

Avenanthramides and Phenolic Acids from Oats Are Bioavailable and Act Synergistically with Vitamin C to Enhance Hamster and Human LDL Resistance to Oxidation^{1,2}

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ABSTRACT The intake of phenolic acids and related polyphenolic compounds has been inversely associated with the risk of heart disease, but limited information is available about their bioavailability or mechanisms of action. Polyphenolics, principally avenanthramides, and simple phenolic acids in oat bran phenol-rich powder were dissolved in HCl:H₂O:methanol (1:19:80) and characterized by HPLC with electrochemical detection. The bioavailability of these oat phenolics was examined in BioF1B hamsters. Hamsters were gavaged with saline containing 0.25 g oat bran phenol-rich powder (40 μ mol phenolics), and blood was collected between 20 and 120 min. Peak plasma concentrations of avenanthramides A and B, *p*-coumaric, *p*-hydroxybenzoic, vanillic, ferulic, sinapic, and syringic acids appeared at 40 min. Although absorbed oat phenolics did not enhance ex vivo resistance of LDL to Cu²⁺-induced oxidation, in vitro addition of ascorbic acid synergistically extended the lag time of the 60-min sample from 137 to 216 min ($P \leq 0.05$), unmasking the bioactivity of the oat phenolics from the oral dose. The antioxidant capability of oat phenolics to protect human LDL against oxidation induced by 10 μ mol/L Cu²⁺ was also determined in vitro. Oat phenolics from 0.52 to 1.95 μ mol/L increased the lag time to LDL oxidation in a dose-dependent manner ($P \leq 0.0001$). Combining the oat phenolics with 5 μ mol/L ascorbic acid extended the lag time in a synergistic fashion ($P \leq 0.005$). Thus, oat phenolics, including avenanthramides, are bioavailable in hamsters and interact synergistically with vitamin C to protect LDL during oxidation. J. Nutr. 134: 1459–1466, 2004.

KEY WORDS: • antioxidants • avenanthramides • bioavailability • oats • phenolics

Studies showing an inverse association between the intake of polyphenolic compounds, particularly flavonoids from fruits and vegetables, and cardiovascular disease risk suggest that a beneficial effect may be observed from other foods containing these compounds (1–3). For example, polyphenolics have been identified in several grains, including wheat, rice, corn, and oats (4). These phytochemicals have a range of biological activities, including antiatherosclerotic, anti-inflammatory, and antioxidant effects (5). Similar to their actions in other foods, simple phenolic acids and polyphenolic compounds

from oats (referred to here as oat phenolics) may serve as potent antioxidants via scavenging reactive oxygen and nitrogen species and/or by chelating transition minerals both in plants and in those animals that consume them (6).

Because most phenolics are located in the bran layer of grains (7), oats (*Avena sativa* L.), which are normally consumed as whole-grain cereal, could be a significant dietary source of these compounds (8). Several oat phenolics have been identified, including ferulic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, vanillic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, sinapic acid, triclin, apigenin, luteolin, kaempferol, and quercetin (9,10). These oat phenolics are present as free or simple soluble esters and, to a greater extent, as complex insoluble esters with polysaccharides, proteins, or cell wall constituents (6,8). In addition, Collins (11) isolated and characterized a group of cinnamoylanthranyl alkaloid oat polyphenols, called avenanthramides, which appear to be unique to oats.

The antioxidant capacity of oat phenolics was demonstrated via in vitro studies (12–15). However, few studies have explored the in vivo activity of oat phenolics. Hullless (“na-

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ked") oats fed to cows resulted in a greater stability of their milk against oxidative degradation (16). Similarly, carcasses of broiler chickens fed oats or hullless oats had a lower content of lipid peroxidation products (17,18). However, the antioxidant capacity of serum was not affected in people consuming an oat milk product (19).

To date, no studies have explored directly the bioavailability of oat phenolics and their subsequent effect on antioxidant activity. Therefore, we conducted this study with the following goals: 1) to measure the bioavailability of oat phenolics using a hamster model; 2) to determine the *in vivo* effect of absorbed oat phenolics on the antioxidant capacity of hamsters; and 3) to test *in vitro* the effect of oat phenolics on the resistance of human LDL to oxidation and its potential interactions with vitamin C in this system.

METHODS AND MATERIALS

Chemicals and reagents. The following reagents were obtained from Sigma Chemical: copper sulfate, α -tocopherol, sodium chloride, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, vanillin, vanillic acid, ferulic acid, sinapic acid, sodium phosphate monobasic, sodium phosphate dibasic, Folin Ciocalteu's phenol reagent, and β -glucuronidase type H-2 (containing sulfatase). All organic solvents, glacial acetic acid, ascorbic acid, and potassium bromide were purchased from Fisher Scientific. Food-grade ascorbic acid was from Mallinckrodt, and lithium hydroxide was from Fluka.

Production of oat bran phenol-rich powder. Oat bran was collected from hullless oats passed 3 times through a Satake Rice Machine (type RMB, Satake Engineering). The final weight removed was 20% of the original hullless oats. The oat bran was extracted twice with ethanol:water (80:20, v:v) for 2 h at 35°C with continuous agitation. The extraction slurry was centrifuged at $1250 \times g$ (Inverting Filter Centrifuge, Model HF-600.1, Heinkel Filtering Systems, 5 mm-bag) to provide the supernatant. Food-grade ascorbic acid was added to the supernatant as a processing aid preservative, but was removed during late processing. The supernatant was vacuum concentrated (Alfa-Laval, Model 6 $\times$ 2) at 35–40°C to a thick oat bran extract and then lyophilized (Virtis Model 50-SRC-6, Virtis) to an oat bran phenol-rich powder and stored at –20°C until use.

Measurement of phenolics from oat bran phenol-rich powder. Oat phenolics in oat bran phenol-rich powder were dissolved in HCl:H₂O:methanol (1:19:80). After centrifugation at $11,000 \times g$ for 10 min, an aliquot of the supernatant was dried under purified nitrogen. The residue was reconstituted with the aqueous mobile phase, and the oat phenolics profile was characterized by HPLC equipped with electrochemical detection (ECD)⁴ according to Milbury (20). The quantity of individual oat phenolics was calculated according to concentration curves constructed with authenticated phenolic acid standards and with pure avenanthramide A and B. Phenolic esters were not determined in this study. The total phenolic content of the oat bran powder was also determined using the Folin-Ciocalteu reaction against a gallic acid standard curve and expressed as molar equivalents of gallic acid (21).

Animals. BioF1B strain Golden Syrian Hamsters ($n = 30$; Bio-Breeders), 1 y old, mean body weight 156.7 ± 12.7 g, were housed in cages with a 10-h:14-h light:dark cycle. Hamsters were used due to the similarity of their lipoprotein metabolism to that of humans (22). To increase lipoprotein formation for subsequent collection, hamsters consumed *ad libitum* a nonpurified diet (Harlan) enriched with 10 g coconut oil and 0.5 g cholesterol/100 g diet for 2 wk before the acute oat phenolics feeding experiments (23).

After overnight food deprivation, 30 hamsters were randomly assigned on the basis of their body weight into 6 time point groups: 0, 20, 40, 60, 80, and 120 min. A slurry with 250 mg oat bran

phenol-rich powder containing 40 μ mol phenolics (6.8 mg) was delivered in 1.0 mL of 0.154 mol/L saline via stomach gavage to hamsters anesthetized with Aerrane (Baxter). The same volume of saline was given to hamsters in the baseline control group. The estimated daily polyphenolic intake for a 70-kg body person is 14 mg/kg (24). We chose a dose of 45 mg/kg body weight (40 μ mol oat phenolics/per hamster) because rodents consume 5–6 times more food-based energy than humans on a body weight basis (25). Blood samples from each hamster were collected into tubes containing EDTA via orbital bleeding at selected time points. Plasma samples were collected after whole blood was centrifuged at $1000 \times g$ for 15 min at 4°C. Two aliquots of plasma were stored at –80°C for determination of oat phenolics and antioxidant capacity; the remainder was used immediately for analysis of LDL oxidation. This study was approved by the Animal Care and Use Committee of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University.

Analysis of plasma oat phenolics. Oat phenolics in plasma were measured via HPLC-ECD (20). Briefly, 20 μ L vitamin C-EDTA (1.136 mmol ascorbic acid plus 3.42 μ mol EDTA in 1 mL of 0.4 mol/L NaH₂PO₄) and 20 μ L glucuronidase were added to 200 μ L plasma, and the mixture was incubated at 37°C for 45 min. Oat phenolics were extracted with acetonitrile; the 500- μ L supernatant was removed after centrifugation at $14,000 \times g$ for 5 min, dried under purified nitrogen, and reconstituted in 100 μ L of the aqueous HPLC mobile phase. After centrifugation at $14,000 \times g$ for 5 min, the 50- μ L supernatant was injected into the HPLC for analysis of oat phenolics. Quantification was accomplished using authenticated standards that were spiked into human plasma and processed through the extraction procedure. An internal standard was not used in this study to calculate the recovery rate or for quantification; rather, spiked authenticated standards were used in constructing standard curves that account for extraction losses. We observed an 80% recovery rate for the internal standard (2',3',4'-trihydroxyacetophenone), which has characteristics similar but not identical to the oat phenolic compounds of interest; the recovery rate did not always parallel the recovery rates of authenticated oat phenolic standards during the extraction procedure (data not shown).

Ex vivo antioxidant capacity of oat phenolics. Absorbed oat phenolics were tested *ex vivo* to characterize their antioxidant effect on the resistance of hamster LDL to Cu²⁺-induced oxidation according to a slight modification of the method described by Esterbauer et al. (26). Briefly, LDL was separated from the plasma according to Chung et al. (27) using a Beckman NVT-90 rotor in a Beckman L8-mol/L centrifuge. Salt and EDTA were removed from the sample using a PD-10 column (Amersham Pharmacia Biotech). LDL protein was determined using a BCA protein assay kit (Pierce). Because LDL content in hamster plasma is less than that found in human plasma, 91 nmol/L LDL was oxidized by 5 μ mol/L CuSO₄ with or without the addition 5 μ mol/L ascorbic acid in a total volume of 1.0 mL phosphate buffer (pH 7.4). Formation of conjugated dienes was monitored by absorbance at 234 nm at 37°C over 6 h using a Shimadzu UV1601 spectrophotometer equipped with a 6-position automated sample changer. The results of the LDL oxidation were expressed as lag time (defined as the intercept at the abscissa in the diene-time plot) (28). The total antioxidant capacity of the plasma was measured with the oxygen radical absorbance capacity (ORAC) assay according to a slight modification of the method described by Huang et al. (29).

Synergistic relationship of oat phenolics and vitamin C in the *in vitro* human LDL oxidation. Because the amount of LDL available from hamsters is limited, human LDL was used to confirm the observed synergistic relation between oat phenolics and vitamin C. An added benefit of using human LDL in this assay is the extension of the results in an animal model to future applications for clinical evaluations. Venous blood was obtained at 1400 h from nonfasting healthy adult Caucasian women ($n = 6$), 28–64 y old, with a mean body weight of 63 ± 15 kg, and plasma immediately separated after centrifugation as described above. LDL samples from the first 3 subjects were used to assess the dose-response relation of oat phenolics and from the last 3 subjects for experiments on the interaction between oat phenolics and vitamin C. All LDL experiments were performed on 3 subjects in duplicate. The kinetics of LDL oxidation

⁴ Abbreviations used: C_{max}, maximal concentration; ECD, electrochemical detector; ORAC, oxygen radical absorbance capacity; pca, perchloric acid-treated; RT, retention time; TE, Trolox equivalent; T_{max}, time to maximal concentration.

were monitored after the addition of 10 $\mu\text{mol/L}$ CuSO_4 to 182 nmol/L LDL protein in a total volume of 1.0 mL phosphate buffer (pH, 7.4) and the formation of conjugated dienes monitored as described above. An aliquot of oat phenolics in acidified methanol was dried under nitrogen and redissolved in an equal volume of phosphate buffer (pH 7.4) for testing in the assay. The lowest concentration of oat phenolics (0.52 $\mu\text{mol/L}$) used in the *in vitro* LDL oxidation experiment was selected because it consistently extended the lag time. Additional concentrations of oat phenolics were selected to reflect the concentrations observed in the plasma from the hamster study described above. Oat phenolics were incubated with 182 nmol/L LDL at 37°C for 30 min before initiation of oxidation. When used in the assay, ascorbic acid was dissolved in PBS and added to the reaction immediately before initiation of oxidation. The effect of oat phenolics and ascorbic acid on the resistance of LDL against oxidation was expressed as the lag time increase compared with the lag time of LDL without the addition of oat phenolics or vitamin C.

Statistics. All results are reported as means $\pm$ SD. The Tukey-Kramer honestly significant difference (HSD) test was used after significant differences were obtained by one-way ANOVA in experiments on plasma phenolics in hamsters, *ex vivo* and *in vitro* hamster LDL oxidation, and *in vitro* human LDL oxidation. When variance was unequal, Hartley's test (30) was used and data (including that for ferulic and sinapic acids) were square root-transformed before ANOVA. A paired *t* test was performed to determine the significance of the synergy between oat phenolics and vitamin C in human LDL oxidation by comparing the observed lag time during their coincubation with the expected (calculated) sums of values observed for oat phenolics and vitamin C treatments alone. Differences with $P \leq 0.05$ were considered significant. The JMP IN 4 statistical software package (SAS Institute) was used to perform all statistical analyses.

RESULTS

The total polyphenolic content in the oat bran phenol-rich powder was 162 μmol gallic acid equivalents/g as determined by the Folin-Ciocalteu method. As revealed by a typical HPLC-ECD chromatogram, there were ~ 30 peaks with detectable redox potential (Fig. 1). We identified and quantified 9 phenolics in oat bran phenol-rich powder (in descending order of concentration) as: avenanthramide A (2.50 $\mu\text{mol/g}$),

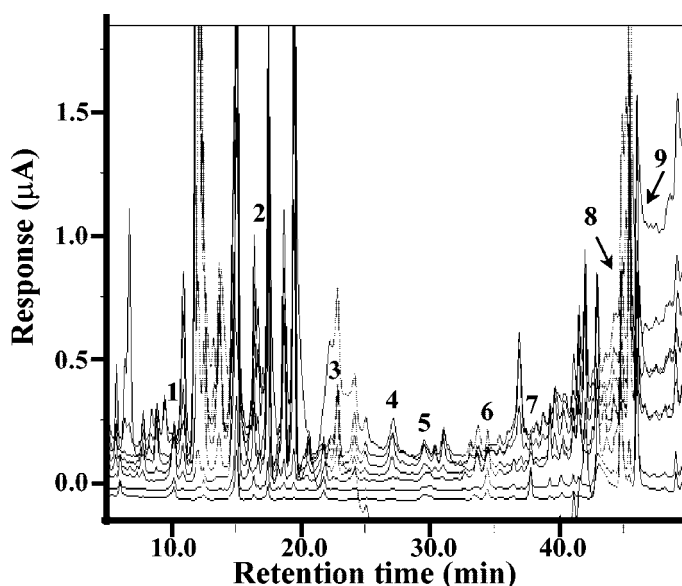


FIGURE 1 HPLC-ECD profile of phenolic acids and avenanthramides in oat bran phenol-rich powder identified by HPLC-ECD. Labeled peaks are: (1) *p*-hydroxybenzoic acid, (2) vanillic acid, (3) syringic acid, (4) *p*-coumaric acid, (5) vanillin, (6) ferulic acid, (7) sinapic acid, (8) avenanthramide A, (9) avenanthramide B.

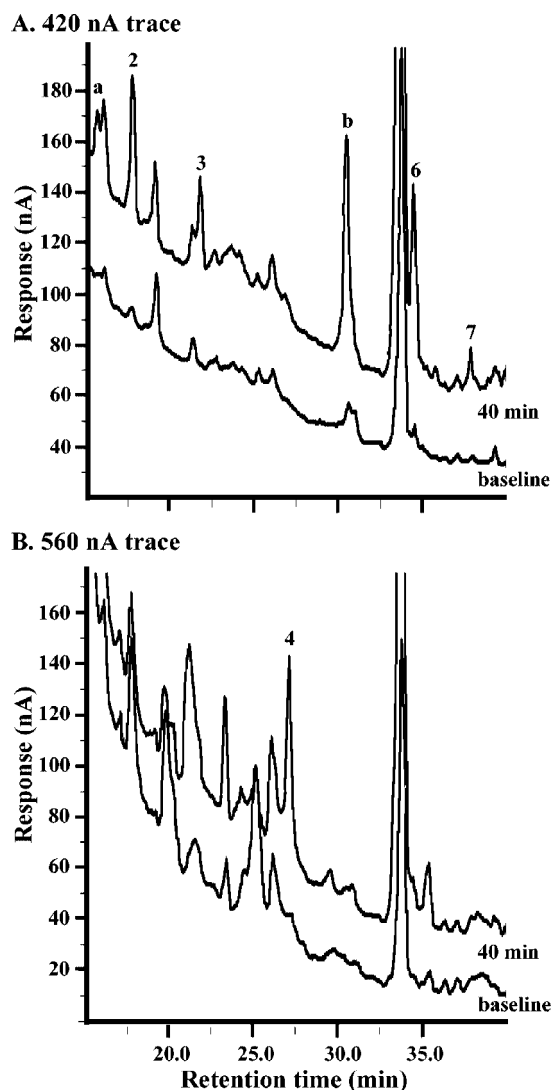


FIGURE 2 HPLC-ECD chromatographs of hamster plasma samples obtained 40 min after administration of 0.25 g oat bran phenol-rich powder in saline, containing 40 μmol phenolics (gallic acid equivalents) and immediately after gavage with saline (baseline). (A) The 420-mV ECD trace. (B) The 560-mV ECD trace. Labeled peaks are: (a) 17.80-min RT compound, (2) vanillic acid, (3) syringic acid, (4) *p*-coumaric acid, (b) 30.95-min RT compound, (6) ferulic acid, (7) sinapic acid.

avenanthramide B (1.97 $\mu\text{mol/g}$), vanillin (2.40 $\mu\text{mol/g}$), *p*-coumaric acid (1.28 $\mu\text{mol/g}$), ferulic acid (0.64 $\mu\text{mol/g}$), vanillic acid (0.53 $\mu\text{mol/g}$), syringic acid (0.39 $\mu\text{mol/g}$), sinapic acid (0.25 $\mu\text{mol/g}$), and *p*-hydroxybenzoic acid (0.03 $\mu\text{mol/g}$). Although oats are rich in vitamin E (8,31), none was detectable by our HPLC method in this oat bran phenol-rich powder because most of tocopherols and tocotrienols are located in the germ and endosperm (31), both of which were eliminated by abrasion milling.

Although there were numerous compounds in oat bran phenol-rich powder, avenanthramide A and B, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, and *p*-hydroxybenzoic acid (not shown in the chromatogram) were bioavailable in hamsters (Figs. 2 and 3). In addition, 2 unknown compounds in plasma were noted at a retention time (RT) of 17.80 and 30.95 min. Comparing the ratio observed of oat phenolic concentrations in oat bran phenol-rich powder and plasma, the compounds syringic, ferulic, and *p*-hydroxy-

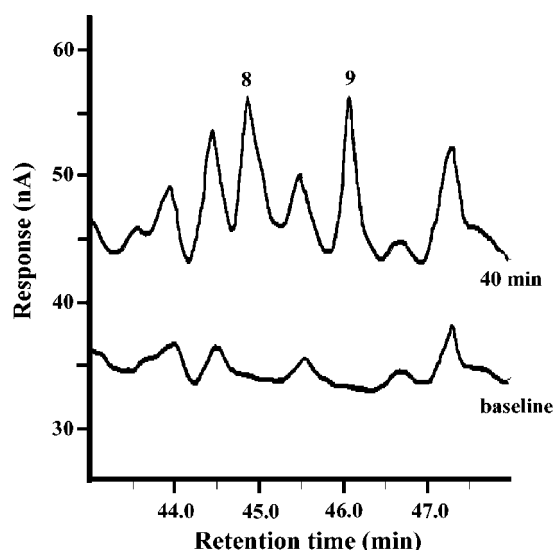


FIGURE 3 HPLC-ECD chromatograph of avenanthramide A (peak 8) and B (peak 9) in hamster plasma obtained immediately after a gavage with saline (baseline, lower trace) and 40 min after administration of 0.25 g oat bran phenol-rich powder in saline, containing 40 μmol phenolics (gallic acid equivalents) (upper trace).

benzoic acids possessed a similar bioavailability, whereas *p*-coumaric acid had at least 10% greater bioavailability (Table 1). Among identified oat phenolics, avenanthramides were found at the highest concentration in the oat bran phenol-rich powder but the lowest concentration in hamster plasma.

On the basis of their pharmacokinetic profile, the maximum plasma concentrations (C_{max}) of *p*-hydroxybenzoic acid, vanillic acid, sinapic acid, syringic acid, ferulic acid, and *p*-coumaric acid ranged from 0.10 to 1.55 $\mu\text{mol/L}$ (Fig. 4). The C_{max} for avenanthramide A and B was 0.04 and 0.03 $\mu\text{mol/L}$, respectively (Fig. 4). The C_{max} of these oat phenolics and the compound identified at 30.95 min RT was reached at 40 min (T_{max}). In contrast, the compound identified at 17.80 min RT had a T_{max} at 80 min. At 120 min, the plasma concentrations of these 10 compounds did not differ from the baseline reference.

Absorbed oat phenolics did not change the resistance of hamster LDL collected at 40 and 60 min against Cu^{2+} -induced oxidation (Fig. 5). However, after 5 $\mu\text{mol/L}$ ascorbic acid was added to the assay mixture, LDL collected at 60 min had a

58% longer lag time than that collected at baseline (216 and 137 min, respectively; $P \leq 0.05$). The ORAC assay for total antioxidant capacity, expressed as $\mu\text{mol/L}$ Trolox equivalent (TE), was measured in plasma ($\text{ORAC}_{\text{total}}$) and protein-precipitated, perchloric acid-treated plasma (ORAC_{pca}). Absorbed oat phenolics did not change the $\text{ORAC}_{\text{total}}$ (8274 ± 1243 and $7079 \pm 777 \mu\text{mol/L TE}$) or ORAC_{pca} (1284 ± 123 and $1081 \pm 171 \mu\text{mol/L TE}$) in samples collected at baseline and 40 min, respectively.

The antioxidant activity of oat phenolics in vitro was apparent through a dose-dependent increase in the resistance of human LDL against Cu^{2+} -induced oxidation ($P \leq 0.0001$) (Fig. 6). The lowest concentration of oat phenolics tested for this effect was 0.52 $\mu\text{mol/L}$ gallic acid equivalents which resulted in a lag time 9.6 ± 1.7 min greater than that of the control absent oat phenolics. Oat phenolics doses of 0.78, 1.3, and 1.95 $\mu\text{mol/L}$ further extended the lag time by 12.8 ± 2.1 , 21.8 ± 2.4 , and 37.5 ± 3.3 min, respectively. The addition of ascorbic acid alone at 2.5 and 5.0 $\mu\text{mol/L}$ increased the lag time by 11.8 ± 2.6 and 46.3 ± 3.5 min, respectively (Fig. 7). A 1-fold synergy (i.e., an observed value twice the expected value from additive calculation) was observed with oat phenolics and the 5.0 $\mu\text{mol/L}$ ascorbic acid dose, but no such interaction was found with the 2.5 $\mu\text{mol/L}$ dose ($P \leq 0.005$).

DISCUSSION

In addition to their protein and micronutrient content, whole grains contain an array of phytochemicals that may contribute substantially to the total intake of dietary antioxidants. Although typically consumed in lower quantities than grains such as rice and wheat, oats are normally consumed as a whole-grain cereal; thus, the antioxidant-rich portion of the grain is retained. Among other potential health benefits, these constituents may contribute to the reduction in risk of cardiovascular disease associated with whole-grain intake as found in several observational studies (32–35). Interestingly, the antioxidant capacity of oats had been recognized many years ago with their use as additives in food and beverage products to preserve their quality (36,37).

Using HPLC-ECD to analyze phenolics of oat bran phenol-rich powder, we identified (in descending order of concentration) avenanthramide A and B, vanillin, ferulic acid, *p*-coumaric acid, vanillic acid, syringic acid, sinapic acid, and *p*-hydroxybenzoic acid. These results are consistent with those of Peterson et al. (9) and Daniels and Martin (10). However, caffeic acid, protocatechuic acid, tricetin, apigenin, luteolin,

TABLE 1

Relative bioavailability of 8 phenolics in hamsters fed 40 μmol total phenolics of oat bran phenol-rich powder

Oat phenolics	Oral dose ¹ μmol	Plasma C_{max} ² $\mu\text{mol/L}$	Plasma C_{max} /oral dose ($\mu\text{mol/L}$)/ μmol	Apparent relative bioavailability ³
<i>p</i> -Coumaric acid	0.32	1.55 ± 0.91	4.84 ± 2.84	100
Sinapic acid	0.06	0.26 ± 0.38	4.30 ± 6.30	89.5
Syringic acid	0.10	0.38 ± 0.25	3.80 ± 2.50	78.5
<i>p</i> -Hydroxybenzoic acid	0.03	0.10 ± 0.04	3.33 ± 1.33	68.8
Ferulic acid	0.50	1.20 ± 1.08	2.40 ± 2.60	49.5
Vanillic acid	0.13	0.15 ± 0.05	1.20 ± 0.38	23.8
Avenanthramide A	0.63	0.04 ± 0.03	0.06 ± 0.05	1.3
Avenanthramide B	0.49	0.03 ± 0.02	0.06 ± 0.04	1.3

¹ Oral dose is the absolute amount of each phenolic compound fed to each hamster.

² Values are means $\pm$ SD, $n = 5$.

³ The ratio of plasma C_{max} /oral dose for *p*-coumaric acid was arbitrarily set at 100.

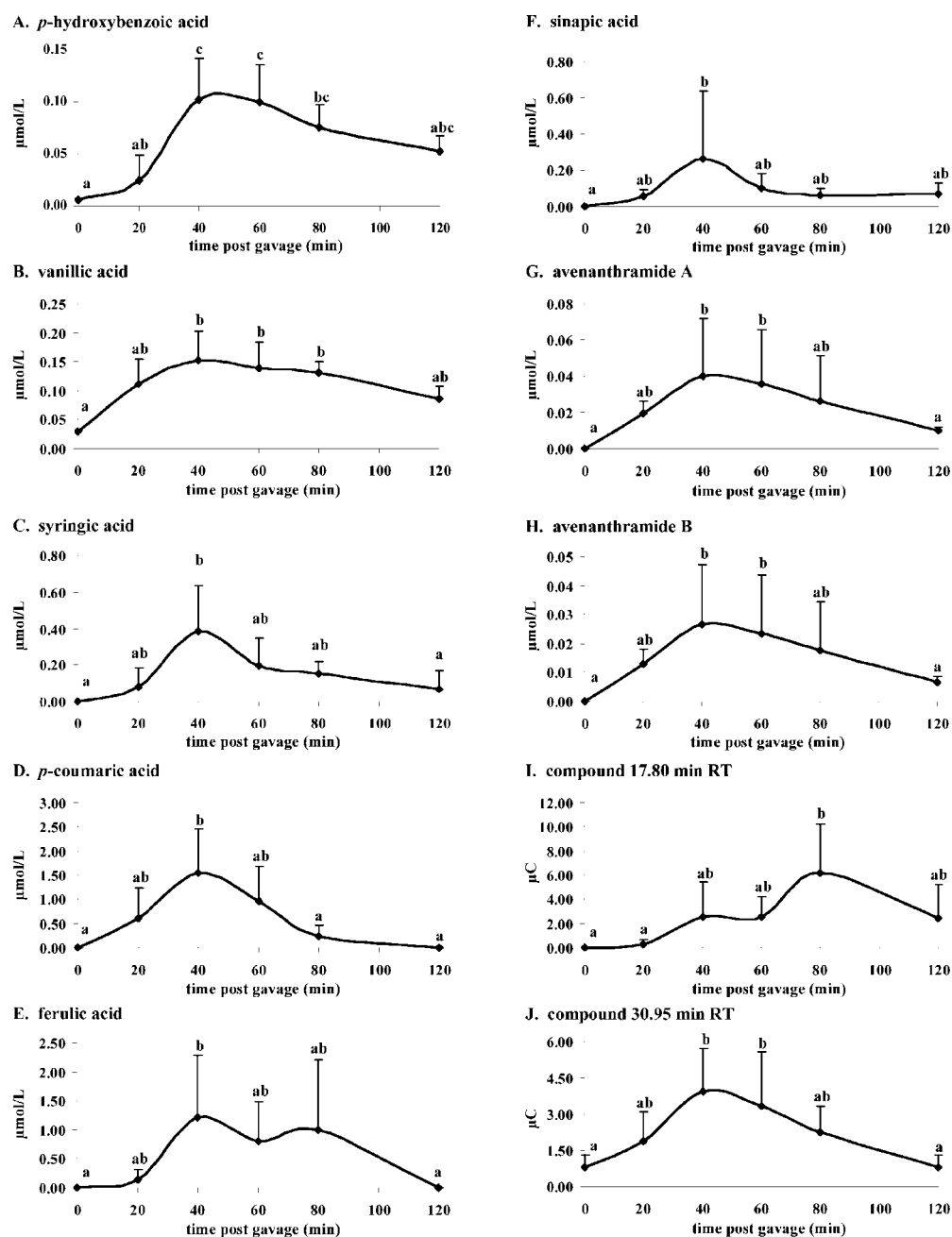


FIGURE 4 Time course of oat phenolic compounds in the plasma of hamsters administered 0.25 g oat bran phenol-rich powder in saline containing 40 μ mol phenolics (gallic acid equivalents). Values are mean $\pm$ SD, $n = 5$. In panels I and J, μ C is the area under the ECD trace with time. Means in each panel without a common letter differ, $P \leq 0.05$.

kaempferol, and quercetin were not found in the oat bran phenol-rich powder by our HPLC-ECD method. We identified 2 of the 6 reported oat avenanthramides (11,15) by HPLC-ECD using authenticated standards. Our chromatographic results suggest that there are numerous other antioxidant phytochemicals in oat bran that remain to be identified and fully characterized, such as phenolic esters (9). The absence of free caffeic acid and some other oat phenolics from our material, in contrast to reports by others (9,10), is likely due to the different methods employed to isolate these compounds, including factors such as extraction solvent, heating, and esterase activity. As noted, the oat bran phenol-rich powder employed in our studies contained no vitamin E or other tocopherols as detected by HPLC and reported by Peterson (31); thus, they are not a source of the antioxidant activity noted in our experiments.

Oat phenolics from the oat bran phenol-rich powder were

found to be bioavailable in hamsters. Ji et al. (38) recently reported that the dietary administration of a synthetic avenanthramide had an antioxidant effect in selected tissues of exercised rats. Vanillic, *p*-hydroxybenzoic, sinapic, ferulic, and *p*-coumaric acids from other food sources were found previously to be bioavailable (39–42), but our results appear to be the first to identify syringic acid and avenanthramides in plasma and suggest their bioactivity. The T_{max} of the phenolic acids and avenanthramides in hamsters were reached at 40 min and essentially eliminated by 120 min. *p*-Coumaric acid was the most bioavailable among the identified oat phenolics. In contrast, although the polyphenolic avenanthramides had the greatest concentration in the oat bran phenol-rich powder, their apparent relative bioavailability was only 5% of the least bioavailable phenolic acid (vanillic acid). Although *p*-coumaric acid is the most bioavailable phenolic acid, the apparent relative bioavailabilities among phenolics might be influenced

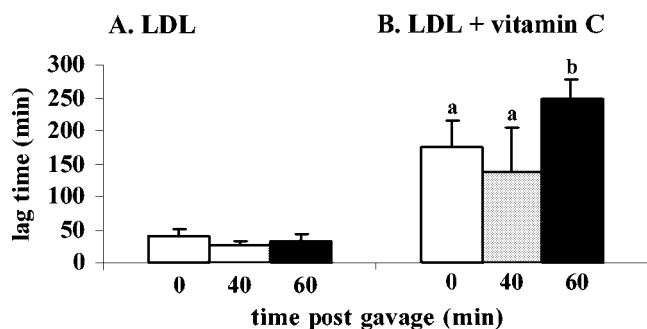


FIGURE 5 Lag time to Cu^{2+} -induced oxidation of hamster LDL obtained at 0, 40, and 60 min after administration of 0.25 g oat bran phenol-rich powder in saline, containing 40 μmol phenolics (gallic acid equivalents) without (A) or with (B) 5 $\mu\text{mol/L}$ ascorbic acid added in vitro. Values are means $\pm$ SD, $n = 5$. Means in the same category without a common letter differ, $P \leq 0.05$.

by the distribution and/or biotransformation of phenolic acids in the hamsters. For example, vanillin was the richest phenolic acid in oat bran phenol-rich powder, but none was observed in plasma, possibly due to its conversion to vanillic acid in vivo (43). Because the concentrations of the oat phenolics were measured only in plasma, it is not possible to determine from this study to what extent these compounds were distributed to other tissues. The C_{max} of the unidentified 17.80- and 30.95-min RT compounds was achieved at 80 and 40 min, respectively. The 17.80-min RT compound may be a metabolite because its T_{max} was substantially delayed relative to other oat phenolics, and it was not present in baseline plasma. In addition to hepatic metabolism, the biotransformation of polyphenolics by colonic microflora was demonstrated (44–46). In contrast, the 30.95-min RT compound was likely produced endogenously because it was present, albeit at a lower concentration, in the baseline plasma. The identification of these compounds could not be achieved without authenticated standards by our HPLC-ECD method; therefore, an effort is underway to identify these and other oat phenolic metabolites using HPLC-MS.

In vitro studies of ferulic, syringic, and other phenolic acids clearly reveal the capacity of these compounds to bind to LDL and increase its resistance against oxidation (47,48). We evaluated the potential antioxidant activity of the oat phenolics using an ex vivo hamster LDL oxidation model and found no apparent change in the lag time after induction by Cu^{2+} . This

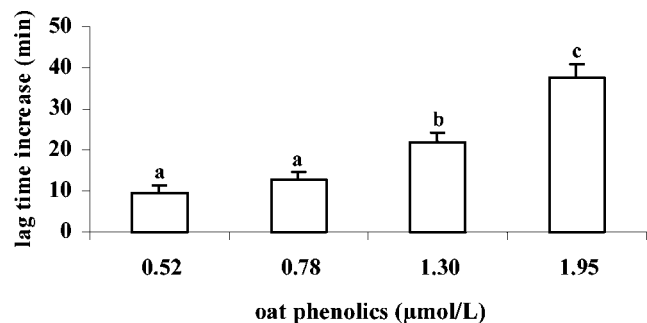
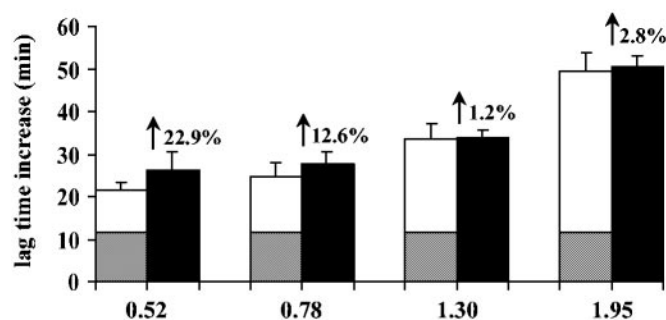


FIGURE 6 Effect of oat phenolics on increased lag time to Cu^{2+} -induced oxidation of human LDL in vitro; 182 $\mu\text{mol/L}$ LDL was oxidized by 10 $\mu\text{mol/L}$ Cu^{2+} with addition of oat phenolics. Lag time of control (no added oat phenolics) was 49.3 ± 3.7 min. Values are means $\pm$ SD, $n = 3$. Means without a common letter differ, $P \leq 0.0001$.

A. 2.5 $\mu\text{mol/L}$ ascorbic acid



B. 5 $\mu\text{mol/L}$ ascorbic acid

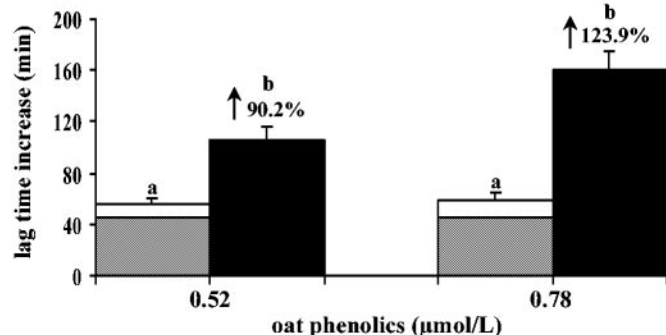


FIGURE 7 The synergistic effect of oat phenolics and vitamin C on the increased lag time of human LDL oxidation in vitro; 182 $\mu\text{mol/L}$ LDL was oxidized by 10 $\mu\text{mol/L}$ Cu^{2+} with the addition of oat phenolics, vitamin C, or oat phenolics and vitamin C combined. Values are means $\pm$ SD, $n = 3$. Means without a common letter differ, $P \leq 0.005$. Lag time of control (no added oat phenolics or ascorbic acid) (A) was 45.5 ± 0.7 min and (B) 47.7 ± 1.5 min. Open bar (oat phenolics) and hatched bar (ascorbic acid) are stacked to illustrate expected values by calculation of the additive effect of individual ingredients. Solid bar represents the observed effect of oat phenolics + ascorbic acid. The percentage value above the solid bar indicates the observed synergistic increase of combined antioxidants over expected calculated values of the individual ingredients.

lack of an effect might be due to an inadequate concentration of the oat phenolics in the plasma or to their biotransformation [hepatic phase 2 enzymes have been shown to reduce the antioxidant capacity of polyphenolics relative to their parent compounds (49,50)]. Although these results appear in contrast to our in vitro results with oat phenolics in human LDL, it is important to note that the ex vivo assay reflects the action of only those bioavailable oat phenolics that remain associated with the LDL through its isolation process.

Despite no apparent change in the resistance of LDL to oxidation ex vivo, the oat phenolics had a subtle action on the lipoprotein that was indicated by their interaction with vitamin C. When ascorbic acid was added in vitro, an increase in the lag time was observed compared with its respective control. This increase was synergistic in nature, i.e., the lag time was greater than the calculated additive effect of the antioxidants, although the mechanism for such an interaction has yet to be elucidated. This synergistic relationship is consistent with that reported between isoflavones and vitamin C on LDL in vitro (51). Interestingly, the synergy appears only in LDL collected at 60 min rather than at 40 min, the T_{max} of most of the oat phenolics. This time difference in action may be due to an equilibration period between peak plasma and LDL con-

centrations or the duration necessary for the oat phenolics to bind and remodel LDL conformation.

The total antioxidant activity of plasma, assessed with the ORAC assay, was not affected by absorbed oat phenolics in hamsters. Although high doses of some flavonoids were shown to increase ORAC values (52), this assay may not be sufficiently sensitive to detect the changes obtained in this study against the background antioxidant activity contributed by protein, urate, and other redox constituents in plasma as suggested by Ninfali and Aluigi (53).

In addition to the hamster model, we examined the interaction between the oat phenolics and vitamin C on human LDL in vitro. Oat phenolics increased the resistance of human LDL to oxidation in a dose-dependent fashion within concentrations that were achieved in hamsters. Whether these concentrations can be achieved and maintained in humans is not known. A synergy between the oat phenolics and ascorbic acid was evident at selected doses of the vitamin. These results are consistent with the observation of a synergy between isoflavones and ascorbic acid as reported by Hwang et al. (51) who found as much as a 5-fold increase in lag time over the calculated effect. We also noted a synergy between vitamin E and phenolic compounds from almond bran (54). The mechanism(s) for this interaction has not been established, although a regeneration of vitamins C and E by polyphenolics was proposed as contributing to this effect (51,55). Hwang et al. (51) also suggested that polyphenolics may stabilize the LDL particle structure via a dynamic interaction with its apoprotein-B domain. Further, as suggested by the absence of an effect with our low vitamin C concentration (2.5 $\mu\text{mol/L}$), ascorbic acid may also contribute to a synergy via its inhibition of the decomposition of lipid peroxides and/or prevention of Cu^{2+} binding to LDL.

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