*Lead Article*

# Nuts and oxidation: a systematic review

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*In recent years, nuts have received special attention because of their potential role in preventing cardiovascular disease. Because nuts are very rich in total fat that can potentially be oxidized and their skins contain several antioxidants, studies have been conducted to evaluate the potential effect of nut consumption on oxidative stress. This review evaluates the in vitro and in vivo studies conducted in animals or humans to analyze the effect of nuts on oxidation.*



## INTRODUCTION

Epidemiological studies have associated frequent nut consumption with a reduced risk of coronary heart disease (CHD), type 2 diabetes, or death by all-cause mortality.[1] Several clinical trials conducted in healthy, hypercholesterolemic, or diabetic individuals, using different types of nuts and study designs, showed improvement in the plasma lipid profile after nut consumption.[2] The favorable fatty acid profile of nuts means that nut consumption has a beneficial effect on plasma lipids and lipoproteins (Table 1). This appears to be one of the main mechanisms accounting for cardiovascular benefits.[3] However, it has been suggested that other mechanisms also contribute to the observed reduction in CHD risk,[3] for example, a decrease in the susceptibility to low-density lipoprotein (LDL) oxidation, a decrease in the inflammatory process, or improvement of endothelial function.[4] Because of their healthy effects, nuts have been included in dietary guidelines published by the United States, Canada, and Spain.[5–9] Likewise, the United States Food and Drug Administration (FDA) issued a claim that nuts and nut-containing products protect cardiovascular health.[10]

A plethora of physiological disorders and degenerative diseases have been related to oxidative stress,[11–13] which has a key role in atherosclerosis and coronary heart disease.[14] Oxidative modification of LDL is thought to play an important role in the development of atherosclerosis.[14–16] Of the fats, polyunsaturated fatty acids (PUFA) are the most susceptible to oxidation[17,18] and the PUFA content of nuts (principally walnuts) may lead to an increase in LDL oxidation.[19] However, emerging evidence indicates that some bioactive compounds in nuts can probably counteract the pro-oxidant effect of PUFA on LDL.[20–22] This is important because nuts are a rich source of many antioxidants that may protect PUFA in vivo against oxidative modification.[23]

Several methods have been used to assess the total antioxidant capacity (TAC) of nuts: for example, FRAP (ferric-reducing plasma ability),[24–27] ORAC (oxygen radical absorbance capacity),[28] TRAP (total radical antioxidant parameter) or TEAC (trolox equivalent antioxidant capacity) assays.[27] TEAC and ORAC are based on the antioxidant's ability to react with or neutralize free radicals generated in the assay systems, whereas FRAP measures the reduction of $Fe^{3+}$ (ferric iron) to $Fe^{2+}$ (ferrous iron) in the presence of antioxidants. In contrast, the TRAP assay is based on the protection provided by antioxidants during a controlled peroxidation reaction.[27] The TAC measure was considered appropriate for assessing the cumulative antioxidant properties of plant foods.[27] However, the impossibility of comparing results obtained with different methodologies has seriously limited understanding of the role of TAC in disease prevention.[29] The FRAP assay showed that walnuts have the highest antioxidant capacity (more than 20 mmol/100 g) followed by pecans, chestnuts, peanuts, and pistachios (between 8.3

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*Table 1* **Fatty acid composition of nuts.**

| Nut | SFA (g/100g) | MUFA (g/100g) | PUFA (g/100g) |
|---|---|---|---|
| Almonds | 3.73 | 30.89 | 12.07 |
| Cashews | 7.78 | 23.80 | 7.84 |
| Hazelnuts | 4.46 | 45.65 | 7.92 |
| Peanuts | 6.83 | 24.43 | 15.56 |
| Chestnuts | 0.42 | 0.78 | 0.89 |
| Walnuts | 6.13 | 8.93 | 47.17 |
| Brazil nuts | 15.14 | 24.55 | 20.58 |
| Macadamia nuts | 12.06 | 58.88 | 1.50 |
| Pecans | 6.18 | 40.80 | 21.61 |
| Pine nuts | 9.38 | 22.94 | 25.67 |
| Pistachios | 5.44 | 23.32 | 13.45 |

*Abbreviations*: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
Data from US Department of Agriculture Nutrient Database for Standard Reference, Release 21.[69]

and 1.3 mmol/100 g) and then hazelnuts, almonds, Brazil nuts, macadamias, pine kernels, and cashews (between 0.3 and 0.7 mmol/100 g).[25]

Nuts have different types of antioxidants (Table 2). For instance, almonds contain flavonoids such as catechins, flavonols, and flavonones in their aglycone and glycoside form,[30] while peanuts and pistachios contain flavonoids and have the highest concentration of resveratrol in comparison with the other nuts.[31] Walnuts contain a wide range of polyphenols and tocopherols[20] and cashews have alkyl phenols as the principal antioxidant.[32] The phytochemicals contained in nuts could work in synergy with other important nut constituents to promote antioxidant activities.[33]

In recent years, ambiguous results have been published about the possible effect of nuts on oxidative stress status. The purpose of this article is to review human trials, studies conducted on animals, and studies conducted using in vitro assays to evaluate the effect of nuts on oxidative stress parameters.

## Effects of tree nut extracts on oxidative stress in in vitro studies

All of the six studies using in vitro assays that were analyzed in this review showed an improvement in most of the oxidative stress biomarkers assessed with several types of tree nut extracts on human plasma or LDL particles, bovine liver microsomal membranes, or plasmid DNA. Four studies that evaluated lipid peroxidation as the conjugated diene (CD) formation on human LDL particles after incubation with walnut extracts,[34] almond-skin flavonoids[35] or polyphenols,[36] or pistachio hydrophilic extracts[37] found a significant increase in the lag time and therefore greater inhibition of lipid peroxidation in LDL. Anderson et al.[34] also found a significant

*Table 2* **Phytochemical compounds of nuts with antioxidant effects.**

| Nut* | Phytoesterols† (mg/100 g) | Phenols | | | Carotenoids† (µg/100g) | Vitamin E† | | |
|---|---|---|---|---|---|---|---|---|
| | | Proanthocyanidins‡ (mg/100 g) | Flavonoids§ (mg/100 g) | Resveratrol¶ (µg/100 g) | | α-tocopherol (mg/100 g) | β-tocopherol (mg/100 g) | γ-tocopherol (mg/100 g) |
| Almonds | 141 | 184.02 | 15.24 | NA | 2 | 26.22 | 0.29 | 0.65 |
| Cashews | NA | 8.68 | 1.98** | NA | 22 | 0.90 | 0.03 | 5.31 |
| Hazelnuts | 96 | 500.66 | 11.74 | NA | 106 | 15.03 | 0.33 | 0.00 |
| Peanuts | 220 | 15.62** | 0.66 | 84 | 0 | 8.33 | NA | NA |
| Chestnuts | 22 | 0.05** | 0.02 | NA | NA | NA | NA | NA |
| Walnuts | 72 | 67.25 | 2.71 | NA | 21 | 0.70 | 0.15 | 20.83 |
| Brazil nuts** | NA | 0.00 | 0.00 | NA | 0 | 5.73 | 0.00 | 7.87 |
| Macadamia nuts | 116 | 0.00** | 0.00 | NA | NA | 0.54 | 0.00 | 0.00 |
| Pecans | 102 | 494.05 | 34.01 | NA | 55 | 1.40 | 0.39 | 24.44 |
| Pine nuts** | NA | 0.00 | 0.49 | NA | NA | NA | NA | NA |
| Pistachios | 214 | 237.34 | 14.37 | 115 | 332 | 2.30 | 0.00 | 22.60 |

* Nuts are raw unless indicated otherwise.
† Data from US Department of Agriculture Nutrient Database for Standard Reference, Release 21.[69]
‡ Data from US Department of Agriculture Database for the Proanthocyanidin Content of Selected Foods.[70] Total nut carotenoids are the sum of β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthin.
§ Data from US Department of Agriculture Database for the Flavonoid Content of Selected Foods.[71] Total nut flavonoids are the sum of anthocyanidins, flavan-3-ols, flavanones, flavones, and flavonols.
¶ Data from Tokusoglu et al. (2005).[72]
** Nuts are not raw. Cashews are "cashew-oil-roasted, without salt added"; peanuts are "all types, oil-roasted, with salt"; chestnuts are "dried, unpeeled"; Brazil nuts are "dried, unblanched"; Macadamia nuts are "dry roasted, without salt added"; pine nuts are "Pinyon, dried" in the corresponding USDA Database.
*Abbreviations*: NA, not available.

reduction in the formation of thiobarbituric acid reactive substances (TBARS) after 4 h of human plasma incubation in the presence of 100 and 150 μmol/L gallic acid equivalents of walnut phenolic extracts.

By inhibiting CD formation, two studies assessed lipid peroxidation in human LDL; one study used whole almonds, the brown skin of almonds, or extracts of the green shell cover of almonds,[38] while the other used whole hazelnuts or byproducts of hazelnuts.[39] Wijeratne et al.[38] observed effective inhibition of human LDL oxidation with extracts of whole almond seed, brown skin, or green shell cover at 10, 50, and 100 ppm quercetin equivalent doses after 20 h of incubation in comparison to quercetin, which was used as the reference control compound. Shaihidi et al.[39] also observed that extracts of hazelnut byproducts (skin, shell, green leafy cover, tree leaf) significantly decreased lipid peroxidation after 22 h of incubation compared to hazelnut kernel by increasing the percentage of inhibition of CD formation in human LDL.

Gentile et al.[37] investigated how the lipophilic and hydrophilic extract of a Sicilian variety of pistachio protected against lipid peroxidation in a study that evaluated TBARS formation on bovine liver microsomes. The results showed that, in the presence of hydrophilic pistachio extract, TBARS formation decreased significantly in bovine liver microsomes compared to those microsomes incubated in the absence of pistachio extract.

Finally, two in vitro studies evaluated the oxidative damage of DNA by monitoring the percentages of retention of supercoiled DNA with a tree-nut extract on plasmid DNA of *Escherichia coli*.[38,39] Both studies showed that incubation for 1 h with whole almonds, brown skin of almonds, or green shell extracts of almonds,[38] or with the kernel (with skin), the skin, the hard shell, the green leafy cover, or leaf extracts of hazelnuts[39] had a greater protective effect against strand breaking in supercoiled DNA than incubation with the control compounds quercetin or catechin.

All six of these studies suggest that tree-nut extracts possess an in vitro antioxidant capacity and are an excellent source of natural antioxidants with potential capacity for modulating oxidative stress.

### Effect of nuts on oxidation in animal studies

Few studies have evaluated the effect of nut consumption on oxidation in animal models. In fact, only six experimental studies (four chronic and two acute) have analyzed the effect of nuts on lipid peroxidation, antioxidant enzymatic activity, and cholesterol oxidation products. Of these, five were conducted in rodents and one in rabbits.

Similar non-significant chronic effects were observed in two animal studies that measured lipid peroxidation and antioxidant enzymatic activities in aortic tissues after animals had been fed with diets enriched with walnuts. In male Golden Syrian hamsters, Davis et al.[40] assessed how 12 experimental high-fat atherogenic diets with increasing concentrations of whole walnuts (61–150 g/kg of feed) or α-tocopherol (8.1–81 mg/kg), and single diets with either walnut oil (32 g/kg) or pure γ-tocopherol (81 mg/kg), affected some atherosclerosis-related markers. The content of carbohydrates, proteins, fat, fiber, vitamin, and mineral for each diet was maintained at a constant level throughout the 12 experimental diets. No significant differences in aortic Mn and Cu/Zn superoxide dismutase or bilidervin reductase protein levels were observed after 26 weeks between hamsters fed with walnuts and those fed with α-tocopherol- or γ-tocopherol-enriched diets. However, the increase in dietary walnuts was associated with a significant inverse, dose-dependent effect in the aortic endothelin (ET-1) levels, while they were not affected by dietary α-tocopherol. The authors suggested that the beneficial effects on CVD risk observed in this study after walnut consumption were not due to changes in oxidative stress but were partly due to ET-1–related effects on the endothelial processes. Likewise, Iwamoto et al.[41] observed no significant changes in the levels of cholesterol oxidation endproducts in the abdominal or thoracic aorta of apolipoprotein-E (apo-E)-deficient mice after consumption of a walnut-oil-enriched diet compared to mice fed with a high-linoleic safflower oil-enriched diet. However, female mice that were fed the walnut oil-enriched diet had a greater lesion area in the aortic root than those on the safflower oil diet. These observations have been attributed by the authors to the high α-linolenic content in walnuts and the specificity of the apo-E-deficient mice model studied.

In contrast, Aksoy et al.[42] evaluated the effect of pistachio consumption on serum paraoxonase-1 (PON1) and arylesterase activities. A total of 36 rats were randomly allocated to one of three diets for 10 weeks: a diet enriched with 2.5 g/day of pistachios (20% of the total caloric intake), a diet enriched with 5 g/day of pistachios (40% of the total caloric intake), or a control diet. The rats that consumed 20% of their daily energy in the form of pistachios exhibited a significant increase in PON1 and arylesterase activities compared to controls. However, increased antioxidant activity was mitigated when pistachio intake was increased to 40% of daily caloric intake, but it was still higher than that of the control group. Hatipoglu et al.[43] demonstrated that hazelnut oil has an effect on lipid peroxidation in rabbits fed with a hypercholesterolemic diet. After 14 weeks of nut consumption, plasma, liver, and aorta lipid peroxidation (measured by malondialdehyde [MDA] levels and CD formation) were significantly lower than in rabbits that did not consume

hazelnuts. However, no differences were observed in glutathione, glutathione peroxidase, or glutathione transferase activities in liver between diets that contained hazelnut oil and those that did not. The authors suggested that hazelnut oil may have antiatherogenic potential, which could be related to its reducing effect on oxidative stress biomarkers and, especially, on LDL oxidation.

Both acute studies showed that oxidative stress biomarkers improved after rodents consumed walnuts[44] or flavonoid extracts from almond skin.[35] In hamsters, a significant postprandial increase in the plasma concentrations of catechin, epicatechin, and flavonoids was associated with a longer lag time of the LDL oxidation 120 min after the ingestion of 40 μM of gallic acid equivalents (6.8 mg of an almond skin flavonoid extract) than in hamsters that did not ingest the almond extract.[35] These results suggest that this flavonoid extract is bioavailable and acts in synergy with vitamins C and E to protect LDL against oxidation.[35] The second acute study was carried out with rats.[44] After a fasting period, animals in the control group were given access to rodent chow, whereas the nut group was given walnut bulk. After 5.5 h of walnut consumption, the postprandial serum antioxidant capacity (measured by TEAC and FRAP) increased significantly in comparison to that of the control group. This beneficial effect was related to the increased levels of blood melatonin secondary to the ingestion of walnuts.

## SYSTEMATIC REVIEW OF THE EFFECT OF NUTS ON BIOMARKERS OF OXIDATIVE STRESS IN CONTROLLED FEEDING TRIALS OF HUMANS

### Literature search method

The data for this review were obtained from articles identified through the PubMed[45] and Web of Science[46] databases (from January 1990 to January 2009) and from reference lists of other relevant publications selected for review. Search terms used included MeSH terms (PubMed): "nuts" [Mesh] AND "humans" [Mesh] refined by the type of article (controlled clinical trial, randomized controlled trial) in "All Fields" as tag terms. The key words used in Web of Science were: (oxidat*) AND (almond* OR cashew* OR hazelnut* OR macadamia* OR peanut* OR pistachio* OR walnut* OR chestnut* OR pecan* OR pine nut OR Brazil nut OR filbert* OR hickory*), refined by: document type, subject areas, and publication period.

Studies were selected using the following criteria: 1) They had to be randomized, controlled, clinical feeding trials. 2) The intervention diet had to be supplemented with at least one of the following types of nut: almond, cashew, hazelnut, macadamia, peanut, pistachio, walnut, chestnut, pecan, pine nut, and Brazil nut. 3) They had to be published in a scientific journal between January 1990 and January 2009. 4) They had to be original. 5) They had to evaluate the effects of nuts on at least one of the following oxidative stress biomarkers: in vivo antioxidant capacity, oxidized LDL, MDA concentrations, plasma or urine isoprostane concentrations, CD formation, antioxidant non-enzymatic and enzymatic activities or DNA damage were the primary or secondary objectives.

Of the 681 articles identified by Web of Science,[46] 72 were excluded because of document type (review, note, letter, proceedings paper, or meeting abstract), 343 because of subject area, and nine because of time span. Of the 257 articles screened (titles and abstracts), 235 were excluded for the following reasons: 155 were irrelevant to the topic, 24 assessed the antioxidant or lipid content of nuts, 20 used animal models, and 36 were in vitro studies. The 22 remaining studies were conducted in humans. Of these 22 studies, five did not meet the inclusion criteria. Therefore, 17 studies from this database were included in our review.

Of the 45 human studies identified by PubMed,[45] 37 did not meet the inclusion criteria. After six duplicates were removed, two studies from this database were included in the review.

The reference lists of other relevant publications selected for review revealed five publications. Of these, two were not included in the review because they were not randomized studies[47,48] and two were not included because nuts were not used as a simple food but as part of a whole diet and the amount of nuts used was not specified.[49,50] Consequently, 20 clinical trials were identified that were suitable for inclusion in the present systematic review. Of these, five evaluated antioxidant capacity, 16 evaluated parameters related to lipid peroxidation, five evaluated antioxidant enzymatic and non-enzymatic activities, and two evaluated markers related to DNA damage.

Once the articles had been selected, the following significant data were extracted: author and year of publication, number and gender of the participants, type of individuals studied (i.e., healthy, hypercholesterolemic, smokers, non-smokers, high CHD risk, type 2 diabetes, or metabolic syndrome patients), type of study, length of the interventions (weeks), interventions used, dose of nuts administered (g/day), and the results in relation to the oxidative stress biomarkers evaluated.

### Results of the studies reviewed

*Individuals studied.* Almost all the 20 studies analyzed were carried out in small population samples that were heterogeneous as far as gender, age, and health status were concerned (Tables 3 and 4). The two studies that contained the highest numbers of individuals were by Davis

*Table 3* **Clinical trials evaluating the chronic effect of nut consumption on oxidative stress biomarkers.**

| Reference | N (M/F) Type of individuals | Type of study, (length of the intervention) | Control group | Intervention groups | Dose of nut (g/day) | Lipid peroxidation | | | Antioxidant non-enzymatic and enzymatic activity | DNA damage |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Serum oxidized LDL | MDA | Conjugated diene formation in LDL | | |
| Zambón et al. (2000)[20] | 49 (23/26) HC | Crossover (6 wk each period) | Mediterranean diet | Mediterranean diet + walnuts | 41–56 | NE | NE | ↓ lag time (NS) | NE | NE |
| Hargrove et al. (2001)[53] | 22 (9/13) Healthy | Crossover (3.5 wk each period) | American diet | a) Low-fat, step-II diet<br>b) High MUFA (olive oil)<br>c) High MUFA (peanut oil)<br>d) High MUFA (peanuts + peanut butter) | <br><br>c) 17.0<br>d) 18.3 + 18.0 | NE | NE | ↑ lag time in peanut oil (NS) and in peanut + peanut butter ($P < 0.05$) = Vmax in peanut oil (NS) and in peanut + peanut butter (NS)<br>↑ Cmax in both peanut treatments (NS) | NE | NE |
| Muñoz et al. (2001)[62] | 6 (6/0) HC | Crossover (6 wk each period) | Mediterranean diet | Mediterranean diet + walnuts | 41–56 | NE | NE | ↓ lag time (NS)<br>↑ Vmax ($P = 0.012$)<br>↑ Cmax (NS) | NE | NE |
| Hyson et al. (2002)[54] | 22 (10/12) Healthy | Crossover (6 wk each period) | Control diet | a) Control diet + almond oil replacing other sources of fat<br>b) Control diet + almonds replacing other sources of fat | 66 ± 5 | NE | NE | ↑ lag time (NS)<br>↓ Vmax (NS)<br>↑ Cmax (NS) | NE | NE |
| Iwamoto et al. (2002)[21] | 40 (20/20) Healthy | Crossover (4 wk each period) | Japanese diet | Japanese diet + walnuts | 44–58 | NE | NE | ↓ lag time (NS) | NE | NE |
| Jenkins et al. (2002)[63] | 27 (15/12) HC | Crossover (4 wk each period) | Low-fat, step-II diet | a) Low-fat diet + almonds<br>b) Low-fat diet + half-dose of almonds | a) 73<br>b) 37 | NE | NE | ↓ Cmax ($P \leq 0.01$) | NE | NE |
| Ros et al. (2004)[22] | 20 (8/12) HC | Crossover (4 wk each period) | Mediterranean diet | Mediterranean diet + walnuts | 40–65 | ↓ (NS) | ↓ in plasma (NS) | ↓ lag time (NS) | NE | NE |
| Haddad et al. (2006)[59] | 24 (14/10) Healthy | Crossover (4 wk each period) | Isocaloric, step-I diet | Isocaloric, step-I diet + pecans | 72 | NE | ↓ in plasma ($P < 0.014$) | NE | NE | NE |
| Jia et al. (2006)[61] | 30 (30/0) Smokers | Parallel (4 wk) | Chinese diet | a) Chinese diet + low-dose of almond powder supplement<br>b) Chinese diet + high-dose of almond powder supplement | a) 84<br>b) 168 | NE | ↓ in plasma ($P < 0.05$) in both doses | NE | ↑ in plasma SOD (NS) in both doses<br>↑ in plasma GSH-Px (NS) in both doses | ↓ urine 8-OHdG ($P < 0.05$) in both doses<br>↓ % tail DNA ($P < 0.05$) in high dose |
| Kocyigit et al. (2006)[55] | 44 (24/20) Healthy | Parallel (3 wk) | Isocaloric low-fat diet | Isocaloric low-fat diet + pistachio | 65–75 | NE | ↓ in plasma ($P < 0.05$) | NE | NE | NE |

*Table 3* **Continued**

| Reference | N (M/F)<br>Type of individuals | Type of study, (length of the intervention) | Control group | Intervention groups | Dose of nut (g/day) | Lipid peroxidation | | | Antioxidant non-enzymatic and enzymatic activity | DNA damage |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Serum oxidized LDL | MDA | Conjugated diene formation in LDL | | |
| Canales et al. (2007)[66] | 22 (12/10)<br>High CHD risk | Crossover<br>(5 wk each period) | Control diet | Control diet + walnut-paste-enriched meat and sausage | 21.4 | NE | ↓ in erythrocytes ($P = 0.05$) | NE | ↑ catalase* ($P = 0.05$)<br>↑ SOD* (NS)<br>↑ PON1* (NS)<br>↑ total glutathione* ($P < 0.001$)<br>↑ GSH* (NS)<br>↑ GSSG* ($P < 0.01$)<br>↓ GSH/GSSG* ($P < 0.05$) | NE |
| Davis et al. (2007)[51] | 64 (29/35) MetS | Parallel<br>(8 wk) | South African isocaloric diet (SAID) | a) SAID supplemented with walnuts<br>b) SAID supplemented with cashews | 63–108 | NE | NE | NE | ↓ plasma GSH (NS)<br>↓ plasma GSSG (NS)<br>↑ plasma GSH/GSSG (NS) | NE |
| Li et al. (2007)[52] | 60 (60/0)<br>Smokers and non-smokers | Crossover<br>(4wk each period) | Chinese diet + pork meat | Chinese diet + almond powder supplement | 84 | NE | ↓ in urine ($P < 0.05$) in smokers | NE | ↑ plasma SOD ($P < 0.05$) in smokers<br>↑ plasma GPx ($P < 0.05$) in smokers<br>↓ plasma Catalase (NS) in smokers | In smokers:<br>↓ urine 8-OHdG ($P < 0.05$)<br>↓ % tail DNA ($P < 0.05$) |
| Nus et al. (2007)[67] | 23 (14/9)<br>High CHD risk | Crossover<br>(5 wk each period) | Control diet | Control diet + walnut-paste-enriched meat and sausage | 21.4 | (NS) | ↓ in erythrocytes ($P = 0.031$) of PON1-192R polymorphism carriers | NE | NE | NE |
| Jenkins et al. (2008)[64] | 27 (15/12)<br>HC | Crossover<br>(4 wk each period) | Low-fat diet | a) Low-fat diet + full-dose almond<br>b) Low-fat diet + half-dose almond | a) 73<br>b) 37 | NE | ↓ in serum ($P = 0.04$) in full dose | NE | NE | NE |
| Thomson et al. (2008)[58] | 59 (30/29)<br>Healthy | Parallel<br>(12 wk) | Normal diet | Normal diet + Brazil nuts | 8 | NE | NE | NE | ↑ GPx in whole blood ($P < 0.001$)<br>↑ GPx in plasma ($P = 0.002$) | NE |

* Measured in erythrocytes.

*Abbreviations*: 8 OH dG, 8-hydroxy-deoxyguanosine; % tail DNA, DNA strand breaks; CHD, coronary heart disease; Cmax, maximum concentration; F, female; GPx, glutathione peroxidase activity; GSH, reduced glutathione concentration; GSH:GSSG, reduced glutathione/oxidized glutathione ratio; GSH-Px, glutathione peroxidase activity; GSSG, oxidized glutathione; HC, hypercholesterolemic; LDL, low density lipoprotein; M, male; MDA, malondialdehyde; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; N, number; NE, non-evaluated; NS, non-significant; *P*, value of the difference between nuts and control diet; PON1, paraoxonase-1; PON1-192R, subjects carrying arginine at least in one allele of position 192 polymorphism; SOD, superoxide dismutase activity; Vmax, maximum velocity; wk, weeks.

*Table 4* **Clinical trials evaluating the acute effect of nut consumption on oxidative stress biomarkers.**

| Reference | N (M/F), type of individuals | Type of study (length of intervention) | Control group | Intervention groups | Dose of nuts (g/day) | Antioxidant capacity | Lipid peroxidation | | | Conjugated diene formation in LDL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Serum oxidized LDL | MDA | Plasma total isoprostanes | Lag time | Vmax | Cmax |
| Cortés et al. (2006)[56] | 24 (20/4) Healthy and HC | Crossover (1 day each test meal) | High-fat meal + olive oil | High-fat meal + walnut | 40 | NE | ↓ (NS) | NE | NE | (NS) | (NS) | (NS) |
| Jenkins et al. (2006)[57] | 15 (7/8) Healthy | Crossover (1 day each test meal) | 97 g of white bread | 97 g of white bread + almonds | 60 | Serum posprandial TAC (NS) | NE | NE | NE | NE | NE | NE |
| Berry et al. (2008)[60] | 20 (20/0) Healthy | Crossover (1 day each test meal) | Muffins with 50 g of sunflower oil blend | Muffins with whole almond seed macroparticles<br>Muffins with almond oil + defatted almond flour | 96.5<br>50.0 + 47.0 | NE<br>NE | NE<br>NE | NE<br>NE | (NS)<br>(NS) | NE<br>NE | NE<br>NE | NE<br>NE |

*Abbreviations:* Cmax, maximum concentration; F, female; HC, hypercholesterolemic; LDL, low density lipoprotein; M, male; MDA, malondialdehyde; N, number; NE, non-evaluated; NS, non-significant; *P*, value of the difference between nuts and control diet; TAC, total antioxidant capacity; Vmax, maximum velocity.

et al.,[51] who investigated the effect of walnut and cashew consumption on the antioxidant status of 64 subjects with metabolic syndrome, and by Li et al.,[52] who investigated the effect of almonds on 60 male smokers and non-smokers. In total, these 20 studies included a total of 656 individuals (400 men and 256 women). Some of the studies were performed on healthy individuals,[21,53–60] while others were performed on smokers[52,61] and non-smokers,[52] hypercholesterolemic,[20,22,56,62–64] type 2 diabetic[65] or metabolic syndrome patients,[51] or individuals at high risk of CHD.[66,67]

*Study design and length.* Most of the studies were crossover clinical trials ($n = 15$), but five used a parallel design.[51,55,58,61,65] The two types of clinical trials were of varying duration. In the crossover studies, the intervention period lasted between 3.5 and 6 weeks. In the parallel studies, the longest intervention period was 24 weeks[65] and the shortest was 3 weeks.[55] Three crossover studies (Table 4) evaluated the acute effect of a meal containing nuts, so the intervention was conducted in a single day.[56,57,60]

*Intervention and control treatments.* Nuts were administered in different types, doses, and presentations. The nuts evaluated were walnuts,[20–22,51,56,62,65–67] peanuts,[53] almonds,[52,54,57,60,61,63,64] pistachios,[55] cashews,[51] pecans,[59] and Brazil nuts.[58] The total dose of nuts used in most clinical trials was between 17 and 168 g/day, except in the study by Thomson et al.,[58] which evaluated the effect of only 8 g of Brazil nuts per day. In most studies, raw nuts were administered in the context of a meal or diet ($n = 15$), although in some, the nuts were dry roasted ($n = 1$), cooked ($n = 3$), or in the form of butter ($n = 1$), oil ($n = 3$), flour ($n = 1$), or powder ($n = 2$). Comparisons were made with control diets or meals in which the individuals were asked not to consume nuts, nut butter, or nut oil of any kind. Some studies compared the effect of different doses of nuts.[53,60,61,63,64]

In six trials, the diet was totally controlled and meals were provided in a canteen or a metabolic kitchen.[21,51–53,59,61] In five of these trials, both the control and the intervention diets were isocaloric.[21,51–53,59] In seven studies, patients received dietary recommendations so that the intervention group and the control group received isocaloric diets.[20,22,53–55,62] This was achieved by replacing energy from monounsaturated fatty acids (MUFA) or total fat in the control group by nuts or nut oil in the interventional group. In eight studies, both the control group and the intervention group received the usual diet or a low-fat diet. However, only the intervention group received nuts as a supplement; the control group was given instructions to avoid them.[52,58,59,61,63,64,66,67] Basal diets were quite different among the studies: in three studies, a Mediterranean

diet was administered,[20,22,62] in eight cases, the diet was Asian or low-fat,[21,52,55,59,61,63–65] and in all others, the diet was a habitual or Western type.[51,53,54,58,66,67] In the trials that evaluated the acute effect of nut consumption, nuts were administered in the context of a meal. In two studies, the fat content in the control meal (olive[56] or sunflower oil[60]) was replaced by fat in the form of nuts. In another study, almonds were administered as a supplement to the meal in the intervention group.[57]

*Effect of nuts on the antioxidant capacity.* The results from studies evaluating the effect of nuts on antioxidant capacity are mixed. In the parallel-design study by Kocyigit et al.,[55] 44 healthy individuals were randomly assigned to either an isocaloric regular diet or a whole-pistachio diet for 3 weeks. In the pistachio group, the authors observed a significant increase in the plasma antioxidant potential, measured as the capacity to inhibit TBARS production. Using the ORAC method, another chronic study showed no significant effect on the plasma antioxidant capacity after the whole-walnut or cashew diet interventions in subjects with metabolic syndrome.[51] In a parallel, randomized, controlled trial, Tapsell et al.[65] assessed the total plasma antioxidant capacity in type 2 diabetes patients and found no significant difference in total plasma antioxidant capacity after 24 weeks of walnut consumption as a supplement in the context of a low-fat diet.[65] The postprandial serum total antioxidant capacity was also evaluated by using an acute clinical trial with a randomized crossover design.[57] In this study, the postprandial antioxidant capacity of serum was not significantly influenced by the consumption of 60 g of raw, unblanched almonds added as a supplement to the basal meal.

*Effect of nuts on serum oxidized LDL.* All of the studies evaluating lipid peroxidation by measuring the oxidized LDL in serum observed that nut consumption induced no significant changes.[22,56,67] Using a crossover design, Ros et al.[22] evaluated the effect of walnut consumption in 20 hypercholesterolemic patients. Patients were randomly allocated to two isocaloric dietary interventions in which nuts replaced other sources of MUFA in the context of a Mediterranean diet. Although levels of oxidized LDL particles in serum showed a non-significant decrease after 4 weeks of a walnut-diet intervention, no significant differences in oxidized LDL were observed between the two intervention diets. The same non-significant tendency to lower the serum oxidized LDL levels was observed by Cortés et al.[56] in healthy and hypercholesterolemic individuals. They used a crossover study to compare the postprandial effect of one high-fat meal with another in which fat content in the form of olive oil was replaced by walnuts.

Finally, Nus et al.[67] used a crossover design study in which walnut-enriched restructured steaks and sausages were added to the regular diets of patients at high risk for CHD for 5 weeks. They observed no significant changes in oxidized LDL.

*Effect of nuts on malondialdehyde concentration.* Lipid peroxidation, measured by the mean of MDA concentrations, decreased in almost all the studies (7/8) that evaluated the effect of dietary nut consumption on this oxidation parameter. In these studies, MDA concentrations were measured in four biologically different samples: plasma, urine, serum, and erythrocytes. This favorable effect on MDA concentrations was observed not only in subjects with metabolic stress,[52,61,64–67] but also in healthy individuals.[55,59]

Only two studies have evaluated the effect of almond consumption on smokers. In one study with a parallel design, 30 male smokers were randomly assigned to a standard diet or a diet enriched with 84 or 168 g/day of powdered almonds. All the patients on an almond-enriched diet experienced a significant decrease in plasma MDA concentrations and a better response in other oxidation parameters.[61] This beneficial effect on lipid MDA urine concentrations was also observed by the same group of researchers using a crossover study in another series of 60 smokers.[52]

The effect of nuts was also analyzed in hypercholesterolemic patients[64] and in subjects at high risk of CHD.[66,67] In a crossover study, the serum MDA concentrations of hypercholesterolemic patients decreased significantly more when the patients consumed 73 g of whole almonds than when they consumed half this amount or no almonds at all.[64]

Two studies measured MDA concentrations in erythrocytes to evaluate the effect of walnut consumption.[66,67] Both clinical trials were conducted by the same group of investigators on patients at high risk of CHD, and both studies used the same crossover design. In periods of 5 weeks, patients received walnut-enriched restructured steaks and sausages in the context of their usual diet, or steaks and sausages without walnuts. In both studies, erythrocyte MDA concentrations decreased significantly during the walnut-enriched-meat-diet period compared to the period without walnuts. It should be pointed out that in the study conducted by Nus et al.[67] this decrease in lipid peroxidation was only observed in patients with the PON1-192R polymorphism, suggesting that this polymorphism modulates the effect of walnut consumption on lipid peroxide in LDL and, therefore, probably the risk of CHD by this mechanism.

*Effect of nuts on urine isoprostane concentrations.* Only two of the 20 studies analyzed in this review evaluated

lipid peroxidation by measuring the 24-h urine isoprostane concentration. In a crossover clinical trial carried out in 27 hypercholesterolemic men and women, Jenkins et al.[64] showed that this biomarker of fat oxidation was significantly lower when patients received the almond supplement in the context of a low-fat diet than when they did not receive it. It should be noted that after creatinine correction, urinary isoprostane outputs during almond supplementation were lower than during the control diet.

However, using an acute, randomized, crossover design, postprandial total 8-isoprostane-$F_2\alpha$ plasma concentrations were not significantly influenced by the consumption of almond test muffins baked with either 96.5 g of almond seeds or with 50 g of almond oil and 47 g of defatted almond flour.[60]

*Effect of nuts on lipid peroxidation measured by CD formation in LDL particles.* Several indexes can be used to measure the kinetics of the CD formation and thus describe the potential of the LDL particles to oxidate: the lag time, defined as the interval between the intercept of the linear least-square slope of the curve with the initial-absorbance axis; the maximal rate of oxidation (Vmax) index, calculated from the slope of the absorbance curve during the propagation phase; and the maximal amount of dienes (Cmax) formed.[68]

Of the 20 articles selected, eight focused on the lag time, Vmax, and/or Cmax formation to evaluate the effect of nut consumption on lipid peroxidation in LDL particles. All the studies were conducted on healthy and hypercholesterolemic patients, and all used a crossover clinical trial to analyze the effect of consuming different types of nut-enriched diets. Four of these studies measured the three CD indexes in LDL particles,[53,54,56,62] whereas three other studies evaluated lipid peroxidation by measuring only the lag time.[20–22] Only one measured the Cmax index.[63]

Some of these studies found an improvement in CD formation, whereas others failed to find any significant effects. Yet others have described a worse response after nut consumption. The equivocal effects observed in these studies are largely dependent on the type of nuts investigated.

Four chronic clinical trials studied the effect of walnut consumption on CD formation using a crossover design. Three of these studies were conducted by the same group of investigators on hypercholesterolemic patients[20,22,62] and they analyzed the effect of two Mediterranean isocaloric diets in which MUFA-rich foods were replaced by walnuts. Iwamoto et al.,[21] however, studied a group of healthy women and compared the effect of two isocaloric Japanese diets in which other sources of fat were replaced by walnuts. In all of these studies, response tended to be worse during the walnut consumption period. Muñoz et al.[62] described a significant increase in the Vmax associated with a non-significant increase in the Cmax index during the Mediterranean diet with walnuts period. The effect of walnut consumption was also studied under acute conditions in hypercholesterolemic patients by Cortés et al.[56] The lag time and Vmax indexes showed no significant differences in lipid peroxidation after meals containing walnuts and isocaloric meals containing the same amount of fat in the form of olive oil.

Mixed results were also observed when the effect of almond consumption on healthy and hypercholesterolemic patients was analyzed. Lipid peroxidation decreased significantly in hypercholesterolemic patients after a low-fat diet supplemented with 73 or 37 g of almonds in comparison with a control, low-fat diet.[63] In contrast, Hyson et al.[54] showed a non-significant effect on CD kinetics of a whole-almond diet compared to a diet containing the same calories in the form of almond oil or after the control diet (without almonds).

Hargrove et al.[53] evaluated how a low-fat or various monounsaturated-enriched diets (with olive oil, peanut oil, and peanuts plus peanut butter) affected LDL oxidative susceptibility in vitro. In comparison to a typical American diet, a significant increase in LDL lag time was reported in healthy subjects after they had consumed a low-fat diet or high-MUFA diets containing different sources of MUFA.

*Effect of nuts on antioxidant non-enzymatic and enzymatic activity.* The effect of nut consumption on antioxidant non-enzymatic or enzymatic activity was measured in five studies. Positive effects were observed in three of them and no statistically significant effect in two. In one crossover study, antioxidant defense mechanisms in male smokers increased significantly after the subjects consumed almond powder.[52] The same study also reported an increase in superoxide dismutase and glutathione peroxidase enzymatic activities when subjects consumed the almond-powder supplement. Canales et al.[66] also showed general improvement in the antioxidant status (an increase in the erythrocyte catalase activity and a decrease in the glutathione/oxidized glutathione ratio) of high-coronary-risk patients after they consumed a diet containing walnut-enriched meat. These beneficial effects on antioxidant enzymatic activities were also reported in the parallel study conducted by Thomson et al.[58] In comparison with the control groups (one of which was receiving a seleniomethionine tablet and the other a placebo), a significant increase in total blood and plasma glutathione peroxidase activities was reported in healthy subjects after their diet was supplemented with small amounts of Brazil nuts for a 12-week period.

Finally, no differences in the plasma glutathione or oxidized glutathione levels were observed in metabolic syndrome patients after they had consumed a control diet or an isocaloric diet with or without a walnut or a cashew supplement.[51] Similar results were reported by Jia et al.[61] in a parallel study analyzing the effect of an almond-powder supplement in smokers.

*Effect of nuts on oxidative DNA damage.* Oxidative DNA damage was analyzed in only two studies, which were conducted in healthy male smokers after they had consumed a supplement of whole-almond powder. Both parameters analyzed in these studies – the percentage of DNA strand breaks in lymphocytes and 8-hydroxy-deoxyguanosine urine concentrations – decreased significantly after subjects had consumed a diet containing almonds. This finding suggests almonds can help decrease the oxidative stress mediated by tobacco.[52,61]

## CONCLUSION

Nuts comprise a complex food group and are rich in bioactive constituents that can affect cardiovascular health. In vitro studies have shown that incubating cells with extracts from several tree nuts (some of them very rich in polyphenols) can inhibit oxidative susceptibility. This may be due to the phytochemical compounds they contain. However, in experimental studies conducted with animals, or in human clinical trials, the consumption of several types of nuts as a whole food was not observed to have any consistent positive effects on oxidation status. Most of the studies that showed a potential beneficial effect of nuts on oxidation focused on almonds, pistachios, Brazil nuts, or peanuts, all rich sources of MUFAs. This beneficial effect on oxidation was not found consistently in walnut trials performed on humans. However, no deleterious effects on oxidation were reported in any of the published studies. All in all, the studies suggest that, although some whole nuts are susceptible to oxidation because of their PUFA content – which is particularly high in walnuts – the potential antioxidant activity of the polyphenols, phytosterols, and other antioxidants contained in nuts, particularly in the skin, may counteract the pro-oxidant effects of fat, thus preventing potentially adverse effects on oxidation.

Further studies on nuts fully characterizing the antioxidant content, bioaccesibility, bioavailability, metabolism, and elimination in humans will be necessary in the future. The possible interactions between different nut antioxidant products and other important nut constituents that promote antioxidant activities also need to be explored. Such studies may help elucidate the mechanisms explaining the effect of consuming nuts or their byproducts on oxidative stress status in humans.


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