by means of enzymatic procedures. Apo A-I and Apo B were determined by inmunoturbidimetry. High-density lipoprotein-cholesterol (HDL)-C was measured by analyzing the supernatant obtained following precipitation of a plasma aliquot with dextran sulfate-Mg^{2+}. Low-density lipoprotein-cholesterol (LDL)-C levels were estimated using the Friedewald formula based on TC, TG, and HDL-C concentrations. Plasma glucose concentrations were measured with an Architect-CG16000 analyzer (Abbott Diagnostics, Tokyo, Japan) by the hexoquinase method. Plasma insulin concentrations were measured by chemoluminesence with an Architect-I2000 analyzer (Abbott Diagnostics, Tokyo, Japan).

Study of endothelial function using laser Doppler

A laser Doppler linear Periflux 5000 (Perimed S.A., Stockholm, Sweden) was used to measure ischemic reactive hyperemia (IRH). The methodology has been published previously elsewhere (Delgado-Lista et al. 2010; Fuentes et al. 2008; Ruano et al. 2005) where we found an inter-study variability of 8.85% and an intra-study variability of 8.7%.

Briefly, capillary flow of the second finger of the dominant arm of the patient was assessed for 1 min before (t0) and after (td) applying 4 min of ischemia to the arm by means of a sphygmomanometer, and the ischemic reactive hyperemia was obtained IRH= (AUC_{td} 2 AUC_{t0})×100AUC_{t0}. This calculation was carried out using the basal determinations and at 2 and 4 h after consumption of the breakfast.

Coenzyme Q determination

CoQ levels were carried out in plasma samples according to the method described by Santos-Gonzalez et al. (2007). The quantification of these substances was perfomed by reversed-phase HPLC. Separation was performed at 1 mL/min in a C18 column (5 μm particles, 5×0.45 cm) and with a mobile phase that consisted of a mixture of methanol and n-propanol (1:1) containing lithium perchlorate (2.12 g/L). Monitoring was carried out with a Coulochem II electrochemical detector (ESA, Chelmsford, MA) fitted with a Model 5010 analytical cell with the electrodes set at potentials of −500 and +300 mV. CoQ was detected from the signal obtained at the second electrode. Eluted compounds were quantified by integration of peak areas and comparison with an external CoQ_{10} standard (Sigma Aldrich, Madrid, Spain).

Determination of oxidative stress biomarkers

For the measurement of oxidized LDL (oxLDL) we used a commercial non-competitive ELISA (Mercodia, Uppsala, Sweden), intra-assay coefficient of variation of 5.5%, and inter-assay coefficient of variation of 6.2%. Oxidized apolipoprotein B-100 in the sample reacts with anti-oxidized apolipoprotein B-100 antibodies bound to microtitration wells and peroxidase-conjugated anti-oxidized LDL antibodies in the solution (Holvoet et al. 2008; Stanhope et al. 2009). The absorbance was evaluated in an EIA plate reader (DTX 880 Multimode Detector; Beckman Coulter) at a wavelength of 450 nm.

Nitric oxide (NO) is a free gas produced endogenously by a variety of mammalian cells. This molecule induces vasodilatation and inhibits platelet aggregation and adhesion to the vascular endothelium. Total nitrite (nitrite and nitrate) was used as an indicator of NO production assayed following the Griess method (Ricard-Jané D and Lopez-Tejero 2002), intra-assay coefficient of variation of 6.1%, and inter-assay coefficient of variation of 7.7%. The reaction was monitored at 540 nm (UV-1603 spectrophotometer, Shimadzu).

Lipid peroxidation is a mechanism of cellular and molecular injury. Plasmatic levels of lipid peroxidation products (LPO) were determined using the Bioxytech® LPO-586 Kit (OXIS International Inc., Portland, USA) (Erdelmeier et al. 1998; Bouhafs et al. 2003), intra-assay coefficient of variation of 5.8%, and inter-assay coefficient of variation of 7.2%. The kit uses a chromatogenic reagent which reacts with malondialdehyde+4-hydroxyalkenals. The absorbance was evaluated in a spectrophotometer (UV-1603; Shimadzu) at a wavelength of 586 nm.

Protein carbonyl (PC) content was carried out in plasma samples using the method of Levine et al. (1990), intra-assay coefficient of variation of 4.7%, and inter-assay coefficient of variation of 8.0%. The carbonyls were evaluated in a spectrophotometer (UV-1603 Shimadzu, Kyoto, Japan) at 360 nm.

Nitrotyrosine is a product of tyrosine nitration, and it may be used by an indicator of cell damage and inflammation caused by NO in cells. Nitrotyrosine was quantified by a "sandwich" enzyme-linked immunosorbent assay Nitrotyrosine-EIA (Hycult Biotech, The Netherlands), intra-assay coefficient of variation of 6.5%, and inter-assay coefficient of variation of 8.5%. A standard curve was constructed by incubating in the wells serial dilutions of 4.5 mol/L nitrotyrosine standard in duplicate. The nitrotyrosine concentrations of the samples were estimated from the standard curve (Sakano et al. 2009). The reaction was monitored at 450 nm (UV-1603 spectrophotometer, Shimadzu).

Antioxidant enzyme activities

Catalase (CAT) activity was measured spectrophotometrically (UV-1603 spectrophotometer, Shimadzu) in plasma samples according to Aebi et al. (1984) by recording the rate of decomposition of H_2O_2 at 240 nm, intra-assay coefficient of variation of 6.9%, inter-assay coefficient of variation of 7.1%. H_2O_2 (10 mM) was used as reagent. Total superoxide dismutase (SOD; E.C., 1.15.1.1) activity was determined by colorimetric assay in plasma at wavelength of 525 nm (UV-1603 spectrophotometer, Shimadzu) according to the laboratory method described by Nebot et al. (1993), intra-assay coefficient of variation of 7.3%, inter-assay coefficient of variation of 8.7%. Glutathione peroxidase (GPx; E.C., 1.11.1.9) activity was evaluated in plasma by the Flohé and Gunzler method (Flohe and Gunzler 1984; Zhu et al. 2008), intra-assay coefficient of variation of 6.5%, and inter-assay coefficient of variation of 7.2%. The GPx assay is based on the oxidation of NADPH to NAD+, catalyzed by a limiting concentration of glutathione reductase, with maximum absorbance at 340 nm. The absorbance was evaluated in a Shimadzu UV-1603 spectrophotometer (Kyoto, Japan).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS 17.0 for Windows Inc., Chicago, IL) was used for the statistical comparisons. The Kolmogorov–Smirnov test did not show a significant departure from normality in the distribution of variance values. In order to evaluate data variation, Student's *t* test and an analysis of variance for repeated measures (ANOVA) was performed, followed by Bonferroni's correction for multiple comparisons. We studied the statistical effects of the type of fat meal ingested, independent of time (represented by $p1$), the effect of time (represented by $p2$), and the interaction of both factors, indicative of the degree of the postprandial response in each group of subjects with each fat meal (represented by $p3$). Differences were considered to be significant when $p<0.05$. All data presented in text and tables are expressed as means±standard error (±SE).

Results

Lipid analysis, biochemical determination, and study of endothelial function

The baseline characteristics of the 20 participants who completed the three dietary intervention periods showed that males had higher height and waist circumference, TG, and Apo B than females. We did not find any other differences by gender (Supporting Table S1).

At the end of each period of dietary intervention fasting plasma concentrations of TC ($p<0.001$), LDL-C ($p=0.013$), ApoB ($p=0.017$), and ApoA-I ($p=0.002$) were higher after participants consumed the SFA diet than when they consumed the other diets (Table 2). Those of TG, HDL-C, glucose, insulin did not differ after the three periods but the change in IRH was greater after the Med+CoQ diet period than after the Med and SFA diet periods ($p=0.028$) (Table 2).

Differences in the parameter levels (increases or decreases) measured between time 0 and the postprandial phase are shown in Table 3. Throughout the whole period of postprandial lipemia, SFA diet induced a significantly greater postprandial decrease in plasma HDL-C levels compared to the Med and Med+CoQ diets ($p=0.019$). Changes in postprandial plasma HDL-C levels were not detected when the participants consumed the Med or Med+CoQ diets. No significant postprandial differences were observed in plasma TG, LDL-C, ApoB, ApoA-I, glucose, and insulin levels between the three diets during the postprandial phase. At 2 h after the intake of the Med and Med+CoQ diets, we observed a greater postprandial increase in IRH than after the SFA diet ($p=0.035$). This effect remained significant 4 h after consumption of the Med and Med

Table 2 Fasting plasma lipids, apolipoproteins, glucose, insulin, and PC levels and capillary flow for each intervention period

	Med diet, n=20	Med+CoQ diet, n=20	SFA diet, n=20	P
TC (mmol/L)	4.65±0.13a	4.73±0.14a	5.21±0.17b	<0.001
TG (mmol/L)	1.10±0.01	1.15±0.12	1.16±0.09	0.675
HDL-C (mmol/L)	1.31±0.06	1.34±0.06	1.40±0.07	0.069
LDL-C (mmol/L)	2.80±0.12a	2.82±0.06a	3.19±0.17b	0.013
Apo B (g/L)	0.83±0.04a	0.85±0.04a	0.91±0.05b	0.017
Apo A-I (g/L)	1.44±0.05a	1.47±0.05a	1.56±0.06b	0.002
Glucose (mmol/L)	4.97±0.12	5.06±0.17	5.15±0.22	0.440
Insulin (pmol/L)	74.12±17.15	103.32±36.02	61.92±9.96	0.470
Total nitrite (μmol/L)	64.75±9.02a	84.06±12.91b	51.41±8.01c	0.021
IRH (% baseline)	88.26±13.72a	75.94±9.92a	59.61±8.09b	0.028
PC (μmol/L×10^{-3})	4.21±0.22ab	3.16±0.13a	5.19±0.31b	0.022

All values are ±SE.
Main effect of diet by repeated measures ANOVA.
Different lowercase letters are significantly different

+CoQ diets compared with the SFA diet (p=0.023) (Fig. 1a). In addition, at 4 h after the Med+CoQ diet we observed a greater postprandial increase in IRH compared with the Med diet (p=0.017).

Metabolic parameter levels

We observed a higher fasting plasma CoQ concentration (p<0.001) after the intake of the Med+CoQ diet compared with the Med and SFA diets (Fig. 2a). At 2 and 4 h after consumption of the Med+Q diet, we observed a greater postprandial increase in plasma CoQ levels compared with the Med and SFA diets (p=0.018 and p=0.032, respectively). No significant differences were detected in postprandial CoQ levels at 2 and 4 h between the Med and SFA diets.

Fasting plasma total nitrite levels were greater after the intake of the Med+CoQ diet compared with the other diets

Table 3 Differences in the postprandial plasma lipids, apolipoproteins, glucose, and insulin levels (increases or decreases) according to the consumption of the different diets.

	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Apo A-I (g/L)	Apo B (g/L)	Glucose (mmol/L)	Insulin (pmol/L)
Δ 2 h							
Med diet, n=20	1.06±0.14	−0.060±0.03	−0.44±0.12	−0.017±0.04	−0.017±0.02	0.72±0.15 $^{\gamma}$	160.58±58.40 $^{\gamma}$
Med+CoQ diet, n=20	0.82±0.16	−0.045±0.02	−0.35±0.10	−0.037±0.04	−0.020±0.02	0.09±0.01*	132.95±56.54*
SFA diet, n=20	0.74±11.18	−0.134±0.03*	−0.34±0.09	−0.050±0.02	−0.035±0.01	0.44±0.16*	137.76±36.66*
Δ 4 h							
Med diet	0.88±0.14	−0.056±0.01a	−0.31±0.09	−0.073±0.04	−0.054±0.02	−0.15±0.09	6.24±24.61
Med+CoQ diet	0.70±0.16	−0.041±0.02a	−0.27±0.07	−0.009±0.02	−0.017±0.01	−0.71±0.28	−13.85±19.30
SFA diet	0.82±0.13	−0.211±0.05b	−0.38±0.06	−0.026±0.02	−0.030±0.01	−0.48±0.08	57.04±33.58
Global analysis							
Diet effect	0.235	0.019	0.841	0.811	0.700	0.455	0.263
Time effect	0.300	0.026	0.168	0.838	0.132	0.007	0.002
Diet×time interaction	0.348	0.031	0.552	0.074	0.091	0.941	0.557

Values are means±SE; n=20. Differences in the parameter levels between time 0 and time 2 are shown as Δ 2 h (value of time 2 minus value of time 0), and between time 0 and time 4 are shown as Δ 4 h (value of time 4 minus value of time 0). Data were analyzed using ANOVA for repeated measures. $p1$, diet effect; $p2$, time effect; $p3$, diet×time interaction. p<0.05 (ANOVA for repeated measures). Means in a row with lowercase letters without a common letter differ (diet effect)

* p<0.05, Δ 2 h vs Δ 4 h SFA-enriched diet (time effect)

*p<0.05, Δ 2 h vs Δ 4 h Med+CoQ diet (time effect)

$^{\gamma}$ p<0.05, Δ 2 h vs Δ 4 h diet (time effect)

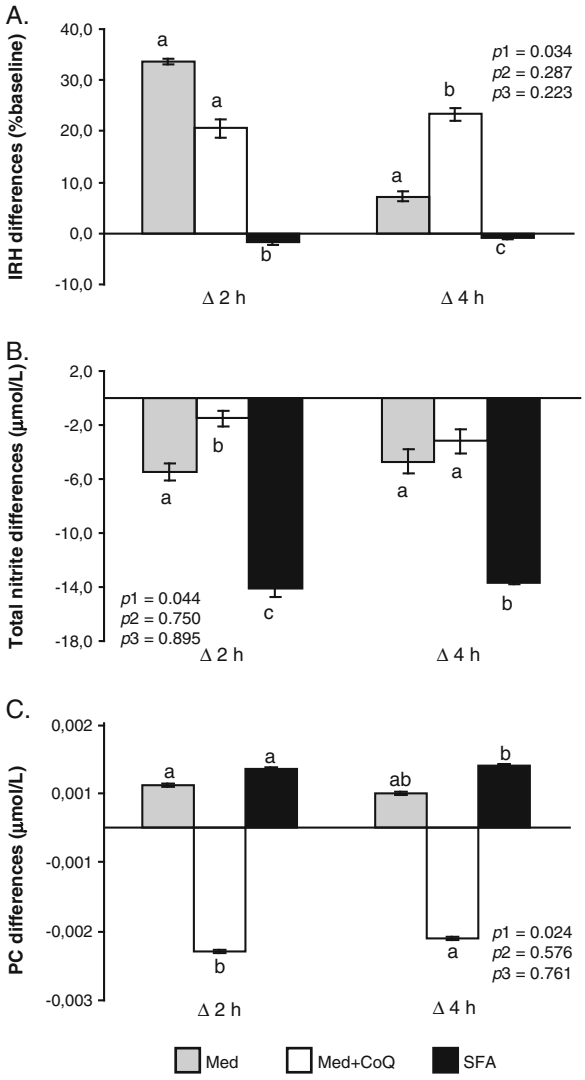

Fig. 1 Differences in **A** IRH change, **B** total nitrite levels in plasma, and **C** plasma PC levels according to the type of fat consumed during the postprandial phase. Differences in the parameter levels between time 0 and time 2 are shown as Δ 2 h (value of time 2 minus value of time 0), and between time 0 and time 4 are shown as Δ 4 h (value of time 4 minus value of time 0). Data were analyzed using ANOVA for repeated measures. All values represent the mean±standard errors (SE). *Bars* with different superscript letters depict statistically significantly differences ($p<0.05$). $p1$, diet effect; $p2$, time effect; $p3$, diet×time interaction

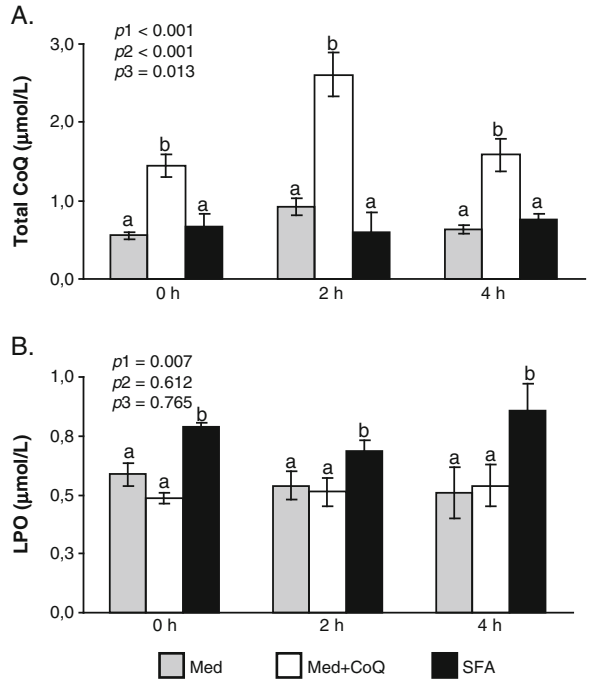

Fig. 2 Postprandial plasma levels in **A** total CoQ and **B** LPO according to the type of fat significantly differences ($p<0.05$). $p1$, diet effect; $p2$, time effect; $p3$, diet×time interaction

and were greater after intake of the Med diet that the SFA diet ($p<0.05$) (Table 2). Along the postprandial phase, SFA diet produced a significantly greater postprandial decrease in total nitrite levels in plasma compared to the Med and Med+CoQ diets ($p=0.044$). Furthermore, at 2 h after the intake of the Med diet we found a greater postprandial decrease in total nitrite levels in plasma compared to the Med+CoQ diet ($p=0.031$). No significant differences were detected in total nitrite levels at 4 h after the intake of the Med and Med+CoQ diets (Fig. 1b).

Biomarkers of oxidative stress

Fasting plasma concentration of oxLDL was lower after participants consumed the Med+CoQ diet than when they consumed the other two diet period ($p<0.001$) (Table 4). However, no significant differences were found between the three diets in postprandial plasma oxLDL levels (Table 4).

We observed lower fasting plasma PC levels ($p=0.022$) after consumption of the Med+CoQ diet as compared to the intake of the SFA diet (Table 2). At 2 h after the intake of the Med+CoQ diet, we observed a greater postprandial decrease in plasma PC levels with respect to the other diets ($p=0.011$). This effect remained significant 4 h after consumption of the Med+CoQ diet compared with the SFA diet ($p=0.013$) (Fig. 1c).

Fasting plasma LPO levels were lower after participants consumed the Med or Med+CoQ diets and fasting plasma nitrotyrosine levels were lower after participants consumed the Med+CoQ diet than when

Table 4 Postprandial plasma biomarkers of oxidative stress levels and antioxidant enzyme activities according to the type of fat consumed

	oxLDL (U/L)	Nitrotyrosine (nmol/L)	SOD activity (U/mL)	CAT activity (U/dLx10)	GPx activity (nmol/mL/min)
0 h					
Med diet, *n* =20	59.98±3.12ab	4.87±0.37ab[1]	1.45±0.08a[1]	0.68±0.12a	52.10±2.05a[1]
Med+CoQ diet, *n*=20	54.65±3.89a	4.17±0.22a[1]	0.95±0.01b[1]	0.32±0.03b	50.71±5.02a[1]
SFA diet, *n*=20	69.98±5.01b	8.38±0.38b[1]	3.08±0.43c[1]	0.87±0.17a	55.93±8.02a[1]
4 h					
Med diet	57.90±4.09a	6.45±3.11a[2]	4.71±0.48a[2]	0.71±0.12ab	70.31±3.29a[2]
Med+CoQ diet	59.76±3.26a	7.51±3.11a[2]	3.91±0.41a[2]	0.54±0.06a	60.01±3.92b[2]
SFA diet	62.69±4.53a	11.34±4.66b[2]	6.35±0.43b[2]	1.11±0.14b	91.35±6.57c[2]
Global analysis					
Diet effect	0.025	0.041	<0.001	0.002	<0.001
Time effect	0.336	0.045	<0.001	0.173	<0.001
Diet×time interaction	0.354	0.652	0.814	0.645	0.091

Means in a row with lowercase letters without a common letter differ (diet effect) and without a common number differ (time effect)

[1] Values are means±SE. All parameters were measured in plasma samples at 0 h and 4 h after consumption of the diet.

[2] Data were analyzed using ANOVA for repeated measures. *p*1, diet effect; *p*2, time effect; *p*3, diet×time interaction. $p<0.05$ (ANOVA for repeated measures).

they consumed the SFA diet ($p<0.05$) (Table 4 and Fig. 2b, respectively). At 2 and 4 h after the SFA diet, we found higher postprandial plasma LPO levels compared with the Med and Med+CoQ diets ($p=0.008$ and $p=0.003$, respectively) (Fig. 2b). In addition, at 4 h after the SFA diet, we found higher postprandial plasma nitrotyrosine levels compared with the Med and Med+CoQ diets ($p=0.041$) (Table 4).

Antioxidant enzyme activities

After intake of the Med+CoQ, fasting plasma SOD and CAT activities were lower than after intake of the other two diets. Fasting plasma SOD activity was lower after participants consumed the Med diet than when they consumed the SFA diet ($p<0.05$). There were no significant changes in fasting plasma GPx enzyme activity after intake of any of the three diets (Table 4). At the postprandial phase, we observed significantly lower plasma SOD and CAT activities at 4 h after the consumption of the Med+CoQ diet compared with the SFA diet ($p<0.001$, $p=0.002$, respectively). No significant differences were detected in postprandial plasma SOD and CAT activities at 4 h after the intake of Med and Med+CoQ diets. At 4 h after the intake of the Med+CoQ diet we found a lower postprandial plasma GPx activity compared with the Med and SFA diets ($p=0.042$) while at 4 h after the intake of the Med diet we observed a lower postprandial plasma GPx activity than after the SFA diet ($p<0.001$) (Table 4).

Discussion

Fasting is not the usual physiological state of the modern human being, which spends most of the time in the postprandial state. Therefore, the assessment of the postprandial state may be more relevant to identify disturbances in metabolic pathways related to oxidative stress than measures taken in the fasting state. Moreover, our study is important particularly in view of the lack of randomized controlled trials assessing the postprandial effects of the Mediterranean diet and the potential benefits obtained when this diet is supplemented with an endogenous antioxidant such as CoQ.

Our study supports that the Med diet improves postprandial oxidative stress with a higher increase in capillary flow and in NO levels, lower LPO and nitrotyrosine levels and GPx activity, and a lower postprandial decrease in HDL-C than the SFA diet.

Moreover, the addition of CoQ had an additive effect on the Med diet since the participants that consumed this diet showed a greater postprandial decrease in PC levels, SOD, CAT, and GPx activities and a greater increase in capillary flow and NO levels with respect to the other diets.

A recent PREDIMED substudy demonstrated that adherence to the Mediterranean diet was associated with a reduced fasting oxidative status compared with the low-fat diet (Fitó et al. 2007; Razquin et al. 2009). (Covas 2007) reported the effects of high phenolic olive oil consumption on decreasing lipid oxidative damage. Furthermore, we have demonstrated that the high-monounsaturated diet improves postprandial oxidative stress compared with low-fat, high complex carbohydrate and high saturated fatty acid diets in patients with MetS (Perez-Martinez et al. 2010), which is in agreement with our results obtained after intake of the Med diet rich in virgin olive oil. Moreover, we also found an improvement in capillary flow along the postprandial phase after consumption of the Med diet. Olive oil-rich diets have been proven to improve the flow-mediated (endothelial-dependent) dilatation in healthy subjects (Karatzi et al. 2008). This improvement in capillary flow was even more evident when the Med diet was supplemented with CoQ, which is consistent with other studies showing that dietary supplementation with CoQ reduce blood pressure (Hodgson et al. 2002). Also, it is known that capillary flow is largely NO dependent (Bertuglia 2008). The improved bioavailability of NO after ingestion of the Med+CoQ, as shown in our study, may be an indirect effect of a lower production of superoxide anion due to CoQ supplementation, which also coincides with lower levels of nitrotyrosine.

Although previous studies showed that the use of antioxidants in the diet improves cellular oxidative stress (Bañuls et al. 2010; Kar et al. 2009) the current controlled intervention study is, to our knowledge, the first to examine the effect of a Med diet supplemented with an endogenous antioxidant such as CoQ during the postprandial state. It has been reported that CoQ supplementation suppressed PC levels in mice (Wadsworth et al. 2008) which coincides with our results because we observed a significant decrease in PC levels after consumption of Med+CoQ diet compared with either the non-supplemented Med diet or the SFA-enriched diet. In addition, low oxLDL and LPO levels obtained after the intake of the Med+CoQ compared with the other diets is indicative of lower oxidative damage due to an antioxidant environment provided by CoQ supplementation (Quiles et al. 2005).

In response to oxidative stress, cells attempt to fortify their antioxidant arsenal as the first defense line. SOD, GPx, and CAT are considered primary antioxidant enzymes, since they are involved in direct elimination of ROS. They protect cells against ROS produced during normal metabolism and after an oxidative insult. Antioxidant defense systems work cooperatively to alleviate the oxidative stress caused by enhanced free radical production. SOD metabolizes and protects the cells against O_2^--mediated lipid peroxidation while CAT and GPx act on H_2O_2 and/or ROOH by decomposing them, thereby neutralizing their toxicity. Any changes in one of these systems may break the equilibrium and cause cellular damage (Arsova-Sarafinovska et al. 2009). Previous studies have shown that the expression of SOD is upregulated by superoxide (Mates et al. 1999). Thus, the decrease in SOD activity observed along the postprandial state of the Med+CoQ diet with respect to the other diets could be explained on the basis of lower generation of superoxide by supplementing the Med diet with CoQ. Moreover, CoQ deficiency interferes with assembly or stability of the respiratory chain enzymes leading to unbalanced oxidative phosphorylation and enhanced ROS production (Quinzii et al. 2008). Therefore, the use of CoQ could enhance respiratory chain activity with subsequent minor ROS production (Imada et al. 2008). CAT and GPx activities showed the lowest postprandial levels in Med+CoQ diet with respect to the other diets. Presumably, CoQ supplementation contributed to a reduction in the production of H_2O_2 and in this way suppressed both enzymes for which H_2O_2 serves as a substrate (Stachowska et al. 2005).

A limitation of the present study is that, in our model, we only studied the effects of the different dietary models after a chronic dietary period, but not those other which could be present after a single meal. In other words, part of the results described here could be theoretically presented after a single meal (with/without CoQ supplementation). To uncover this point, new studies must be performed comparing the postprandial state effects of these diets after a single meal and a chronic intervention. Another limitation of this study is that we did not study if supplementation with CoQ of the saturated fatty acid-rich diet may

partly reduce the negative effects of this dietary model on postprandial oxidative stress.

In conclusion, our results support the antioxidant effect of Med diet rich in olive oil and that exogenous CoQ supplementation in synergy with a Med diet improves the postprandial oxidative stress in elderly men and women. This fact may have favorable effects on the aging process and may have a link with the benefits of consuming Med diet on the prevalence of age-related conditions such as cardiovascular and neurodegenerative diseases. Moreover, the favorable biological effects shown with the supplementation with CoQ may be a good starting point for assessing the effects of CoQ chronic supplementation on clinical features associated to age.

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References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Arsova-Sarafinovska Z, Eken A, Matevska N, Erdem O et al (2009) Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clin Biochem 42(12):1228–1235

Bañuls C, Martínez-Triguero ML, López-Ruiz A, Morillas C, Lacomba R, Víctor VM, Rocha M, Hernández-Mijares A (2010) Evaluation of cardiovascular risk and oxidative stress parameters in hypercholesterolemic subjects on a standard healthy diet including low-fat milk enriched with plant sterols. J Nutr Biochem 21 (9):881–886

Bertuglia S (2008) Intermittent hypoxia modulates nitric oxide-dependent vasodilation and capillary perfusion during ischemia-reperfusion-induced damage. Am J Physiol Heart Circ Physiol 294:1914–1922

Bouhafs RK, Samuelson A, Jarstrand C (2003) Lipid peroxidation of lung surfactant due to reactive oxygen species released from phagocytes stimulated by bacteria from children with cystic fibrosis. Free Radic Res 37:909–917

Covas MI (2007) Olive oil and the cardiovascular system. Pharmacol Res 55(3):175–186

Delgado-Lista J, Garcia-Rios A, Perez-Martinez P et al (2010) Gene variations of nitric oxide synthase regulate the effects of a saturated fat rich meal on endothelial function. Clin Nutr. doi:10.1016/j.clnu.2010.08.006

Erdelmeier I, Gerard-Monnier D, Yahan JC, Chaudière J (1998) Reactions of N-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. Chem Res Toxicol 11:1184–1194

Fitó M, Guxens M, Corella D et al (2007) Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. Arch Intern Med 167(11):1195–1203

Flohe L, Gunzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–121

Fuentes F, Lopez-Miranda J, Perez-Martinez P et al (2008) Chronic effects of a high-fat diet enriched with virgin olive oil and a low-fat diet enriched with alpha-linolenic acid on postprandial endothelial function in healthy men. Br J Nutr 100(1):159–165

Hodgson JM, Watts GF, Playford DA, Burke V, Croft KD (2002) Coenzyme Q(10) improves blood pressure and glycaemic control: a controlled trial in subjects with type 2 diabetes. Eur J Clin Nutr 56:1137–1142

Holvoet P, Lee DH, Steffes M, Gross M, Jr Jacobs Dr (2008) Association between circulating oxidized low-density lipoprotein and incident of the metabolic syndrome. JAMA 299(19):2287–2293

Human Nutrition Information Service, Department of Agriculture (1987) Composition of foods. US Government Printing Office, Washington, DC

Imada I, Sato EF, Kira Y, Inoue M (2008) Effect of CoQ homologues on reactive oxygen generation by mitochondria. Biofactors 32(1–4):41–48

Kar P, Laight D, Rooprai HK, Shaw KM, Cummings M (2009) Effects of grape seed extract in type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. Diabet Med 26(5):526–531

Karatzi K, Papamichael C, Karatzis E, Papaioannou TG, Voidonikola PT, Vamvakou GD, Lekakis J, Zampelas A (2008) Postprandial improvement of endothelial function by red wine and olive oil antioxidants: a synergistic effect of components of the Mediterranean diet. J Am Coll Nutr 27 (4):448–453

Levine RL, Garland D, Oliver CN et al (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186:464–478

Mates JM, Perez-Gomez C, Nunez de Castro I (1999) Antioxidant enzymes and human diseases. Clin Biochem 32:595–603

Nebot C, Moutet M, Huet P, Xu JZ, Yadan JC, Acudiere J (1993) Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. Anal Biochem 214(2):442–451

Perez-Martinez P, Garcia-Quintana JM, Yubero-Serrano EM, Tasset-Cuevas I, Tunez I, Garcia-Rios A, Delgado-Lista J, Marin C, Perez-Jimenez F, Roche HM, Lopez-Miranda J (2010) Postprandial oxidative stress is modified by dietary fat: evidence from a human intervention study. Clin Sci 119(6):251–261

Quiles JL, Ochoa JJ, Battino M, Gutierrez-Rios P, Nepomuceno EA, Frías ML, Huertas JR, Mataix J (2005) Life-long supplementation with a low dosage of coenzyme Q10 in

the rat: effects on antioxidant status and DNA damage. Biofactors 25(1–4):73–86

Quinzii CM, López LC, Von-Moltke J, Naini A, Krishna S, Schuelke M, Salviati L, Navas P, DiMauro S, Hirano M (2008) Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ10 deficiency. FASEB J 22(6):1874–1885

Razquin C, Martinez JA, Martinez-Gonzalez MA, Mitjavila MT, Estruch R, Marti A (2009) 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. Eur J Clin Nutr 63(12):1387–1393

Ricard-Jané D LM, Lopez-Tejero X (2002) Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantifications by the Griess method. Nitric Oxide 6:178–185

Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martínez P, Lozano A, Gomenz P, Jimenez Y, Perez-Jimenez F (2005) Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol 46:1864–1868

Sakano N, Takahashi N, Wang DH, Sauriasari R, Takemoto K, Kanbara S, Sato Y, Takigawa T, Takaki J, Ogino K (2009) Plasma 3-nitrotyrosine, urinary 8-isoprostane and 8-OHdG among healthy Japanese people. Free Radic Res 43:183–192

Santos-González M, Gómez-Díaz C, Navas P, Villalba JM (2007) Modifications of plasma proteome in long-lived rats fed on a coenzyme Q_{10}-supplemented diet. Exp Gerontol 42(8):798–806

Stachowska E, Wesołowska T, Olszewska M, Safranow K, Millo B, Domański L, Jakubowska K, Ciechanowski K, Chlubek D (2005) Elements of Mediterranean diet improve oxidative status in blood of kidney graft recipients. Br J Nutr 93(3):345–352

Stanhope KL, Schwarz J, Keim NL, Griffen SC, Bremer AA, Graham JL et al (2009) Consuming fructose sweeted, not glucose sweeted, veverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 119(5):1322–1334

Trichopoulou A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348(26):2599–2608

Turunen M, Olsson J, Dallner G (2004) Metabolism and function of coenzyme Q. Biochim Biophys Acta 1660(1–2):171–199

Varela G (1980) Tablas de composición de alimentos. (Food composition tables). Instituto de Nutrición CSIC, Madrid

Visioli F, Galli C (2001) The role of antioxidants in the Mediterranean diet. Lipids 36(suppl):S49–S52

Wadsworth TL, Bishop JA, Pappu AS, Woltjer RL, Quinn JF (2008) Evaluation of coenzyme Q as an antioxidant strategy for Alzheimer's disease. J Alzheimers Dis 14(2):225–234

Zhu H, Zhang L, Amin AR, Li Y (2008) Coordinated upregulation of a series of endogenous antioxidants and phase 2 enzymes as a novel strategy for protectig renal tubular cells from oxidative and electrophilic stress. Exp Biol Med (Maywood) 233(6):753–765