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Pecans Acutely Increase Plasma Postprandial Antioxidant Capacity and Catechins and Decrease LDL Oxidation in Humans[1–3]

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Abstract

Bioactive constituents of pecan nuts such as γ-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 ± 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 γ-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 ± SEM; 95.1 ± 30.6 nmol/L) and 2 h (116.3 ± 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. J. Nutr. 141: 56–62, 2011.

Introduction

The pecan (*Carya illinoinensis*) is a nut native to southcentral 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.

The phenolic composition of pecans is complex and largely unknown. Pecans have been identified as a source of flavonoids, particularly the flavan-3-ol monomers (+)-catechin (C)[6] and (-)-epicatechin (EC) and their polymers, the proanthocyanidins (6). In nuts, flavonoids occur in the endothelial layer of the seed and provide protection against microbial pathogens and insect pests. Food assays rate pecans as highest among other commonly consumed nuts in total phenolic compounds at 20.2 ± 1.03 mg gallic acid equivalents (GAE)/g and antioxidant capacity at 179.4 μmol Trolox equivalents (TE)/g (7). In vitro studies have classified flavan-3-ols as powerful antioxidants capable of scavenging both reactive oxygen and nitrogen species (8). Currently, to our knowledge, no data exists on the bioavailability of nut flavanols or their contribution to in vivo antioxidant status.

In addition, pecan nuts have the particularity of being rich in γ-tocopherol and thus can serve as a food candidate for exploring the effect of γ-tocopherol–rich food on postprandial oxidative responses. In a randomized clinical trial, we observed that a pecan-enriched diet improved fasting concentrations of

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[6] Abbreviations used: AUC, area under the curve; C_{max}, maximum concentration; T_{max}, 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.

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γ-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 β-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 ± 3.4 kg/m^2) 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 α-tocopherol but relatively large amounts of γ-tocopherol, refined olive oil contains moderate amounts of α-tocopherol but practically no γ-tocopherol (10). Although the comprehensive phenolic acid composition of pecans is largely unknown, Gu et al. (6) reported that pecans contain 17.2 ± 2.5 mg/100 g of flavan-3-ol monomers and 494 ± 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.

Sample collection and storage. Blood samples were drawn via venipucture 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.

TABLE 1 Nutrient composition of test meals[1]

	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,[2] *mg*	—	445
Flavan-3-ol monomers,[3] *mg*	—	14.4
Catechin	—	6.5
EGC	—	5.1
Epigallocatechin gallate	—	2.1
EC	—	0.7
Total phenolic compounds, *mg GAE*	13[4]	1815[5]

[1] Nutrient composition values were obtained from USDA Database for standard reference unless otherwise specified (10).
[2] Proanthocyanidin database (44).
[3] Flavonoid database (37).
[4] Mostly tyrosol and hydroxyl-tyrosol (11).
[5] From (7).

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-Ciocalteau 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 peroxyl radicals (16). Fluorescein is used as the target molecule for free radical attack, 2,2′-azobis(2-amidinopropane) dihydrochloride as peroxyl 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 gallocatechin gallate (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 coulochem 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 β-glucoronidase 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 5μ ODS (150 × 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.

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

Statistical analysis. Data are expressed as least square means (LSM) ± 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 × 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_{max}) and time to reach C_{max} (T_{max}) 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.

Plasma tocopherols. Although plasma concentrations of α-tocopherol did not vary over time after the 3 meals (**Fig. 2***A*), those of γ-tocopherol (Fig. 2*B*) 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 ($P < 0.001$).

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. 3***A*). 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 α-tocopherol in oil. On the other hand, postprandial triglyceride concentrations increased steadily following all 3 test meals ($P < 0.001$) and

TABLE 2 Changes in plasma biomarkers after participants consumed control, whole pecan, and blended pecan meals[1]

	Test meals		
Biomarkers, $AUC_{(0-5\ h)}$	Control	Pecans, blended	Pecans, whole
Total polyphenols, *mmol GAE/L·h*	6.91 ± 0.92[a]	8.25 ± 0.90[b]	7.67 ± 0.90[a]
ORAC (hydrophilic), *mmol/L·h*	7.28 ± 0.31[a]	7.72 ± 0.30[b]	7.80 ± 0.30[b]
ORAC (lipophilic), *mmol/L·h*	3.73 ± 0.16[a]	4.12 ± 0.16[b]	4.15 ± 0.16[b]
FRAP, *mmol/L·h*	8.43 ± 0.52	9.24 ± 0.48	9.19 ± 0.48
Uric acid, *mmol/L·h*	1.62 ± 0.16	1.69 ± 0.18	1.66 ± 0.17
α-Tocopherol, *μmol/L·h*	113 ± 12	105 ± 12	109 ± 12
γ-Tocopherol, *mmol/L·h*	13.1 ± 1.5	14.0 ± 1.1	14.7 ± 1.4

[1] Values are LSM ± SEM, n = 16. Means in a row with superscripts without a common letter differ, $P < 0.05$.

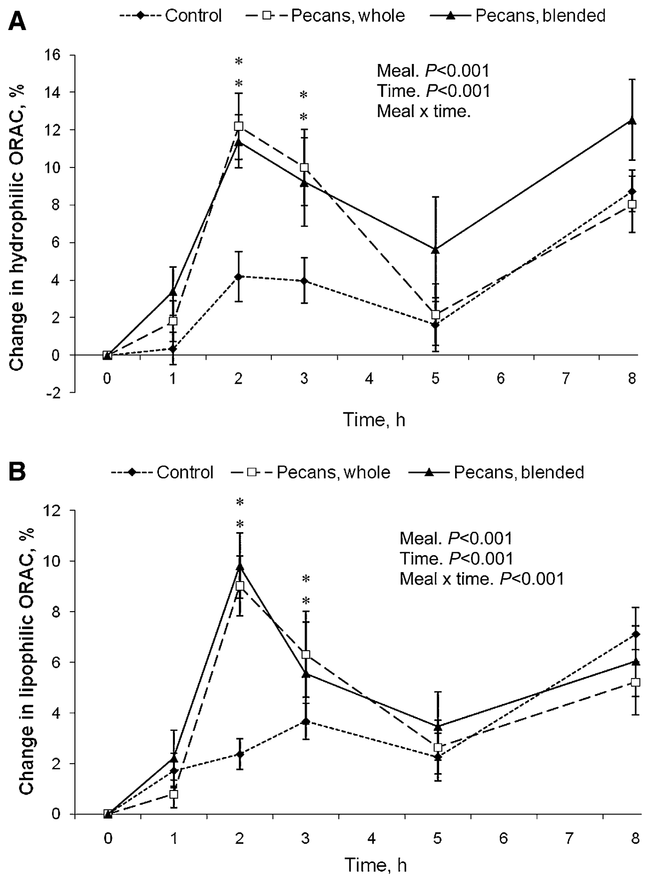

FIGURE 1 Percent change from baseline of plasma hydrophilic (*A*) and lipophilic (*B*) ORAC after participants consumed control, whole pecan, and blended pecan meals in random order. Data are LSM ± SEM, n = 16. *Different from baseline, $P < 0.001$.

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. 3*B*).

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 ECGC in pecans was less than that of C or EC, the highest $AUC_{(0\text{–}5h)}$ and C_{max} 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**).

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**).

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 postingestion plasma antioxidant

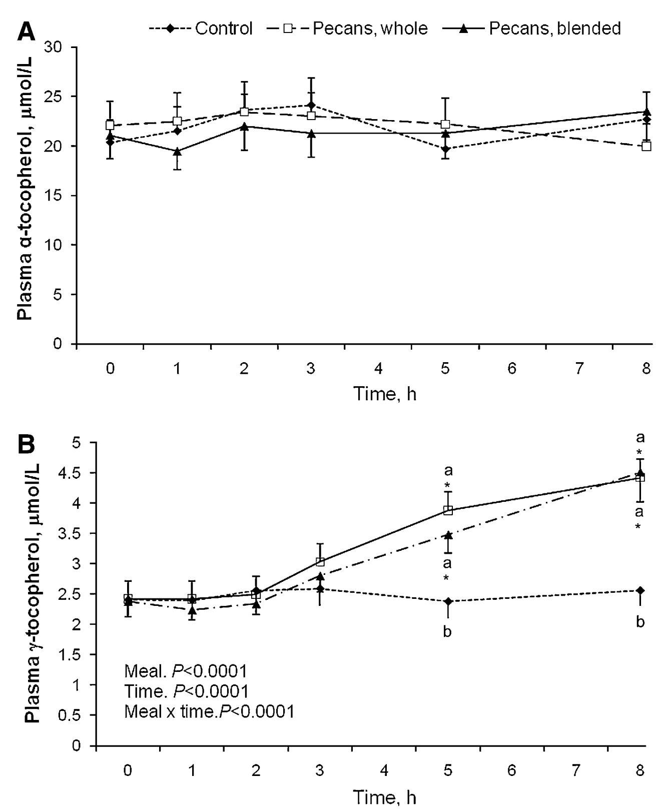

FIGURE 2 Plasma concentrations of α-tocopherol (*A*) and γ-tocopherol (*B*) after consumption of test meals. Values are LSM ± SEM, n = 16. Labeled means at a time without a common letter differ, $P < 0.001$. *Different from baseline, $P < 0.001$.

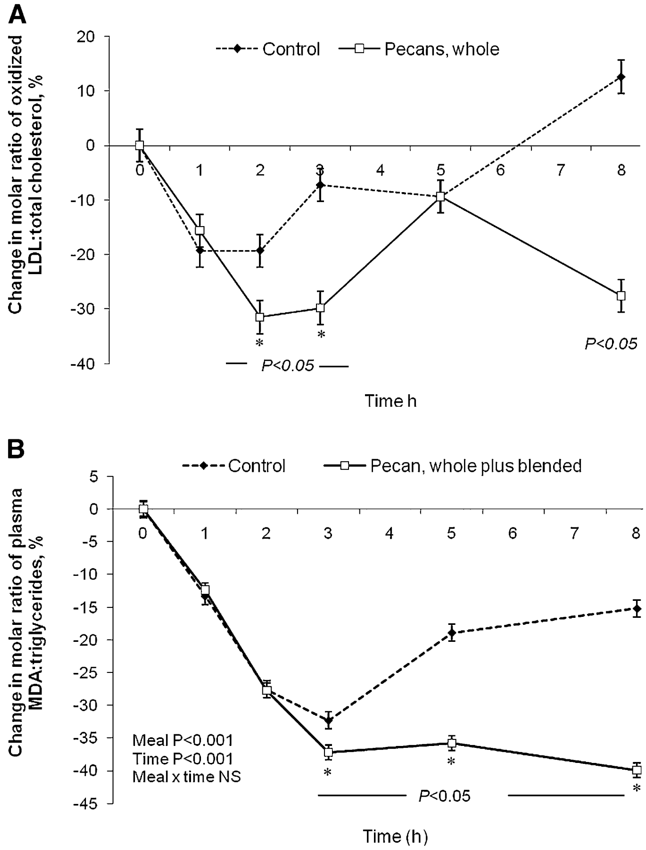

FIGURE 3 Percent change from baseline in ratios of (*A*) oxidized LDL:total cholesterol after participants consumed the control and whole pecan test meals and (*B*) plasma MDA:triglycerides following the control and combined whole and blended pecan test meals (pooled data). Values are mean ± SEM, $n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

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 γ-tocopherol concentrations. It has been established that γ-tocopherol is rapidly metabolized following ingestion and fasting plasma levels are a fraction of those of α-tocopherol (29). The concentrations of the γ- isomer steadily increased following consumption of both the whole and blended pecan meals, reaching approximately twice the fasting level at 8 h postingestion ($P < 0.001$). Although somewhat temporary, these postprandial increases in γ-tocopherol are physiologically relevant. In vitro experiments and studies in animals suggest that the antioxidant activity of γ-tocopherol exceeds that of the α-isomer (30) and that γ-tocopherol efficiently traps reactive oxygen and nitrogen radicals (31), inhibits LDL oxidation (32), and is antiinflammatory (33,34). In humans with metabolic syndrome, γ-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 interindividual 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

TABLE 3 Pharmacokinetic parameters for selected plasma flavan-3-ol monomers following consumption of 90 g of whole pecans[1]

Flavan-3-ol monomer	Dose, *μmol*	$AUC_{(0–5h)}$, *nmol/L·h*	C_{max}, *nmol/L*	T_{max}, *h*	Bioavailability,[2] *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[3]	78 ± 40	35 ± 19	4.3 ± 0.7	—
GCG	NA	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

[1] Values are mean ± SEM, $n = 12$.
[2] Calculated as $AUC_{(0–5\ h)}$ divided by dose (AUC/dose).
[3] NA, Not available.

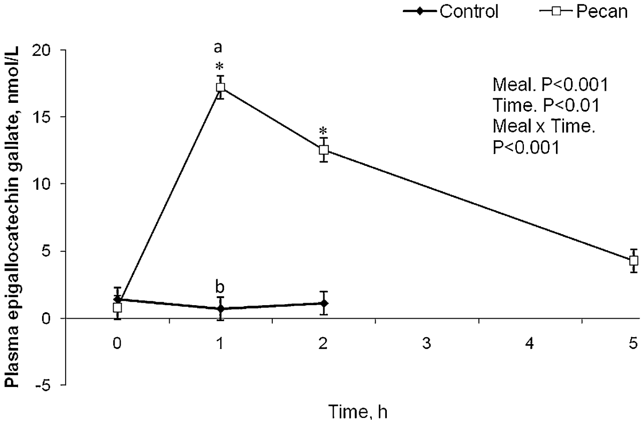

FIGURE 4 Plasma epigallocatechin gallate concentrations in participants before (0 h) and after control and whole pecan test meals. Data were log10-transformed prior to analysis. Values are LSM ± SEM, n = 12. Labeled means at a time without a common letter differ, $P < 0.05$. *Different from baseline,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

lipophilic ones. The increase in plasma catechins and γ-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 α-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 γ-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 γ-tocopherol, catechins, or both acting in synergy remains to be determined.

TABLE 4 Urinary excretion of phenylacetic acids following consumption of control and whole pecan test meals[1]

	Control	Pecans, whole
3,4-Dihydroxyphenylacetic acid	*nmol/mol creatinine*	
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*

[1] 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).

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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.

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