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RESEARCH PAPER

Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation

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Abstract

Background: Nuts have been shown to have beneficial effects on human health due to the healthy fat content; however, the effect of antioxidants (i.e. polyphenols) in nuts have not been fully investigated. The present study aimed to assess the immediate effect of a polyphenol-rich meal (75% of energy from nuts: walnuts or almonds) and a polyphenol-free meal on plasma polyphenol content, antioxidant capacity and lipid peroxidation in healthy volunteers.
Methods: Thirteen subjects participated in a randomized, crossover, intervention study. After an overnight fast, walnuts, almonds or a control meal in the form of smoothies were consumed by study subjects. Each subject participated on three occasions, 1 week apart, consuming one of the smoothies each time. Blood samples were obtained at fasting and then at intervals up to 3.5 h after consumption of the smoothies.
Results: There was a significant increase in plasma polyphenol concentration following both nut meals, with peak concentrations being achieved at 90 min, and with a walnut meal having a more sustained higher concentration than an almond meal. The plasma total antioxidant capacity reached its highest point at 150 min postconsumption of the nut meals, and was higher after the almond compared to walnut meal. A gradual significant ($P < 0.05$) reduction in the susceptibility of plasma to lipid peroxidation was observed 90 min after ingestion of the nut meals. No changes were observed following consumption of control meal.
Conclusions: Consumption of both nuts increased plasma polyphenol concentrations, increased the total antioxidant capacity and reduced plasma lipid peroxidation.

Introduction

A correlation between nut consumption and a reduced incidence of ischaemic heart disease has been reported in several large epidemiological studies (Fraser *et al.*, 1992; Kushi *et al.*, 1996; Hu *et al.*, 1998). Human intervention studies on nuts show that most of these cardio-protective effects of nuts are brought about via reducing both total and low-density lipoprotein (LDL) cholesterol, largely due to the high unsaturated fatty acid content of these nuts (Feldman, 2002; Strahan, 2004). Although it is known that, for every 1% decrease in plasma LDL cholesterol, there is a 2% reduction in cardiovascular disease (CVD) risk (Levine *et al.*, 1995), the overall risk reduction with frequent nut consumption is greater than that accounted for by changes in blood lipids alone (Jenkins *et al.*, 2002). Nuts have other nutrients, such as arginine, fiber, vitamin E and magnesium (Sabaté & Fraser, 1994; Kris-Etherton *et al.*, 1999; Hu, 2003), and nonnutrient phytochemicals such as polyphenols (Anderson *et al.*, 2001; Hu, 2003; Wu *et al.*, 2004) that can also contribute to lowering the risk for CVD by mechanisms beyond that of cholesterol lowering (Hu, 2003).

Both *in vitro* and *in vivo* studies have shown that polyphenols from red wine (Teissedre *et al.*, 1996), green

tea (Yoshida *et al.*, 1999) and chocolate (Waterhouse *et al.*, 1996) can decrease the risk of CVD by increasing the plasma antioxidant capacity and inhibiting LDL peroxidation (Whitehead *et al.*, 1995; Serafini *et al.*, 1996, 2003; Rein *et al.*, 2000; Seeram *et al.*, 2006). Walnuts and almonds are the two most commonly consumed nuts in the USA (ERS/USDA Briefing Room, 2004) as well as in European countries, including UK (Jenab *et al.*, 2006). Jenab *et al.* (2006) showed that the three most popular tree nuts in the European countries including UK were walnuts, almonds and hazelnuts, respectively, and this study also showed in the European countries in general, tree nuts were more widely consumed than peanuts or seeds. Walnuts and almonds contain favourable levels of total polyphenols relative to other polyphenol rich foods, when compared on a per serving basis (Anderson *et al.*, 2001). A handful of walnuts (50 g) has significantly more phenolics than 236 mL (8 fl oz). of glass of apple juice (Pearson *et al.*, 1999), and a 148 mL (8 fl oz). glass of red wine (Frankel *et al.*, 1995).

However, little is known about the polyphenols from nuts, especially their bioavailability and their ability to provide antioxidant protection in humans. Therefore, the present study primarily aimed to investigate the acute effect of ingesting almonds and walnuts on plasma total polyphenols, antioxidant capacity and lipid perxidation in healthy human volunteers.

Materials and methods

Subjects

Healthy subjects, seven men and seven women aged 19–65 years, were recruited using advertisement flyers from Loma Linda University and surrounding areas. Subjects underwent a screening process including a person-to-person interview with the investigator. Presence of any chronic disease, the use of special diets, pregnancy, lactation, smoking, body mass index ≥ 25, the use of any medications or dietary supplements, allergies or sensitivity to nuts were considered as exclusion criteria. Of the fourteen subjects who started the study, 13 completed the study and one person dropped-out because of a lack of time. Sample size was calculated for a three-treatment, three-period crossover design using SAS, version 8.0 (SAS Institute Inc., 1999), based on the method recommended by Senn (1993). The sample size calculation was based on an estimate of the SD of the treatment difference (the difference between treatment and control measurements of plasma phenols taken on an individual) of 2.5 mg L^{-1} obtained from previous research conducted by O'Byrne *et al.* (2002). At the $P < 0.05$ level of significance, a sample size of six subjects will provide >90% power to detect a 15.0 mg L^{-1} change in plasma phenol concentration.

The study was approved by Institution Review Board (IRB) of Loma Linda University and all participants provided their written informed consent.

Study design

A randomized (3×3) cross-over design with 1 week washout period between treatments (walnut, almond and control) was used. On the test day, subjects arrived after a 12-h overnight fast and a baseline blood sample was drawn. Then, according to the randomization schedule, they were provided with one of three test meals for breakfast, served in the form of a smoothie that was consumed within 10–15 min. Blood samples were collected at 30, 90, 150 and 210 min after consumption of the test meals. Participants were asked not to consume any nuts, fruits, vegetables, chocolate (any cocoa containing product), coffee, tea, juice (fruit or vegetable) and wine 24 h prior to the clinic visit and during the study. Water was consumed *ad libitum*.

Test meals

Test meals were in form of smoothies all nutrients were blended in water. For the nut-containing meals, nuts provided 75% of energy intake (approximately 10 kcal kg^{-1} body weight) and the remaining 25% of energy came from a refined carbohydrate source (polycose). For the control meal, macronutrient composition similar to nut-containing meals was calculated and food sources were carbohydrate (polycose), fat (whipping cream and coconut oil) and protein (purified whey protein). Water was used depending on person's body weight and ranged between 236–473 mL (8–16 fl oz).. Both meals, treatment and control were fully tolerated by our study participants and no one refused to consume the entire smoothie. Table 1 presents the diet composition of the three test meals. Diet composition including macro and micronutrient contents of test meals were calculated by using Nutrition Data System Research software, version 2005 from University of Minnesota.

Chemical analysis

Blood samples were collected and plasma separated by centrifugation (1500 ***g*** at 4 °C for 10 min) and aliquots stored at−80 °C until analyses.

Plasma polyphenol assay

Total phenolic content in plasma was measured by the Folin–Ciocalteau method (Serafini *et al.*, 1998) to remove protein interferences. Total phenolic concentrations of plasma samples were determined after a procedure of acid extraction/hydrolysis, and protein precipitation with

Table 1 Nutrient composition for the three test meals (700 kcal each) based on a 70 kg body weight volunteer

	Walnut (81 g)	Almond (91 g)	Control
Macronutrients (% energy)			
Fat	65.5	57.5	60
Saturated fatty acid	6.5	5	51
Monounsaturated fatty acid	9.5	38	6
Polyunsaturated fatty acid	49.5	14.5	3
Carbohydrate	30	32.5	31
Protein	5.5	9	7
Vitamin E*	0.9	23	0.5
Polyphenol†	44.37 ± 4.06	13.39 ± 2.83	0.13 ± 0.01

*Milligram (mg) of total alpha-tocopherol in 700 kcal test meals.
†Total polyphenol data expressed as gallic acid equivalents per gram (mg g^{-1} GAE).

0.75 mol L^{-1} metaphosphoric acid (MPA). For hydrolyzing the conjugated forms of polyphenols, hydrochloride acid was added to the sample, followed by sodium hydroxide in methanol. This step breaks the links of polyphenols with lipids and provides a first extraction of polyphenols. For removing plasma proteins, MPA was used in this procedure. The final extraction of polyphenols was performed by adding 1 : 1 (v/v) solution of acetone:water. The results were expressed as the gallic acid equivalent (GAE) in mg L^{-1}.

Antioxidant capacity assay

Two methods were applied to measure the antioxidant capacity of plasma: the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996) and the oxygen radical absorbance capacity (ORAC) (Cao & Prior, 1999) and recently modified method (Prior *et al.*, 2003).

FRAP assay

The FRAP assay takes advantage of electron transfer reactions. This method measures the ferric reducing ability of plasma. At low pH, when a ferric tripyridyltriazine (Fe III-TPTZ) complex is reduced to the ferrous (Fe II) form, an intense blue colour with an absorption maximum at 593 nm develops (Benzie & Strain, 1996). Test conditions favour reduction of the complex, and thereby colour development, provided that a reductant (antioxidant) is present. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma and with solutions containing antioxidants. The results are expressed as μmol L^{-1} of antioxidant power.

ORAC assay

The ORAC method with fluorescein as fluorescent probe was used as (Prior *et al.*, 2003). The method involves extraction and analysis of hydrophilic and lipophilic antioxidants in plasma. The ORAC method is based on the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as 2,2′-azobis (2-amidino-propane) dihydrochloride. This assay utilizes a biological relevant radical source and is the only method that combines both inhibition time and degree of inhibition into simple quantity.

The assay uses area under the curve for quantification. The $ORAC_{FL}$ values were calculated by using a quadratic regression equation ($y = ax^2 + bx + c$) between the Trolox or sample concentration and net area under the FL decay curve. Data are expressed as Trolox equivalents (TE) per g or mL of sample (μmol g^{-1} or μmol mL^{-1}). The area under curve (AUC) was calculated as $AUC = [0.5 + (f_1/f_0) + (f_n/f_0)] \times CT$ where f_1 = fluorescence reading at cycle 1, f_n = fluorescence reading at cycle n, and CT = cycle time in minutes. The data were analysed using Microsoft Excel (Microsoft, Redmond, WA, USA).

Lipid peroxidation assay

The Thiobarbituric Acid Reactive Substances (TBARS) assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); forming a MDA-TBA_2 adduct that absorbs strongly at 532 nm. This reaction is the most popular method for estimating MDA in biological samples. TBARS are expressed as malondialdehyde equivalents (Wang *et al.*, 2000).

Statistical analysis

Results in the text and tables are expressed as the mean ±SD. Changes between baseline (0 h) and other time points among treatment groups were examined using repeated-measures analysis of variance, with control for multiple measurements on the same subjects. Where appropriate, multiple comparisons were made using Tukey–Kramer corrections. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SAS for Windows (SAS Institute Inc., 1999).

Results

Plasma total polyphenol, antioxidant capacity and lipid peroxidation results are provided in Table 2. Plasma polyphenol concentration significantly increased within 30 min of the test meals with both walnut and almond, reaching peak levels by 90 min after ingestion (224.3 ± 2.57 mg L^{-1} GAE for walnut diet and 238.48 ± 2.71 mg L^{-1} GAE for almond meal). No significant increase was observed after the consumption of control meal. The increase in plasma polyphenol levels following the intake of the walnut meal

was more constant, and reached a steady state after 90 min, and remained higher than baseline even at 210 min. For the almond meal, following the 90 min peak, levels returned to baseline by 210 min (Figs 1 and 2). Both nut meals demonstrated a significant increase in plasma antioxidant capacity compared to the control meal (Table 2). With respect to plasma lipid peroxidation, consumption of the walnut and almond meals resulted in a gradual reduction in TBARS 90 min after ingestion (Table 2). By contrast, no changes were observed for the control group.

The antioxidant capacity in both treatment groups reached a peak value at 150 min and started to decline by 210 min. At 150 min after consumption of the walnut meal, both lipophilic and hydrophilic ORAC components showed an increase of 123% and 201% in plasma antioxidant capacity over baseline and, for the almond meal, the increase was 363% (Fig. 2). The results for the control meal showed no significant change over baseline at any time point.

Coefficients of variation at baseline for all study assays were 13.98 for plasma polyphenol measurement, 10.49 for FRAP, 20.11 for lipophilic ORAC, 15.74 for hydrophilic ORAC and 28.17 for TBARS. This degree of variation might explain the great variability in baseline values of

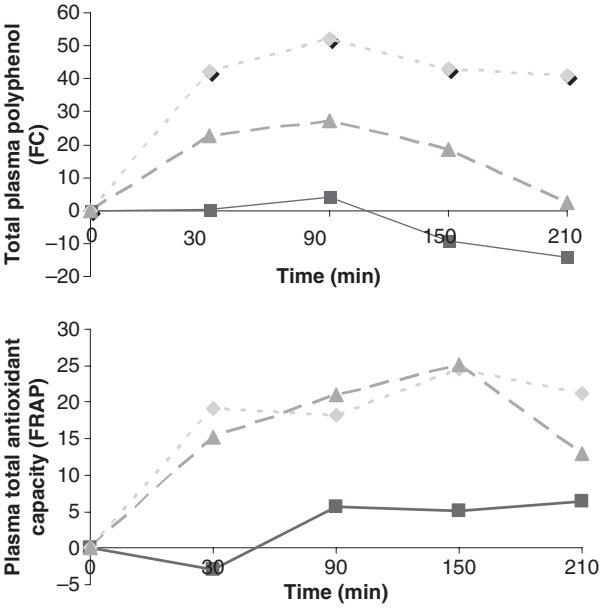

Figure 1 Effect of control (■), walnut (♦) and almond (▲) meals on total plasma polyphenols measured by Folin–Ciocalteau (FC) and plasma total antioxidant capacity measured by the ferric ion reducing antioxidant power (FRAP) method. Values are expressed as the percent change of the individual increments over baseline.

Table 2 Plasma total polyphenols concentrations, total antioxidant capacity and lipid peroxidation in human subjects before and after consuming walnut, almond and control meals

Test meal	Time (min)	Plasma polyphenol (mg L^{-1} GAE)†	Plasma total antioxidant capacity (μmol L^{-1})			Plasma lipid peroxidation (μmol L^{-1} MDA)‡
		FC	ORAC hydrophilic	ORAC lipophilic	FRAP	TBARS
Control	0	200.3 ± 2.6	54.3 ± 1	243.7 ± 1.5	551 ± 3.9	9.2 ± 1.4
	30	200.7 ± 2.5	64.4 ± 1	388.4 ± 1.2	535.1 ± 8.9	11.2 ± 1.7
	90	208.4 ± 2.45	65.3 ± 0.9	356.9 ± 1.9	582.6 ± 4.6	10.5 ± 1.6
	150	181.2 ± 2.7	67.5 ± 0.9	280 ± 1.3	578.9 ± 4.9	10.5 ± 1.5
	210	171.6 ± 3.1	65.9 ± 1.1	262.5 ± 3.1	586.8 ± 5.7	10.8 ± 1.8
Walnut	0	147.6 ± 2.7	58.6 ± 1	113.7 ± 4.5	510 ± 9	9.1 ± 1.3
	30	209.7 ± 2.5*	77 ± 1.1	194.4 ± 1.2	608.1 ± 6*	10.2 ± 1.3
	90	224.4 ± 2.6*	103.9 ± 3.3*	332.2 ± 2.2*	602.9 ± 5.9*	6.4 ± 0.9*
	150	210.9 ± 2.5*	130.5 ± 3.4*	455.3 ± 3.8*	635 ± 7.1*	7.4 ± 1
	210	208.2 ± 2.5*	100.8 ± 2.4*	324.1 ± 1.8*	618.3 ± 5.4*	7.6 ± 1.1
Almond	0	187.2 ± 2.5	57.5 ± 1.4	123.7 ± 3.7	525.2 ± 9.8	7.3 ± 0.3
	30	230.1 ± 2.7*	102.5 ± 3.6*	215.6 ± 2.5	604.8 ± 8.2*	7.6 ± 0.7
	90	238.5 ± 2.7*	152.9 ± 4.6*	375.9 ± 1.9*	635.8 ± 5.9*	5.2 ± 0.9*
	150	221.6 ± 3.4	266.2 ± 8.7*	472.5 ± 3.8*	657.4 ± 6.7*	6.2 ± 0.5
	210	191.7 ± 2.9	200.4 ± 6.4*	256.2 ± 2.1	593.3 ± 5.7*	6.1 ± 0.8

FC, Folin–Ciocalteau; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing ability of plasma; TBARS, thiobarbituric acid reactive substances.

Data are presented as the mean ± SD.

*Significantly different ($P < 0.05$) from baseline.

†Gallic acid equivalents.

‡Malondialdehyde equivalents.

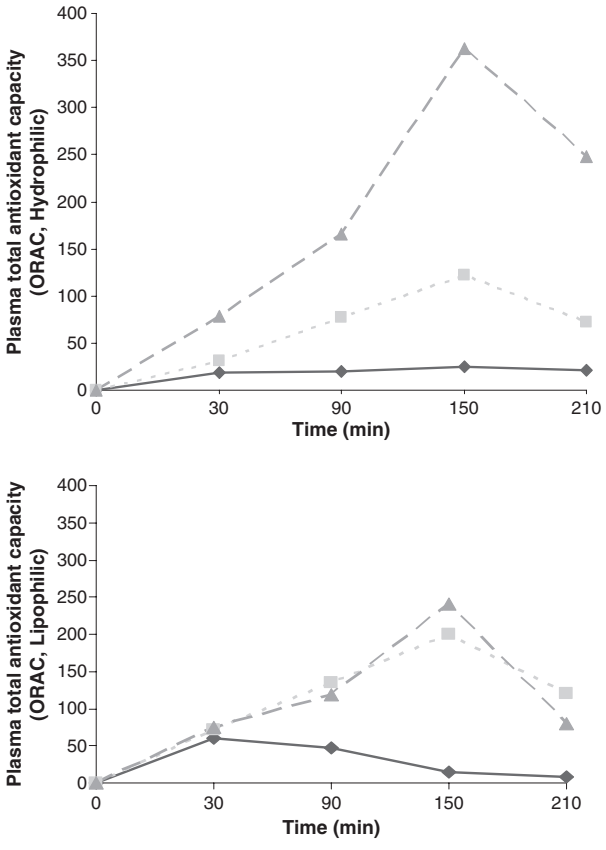

Figure 2 Effect of control (♦), walnut (■) and almond (▲) meals on plasma total antioxidant capacity measured by oxygen radical absorbance capacity (ORAC). Values are expressed as the percent change of the individual increments over baseline.

three groups, which are drastically different from each other at baseline.

Changes in antioxidant capacity of plasma in response to the three test meals over time were calculated as area under the curve (AUC). The AUC for the different assays of antioxidant capacity is presented in Table 3. Data are presented for the time period of 0–210 min. The AUC of the ORAC assay for hydrophilic components was significantly higher for both walnuts and almonds compared to control, and lower for walnuts compared to almonds. No significant difference was observed for FRAP and ORAC lipophilic.

Discussion

For the first time the acute effects of consuming a nut meal (almond and walnut) on plasma polyphenol, antioxidant capacity and lipid peroxidation has been studied in healthy volunteers. We observed that both nut meals increased total plasma polyphenol levels with a peak concentration being achieved at approximately 90 min. Both

Table 3 Diet comparisons of overall antioxidant capacity in plasma of human subjects as indicated by the area under the curve (AUC, 0–210 min) following three different meals

	Methods		
Meal comparison	FRAP	ORAC, lipophilic	ORAC, hydrophilic
Walnut versus control	2736	805	700.7*
Almond versus control	3839	1135	2108.9*
Walnut versus almond	−1103	−330	−1408.2*

FRAP, ferric reducing ability of plasma; ORAC, oxygen radical absorbance capacity.
*Significantly different using paired *t*-test ($P < 0.05$).

almond and walnut meals also increased plasma antioxidant capacity at the same time as reducing lipid peroxidation products in the plasma. Although the plasma polyphenol levels remained high for a longer time following the walnut compared to the almond meal, the total antioxidant capacity was greater following the almond meal. In our study, we observed a great variability in baseline values in all three groups. This could be due to biological variability or subjects' noncompliance at baseline, which might be due to not refraining from polyphenol-containing foods completely or even measurement error. Although subjects on the control meal started the study with greater baseline plasma polyphenol concentrations compared to those on the treatment meal, the increase observed after consumption of nut smoothies in the treatment meal group were greater compared to the control meal group.

Plasma polyphenols levels following ingestion of foods such as fruits and vegetables (Fuhrman *et al.*, 2005), tea (Henning *et al.*, 2004, 2005), wine (Duthie *et al.*, 1998), olive oil (Covas *et al.*, 2003) and chocolate (Serafini *et al.*, 2003) have been previously reported. According to these studies, after consumption of approximately 1–2 servings of polyphenol rich foods, a plasma peak concentration of polyphenols occurred between 30–60 min, except following olive oil consumption, which resulted in a peak plasma polyphenol concentration at approximately 120 min postingestion. Our results are consistent with these findings in that the plasma polyphenol concentration increased following ingestion of the walnut and almond meal (containing 80–90 g or approximately 3–3.5 oz walnut or almond), reaching the peak concentration at 90 min. Although we cannot infer much on the mechanisms and the sites of absorption of polyphenols in humans from our data because the peak concentration was achieved by 90 min postconsumption of the nut meals, we speculate that the absorption of polyphenols might have mostly occurred at the upper tract of

gastrointestinal system. To completely understand the kinetics of polyphenols in humans, future studies should include measurement of some of the urinary metabolites. Between the two nuts, the plasma polyphenol concentration remained higher for a longer duration following walnut ingestion. The reason for this sustainability might be explained by the higher amount of polyphenols found in walnuts compared to almonds per serving. Walnuts have favourable levels of total phenols relative to other foods that are reported to have high phenolic and antioxidant levels, when compared on a per serving basis (Anderson *et al.*, 2001).

In most previous studies, the presence of high plasma concentrations of polyphenols was accompanied by a significant increase in plasma total antioxidant capacity (Lauder, 1992; Serafini *et al.*, 1998; Langley-Evans, 2000). The antioxidant potential in humans responds to the oral ingestion of phenol-rich beverages such as red wine (Nigdikar *et al.*, 1998) and tea (Serafini *et al.*, 1996). Consistent with these findings, we showed an increase in plasma total antioxidant capacity following ingestion of both nut meals. However, although the plasma polyphenol concentration was higher after the walnut meal, the total antioxidant capacity was greater following the almond meal. This can be partly explained by the additive effect of different antioxidants found in almonds, including vitamin E. Plasma vitamin E concentrations were not measured in our study population due insufficient plasma for this measurement. For a more accurate conclusion, we recommend that vitamin E is measured in future studies. According to the USDA Nutritional composition data (http://www.nal.usda.gov/fnic/foodcomp/search/), 28 g (1 oz). of almonds contains 7.3 mg of vitamin E compared to other nuts, which contain less than 2 mg per 28 g (1 oz).. Many studies (Spiller *et al.*, 2003; Jambazian *et al.*, 2005) have demonstrated an increase in plasma antioxidant capacity and vitamin E levels after the consumption of a handful of almonds.

Consumption of a diet rich in polyphenols not only is shown to increase plasma polyphenol levels, but also reduces the susceptibility of plasma to lipid peroxidation (Nigdikar *et al.*, 1998). In line with these findings, we observed a reduction in lipid peroxidation products in plasma following consumption of both the nut meals. Polyphenols are known for their antioxidant properties by mechanisms that reduce the generation of superoxide anions (Robak & Gryglewski, 1988), hydroxyl radicals (Husain *et al.*, 1987) and lipid peroxyl radicals (Torel *et al.*, 1986). By reducing susceptibility to lipid peroxidation and increasing antioxidant capacity, the risk of cardiovascular disease can be reduced (Diaz *et al.*, 1997).

The top three most consumed nuts in the USS are almonds, walnuts and pecans (ERS/USDA Briefing Room, 2004) and adding walnuts and almonds to the diet is a simple way to increase both dietary and serum polyphenols and antioxidant capacity. A moderate to high amount of nut intake has been demonstrated through epidemiological studies to confer protection against coronary heart disease (Sabate, 1999; Maguire *et al.*, 2004; Strahan, 2004). The major mechanism explaining these cardio-protective effects of nuts is through the favourable modification of blood lipid risk factors. However, the present study provides evidence that cardiovascular risk reduction may go beyond the blood lipid lowering and may be in part due to the antioxidant protection offered by nuts. Being a whole food, nuts such as almonds and walnuts are a combination of several vitamin and nonnutrient antioxidants, which synergistically may offer a wider range of antioxidant protection (Maguire *et al.*, 2004). The findings obtained in the present study provide further support to the existing recommendations made by the American Heart Association for including at least 28–56 g (1–2 oz). of nuts daily as part of a healthy diet for the heart.

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Conflict of interest, source of funding and authorship

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ST wrote the seed grant proposal for obtaining funding for this study, gathered the data and wrote the first draft of the paper. EH and JS conceptualized and designed the study, and critically reviewed the different drafts of the manuscript. JB was the statistician and SR helped with data collection and analysis. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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