Original Article

Effect of nut consumption on oxidative stress and the endothelial function in metabolic syndrome

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ARTICLE INFO

Article history:
Received 10 September 2009
Accepted 16 December 2009

Keywords:
Nuts
Antioxidant capacity
Lipid oxidation
DNA damage
Endothelial function
Metabolic syndrome

SUMMARY

Background & aims: Oxidative stress has a key role in atherosclerosis, cancer and other chronic diseases. Some bioactive compounds in nuts have been implicated in antioxidant activities.

Objective: We assessed how nut consumption affected several markers of oxidation and endothelial function (EF) in metabolic syndrome (MetS) patients.

Patients and methods: A randomized, controlled, parallel feeding trial was conducted on 50 MetS adults who were recommended a healthy diet supplemented or not with 30 g of mixed nuts (Nut and Control groups, respectively) every day for 12 weeks. The plasma antioxidant capacity (AC), oxidized LDL (oxLDL), conjugated diene (CD) formation, urine 8-isoprostanes, DNA damage assessed by yield of urine 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), and EF assessed by peripheral artery tonometry (PAT) and biochemical markers, were measured at baseline and the end of the intervention.

Results: No significant differences in changes between groups were observed in AC, oxLDL, CD, 8-isoprostanates or EF during the intervention, whereas the reduction in DNA damage was significant in the Nut group compared to Control group (P < 0.001).

Conclusion: Nut consumption has no deleterious effect on lipid oxidation. The decrease in DNA damage observed in this study could contribute to explain the beneficial effects of regular nut consumption on some MetS features and several chronic diseases.

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1. Introduction

Epidemiological studies have consistently demonstrated that frequent nut consumption is associated with reduced risk of developing coronary heart disease (CHD), type 2 diabetes, or death by overall mortality causes. It has been claimed that the favourable effects of nuts on the plasma lipoprotein profile is the main mechanism that explains the marked reduced risk of CHD observed in cohort studies. However, it has been suggested that other mechanisms of action, such as decreasing inflammation or improving EF, are important for explaining the beneficial effect of nuts on cardiovascular health.

Because of their increasingly recognized healthy benefits, nuts are currently included in several dietary guidelines worldwide and have been proposed as a component of optimal diets for CHD prevention.
However, nuts are fatty foods and presumably for this reason, until recently, were ignored or treated with a great deal of caution on most dietary recommendations. One of the possible deleterious effects of chronic nut consumption may be because nuts, especially walnuts, very rich in polyunsaturated fatty acids (PUFA). Of the various fatty acids, PUFA are the most susceptible to oxidation. In fact, a diet enriched with linoleic acid increases LDL oxidation in both human and animal experiments. This is important because current evidence indicates that oxidative damage plays a key role in atherosclerosis, cancer and other chronic diseases.

However, emerging evidence indicates that some bioactive compounds in nuts probably counteract the pro-oxidant effect of PUFA on LDL and have elicited some cardioprotective effects. Several phytochemicals that have been shown to be common in nuts—e.g. polyphenols and phytoestersols—have a variety of bioactions that have been implicated in antioxidant activities. These phytochemicals may work in synergy with other important nut constituents such as antioxidant vitamins (α-tocopherol, γ-tocopherol) and minerals that decrease the oxidative damage to lipids, proteins, and lipoproteins and slow down the progression of the atherosclerosis plaque.

However, the effect of nut consumption on oxidative stress has been analyzed in only a few clinical trials, and only in eight was the main outcome related to oxidation. These studies have been conducted in different populations, using different types of nuts, and oxidative damage has been explored using different techniques and approaches, which explains the contradictory results obtained. The effect of nut consumption on EF has also been poorly studied and the results are contradictory.

Therefore, the aim of our study is to evaluate the effect of nut consumption on several steps of the cascade oxidation and endothelial dysfunction in a non diabetic MetS population. The lipid profile, the insulin resistance and the inflammatory responses from this feeding trial have been previously described.

2. Material and methods

2.1. Subjects

Sixty-one male and female volunteers, aged between 18 and 65 y old with MetS from three Primary Care Centres (Alcover, Riudoms and Reus) of our zone and the University Hospital Sant Joan, Reus (Spain) were screened. To be enrolled the subjects had to have at least three of the following MetS components as defined by the updated Adult Treatment Panel (ATP III) criteria: (a) waist circumference >102 cm for men and >88 cm for women, (b) triglycerides (TG) >1.7 mmol/L or drug treatment for elevated TG, (c) HDL-cholesterol concentrations <0.9 mmol/L in men and <1.1 mmol/L in women or drug treatment for reduced HDL-cholesterol, (d) elevated systolic blood pressure >130 mmHg or elevated diastolic blood pressure >85 mmHg or treatment with antihypertensive drugs, and (e) fasting glucose ≥100mg/dL or drug treatment for elevated glucose. Subjects were excluded if they had nut allergy, established type 2 diabetes mellitus with or without oral or insulin treatment, acute or chronic infection, inflammatory disease or cancer; leukocytosis at the beginning of the study (>10,000 cells x 10⁹). Other reasons for exclusion were anti-inflammatory, corticosteroid hormonal or antibiotic drug treatment; alcoholism or active drug dependence; restrictive diet during the 3 mo prior to the study; body mass index (BMI) >35 kg/m², or a recent weight change of >5 kg in the previous three months.

The University Hospital Sant Joan of Reus Ethics Committee approved the protocol and all participants provided written informed consent. All procedures were in compliance with the Helsinki Declaration principles.

2.2. Study design

A randomized, controlled, parallel, interventional feeding trial was designed for 12 wk. At the pre-inclusion visit, a medical clinical history was taken, a physical examination performed and biochemical analyses made to verify health status and compliance with the inclusion criteria. Subjects were stratified by sex and age (<50 y or ≥50 y) and were randomly assigned to the Control group (qualitative recommendations according to the American Heart Association dietary guidelines) or the Nut group (the same dietary recommendations enriched with a daily supplement of 30 g of mixed raw nuts with skin [15, 7.5 and 7.5 g/d of walnuts, almonds and hazelnuts, respectively], which were provided free. At wk 0, 4, 8 and 12 a dietician assessed all the subjects and provided dietetic support so that participants could comply with dietetic recommendations. They were also asked not to change their habitual physical activity or smoking level during the study period. Every 4 wk, both groups of patients filled in a 3-day food record to assess their compliance with the dietetic recommendations. In addition, the returned empty bags of nut and α-linolenic plasma concentrations were determined as markers of adherence for the nut group. Fasting blood samples were obtained and the endothelial function was evaluated at baseline and the end of the study.

The dietary recommendations given to the participants in both the Control and the Nut group were that they should eat a diet rich in vegetables and fruits; select whole-grain, high fiber foods; eat fish at least twice a week; limit saturated or trans food and cholesterol; select fat-free and low-fat dairy products; cut down on foods containing partially hydrogenated vegetable oils; cut down on soft drinks and foods with added sugar; choose and prepare foods with little or no salt; and limit alcohol intake.

2.3. Blood pressure, anthropometric measurements and body composition

Systolic and diastolic blood pressures were measured using a blood pressure monitor electronic device (Omron model 705IT). Body wt was measured using a Tanita TBF-300 weight scale electronic device (Tanita®, Tokyo, Japan) while the subjects were minimally clothed and not wearing shoes. Measurements were performed in standard conditions. Height was measured with a wall-mounted stadiometer in a standing position, without shoes or hair ornaments, and recorded to the nearest 0.1 cm. Waist circumference was measured midway between the lower rib margin and the iliac crest with the subject standing and wearing only underwear, at the end of gentle expiration. BMI was calculated.

2.4. Diet and physical activity

Total energy and macronutrient content were calculated using Spanish food composition tables. To evaluate the level of physical activity throughout the study the Minnesota Leisure Time Physical Activity questionnaire validated for Spanish population was applied.

2.5. Blood collection and biochemical measurements

At baseline and the end of the intervention, 12-h fast serum and plasma samples were collected into EDTA vacutainer tubes and separated by centrifugation at 2500 rpm for 10 min at 4 °C. Then samples were aliquoted into cryovials and stored at −80 °C until they were analyzed. Serum concentrations of TG, total cholesterol...
(TC), and HDL-cholesterol were measured using standardized clinical laboratory techniques. LDL-cholesterol was calculated using the Friedewald equation (LDL-cholesterol (mmol/L) = TC-TG/2.2-HDL-cholesterol).

2.5.1. Plasma antioxidant capacity

Plasma samples were diluted 500-fold in 75 mM potassium phosphate buffer (pH 7.4) before analysis. The reaction mode used 20 μL of the sample and 370 μL of fluorescein, 48 mM in a multwell plate. The reagents were mixed and incubated for 10 s before the initial fluorescence was recorded. Peroxyl radicals were generated by 10 μL of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) reagent. Fluorescence was taken every minute for 120 min at 485 nm (λex) and 538 nm (λem) wavelength on a Fluoroskan Ascent fluorescence plate reader (Labsystems, Helsinki, Finland). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard. The final oxygen radical absorbance capacity (ORAC) values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescein decay curve and were expressed as Trolox Equivalents per litre of plasma (mmol TE/L plasma).8,10

2.5.2. Plasma LDL oxidation

oxLDL was determined by a monoclonal antibody-based immunoassay commercially available ELISA kits (Mercodia, Uppsala, Sweden). The mean intra- and interassay coefficients of variation were 6.33 and 4.73% respectively.

2.5.3. Plasma conjugated diene formation

PD-10 desalting columns (GE Healthcare, Uppsala, Sweden) were used to remove EDTA from the plasma. Cu²⁺ was used to oxidize plasma lipids and the CD formation was measured at 234 nm, at 37 °C for 5 h as described.20 A total of 50 μL of plasma was eluted through the column with phosphate-buffered saline (PBS) until a final dilution of 1:75. The oxidation was then started in a spectrophotometrical cuvette with 1.5 mL of the eluted sample, 1.47 mL of PBS and 30 μL of 5 mM CuCl₂ solution. From the kinetics profile of each measured sample, several indexes have been determined describing the plasma oxidizability as described.23 Lag phase, defined as the interval (min) between the intercept of the linear least-square slope of the curve with the initial-absorbance axis, was measured. The maximal rate of oxidation (Vmax) was calculated from the slope of the absorbance curve during the propagation phase (expressed as nmol/min/mL of plasma) using the molar absorptivity for CD (ε234 = 29,500 L mol⁻¹ cm⁻¹). The same absorptivity was used to determine the maximal amount of CD produced (Gmax).

2.5.4. Endothelial adhesion molecules

Soluble intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were measured using the standard ELISA kit from Diaclone (Besançon, France). The mean intra- and interassay coefficients of variation were 2.82, 8.15% and 2.27, 5.94% respectively.

2.6. Urinary 8-oxo-dG assay

24 h urine sample was collected at home by patients in polyethylene bottles. The volume of the sample was measured and, after agitation, aliquots (2 × 10 mL) of the homogenized urine were kept at −20 °C until further analysis.

The method for detecting 8-oxo-dG was based on that of Brown RK et al.24 To 1 mL of urine, 100 μL of 3 mol/L Tris-EDTA solution pH 8.6 was added and vortex-mixed for 30 s. The solution was then applied to a Bond Elute C18(OH)SPE (3 mL) column that had been prepared with 3 mL methanol and 3 mL water. The column was washed with 3 mL water followed by 3 mL 2.5% acetonitrile and 1.5% methanol in 10 mM/L borate pH 7.9. The sample was eluted with 3 mL of the same buffer and applied to a Bond Elute strong cation exchange column (3 mL) prepared with 3 mL methanol and 3 mL borate buffer pH 7.9. The 8-oxo-dG was eluted with 2 mL of acetonitrile/methanol buffer in borate and then adjusted to pH 6.0 with 1 mol/L HCl. 4 mL of 50:50 dichloromethane: propane-2-ol was added to the 2 mL of eluent and vortex-mixed for 30 s. The sample was then centrifuged for 10 min at 3500 rpm, the upper aqueous layer aspirated off and 3 mL of organic layer evaporated to dryness under nitrogen at 50 °C. Finally, the sample was reconstituted in 1 mL HPLC running buffer without acetonitrile and 50 μL injected into an HPLC column. To separate 8-oxo-dG, a Waters 515 HPLC pump model was used. This separation was carried out using a 5 μm Spherisorb ODS2 column (4.6 mm × 250 mm) with a flow rate of 1 mL/min. The buffer used was 50 mM/L potassium phosphate pH 5.1 in 5% of acetonitrile, and the retention time was 7.5 min. To assess the optimization and accuracy of the HPLC-EC assay in isolating and detecting 8-oxo-dG, appropriate chromatograms of both samples and standards were recorded at the beginning of each working day.23 The 8-oxo-dG values were expressed as the ratio to creatinine urine concentration given in mmol/mL.

2.7. Urine 8-isoprostane assay

24 h urine samples were frozen at −20 °C until analysis. The 8-isoprostane was extracted from the urine samples before the assay using an immunoaffinity column (cat number 416358; Cayman Chemical Corp). Aliquots of 0.5 mL urine were directly applied to the columns and the columns were washed with 2 mL of 0.1 mol/L PBS followed by 2 washes with 2 mL of water. The levels of urine 8-isoprostanes were determined with a commercial EIA kit from Cayman Chemical (N° 516351). Readings were made at 405 nm in a Multiskan EX, Thermo Labsystems. The results were expressed as ng of 8-isoprostanemes/mmol creatinine.

2.8. In vivo endothelial function measurement

To assess the EF, a finger plethysmograph based on non-invasive PAT technology was used (EndoPAT 2000, Itamar-Medical Ltd, Caesarea, Israel). A thimble-shaped pneumatic probe was placed on the index finger of the hand undergoing hyperaemia testing and a second PAT probe was placed on the contralateral index finger. The probe components were connected to isolated volume reservoirs, which buffer pressure changes and provide a uniform pressure field. The pressure changes accompanying peripheral volume changes are fed to a personal computer, where the signal is band pass-filtered (0.3–30 Hz), amplified, displayed, and stored.24 The PAT hyperaemic response (ENDOPAT index) was automatically calculated at the conclusion of the study as the ratio of the average baseline pulse wave amplitude during reactive hyperaemia to the preocclusion baseline. PAT measurements were analyzed with a computerised, automated algorithm (EndoPAT 2000, Itamar-Medical Ltd, Caesarea, Israel), so this calculated ratio automatically provides an index of EF. Therefore, there is no intraobserver or interobserver variability.

2.9. Statistical analysis

We used the Kolmogorov–Smirnov statistical test to check the normal distribution of variables. For the statistical comparison and depending on the normality data for each parameter measured before and after the 12-wk intervention period, results were expressed as the mean (standard deviation or 95% CI) or median [p25–p75]. Means were analyzed using the unpaired Student’s t-test. The differences between basal and final values were tested
within groups by a paired t-test or the Wilcoxon test as appropriate. The differences between the changes in the values of the two diet groups were tested by an unpaired Student’s t-test or the Mann–Whitney U-test as appropriate. The differences in changes between groups were also tested in the case of the DNA damage measurements by ANCOVA using a general linear model, with body weight changes as a covariate. Differences were considered statistically significant at \( P \leq 0.05 \). All statistical analyses were carried out with the Statistical Package for the Social Sciences, SPSS software for Windows version 15.0 SPSS, Inc.,USA.

3. Results

A total of 61 subjects were assessed for inclusion in the study. Of these, 4 did not meet the inclusion criteria, 4 had severe difficulties in following the study, and 1 had started statine therapy just before the randomization. Of the 52 participants randomized, 2 withdrew from the study for personal reasons. A total of 50 subjects (25 in the Control group and 25 in the Nut group) completed the trial and were included in the statistical analyses (Fig. 1). The average age of the 28 men and 22 women studied was 51.8 y (range 26–63).

The general baseline characteristics of the subjects did not differ significantly between the two intervention groups (Table 1). Most of the subjects included in the study were overweight or obese (BMI between 25 and 35 kg/m²), and 96% of the Nut group and 84% of the Control group were diagnosed with hypertension. 72% and 40% of the population had hypertriglyceridemia or low HDL-cholesterol, respectively. Physical activity was similar in both groups before the intervention period and remained unchanged throughout the study (data not shown).

At baseline, no significant differences between groups were observed in relation to the total daily energy and nutrient intake (Table 2), peripheral oxidative stress, EF biomarkers and the ENDOPAT index measurements of endothelial function \((p > 0.05)\).

When the 3-day records were analyzed, no significant differences were observed in the changes between groups in total energy, carbohydrate or alcohol consumption during the intervention (data not shown). However, participants in the Control group showed a slight increase in total protein intake and a considerable decrease in total fat \((p < 0.01)\), monounsaturated fatty acid (MUFA) \((p < 0.01)\) and PUFA \((p < 0.001)\) consumption compared with the participants in the Nut group, who significantly increased their baseline PUFA intake \((p < 0.05)\).

Adherence to the supplemented nuts measured by counting the empty packages of nuts returned to the investigators was excellent (94%). Adherence was also good when evaluated by objective measurements of nut intake in 27 participants of the sample (\(n = 14\) and \(n = 13\) in the Nut and Control group, respectively). Plasma α-linolenic acid levels significantly increased during the intervention only in the nut group \((p = 0.019)\) from baseline 0.26 to 0.36% (95% CI, −0.02 to 0.18).

Participants in both groups lost weight \((-1.5 \text{ kg [95\% CI, } -2.4 \text{ to } -0.6\text{]}\) and \(-2.2 \text{ kg [95\% CI, } -3.4 \text{ to } -0.9\text{]}\) in the Control and the Nut group, respectively. No differences were observed in changes between groups in body mass index \((P = 0.363)\), arterial systolic \((P = 0.238)\) and diastolic \((P = 0.466)\) blood pressure, or any of the general biochemical lipid profile measurements \((\text{TC, LDL-cholesterol, HDL-cholesterol and TG, all } P > 0.05)\).

Although almost all the oxidative stress biomarkers tended to improve in both interventional groups (Table 3), the DNA damage evaluated by 8-oxo-dG \((p < 0.001)\) reduced significantly only in the Nut group. After this variable had been adjusted for body weight changes using an ANCOVA model, the differences in the changes in the 8-oxo-dG measurements between groups remained significant \((p < 0.001)\). The nut group also tended to have less oxidative stress because of a greater but nonsignificant reduction in plasma oxLDL, \(C_{\text{max}}\), and isoprostane urine excretion.

Although plasma ICAM-1 concentrations decreased during the intervention period in the nut group \((p = 0.038)\), no significant differences were observed in changes between groups in plasma endothelial adhesion molecules or in \(\text{in vivo}\) ENDOPAT index measurements (Table 4).

4. Discussion

The results of our feeding clinical trial show that, despite the adverse pro-oxidant effect of consuming PUFA in nuts, especially high in walnuts, the consumption of a supplement of 30 g of mixed...
Table 1  
General baseline characteristics of the groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nut group (n = 25)</th>
<th>Control group (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>52.9 (8.4)</td>
<td>50.6 (8.4)</td>
<td>0.316</td>
</tr>
<tr>
<td>Sex [n (% men)]</td>
<td>15 (60)</td>
<td>13 (52)</td>
<td>0.569</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>86.4 (80.8–92.0)</td>
<td>79.9 (75.5–84.4)</td>
<td>0.069</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.6 (30.5–32.8)</td>
<td>30.0 (28.6–31.4)</td>
<td>0.065</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>144.6 (138.3–150.9)</td>
<td>137.2 (129.2–145.2)</td>
<td>0.137</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>86.0 (82.7–89.3)</td>
<td>82.0 (77.9–86.0)</td>
<td>0.119</td>
</tr>
<tr>
<td>Physical activity (kcal/d)</td>
<td>449.3 (230.9–667.7)</td>
<td>380.0 (248.7–511.3)</td>
<td>0.578</td>
</tr>
</tbody>
</table>

Values are means (95% CI). Statistical significance determined by the unpaired t-test.

Table 2  
Baseline energy and nutrient intake for each intervention group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nut group (n = 25)</th>
<th>Control group (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>2094 (1865–2322)</td>
<td>1993 (1801–2186)</td>
<td>0.493</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>18.9 (18.0–20.2)</td>
<td>18.6 (17.2–20.0)</td>
<td>0.748</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>35.5 (33.1–38.0)</td>
<td>35.7 (33.2–38.1)</td>
<td>0.933</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>10.5 (9.4–11.5)</td>
<td>10.5 (9.4–11.7)</td>
<td>0.931</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>5.4 (4.7–6.1)</td>
<td>5.0 (4.4–5.5)</td>
<td>0.353</td>
</tr>
<tr>
<td>MUFAs (%)</td>
<td>16.6 (15.4–18.5)</td>
<td>17.0 (15.6–18.5)</td>
<td>0.708</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>413.8 (385–441)</td>
<td>42.4 (40.0–45.0)</td>
<td>0.533</td>
</tr>
<tr>
<td>Fiber (%/day)</td>
<td>20.5 (17.3–23.7)</td>
<td>19.5 (15.9–23.1)</td>
<td>0.679</td>
</tr>
<tr>
<td>Alcohol (%/day)</td>
<td>4.3 (2.0–6.8)</td>
<td>3.3 (0.9–5.7)</td>
<td>0.546</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFAs, mono-unsaturated fatty acids.
Values are means (95% CI). Statistical significance determined by the unpaired t-test.

nuts (walnuts, almonds and hazelnuts) in a context of a healthy diet for 12 wk did not produce a deleterious effect on oxidative stress biomarkers or the endothelial function in MetS patients compared with patients consuming the same healthy diet without nuts. Moreover, DNA damage (measured by the 8-oxo-dG urinary excretion), a chronic disease related biomarker, was significantly reduced in the Nut group compared to the Control group during the intervention.

Several studies have investigated the possible effect of nut consumption on oxidation in healthy humans, and patients with hypercholesterolemia or at high cardiovascular risk. These studies are very controversial because some of them show possible benefits but other neutral or negative effects on oxidation. In addition, in only eight clinical trials oxidation was the primary outcome analyzed after chronic consumption of tree nuts, 25–32 and none of them were conducted in patients with metabolic syndrome. The present study is the first to have evaluated the effect of a supplement of mixed nuts on several lipid and DNA oxidation markers in a group of patients with oxidative stress associated to MetS.

Most of the studies that observed a potential beneficial effect of nut consumption on oxidation focused on almonds, 26,27,29,31,32 pistachios, 30 pecans 28 or peanuts, 25 all rich sources of MUFA.

However, this beneficial effect on oxidation was not consistently reported in walnut trials 10–12,33–36 and response even tended to worsen after walnut consumption replacing other sources of MUFA. 10–12,33

Nuts are fatty foods rich in unsaturated fatty acids mainly MUFA, but also are good sources of PUFA, especially in case of walnuts, 5 which are the main substrate for the oxidation of the LDL particles. 6 For this reason, it has been suggested that nut intake can promote LDL oxidation. 3 However, the principal fat compounds in almonds and hazelnuts are MUFA and they have been associated with reduced susceptibility of LDL to oxidation. 7 For this reason, differences in the type of fat content between nuts may partly explain why almonds, pistachios, pecans and other nuts rich in MUFA have the ability to reduce the oxidation processes whereas walnuts (rich in PUFA) do not.

Nuts also contain tocopherols and several phenolic compounds with remarkable antioxidant potential, mainly located in the pellicle. 27 These bioactive compounds may counteract the pro-oxidant effects of PUFA on LDL oxidation 38 and decrease DNA damage. 39 However, the ability of tree nut extracts to modulate oxidative stress demonstrated by several in vitro assays has not been consistently observed in in vivo animal models or human trials. 38 It seems possible that the antioxidant activity of phytochemical compounds and phytosterols bioavailable in nuts could work with other important nut constituents in an additive and synergistic way to protect against oxidative stress. 8 In our study, this complex antioxidant network might help to explain the decrease observed in some markers of oxidation after consuming nuts.

Of all the oxidative stress by-products, 8-oxo-dG has been one of the most widely studied, probably because it is of considerable pathogenic importance. 40 The presence of 8-oxo-dG in DNA results in substantial genomic instability. Guanine oxidation by ROS generates the hydroxyl radical as the mutagenic and carcinogenic effects of which have been extensively documented. 41 Oxidation adds a hydroxyl group to position C8 of the guanine molecule, leading to the oxidative and mutagenic by product 8-oxo-dG, which is considered to be both an important biomarker of generalized cellular oxidative stress and a repair product. 32 Elevated levels of 8-oxo-dG have also been related to tumour initiation and progression in a variety of animal and human experimental models. 42,43 The products of DNA repair of oxidation damage are excreted into the urine in amounts corresponding to a damage rate of up to 104 modifications in each cell every day. The most abundant of these lesions is 8-oxo-dG, which is validated as the method of choice for oxidative stress associated diseases 39,40 and may be useful for monitoring the beneficial effect of antioxidants in dietary intervention studies.

As mentioned, a remarkably significant improvement in 8-oxo-dG urinary excretion was observed in our MetS patients who consumed the mixed nut supplement compared to those who did not consume it. It has been suggested that this generalized cellular oxidative stress biomarker is a pivotal factor not only for carcinogenesis, but also for aging-associated degenerative diseases. 40 Atherosclerosis or diabetes. 9 In fact, increased levels of the damaged base have been isolated from both nuclear and mitochondrial DNA of hypertensive subjects. 42,43 Enhanced oxidative stress and 8-oxo-dG levels have also been observed in other cardiovascular-associated cardiovascular alterations such as combined hyperlipidemia. 40 The only two studies that have assessed the effect of nut consumption on DNA damage biomarkers were conducted on healthy smokers and they also observed similar improvements after almond consumption. 42,43 However, further studies are needed to elucidate the possible positive effects of chronic nut consumption on diseases related to DNA damage.

Endothelial dysfunction, a critical early event in the pathogenesis of atherosclerosis characterized by the reduced bioavailability of nitric oxide (endothelial vasodilator) and the increased expression of cellular adhesion molecules, is related to perfusion abnormalities and the causation of ischemic events. 47 Nuts are rich sources of bioactive compounds, such as α-linolenic acid, the plant n-3 fatty acid which might favourably influence EF. 48 Only two clinical trials (one chronic and the other acute) have assessed the effect of walnut consumption on endothelial dysfunction. 12,36 They both demonstrated a significant improvement in the EF, which was assessed by brachial artery vasodilation after walnut consumption. Moreover, a significant
It was suggested that these beneficial effects are because of their α-linolenic acid content. However, considerable evidence suggests that some antioxidant vitamins, folic acid or l-arginine (also contained in other nuts) may contribute to the beneficial effects on EF. Because the PAT seems to be a promising operator-independent in vivo method that can be used for clinical assessment of EF, we evaluated the effect of nuts on EF not only by biochemical markers (in vitro CAMs measurements), but also by PAT. In our study we observed a decrease in VCAM-1 was also observed in the chronic feeding study. It was suggested that these beneficial effects are because of their α-linolenic acid content. However, considerable evidence suggests that some antioxidant vitamins, folic acid or l-arginine (also contained in other nuts) may contribute to the beneficial effects on EF. Because the PAT seems to be a promising operator-independent in vivo method that can be used for clinical assessment of EF, we evaluated the effect of nuts on EF not only by biochemical markers (in vitro CAMs measurements), but also by PAT. In our study we observed a decrease in VCAM-1 was also observed in the chronic feeding study.12 It was suggested that these beneficial effects are because of their α-linolenic acid content. However, considerable evidence suggests that some antioxidant vitamins, folic acid or l-arginine (also contained in other nuts) may contribute to the beneficial effects on EF. Because the PAT seems to be a promising operator-independent in vivo method that can be used for clinical assessment of EF, we evaluated the effect of nuts on EF not only by biochemical markers (in vitro CAMs measurements), but also by PAT. In our study we observed a decrease in VCAM-1 was also observed in the chronic feeding study.12

### Table 3

Baseline values and changes within and between groups in antioxidant capacity and biomarkers of oxidative stress during the intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nut group (n = 25)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Control group (n = 25)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Interventional effect</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>Differences&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma ORAC (µmol TE/mL)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>25.46 (22.82–28.12)</td>
<td>0.035</td>
<td>26.60 (23.13–28.88)</td>
<td>0.807</td>
<td>1.13 (−2.48–4.76)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 (−1.92–3.60)</td>
<td></td>
<td>−0.29 (−2.79–2.19)</td>
<td></td>
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<tr>
<td>Oxidative stress biomarkers</td>
<td></td>
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</tr>
<tr>
<td>oxLDL (U/l)</td>
<td>70.67 (61.50–79.85)</td>
<td>0.066</td>
<td>67.64 (57.11–78.19)</td>
<td>0.611</td>
<td>−5.71 (−16.50–5.06)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−7.59 (−15.72–0.53)</td>
<td></td>
<td>−1.87 (−9.39–5.63)</td>
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<tr>
<td>Plasma conjugate diene</td>
<td></td>
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<tr>
<td>Lag phase (min)</td>
<td>15.76 (12.36–19.16)</td>
<td>0.612</td>
<td>14.50 (11.25–17.76)</td>
<td>0.028</td>
<td>−1.98 (−5.62–1.65)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 (−2.16–3.60)</td>
<td></td>
<td>2.70 (0.32–5.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (mmol/min/mL)</td>
<td>23.8 (21.3–26.2)</td>
<td>0.690</td>
<td>26.5 (24.1–29.0)</td>
<td>0.575</td>
<td>−0.2 (−2.6–3.0)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.4 (−2.3–1.6)</td>
<td></td>
<td>−0.6 (−2.7–1.5)</td>
<td></td>
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<tr>
<td>c&lt;sub&gt;max&lt;/sub&gt; (mmol/mL)</td>
<td>1088 (976 to 1346)</td>
<td>0.109</td>
<td>1193 (958–1555)</td>
<td>0.330</td>
<td>−30 (−247.5–134)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−71 (−278–134)</td>
<td></td>
<td>28 (−249–136)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine 8-isoprostanes (ng/mmol creatinine)</td>
<td>221.29 (169.74–272.84)</td>
<td>0.000</td>
<td>225.28 (198.39–252.16)</td>
<td>0.000</td>
<td>−16.95 (−76.26–42.34)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−138.01 (−190.49 to −85.54)</td>
<td>0.000</td>
<td>−126.06 (−150.56 to −91.54)</td>
<td>0.000</td>
<td>−16.95 (−76.26–42.34)</td>
</tr>
<tr>
<td>Urine 8-oxo-dG (nmol/mmol creatinine)</td>
<td>11.90 (9.26–12.73)</td>
<td>0.000</td>
<td>11.96 (11.25–12.66)</td>
<td>0.000</td>
<td>−2.42 (−3.58 to 1.25)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−6.35 (−7.20 to −5.51)</td>
<td>0.000</td>
<td>−3.93 (−4.78 to −3.09)</td>
<td>0.000</td>
<td>−2.42 (−3.58 to 1.25)</td>
</tr>
</tbody>
</table>

ORAC, oxygen radical absorbance capacity; TE, trolox equivalent; oxLDL, oxidized LDL; V<sub>max</sub>, maximal rate of oxidation; c<sub>max</sub>, maximal amount of CD produced; 8-OXO-dG, 8-Oxo-7,8-dihydro-2′-deoxyguanosine.

Values are means (95% CI) or median [p25-p75]. Statistical significance determined by the unpaired t-test.

<sup>a</sup> Changes between baseline and final intervention period.

<sup>b</sup> P value for changes within groups.

<sup>c</sup> Differences in changes between groups (Nut vs Control).

<sup>d</sup> P values for changes between groups.

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### Table 4

Baseline values and changes within and between groups in endothelial function markers and the ENDOPAT index during the intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nut group (n = 25)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Control group (n = 25)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Interventional effect</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Differences&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Endothelial function markers</td>
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<tr>
<td>VCAM-1 (µg/L)</td>
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<tr>
<td>Baseline</td>
<td>1082.04 (921.13–1242.95)</td>
<td>0.062</td>
<td>1179.25 (905.15–1453.34)</td>
<td>0.421</td>
<td>−99.72 (−321.96–225.51)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−42.91 (−220.47–134.65)</td>
<td>0.622</td>
<td>−56.81 (−86.40–200.03)</td>
<td>0.000</td>
<td>−30 (−249–136)</td>
</tr>
<tr>
<td>ICAM-1 (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>609.1 (523.2–696.5)</td>
<td>0.038</td>
<td>566.9 (476.4–657.4)</td>
<td>0.824</td>
<td>−80.63 (−211.1–50.72)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−91.98 (−178.29 to −5.68)</td>
<td>0.105</td>
<td>−11.35 (−74–43.53)</td>
<td>0.222</td>
<td></td>
</tr>
<tr>
<td>ENDOPAT index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.82 (1.65–1.99)</td>
<td>0.165</td>
<td>1.92 (1.69–2.14)</td>
<td>0.231</td>
<td>0.02 (0.25–0.29)</td>
</tr>
<tr>
<td>Change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.11 (−0.28–0.05)</td>
<td>0.000</td>
<td>−0.13 (−0.36–0.091)</td>
<td>0.890</td>
<td></td>
</tr>
</tbody>
</table>

VCAM-1, vascular cell adhesion molecule; ICAM-1, intercellular cell adhesion molecule; ENDOPAT index, hyperaemic response.

Values are means (95% CI). Statistical significance determined by the unpaired t-test.

<sup>a</sup> Changes between baseline and final intervention period.

<sup>b</sup> P value for changes within groups.

<sup>c</sup> Differences in changes between groups (Nut vs Control).

<sup>d</sup> P values for changes between groups.

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Please cite this article in press as: López-Uriarte P, et al., Effect of nut consumption on oxidative stress and the endothelial function in metabolic syndrome, Clinical Nutrition (2010), doi:10.1016/j.clnu.2009.12.008

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compared to subjects not receiving nuts. The decrease in DNA damage observed in the present study may help to explain the beneficial effects of regular nut consumption on some MetS features observed in other studies. These findings are consistent with the beneficial effects of nuts on CHD. Further studies will be required to explore any possible interaction between antioxidants and other constituents of tree nuts that may promote different antioxidant activities.

Conflict of Interest
J. Salas-Salvadó has received research funding from the International Nut Council, Reus, Spain. He is a nonpaid member of the Scientific Advisory Board of the International Nut Council. Mónica Bulló, Patricia Casas-Abestench, Patricia López-Uriarte, Lluís Masana, Rosa Mogués, Marta Romeu, Guillermo Saez and Carmen Tormos, no conflict of interest.

Contribution authors
PLU and JSS wrote the paper; JSS and MB designed the research and had primary responsibility for final content; PLU and PCA conducted research and analyzed data; PLU, PCA, MB, RN, MR, GS and CT conducted experiments of the research. MB, LM, GS, critically reviewed the paper. All authors read and approved the final manuscript.

Acknowledgements
This study was supported by the Spanish Ministry of Education and Science (CICYT-AGL2005-0365), Spanish Ministry of Health (RTIC RD06/0045), and the International Nut Council. Borges S.A. (Reus, Spain) donated the walnuts used in this study; CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) is an initiative of ISCIII. Patricia López-Uriarte is the recipient of a predoctoral fellowship from the Generalitat de Catalunya’s Department of Universities, Research and the Information Society and the European Social Funds. We thank Adriana Gómez-Flores for her collaboration in the acquisition of the nutritional data.

References


