



## CASE STUDY

### Pecans as a Natural Antioxidant

Conducted by Loma Linda University  
Loma Linda, California

Journal of Nutrition 2011

Nutrient Physiology, Metabolism and Nutrient Interactions

1. Chatrapa Hudthagosol <sup>4</sup>
2. Ella Hasso Haddad <sup>4</sup>
3. Katie McCarthy <sup>4</sup>
4. Piwen Wang <sup>5</sup>
5. Keiji Oda <sup>4</sup>
6. Joan Sabaté <sup>4</sup>

#### Author Affiliations

<sup>4</sup> Department of Nutrition, School of Public Health, Loma Linda University, Loma Linda, CA 92350

<sup>5</sup> Center for Human Nutrition, David Geffen School of Medicine, University of California, Los Angeles, CA 90095

To whom correspondence should be addressed. E-mail: ehaddad@llu.edu.

#### Abstract

Bioactive constituents of pecan nuts such as  $\gamma$ -tocopherol and flavan-3-ol monomers show antioxidant properties in vitro, but bioavailability in humans is not known. We examined postprandial changes in plasma oxygen radical absorbance capacity (ORAC) and in concentrations of tocopherols, catechins, oxidized LDL, and malondialdehyde (MDA) in response to pecan test meals. Sixteen healthy men and women (23–44 y, BMI 22.7  $\pm$  3.4) were randomly assigned to 3 sequences of test meals composed of whole pecans, blended pecans, or an isocaloric meal of equivalent macronutrient composition but formulated of refined ingredients in a crossover design with a 1-wk washout period between treatments. Blood was sampled at baseline and at intervals up to 24 h postingestion. Following the whole and blended pecan test meals, plasma concentrations of  $\gamma$ -tocopherols doubled at 8 h ( $P < 0.001$ ) and hydrophilic- and lipophilic-ORAC increased 12 and 10% at 2 h, respectively. Post whole pecan consumption, oxidized LDL decreased 30, 33, and 26% at 2, 3, and 8 h, respectively ( $P < 0.05$ ), and epigallocatechin-3-gallate concentrations at 1 h (mean  $\pm$  SEM; 95.1  $\pm$  30.6 nmol/L) and 2 h (116.3  $\pm$  80.5 nmol/L) were higher than at baseline (0 h) and after the control test meal at 1 h ( $P < 0.05$ ). The postprandial molar ratio of MDA:triglycerides decreased by 37, 36, and 40% at 3, 5, and 8 h, respectively ( $P < 0.05$ ), only when whole and blended pecan data were pooled. These results show that bioactive constituent of pecans are absorbable and contribute to postprandial antioxidant defenses.

#### Introduction

The pecan (*Carya illinoensis*) is a nut native to south central North America and has been valued as a food for centuries. Recent interest in pecans as important constituents of healthful diets derives from 2 lines of evidence. First, epidemiological studies established that the frequency of nut consumption is linked to reduced risk of coronary heart disease (1–3) and second, intervention trials in humans demonstrated that pecan-enriched diets effectively lower blood lipids (4, 5). These beneficial effects have been attributed to the low saturated and high monounsaturated lipid concentrations of pecans. However, aside from fat, pecans contain bioactive components that may potentially reduce the risk of disease by mechanisms not related to blood lipid changes.

In addition, pecan nuts have the particularity of being rich in  $\gamma$ -tocopherol and thus can serve as a food candidate for exploring the effect of  $\gamma$ -tocopherol-rich food on postprandial oxidative responses. In a randomized clinical trial, we observed that a pecan-enriched diet improved fasting concentrations of  $\gamma$ -tocopherol and inhibited TBARS in humans (9). It remains to be determined whether this inhibition can be observed postprandially following the consumption of pecans.

Potential bioactivity *in vivo* is dependent on the absorption, metabolism, and distribution of polyphenols and tocopherols in the body after consumption. To date, little is known about the extent to which bioactive compounds in nuts are absorbed, their metabolism, and their biologic actions. The aim of this study was to determine whether consumption of pecans by human volunteers affected postingestion catechin and tocopherol concentrations, lipid oxidation, and markers of antioxidant capacity of plasma. To assess the influence of the physical state of the nut on bioavailability, both whole pecans and pecans blended with water were used as test meals and compared with a similarly high-fat meal composed of catechin-free refined ingredients.

## Materials and Methods

### Chemicals

Chemicals and solvents were obtained from Sigma Chemical Co. unless otherwise stated. Randomly methylated  $\beta$ -cyclodextrin (Trappsol, pharmacy grade) was obtained from Cyclodextrin Technologies Development. Pecans for the study were a gift from the National Pecan Shellers Association and ~50% of the pecans were shelled immediately prior to use.

### Participants

Sixteen healthy volunteers (10 women and 6 men, age range 23 – 44 y, and BMI  $22.7 \pm 3.4$  kg/m<sup>2</sup>) were recruited to participate in the study. All study participants were in good health as determined by a screening questionnaire. Potential volunteers were excluded after screening if they reported current use of over-the-counter or prescribed medications or dietary supplements, habitually consumed tea or coffee, were smokers, or were allergic or sensitive to nuts. The study protocol was approved by the Institutional Review Board of Loma Linda University and written consent was obtained from each participant.

### Study Design

In a placebo-controlled, 3-way crossover design with a 1-wk washout period between treatments, participants were randomly assigned to consume a test meal of 90 g (~3 servings) whole pecans plus water, 90 g pecans blended with water, or a test meal with an energy, macronutrient, and fluid content equivalent to that of the pecan meals as control. The control meal was composed of refined olive oil, whey protein, white bread, and water (Table 1). Compared with pecans, which contain little  $\alpha$ -tocopherol but relatively large amounts of  $\gamma$ -tocopherol, refined olive oil contains moderate amounts of  $\alpha$ -tocopherol but practically no  $\gamma$ -tocopherol (10). Although the comprehensive phenolic acid composition of pecans is largely unknown, Gu et al. (6) reported that pecans contain  $17.2 \pm 2.5$  mg/100 g of flavan-3-ol monomers and  $494 \pm 86.2$  mg/100 g of total proanthocyanidins. Refined olive oil is devoid of flavan-3-ols and contains small amounts of phenolic compounds, mainly tyrosol and hydroxytyrosol (11).

On the day prior to the experiments, participants were served polyphenol-free meals containing ~50, 20, and 30% of energy as carbohydrate, protein, and fat, respectively, but no fruits, vegetables, nuts, chocolate, juice, coffee, tea, or cocoa (Supplemental Table 1). On the day of the experiment, each participant came to the research clinic in the morning after a 12-h overnight fast and a blood sample (the baseline zero time sample) was obtained. After consuming the test breakfast meal (Table 1) along with an allocated quantity of water in 15–20 min, additional blood samples were collected. Lunch and dinner meals devoid of polyphenols were served at 5 and 10 h following the consumption of the test breakfast. Following the completion of the test breakfast, the consumption of water was not limited, but other beverages were not allowed.

**Table I. Nutrient Composition of Test Meals**

	Control, 97 g	Pecan, 90 g
Energy, kJ	2590	2590
Fat, g	64	65
Saturated	8.8	5.7
Monounsaturated	46.7	36.7
Polyunsaturated	6.7	19.5
Protein, g	8.7	8.3
Carbohydrate, g	12	12
α-Tocopherol, mg	9.2	1.3
γ-Tocopherol, mg	0.5	22.0
Proanthocyanidins, <sup>2</sup> mg	—	445
Flavan-3-ol monomers, <sup>3</sup> mg	—	14.4
Catechin	—	6.5
EGC	—	5.1
Epigallocatechin gallate	—	2.1
EC	—	0.7
Total phenolic compounds, mg GAE	13 <sup>4</sup>	1815 <sup>5</sup>

<sup>1</sup> Nutrient composition values were obtained from USDA Database for standard reference unless otherwise specified (10).

<sup>2</sup> Proanthocyanidin database (44).

<sup>3</sup> Flavonoid database (37).

<sup>4</sup> Mostly tyrosol and hydroxyl-tyrosol (11).

<sup>5</sup> From (7).

### Sample collection and storage

Blood samples were drawn via venipuncture with the use of butterfly needles at baseline (0 h) and at 1, 2, 3, 5, 8, and 24 h after the beginning of the test meals. Blood was collected into two 10-mL vacutainer tubes (Becton Dickinson): one serum and one containing sodium heparin as an anticoagulant. Blood was centrifuged at 1500 × g at 4°C for 10 min and serum and plasma were separated, aliquoted, and frozen at –80°C until analyzed.

Urine was collected in 2 portions. The first morning void on the day of the experiments was discarded and collection was begun thereafter and continued until 1900 h. At that time, a second collection was begun that included the first void the next morning. The urine was measured, aliquoted into vials, and stored at –80°C until analysis.

### Serum lipids and uric acid, and urine creatinine

Serum cholesterol, triglycerides, and uric acid were determined with reagents, controls, and calibrators from Thermo Fisher Scientific and were assayed using the Bio-Tek Synergy HT plate reader. Creatinine kits (Parameter) from R & D Systems were used to determine urinary creatinine.

### Total phenolic acids in plasma and urine

Total phenolic acid concentrations in plasma were measured by the Folin-Ciocalteu reagent on deproteinated samples as described by Serafini et al. (12). Absorbance at 765 nm was monitored by UV-VIS spectrophotometer (Beckman DU 406) equipped with a 6-cell holder. All measurements were done in triplicate and results are expressed as mmol GAE/L.

### Biomarkers of antioxidant capacity

Plasma antioxidant capacity was estimated by the ferric reducing ability (FRAP) method of Benzie and Strain (13). This assay measures the ability of plasma to reduce the colorless reagent Fe(III)-2,4,6-Tri(2-pyridyl)-s-triazine complex to the intense blue Fe(II)-2,4,6-Tri(2-pyridyl)-s-triazine, which is related to the amount of reductant present. The assay was modified for a 96-well plate and run on a Synergy Analyzer (Bio Tek Instruments) with Trolox as standard. Plasma was diluted 5× and the reaction was monitored at a wavelength of 593 for 4 min. The results are expressed as mmol TE/L.

The lipophilic and hydrophilic oxygen radical absorbance capacity (ORAC) were assayed as described and validated by Prior et al. (14) and Hoang et al. (15). This assay provides a measure of both the hydrophilic and lipophilic chain-breaking antioxidant capacity of plasma or urine compared with peroxy radicals (16). Fluorescein is used as the target molecule for free radical attack, 2,2'-azobis(2-amidinopropane) dihydrochloride as peroxy radical generator, and Trolox as control standard. The tests were performed in 48-well microplates (Falcon) using the FLX 800 fluorescent microplate reader (Bio Tek Instruments) with fluorescent filters at an excitation wavelength of 546 nm and an emission wavelength of 565 nm. The reaction was monitored for 1 h and 15 min and the assays were carried out in triplicate. All calculations were made using Microsoft Excel and the data are expressed as mmol TE/L. The lipophilic and hydrophilic oxygen radical absorbance capacity (ORAC) were assayed as described and validated by Prior et al. (14) and Hoang et al. (15). This assay provides a measure of both the hydrophilic and lipophilic chain-breaking antioxidant capacity of plasma or urine compared with peroxy radicals (16). Fluorescein is used as the target molecule for free radical attack, 2,2'-azobis(2-amidinopropane) dihydrochloride as peroxy radical generator, and Trolox as control standard. The tests were performed in 48-well microplates (Falcon) using the FLX 800 fluorescent microplate reader (Bio Tek Instruments) with fluorescent filters at an excitation wavelength of 546 nm and an emission wavelength of 565 nm. The reaction was monitored for 1 h and 15 min and the assays were carried out in triplicate. All calculations were made using Microsoft Excel and the data are expressed as mmol TE/L.

### Plasma flavan-3-ol monomers and urine metabolites

The flavanol monomers [C, EC, epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), EC-3-gallate (ECG), and gallic acid (GCG)] were measured in plasma of 12 participants (6 male, 6 female) following consumption of the whole pecan and control test meals. The determinations were performed by Brunswick Laboratories applying HPLC with a coulchem electrode array detection method according to Lee et al. (17). The values represent total (free and conjugated) catechins, because samples were first hydrolyzed to generate the free form of the phenols. Urine polyphenol metabolites were measured at the Henning laboratory at the Center for Human Nutrition, University of California (Los Angeles, CA) according to a previously published protocol (18, 19). Briefly, urine was incubated with  $\beta$ -glucuronidase and sulfatase (Sigma Chemicals). The pH was adjusted to 3 and extracted twice with ethylacetate. The organic phases were combined, vacuum-dried, and reconstituted in mobile phase for HPLC analysis. The 8 channels of the CoulArray detector were sequentially set at -20, 80, 180, 280, 380, 480, 580, and 680 mV potentials. The main peaks appeared at 380 mV (3,4-dihydroxyphenylacetic acid), 380 mV (3-methoxy-4-hydroxyphenylacetic acid), and 680 mV (4-hydroxyphenylacetic acid). The phenolic acid concentrations were adjusted by the urinary creatinine concentrations.

### Plasma malondialdehyde and oxidized LDL

Reverse phase HPLC was employed to separate and quantify malondialdehyde (MDA) by measurement of an MDA thiobarbituric acid adduct as described by Templar et al. (20). This method involves a deproteinization step prior to thiobarbituric acid incubation and the use of 1,1,3,3-tetraethoxypropane as standard. Adduct separation was performed using an automated Shimadzu HPLC system: LC-10AT pump, SPD-10A UV-VIS detector set at 532 nm, SIL-10AD autoinjection, EZStart 7.2 software, and a Phenomenex C18 HyperClone 5 $\mu$  ODS (150  $\times$  4.6 mm) column. Oxidized LDL concentrations were determined in serum obtained postingestion of whole pecan and control test meals. For this assay, an enzyme-linked immunoassay kit (ALPCO Diagnostics) was used that specifically tests for MDA-modified LDL.

### $\alpha$ - and $\gamma$ -Tocopherol

Plasma tocopherols were determined by normal phase HPLC using the Shimadzu system described above and a Supelcosil LC-DIOL column (250  $\times$  4.6 mm) (Supelco). Assays were carried out according to the method of Kramer et al. (21), which uniquely separates  $\gamma$ - from  $\beta$ -tocopherol. Authentic tocopherol standards and the internal standard tocotrienol were obtained from Matraya.

## Statistical analysis

Data are expressed as least square means (LSM)  $\pm$  SEM unless otherwise noted. A mixed model approach was used to compare LSM differences among test meals adjusting for period effect and treating participants as random effects. To compare changes over time, the mixed model included meal, time, and meal  $\times$  time interaction terms. Because changes over time were closely correlated, whole and blended pecan data were pooled prior to analysis of the MDA/triglyceride variable. Pharmacokinetic parameters were determined. The area under the curve from 0 to 5 h [AUC(0-5h)] was calculated using the linear trapezoidal rule, and the maximum observed plasma concentration (C<sub>max</sub>) and time to reach C<sub>max</sub> (T<sub>max</sub>) were determined directly from the data. To achieve normality, oxidized LDL and catechin parameters were log-transformed prior to statistical analysis. Paired t tests were used to compare variables at baseline (Supplemental Table 2) and urinary metabolite concentrations. Statistical analyses were performed by using SAS version 8 (SAS Institute), all tests were 2-sided, and a value of P < 0.05 was considered significant.

## Results

All 16 study participants completed 3 phases of the study. Baseline concentrations of the variables studied did not differ prior to the 3 test meals (Supplemental Table 2).

### Biomarkers of antioxidant capacity

Compared with the control meal, the AUC(0-5h) of total polyphenols increased following the blended pecan meal and those of hydrophilic- and lipophilic-ORAC increased after ingestion of both the whole pecan and blended pecan test meals (Table 2). The postprandial increases in hydrophilic-ORAC and lipophilic-ORAC were modest at -12 and 10% of baseline, respectively (Fig. 1). The postprandial AUC (0-5h) of FRAP did not differ after the whole pecan, blended pecan, or control test meals.

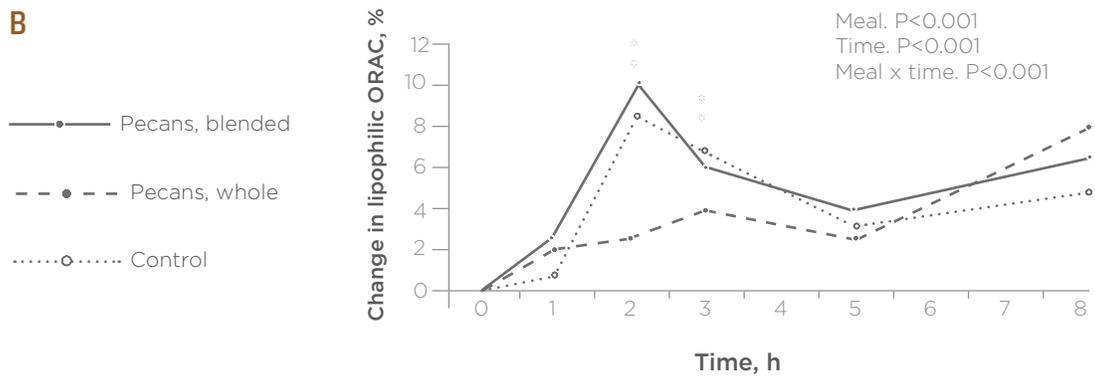
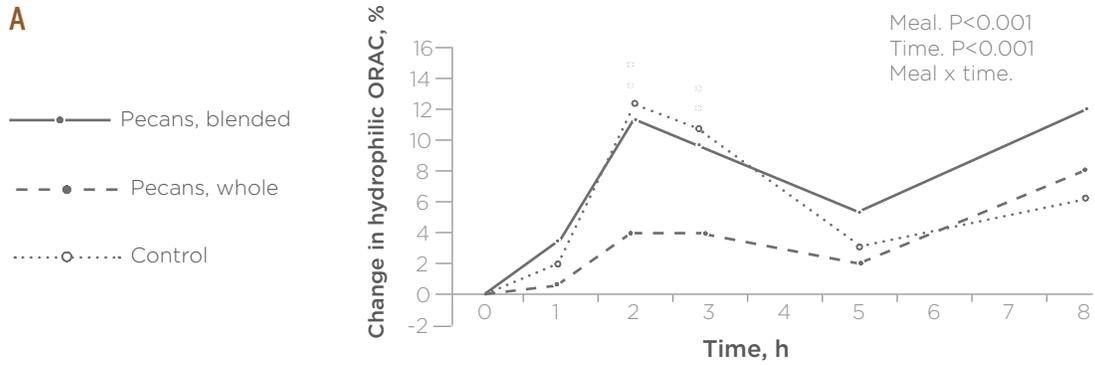
**Table 2. Changes in plasma biomarkers after participants consumed control, whole pecan, and blended pecan meals<sup>1</sup>**

### Test Meals

Biomarkers, AUC(0-5 h)	Control	Pecans, blended	Pecans, whole
Total polyphenols, mmol GAE/L ·h	6.91 $\pm$ 0.92 <sup>a</sup>	8.25 $\pm$ 0.90 <sup>b</sup>	7.67 $\pm$ 0.90 <sup>a</sup>
ORAC (hydrophilic), mmol/L ·h	7.28 $\pm$ 0.31 <sup>a</sup>	7.72 $\pm$ 0.30 <sup>b</sup>	7.80 $\pm$ 0.30 <sup>b</sup>
ORAC (lipophilic), mmol/L ·h	3.73 $\pm$ 0.16 <sup>a</sup>	4.12 $\pm$ 0.16 <sup>b</sup>	4.15 $\pm$ 0.16 <sup>b</sup>
FRAP, mmol/L ·h	8.43 $\pm$ 0.52	9.24 $\pm$ 0.48	9.19 $\pm$ 0.48
Uric acid, mmol/L ·h	1.62 $\pm$ 0.16	1.69 $\pm$ 0.18	1.66 $\pm$ 0.17
$\alpha$ -Tocopherol, $\mu$ mol/L ·h	113 $\pm$ 12	105 $\pm$ 12	109 $\pm$ 12
$\gamma$ -Tocopherol, mmol/L ·h	13.1 $\pm$ 1.5	14.0 $\pm$ 1.1	14.7 $\pm$ 1.4

<sup>1</sup> Values are LSM  $\pm$  SEM, n = 16. Means in a row with superscripts without a common letter differ, P < 0.05

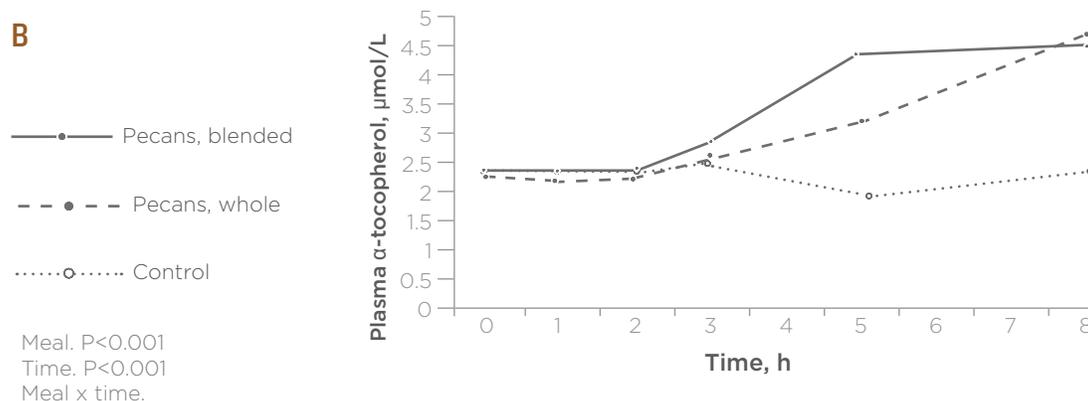
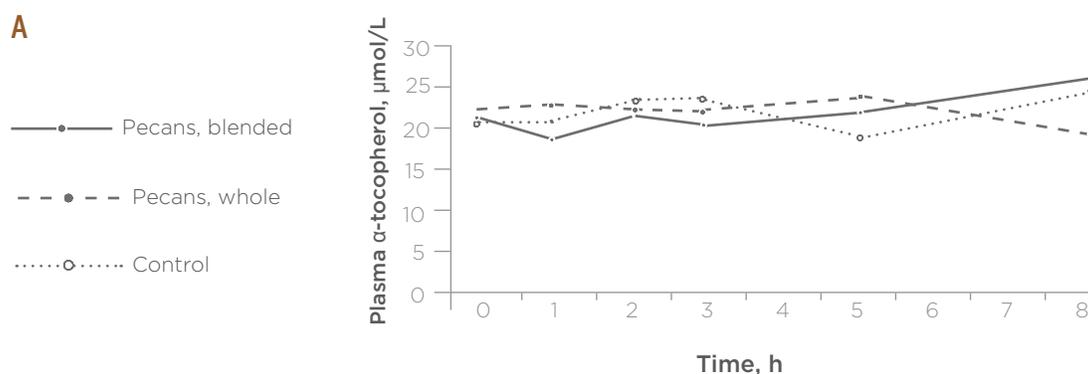
**FIGURE I.** Percent change from baseline of plasma hydrophilic (A) and lipophilic (B) ORAC after participants consumed control, whole pecan, and blended pecan meals in random order. Data are LSM  $\pm$  SEM, n = 16. \*Different from baseline, P < 0.001.



### Plasma tocopherols

Although plasma concentrations of  $\alpha$ -tocopherol did not vary over time after the 3 meals (Fig. 2A), those of  $\gamma$ -tocopherol (Fig. 2B) showed meal, time, and their interaction effects and were significantly higher than baseline and higher than the control meal at 5 and 8 h following both the whole and blended pecan test meals (P < 0.001).

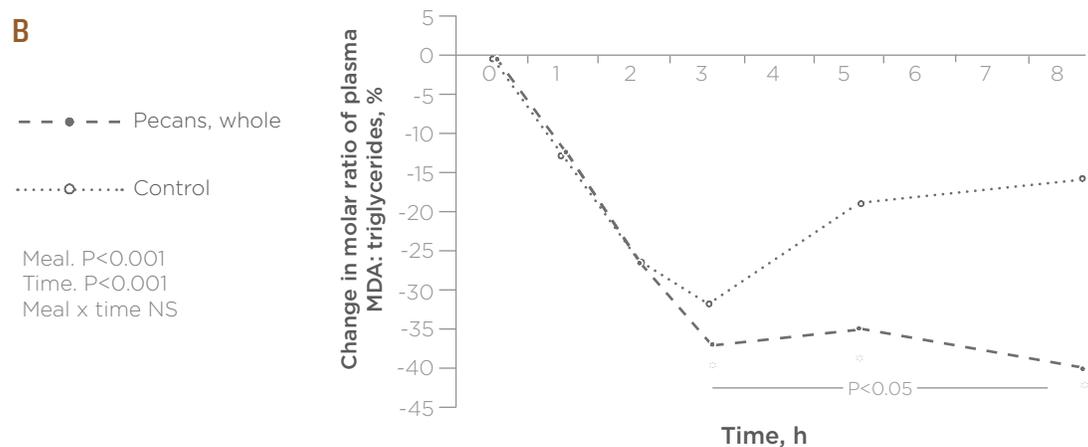
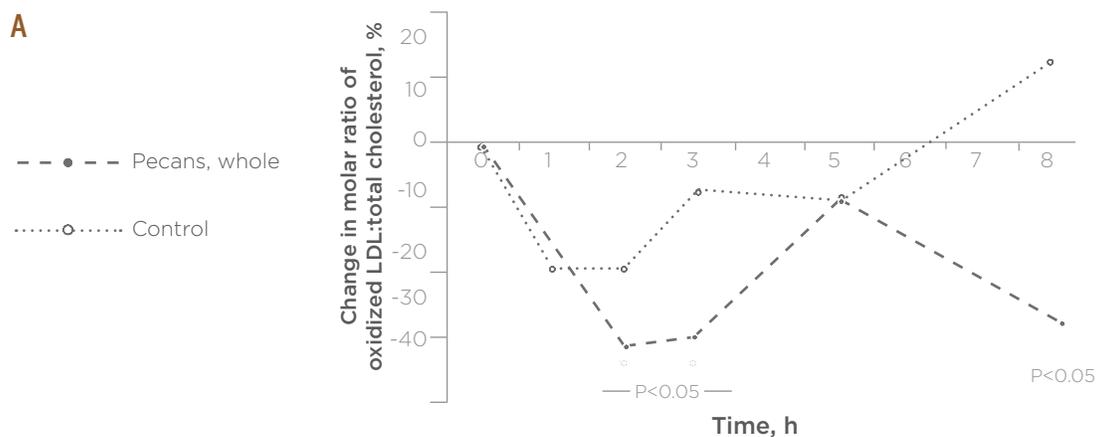
**FIGURE 2. Plasma concentrations of  $\alpha$ -tocopherol (A) and  $\gamma$ -tocopherol (B) after consumption of test meals. Values are LSM  $\pm$  SEM, n = 16. Labeled means at a time without a common letter differ, P < 0.001. \*Different from baseline, P < 0.001.**



### Biomarkers of LDL oxidation and lipid peroxidation

In the current study, serum cholesterol concentrations did not vary from baseline and did not differ following the 3 test meals (data not shown). However, after consumption of the whole pecan meal, oxidized LDL decreased 29.6, 33.3, and 26.3% from baseline at 2, 3, and 8 h, respectively, and the molar ratio of oxidized LDL: total cholesterol decreased 31.5, 29.8, and 27.6% from baseline at 2, 3, and 8 h, respectively (P < 0.05) (Fig. 3A). The molar ratio of oxidized LDL: total cholesterol decreased 19.3% from baseline at 1 and 2 h following the control meal, possibly due to the small amount of tyrosol and  $\alpha$ -tocopherol in oil. On the other hand, postprandial triglyceride concentrations increased steadily following all 3 test meals (P < 0.001) and attained a peak ~1.7 times the baseline levels at 3–4 h. MDA concentrations did not differ over time following the 3 test meals (data not shown). The molar ratio of MDA:triglycerides tended to decrease after all 3 test meals (P > 0.05) and was 37.4, 35.9, and 39.9% lower than baseline at 3, 5, and 8 h after the pecan diets (pooled whole pecan and blended pecan data) (P < 0.05) (Fig. 3B).

**FIGURE 3. Percent change from baseline in ratios of (A) oxidized LDL: total cholesterol after participants consumed the control and whole pecan test meals and (B) plasma MDA: triglycerides following the control and combined whole and blended pecan test meals (pooled data). Values are mean  $\pm$  SEM, n = 16 (A) or 32 (B). \*Different from baseline, P < 0.05.**



### Pharmacokinetic profile of pecan flavan-3-ol monomers (catechins)

The flavanols detected in the highest concentrations in plasma were EGCG, C + EC, EGC, ECG, and GCG (Table 3). Although the concentration of EGCG in pecans was less than that of C or EC, the highest AUC(0-5h) and C<sub>max</sub> observed were for this flavanol. After ingestion of the whole pecan test meal, mean plasma concentrations of EGCG were significantly higher than at baseline at 1 and 2 h and higher than control at 1 h (P < 0.05) (Fig. 4).

**Table 2. Pharmacokinetic parameters for selected plasma flavan-3-ol monomers following consumption of 90 g of whole pecans<sup>1</sup>**

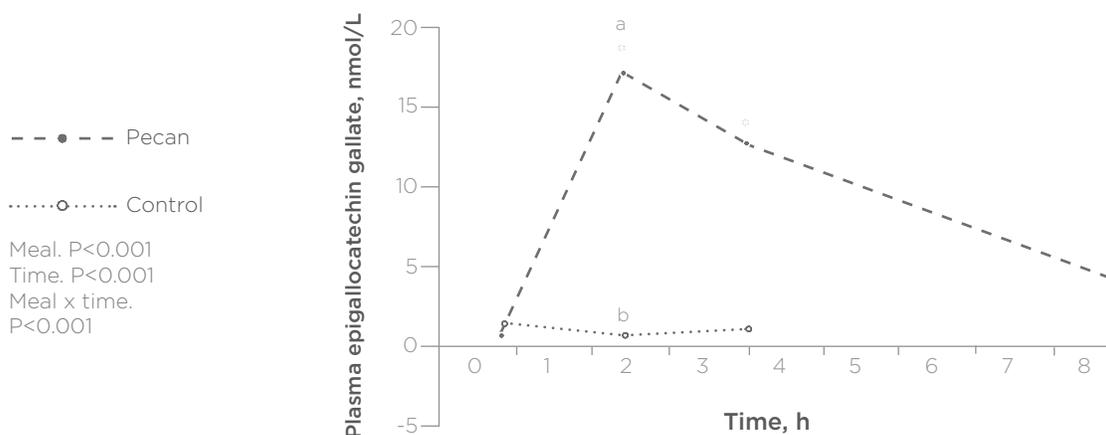
Flavan-3-ol monomer	Dose, $\mu\text{mol}$	AUC(0-5h), nmol/L·h	C <sub>max</sub> , nmol/L	T <sub>max</sub> , h	Bioavailability, <sup>2</sup> AUC/dose
C+EC	15.7	96 ± 24	65 ± 14	5.9 ± 0.9	6.1
EGCG	24.8	337 ± 146	192 ± 70	3.3 ± 0.8	13.6
EGC	16.6	85 ± 42	59 ± 22	2.6 ± 0.9	5.1
ECG	NA	78 ± 40	35 ± 19	4.3 ± 0.7	—
GCG	NA <sup>3</sup>	18 ± 6	11 ± 3	5.1 ± 0.9	—
Sum of C + EC + EGCG + EGC + ECG + GCG	57.1	561 ± 228	298 ± 111	2.7 ± 0.8	9.8

<sup>1</sup> Values are mean ± SEM, n = 12

<sup>2</sup> Calculated as AUC(0-5 h) divided by dose (AUC/dose)

<sup>3</sup> NA, Not available

**FIGURE 4.** Plasma epigallocatechin gallate concentrations in participants before (0 h) and after control and whole pecan test meals. Data were log10-transformed prior to analysis. Values are LSM ± SEM, n = 12. Labeled means at a time without a common letter differ, P < 0.05. \*Different from baseline, P < 0.05.



Bacterial flora in the colon act on unabsorbed flavanol monomers and polymers and on those absorbed and re-excreted through bile to produce the secondary metabolites. In this study, the creatinine-adjusted excretion of 3-methoxy-4-hydroxyphenylacetic acid was significantly higher following the whole pecan test meal in urine collected 13–24 h after consumption of the meal (Table 4).

**Table 4. Urinary excretion of phenylacetic acids following consumption of control and whole pecan test meals<sup>1</sup>**

	Control	Pecans, whole
nmol/mol creatinine		
3,4-Dihydroxyphenylacetic acid		
0–12 h	0.082 ± 0.023	0.092 ± 0.024
12–24 h	0.062 ± 0.016	0.081 ± 0.017
4-Hydroxyphenylacetic acid		
0–12 h	13.0 ± 5.88	19.3 ± 6.55
12–24h	11.6 ± 2.83	11.2 ± 2.94
3-Methoxy-4-hydroxyphenylacetic acid		
0–12 h	1.37 ± 0.26	1.77 ± 0.28
12–24 h	1.27 ± 0.14	1.77 ± 0.14*

<sup>1</sup> Values are LSM ± SEM, n = 6. \*Different from control, P < 0.01

Urine was collected in 2 portions: 1) postintake of test meals and up to 12 h thereafter (0–12 h); 2) 13 h post intake and including the following days' first morning void (12–24 h)

## Discussion

This study was designed to test the hypotheses that bioactive constituents of pecans, mainly tocopherols and flavanol monomers, inhibit postintake plasma lipid oxidation and counteract the prooxidant effect of high-fat meals on LDL, increase antioxidant capacity of the plasma, and are bioavailable. Although also designed to compare whole compared with blended pecans with respect to the above variables, few differences in results between the pecan forms were observed. This is the first study to our knowledge to evaluate the effects of pecan consumption on postprandial antioxidant biomarkers in humans.

Pecans are rich in fat and studies document increased oxidative stress accompanying the increase in triglycerides following high-fat meals (22, 23). In the current study, although plasma triglyceride concentrations increased following the test meals, no differences between interventions or changes from baseline were observed for MDA concentrations. In fact, the molar ratio of MDA:triglycerides was significantly lower than at baseline at 3, 4, and 5 h only after the pecan meals (pooled data). Post-ingestion cholesterol concentrations did not change, but oxidized LDL and the molar ratio of oxidized LDL:cholesterol decreased following the whole pecan test meal. These results suggest that the pecan meals decrease postprandial lipid and cholesterol oxidation more effectively than the control meal and support our first hypothesis. This is in line with studies showing decreased fasting concentrations of MDA and oxidized LDL in association with almond-enriched diets in healthy individuals (24) and in smokers (25). Decreased fasting concentrations of MDA have been observed in an intervention study with pecan-enriched diets in healthy humans (9).

Recently, we reported postprandial increases in plasma antioxidant capacity measured as ORAC and FRAP following test meals containing walnuts or almonds (26). In the current study, the effect of pecans on post-ingestion plasma antioxidant capacity was modest, with hydrophilic- and lipophilic-ORAC showing an increase from baseline of -12 and 10%, respectively, and a higher 5-h AUC postconsumption of both the whole and blended pecan meals. The total phenol AUC (0-5 h) increased only following consumption of the blended pecan meal and antioxidant capacity measured as FRAP did not differ among diets. Antioxidant capacity assays reflect differences in mechanisms and one confounding factor may be the increase in plasma concentrations of uric acid that accompanies some dietary interventions. Postprandial increases in FRAP following ingestion of fruits (27) and wine (28) may be due to increases in plasma uric acid, itself a powerful antioxidant, which occurs as a consequence of the fructose found in those foods. In the current study, no differences were observed in plasma urate among the test meals. The increases in total phenols and ORAC activity partly supports our second hypothesis.

The decrease in lipid peroxidation may be due to the concurrent increase in  $\gamma$ -tocopherol concentrations. It has been established that  $\gamma$ -tocopherol is rapidly metabolized following ingestion and fasting plasma levels are a fraction of those of  $\alpha$ -tocopherol (29). The concentrations of the  $\gamma$ - isomer steadily increased following consumption of both the whole and blended pecan meals, reaching approximately twice the fasting level at 8 h post-ingestion ( $P < 0.001$ ). Although somewhat temporary, these postprandial increases in  $\gamma$ -tocopherol are physiologically relevant. In vitro experiments and studies in animals suggest that the antioxidant activity of  $\gamma$ -tocopherol exceeds that of the  $\alpha$ -isomer (30) and that  $\gamma$ -tocopherol efficiently traps reactive oxygen and nitrogen radicals (31), inhibits LDL oxidation (32), and is anti-inflammatory (33, 34). In humans with metabolic syndrome,  $\gamma$ -tocopherol supplementation inhibited oxidative stress and decreased plasma MDA and lipid peroxides (35).

Besides tocopherols, pecans are also a source of proanthocyanidins chemically derived from the flavan-3-ols building blocks of catechin and EC (36). In pecans, only a small fraction (3-4%) of flavanols exist as monomers and are thus potentially absorbable. Despite the relatively low concentrations of flavanol monomers in pecans (13.2 mg/100 g) compared with brewed green tea (126.6 mg/100 g) (37), measurable increases in plasma concentrations of catechins were detected post pecan ingestion. Concentrations of EGCG were significantly higher following pecan ingestion at 1 and 2 h postprandially compared with fasting levels and higher at 1 h compared with the refined test meal ( $P < 0.05$ ). Although some evidence shows that catechin dimers and trimers may transverse the intestinal cell, there is no unequivocal evidence for their absorption (38). Also, there is no evidence that the longer chain molecules are hydrolyzed within the small intestine prior to absorption and are thus potentially bioavailable. Our data are consistent with those reported by others (39), which showed large inter-individual variations in apparent absorption and peak plasma concentrations of catechins among study participants. However, the pharmacokinetic profile due to pecan consumption, especially  $T_{max}$  and bioavailability estimated as AUC:oral dose, differed markedly from results in green tea for EGCG, with a  $T_{max}$  of 1.8 h and an AUC:dose ratio of only 1.3 h (18). The delayed  $T_{max}$  and apparent higher bioavailability of catechins from pecans may be due to the high-fat content of nuts, which may slow absorption but enhance bioavailability of the catechins, especially the more lipophilic ones. The increase in plasma catechins and  $\gamma$ -tocopherol of the magnitude achieved supports our 3rd hypothesis and is physiologically relevant because of the concurrent increase in plasma antioxidant capacity. The mechanism through which this enhanced bioavailability occurs requires further research.

In the current study, the creatinine-adjusted urinary excretion of the flavanol degradation product 3-methoxy-4-hydroxyphenylacetic acid increased in urine collected 12–24 h following pecan consumption. Catechin moieties not absorbed, and those initially absorbed and subsequently excreted as conjugates in bile, reach the colon. In the colon, the conjugates are hydrolyzed and the aglycones undergo ring fission by colonic microflora to low-molecular weight aromatic acids that can be absorbed and excreted in urine (40). In vitro studies with human fecal microflora demonstrate that simple phenolic acids are formed from proanthocyanidin polymers (18). Phenolic products have been identified in urine following chocolate (41) and cocoa (42) consumption. Recent interest in microbial metabolites of catechins and proanthocyanidins derives from the observation that these phenolic acids are reducing agents that exhibit antiproliferative activity in cancer cell lines (18).

The mechanisms that account for the protective effects of nuts in the diet remain incompletely understood. Oxidative damage is involved in coronary heart disease and other inflammatory and degenerative diseases. The fact that pecans are a rich source of flavan-3-ols and tocopherols that may potentially contribute to antioxidant protection is a plausible explanation for their beneficial health effects. In vitro studies have demonstrated antioxidant synergy between flavonoids and  $\alpha$ -tocopherol (43).

In summary, this randomized crossover trial showed that when pecans are consumed, their catechin monomers, of which EGCG is the most available, are absorbed. The plasma concentration of  $\gamma$ -tocopherol and ORAC activity increased, whereas oxidized LDL and the ratio of MDA:triglycerides decreased following pecan consumption. Whether the improvement in antioxidant status is due to  $\gamma$ -tocopherol, catechins, or both acting in synergy remains to be determined.

## Acknowledgments

C.H., E.H.H., and J.S. designed research; C.H., K.M., and P.W. conducted research; K.O. analyzed data; C.H. and E.H.H. wrote the paper; and C.H. and E.H.H. had primary responsibility for final content. All authors read and approved the final manuscript.

## Footnotes

2 Author disclosures: C. Hudthagosol, E. H. Haddad, K. McCarthy, P. Wang, K. Oda, and J. Sabaté, no conflicts of interest.

1 Supported in part by a grant from the National Pecan Shellers Association, Atlanta, GA.

3 Supplemental Tables 1 and 2 are available with the online posting of this paper at [jn.nutrition.org](http://jn.nutrition.org).

3 Supplemental Tables 1 and 2 are available with the online posting of this paper at [jn.nutrition.org](http://jn.nutrition.org).

6 Abbreviations used: AUC, area under the curve; C<sub>max</sub>, maximum concentration; T<sub>max</sub>, time to reach maximum concentration; C, catechin; EC, epicatechin; ECG, epicatechin-3-gallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; GCG, gallocatechin gallate; LSM, least square means; MDA, malondialdehyde; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalent.

Manuscript received: January 18, 2010.

Initial review completed: February 28, 2010.

Revision accepted: October 15, 2010.

## Literature Cited

1. Fraser GE,  
Sabate J,  
Beeson WL,  
Strahan TM

. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. *Arch Intern Med.* 1992;152:1416–24. Abstract/FREE Full Text

2. Hu FB,  
Stampfer MJ

. Nut consumption and risk of coronary heart disease: a review of epidemiologic evidence. *Curr Atheroscler Rep.* 1999;1:204–9. Medline

3. Kris-Etherton PM,  
Hu FB,  
Ros E,  
Sabate J  
. The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. *J Nutr.* 2008;138:S1746-51. Abstract/FREE Full Text
4. Morgan WA,  
Clayshulte BJ  
. Pecans lower low-density lipoprotein cholesterol in people with normal lipid levels. *J Am Diet Assoc.* 2000;100:312-8. CrossRefMedline
5. Rajaram S,  
Burke K,  
Connell B,  
Myint T,  
Sabate J  
. A monounsaturated fatty acid-rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. *J Nutr.* 2001;131:2275-9. Abstract/FREE Full Text
6. Gu L,  
Kelm MA,  
Hammerstone JF,  
Beecher G,  
Holden J,  
Haytowitz D,  
Gebhardt S,  
Prior RL  
. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr.* 2004;134:613-7. Abstract/FREE Full Text
7. Wu X,  
Beecher GR,  
Holden JM,  
Haytowitz DB,  
Gebhardt SE,  
Prior RL  
. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem.* 2004;52:4026-37. CrossRefMedline
8. Rice-Evans CA,  
Miller NJ,  
Paganga G  
. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med.* 1996;20:933-56. CrossRefMedline
9. Haddad EJP,  
Karunia M,  
Tanzman J,  
Sabate JA  
. Pecan-enriched diet increases  $\gamma$ -tocopherol/cholesterol and decreases thiobarbituric acid reactive substances in plasma of adults. *Nutr Res.* 2006;26:397-402. CrossRef
10. USDA, Agricultural Research Service. 2008. USDA National Nutrient Database for Standard Reference, Release 21 [cited 2009 Dec 2]. Available at: <http://www.ars.usda.gov/ba/bhnrc/ndl>.
11. Samaniego Sanchez C,  
Troncoso Gonzalez AM,  
Garcia-Parrilla MC,  
Quesada Granados JJ,  
Lopez Garcia de la Serrana H,  
Lopez Martinez MC  
. Different radical scavenging tests in virgin olive oil and their relation to the total phenol content. *Anal Chim Acta.* 2007;593:103-7. CrossRefMedline

12. Serafini M,  
Maiani G,  
Ferro-Luzzi A  
. Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J Nutr.* 1998;128:1003-7. Abstract/FREE Full Text
13. Benzie IF,  
Strain JJ  
. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* 1999;299:15-27. Medline
14. Prior RL,  
Hoang H,  
Gu L,  
Wu X,  
Bacchiocca M,  
Howard L,  
Hampsch-Woodill M,  
Huang D,  
Ou B,  
et al  
. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem.* 2003;51:3273-9. CrossRefMedline
15. Huang D,  
Ou B,  
Hampsch-Woodill M,  
Flanagan JA,  
Prior RL  
. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J Agric Food Chem.* 2002;50:4437-44. CrossRefMedline
16. Huang D,  
Ou B,  
Prior RL  
. The chemistry behind antioxidant capacity assays. *J Agric Food Chem.* 2005;53:1841-56. CrossRefMedline
17. Lee MJ,  
Prabhu S,  
Meng X,  
Li C,  
Yang CS  
. An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. *Anal Biochem.* 2000;279:164-9. CrossRefMedline
18. Gao K,  
Xu A,  
Krul C,  
Venema K,  
Liu Y,  
Niu Y,  
Lu J,  
Bensoussan L,  
Seeram NP,  
et al  
. Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity. *J Nutr.* 2006;136:52-7. Abstract/FREE Full Text

19. Wang JS,  
Luo H,  
Wang P,  
Tang L,  
Yu J,  
Huang T,  
Cox S,  
Gao W  
. Validation of green tea polyphenol biomarkers in a phase II human intervention trial. *Food Chem Toxicol.* 2008;46:232-40. [CrossRefMedline](#)  
. Increased plasma malondialdehyde levels in glomerular disease as determined by a fully validated HPLC method. *Nephrol Dial Transplant.* 1999;14:946-51.
20. Templar J,  
Kon SP,  
Milligan TP,  
Newman DJ,  
Raftery MJ
21. Kramer JK,  
Blais L,  
Fouchard RC,  
Melnyk RA,  
Kallury KM  
. A rapid method for the determination of vitamin E forms in tissues and diet by high-performance liquid chromatography using a normal-phase diol column. *Lipids.* 1997;32:323-30. [CrossRefMedline](#)
22. Bae JH,  
Bassenge E,  
Kim KB,  
Kim YN,  
Kim KS,  
Lee HJ,  
Moon KC,  
Lee MS,  
Park KY,  
et al  
. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis.* 2001;155:517-23. [CrossRefMedline](#)
23. Cardona F,  
Tunez I,  
Tasset I,  
Garrido-Sanchez L,  
Collantes E,  
Tinahones FJ  
. Circulating antioxidant defences are decreased in healthy people after a high-fat meal. *Br J Nutr.* 2008;100:312-6. [Medline](#)
24. Jenkins DJ,  
Kendall CW,  
Josse AR,  
Salvatore S,  
Brighenti F,  
Augustin LS,  
Ellis PR,  
Vidgen E,  
Rao AV  
. Almonds decrease postprandial glycemia, insulinemia, and oxidative damage in healthy individuals. *J Nutr.* 2006;136:2987-92. [Abstract/FREE Full Text](#)

25. Li N,  
Jia X,  
Chen CY,  
Blumberg JB,  
Song Y,  
Zhang W,  
Zhang X,  
Ma G,  
Chen J  
. Almond consumption reduces oxidative DNA damage and lipid peroxidation in male smokers. *J Nutr.* 2007;137:2717-22. Abstract/FREE Full Text
26. Torabian S,  
Haddad E,  
Rajaram S,  
Banta J,  
Sabate J  
. Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation. *J Hum Nutr Diet.* 2009;22:64-71. CrossRefMedline
27. Lotito SB,  
Frei B  
. Relevance of apple polyphenols as antioxidants in human plasma: contrasting in vitro and in vivo effects. *Free Radic Biol Med.* 2004;36:201-11. Medline
28. Modun D,  
Music I,  
Vukovic J,  
Brizic I,  
Katalinic V,  
Obad A,  
Palada I,  
Dujic Z,  
Boban M  
. The increase in human plasma antioxidant capacity after red wine consumption is due to both plasma urate and wine polyphenols. *Atherosclerosis.* 2008;197:250-6. CrossRefMedline
29. Dietrich M,  
Traber MG,  
Jacques PF,  
Cross CE,  
Hu Y,  
Block G  
. Does gamma-tocopherol play a role in the primary prevention of heart disease and cancer? A review. *J Am Coll Nutr.* 2006;25:292-9. Abstract/FREE Full Text
30. Christen S,  
Woodall AA,  
Shigenaga MK,  
Southwell-Keely PT,  
Duncan MW,  
Ames BN  
. Gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. *Proc Natl Acad Sci USA.* 1997;94:3217-22. Abstract/FREE Full Text

31. Cooney RV,  
Franke AA,  
Harwood PJ,  
Hatch-Pigott V,  
Custer LJ,  
Mordan LJ  
. Gamma-tocopherol detoxification of nitrogen dioxide: superiority to alpha-tocopherol. *Proc Natl Acad Sci USA*. 1993;90:1771-5. Abstract/FREE Full Text
32. Li D,  
Saldeen T,  
Mehta JL  
. Gamma-tocopherol decreases ox-LDL-mediated activation of nuclear factor-kappaB and apoptosis in human coronary artery endothelial cells. *Biochem Biophys Res Commun*. 1999;259:157-61. CrossRefMedline
33. Jiang Q,  
Ames BN  
. Gamma-tocopherol, but not alpha-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats. *FASEB J*. 2003;17:816-22. Abstract/FREE Full Text
34. Jiang Q,  
Lykkesfeldt J,  
Shigenaga MK,  
Shigeno ET,  
Christen S,  
Ames BN  
. Gamma-tocopherol supplementation inhibits protein nitration and ascorbate oxidation in rats with inflammation. *Free Radic Biol Med*. 2002;33:1534-42. CrossRefMedline
35. Devaraj S,  
Leonard S,  
Traber MG,  
Jialal I  
. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic Biol Med*. 2008;44:1203-8. Medline
36. Prior RL,  
Gu L  
. Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry*. 2005;66:2264-80. CrossRefMedline
37. USDA, Agricultural Research Service 2007. USDA Database for the Flavonoid Content of Selected Foods, Release 2.1 [cited 2009 Dec 2]. Available at: <http://www.ars.usda.gov/services/docs.htm?docid=6231>.
38. Holt RR,  
Lazarus SA,  
Sullards MC,  
Zhu QY,  
Schramm DD,  
Hammerstone JF,  
Fraga CG,  
Schmitz HH,  
Keen CL  
. Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *Am J Clin Nutr*. 2002;76:798-804. Abstract/FREE Full Text

39. Lee MJ,  
Maliakal P,  
Chen L,  
Meng X,  
Bondoc FY,  
Prabhu S,  
Lambert G,  
Mohr S,  
Yang CS  
. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prev.* 2002;11:1025-32. Abstract/FREE Full Text
40. Meng X,  
Sang S,  
Zhu N,  
Lu H,  
Sheng S,  
Lee MJ,  
Ho CT,  
Yang CS  
. Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chem Res Toxicol.* 2002;15:1042-50. CrossRefMedline
41. Rios LY,  
Gonthier MP,  
Remesy C,  
Mila I,  
Lapierre C,  
Lazarus SA,  
Williamson G,  
Scalbert A  
. Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am J Clin Nutr.* 2003;77:912-8. Abstract/FREE Full Text
42. Urpi-Sarda M,  
Monagas M,  
Khan N,  
Llorach R,  
Lamuela-Raventos RM,  
Jauregui O,  
Estruch R,  
Izquierdo-Pulido M,  
Andres-Lacueva C  
. Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography-tandem mass spectrometry. *J Chromatogr A.* 2009;1216:7258-67. CrossRefMedline
43. Pedrielli P,  
Skibsted LH  
. Antioxidant synergy and regeneration effect of quercetin, (-)-epicatechin, and (+)-catechin on alpha-tocopherol in homogeneous solutions of peroxidating methyl linoleate. *J Agric Food Chem.* 2002;50:7138-44. CrossRefMedline
44. USDA, Agricultural Research Service 2004. USDA Database for the Proanthocyanidin Content of Selected Foods [cited 2009 Dec 2]. Available at <http://www.ars.usda.gov/services/docs.htm?docid=5843>.

© 2011 American Society for Nutrition

Manuscript received: January 18, 2010.

Initial review completed: February 28, 2010.

Revision accepted: October 15, 2010.