Avenanthramides Are Bioavailable and Have Antioxidant Activity in Humans after Acute Consumption of an Enriched Mixture from Oats1,2

C.-Y. Oliver Chen,3* Paul E. Milbury,3 F. William Collins,4 and Jeffrey B. Blumberg3

1 Antioxidants Research Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111 and 2 Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food, Ottawa, ON K1A OC6 Canada

Abstract

The consumption of polyphenols is associated with a decreased risk of cardiovascular disease. Avenanthramides (AV), alkaloids occurring only in oats, may have anti-atherosclerotic activity, but there is no information concerning their bioavailability and bioactivity in humans. We characterized the pharmacokinetics and antioxidant action of avenanthamide A, B, and C in healthy older adults in a randomized, placebo-controlled, 3-way crossover trial with 1-wk washout periods. Six free-living subjects (3 mol/L, 3 F; 60.8 ± 3.6 y) consumed 360 mL skim milk alone (placebo) or containing 0.5 or 1 g avenanthamide-enriched mixture (AEM) extracted from oats. Plasma samples were collected over a 10-h period.

Concentrations of AV-A, AV-B, and AV-C in the AEM were 154, 109, and 111 nmol/g, respectively. Maximum plasma concentrations of AV (free + conjugated) after consumption of 0.5 and 1 g AEM were 112.9 and 374.6 nmol/L for AV-A, 13.2 and 96.0 nmol/L for AV-B, and 41.4 and 89.0 nmol/L for AV-C, respectively. Times to reach the Cmax for both doses were 2.30, 1.75, and 2.15 h for AV-A, AV-B, and AV-C and half times for elimination were 1.75, 3.75, and 3.00 h, respectively. The elimination kinetics of plasma AV appeared to follow first-order kinetics. The bioavailability of AV-A was 4-fold larger than that of AV-B at the 0.5 g AEM dose. After consumption of 1 g AEM, plasma reduced glutathione was 2.30, 1.75, and 2.15 h for AV-A, AV-B, and AV-C and half times for elimination were 1.75, 3.75, and 3.00 h, respectively. The elimination kinetics of plasma AV appeared to follow first-order kinetics. The bioavailability of AV-A was 4-fold larger than that of AV-B at the 0.5 g AEM dose. After consumption of 1 g AEM, plasma reduced glutathione was 2.30, 1.75, and 2.15 h for AV-A, AV-B, and AV-C and half times for elimination were 1.75, 3.75, and 3.00 h, respectively. The elimination kinetics of plasma AV appeared to follow first-order kinetics. The bioavailability of AV-A was 4-fold larger than that of AV-B at the 0.5 g AEM dose. After consumption of 1 g AEM, plasma reduced glutathione was 2.30, 1.75, and 2.15 h for AV-A, AV-B, and AV-C and half times for elimination were 1.75, 3.75, and 3.00 h, respectively.
AEM may be antiatherogenic due to their antioxidant, anti-proliferative, and anti-inflammatory activities (13,22,24–27). For example, AV content was associated with the in vitro antioxidant potency of oat extracts (13,24,25); AV-B had ~20% of the antioxidant activity of α-tocopherol in a linoleic acid-oxygen consumption system (28); an AV mixture diminished adhesion molecule expression and inhibited the production of proinflammatory IL-6 and IL-8 as well as monocyte chemoattractant protein in human aortic endothelial cells (29); and AV-C inhibited serum-stimulated smooth muscle cell proliferation in vitro (26). Ji et al. (27) found that, in vivo, synthetic AV-C was a potent antioxidant and decreased exercise-related free radical production in soleus muscle and malondialdehyde production in heart and also increased superoxide dismutase activity in skeletal muscle, liver, and kidneys in rats.

The in vivo efficacy of phytochemicals, including AV, is largely dependent on their bioavailability. Although the results from Ji et al. (27) implicate the bioavailability of AV-C, their presence in vivo was not determined directly. We measured plasma AV-A and AV-B in hamsters administered 0.25 g of an AV-enriched oat bran fraction containing 40 μmol total phenols (30). However, the bioavailability and antioxidant action of AV have not been assessed in humans, so we characterized the pharmacokinetics and in vivo antioxidant activity of AV and other constituents in subjects after they consumed an AV-enriched mixture (AEM) extracted from oats.

Materials and Methods

Chemicals and solvents. The following reagents were obtained from Sigma: BHT, Folin-Ciocalteu phenol reagent, copper sulfate, EDTA, β-glucuronidase type H-2 (containing sulfatase from Helix pomatia), glutathione reductase (type III), NADP, sodium 1-octanesulfonate monohydrate, reduced glutathione, sodium azide, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, 2',3',4'-trihydroxyacetophenone, Tris buffer, and vitamin E (α-tocopherol). All organic solvents, glacial acetic acid, and potassium bromide were purchased from Fisher. Pure synthetic AV-A, AV-B, and AV-C were produced according to the method of Bain and Smalley (31).

AEM preparation. The AEM was prepared from hulled oats developed to contain high concentrations of antioxidants at Agriculture and Agri-Food Canada, ECORC, and it was grown in seed plots in Plessisville, Quebec, during the 2000 crop year.6 The details of AEM preparation have been described previously (29) and the same AEM was used in our study. Briefly, dry, cleaned oats were processed in a Satake pearling mill (type RMB, 36 abrasive roller, 1 mm slit screen) to obtain a 0–21% (by weight) oat bran fraction containing ~520 mg/kg AV-A equivalents. The AEM was produced from this oat bran through procedures consisting of acidified aqueous-ethanol extraction, 2 types of preparative batch chromatography, evaporation, and freeze drying. This semipurified powdered mixture was stored at −20°C until use in the study. In all, 20.0 g of AEM was produced. One gram AEM contained 400 mg GAE. Concentrations of the 3 major components, AV-A, AV-B, and AV-C in the AEM were 154, 109, and 117 μmol/g, respectively (29). The structures of the 3 major AV and their HPLC-electrochemical detection (ECD) chromatogram in AEM are shown in Figure 1 and Figure 2A, respectively.

Subjects. Six healthy older adults (3 males and 3 females) were recruited with the following baseline characteristics: 60.8 ± 3.6 y of age; body weight of 70.9 ± 5.8 kg; systolic and diastolic blood pressure of 127.8 ± 7.7 and 73.5 ± 5.5 mm Hg, respectively, and a BMI of 25.4 ± 1.3. All participants were in good health as determined from a medical history questionnaire, a physical examination, an electrocardiogram test, and normal results of standard clinical laboratory tests. Subjects also fulfilled the following eligibility criteria: 1) no history of cardiovascular, hepatic, gastrointestinal, or renal disease; 2) no alcoholism; 3) no antibiotic or supplemental multivitamin or mineral use for ≥4 wk (and 3 mo for 60 mg vitamin C, 30 IU vitamin E, and/or 70 μg selenium) before the start of the study; and 4) no recent history of smoking. They were asked to consume a low-flavonoid diet for 1 wk before visits, according to the low-flavonoid diet guideline designed by the study diettian, in which all berries, apples, pears, citrus fruits, fruit juices, onions, chocolate, wine, coffee, any kind of tea, beans, nuts, soy products, and most spices were excluded from the daily diet. During their visits, the participants were provided with a low-flavonoid lunch and dinner prepared in the Metabolic Research Unit at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. The results of three 3-d dietary records before each visit indicated subject compliance to the low-flavonoid dietary guideline. The study protocol was approved by the Institutional Review Board of Tufts University Health Sciences/Tufts-New England Medical Center 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, the subjects were randomly assigned to consume 360 mL skim milk alone (placebo) or containing 0.5 or 1 g AV-A extracted from oats. Although the AEM was not completely soluble in skim milk, residual AEM in the glass was rinsed with water and completely consumed by each subject. Each subject was admitted to the Metabolic Research Unit in the morning after a 12-h overnight fast. Following a check of vital signs, an intravenous catheter was inserted into one forearm and a baseline blood sample was obtained. After drinking the test beverage in ~5 min, 8 blood samples were collected at 15, 30, and 45 min and at 1, 2, 3, 5, and 10 h. Lunch and dinner meals, designed to contain low flavonoids and meet the recommended dietary allowances for protein and energy (32), were prepared under the supervision of a diettian. Foods included for lunch were cream of mushroom soup, canned tuna, corn oil, fat-free mayonnaise, white bread, saltine crackers, ginger ale, and angel food cake. Foods included for dinner were turkey breast, cauliflower, corn oil, cooked white rice, baked potato, and butter. The same meals were served during each visit. These meals were provided at 4 and 9 h after consumption of the AEM. The consumption of water, salt, sugar, and ginger ale was unlimited, but food and other beverages were not allowed during the test period.

Sample collection and storage. The blood samples were collected using EDTA vacutainers via the forearm catheter and immediately processed. Whole blood samples were centrifuged at 1000 × g at 4°C for 15 min using a Sorvall RT6000 (Du Pont). Plasma aliquots of 1.5 mL were flushed with N2, stored at 4°C, and used for LDL oxidation assay within 3 d. An aliquot of plasma was treated with an equivalent volume of 10% trichloroacetic acid, and centrifuged at 14,000 × g for 10 min at

6 Identity withheld pending patent application.
4°C, and the resulting supernatant was removed and quickly frozen for GSH analysis. Aliquots of plasma samples were quickly frozen and stored at −80°C until analysis of AV, malondialdehyde (MDA), and glutathione peroxidase (GPx).

**Avenanthramide analysis in plasma.** Concentrations of free plus conjugated AV-A, AV-B, and AV-C in plasma and AEM were measured by the HPLC method of Chen et al. (33). Briefly, 20 μL vitamin C-EDTA (200 mg ascorbic acid plus 1 mg EDTA in 1.0 mL 0.4 mol/L NaH₂PO₄, pH 3.6), 20 μL of 5 mg/L internal standard (2',3',4'-trihydroxyacetophenone), and 20 μL β-glucoronidase/sulfatase (98 units/L β-glucuronidase and 2.4 units/L sulfatase) were added to 200 μL plasma. Following incubation at 37°C for 45 min. Treatment with glucuronidase/sulfatase was used to free AV from glucuronide and sulfate conjugates (34). Free AV in untreated plasma was not assessed due to limited blood volume. After enzyme digestion, AV in plasma were extracted with 500 μL acetonitrile; 500 μL supernatant was removed, dried under purified N₂, and reconstituted in 200 μL of aqueous HPLC mobile phase. Following centrifugation at 14,000 × g for 5 min, 100 μL supernatant was injected into the HPLC equipped with a Zorbax ODS C18 column (4.6 × 150 mm, 3.5 μm) and the Coularray 5600 A detector (ESA). Detection was achieved with potentials applied from 60 to 900 mV at increments of 60 mV. Concentrations of individual AV were determined in duplicate using calibration curves constructed with purified AV standards (R² > 0.999). The detection limit of the column for AV was 0.5 pmol. The limit of quantification for AV-A, AV-B, and AV-C were 74, 50, and 10 nmol/L, respectively. The intra- and interday assay CV, calculated from 6 and 5 AV-A spiked quality control plasma samples, were 3.0 and 9.0%, respectively. The recovery rate for the internal standard, calculated from 54 samples, was 97.0 ± 0.1%.

**Bioavailability of oat avenanthramides**. Plasma reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined by the HPLC-ECD method of Harvey et al. (35) with potentials applied from 60 to 960 mV at 60 mV increments. The concentrations of plasma GSH and GSSG were calculated in duplicate using calibration curves of authentic GSH and GSSG. Absolute quantities of authentic GSH and GSSG on the column ranged from 5 to 250 and 0.5 to 50 pmol, respectively, (R² > 0.995). The intra- and interday assay CV for GSH obtained from 9 quality control plasma samples were 2.72 and 2.88%, and for GSSG they were 6.67 and 8.08%, respectively.

Plasma MDA was measured by a reverse phase HPLC method according to Volpi and Tarugi (36), in which a thiobarbituric acid-MDA conjugate derivative was injected onto a C18 column and fluorometrically quantified at an excitation 515 nm and emission 553 nm. Plasma MDA concentration was calculated using calibration curves of authentic standard (R² > 0.995). The intra- and interassay CV obtained from 6 and 3 quality-control plasma samples were 3.9% and 12.3%, respectively.

Plasma GPx was measured in duplicate by the spectrophotometric method of Pleban et al. (37). The intra- and interassay CV obtained from 20 and 10 samples were 3.4 and 3.2%, respectively.

The resistance of LDL to Cu²⁺-induced oxidation was determined according to the modified method of Esterbauer et al. (38). Briefly, LDL were separated from plasma according to Chung et al. (39) using a Beckman NVT-90 rotor in a Beckman L8-M centrifuge. Following KBr and EDTA removal, using a solid phase extraction PD-10 column (Amersham Pharmacia Biotech), LDL protein was measured using a BCA protein assay kit (Pierce). LDL (182 nmol/L) was oxidized by 10 μmol/L CuSO₄ with (or without) in vitro addition of a final concentration of 6 μmol/L α-tocopherol in a total volume of 1.0 mL phosphate buffer (7.79 mmol/L Na₂HPO₄, 2.59 mmol/L NaH₂PO₄, and 150 mmol/L NaCl, pH 7.4). Concentrations of vitamin E used in these experiments were 9–18% of the plasma concentrations generally found in healthy people (40). α-Tocopherol was dissolved in methanol and subsequently diluted with phosphate buffer before administration; the final concentration of methanol was 0.5%. After the addition of α-tocopherol, LDL was incubated at 37°C for 30 min before initiation of oxidation. Formation of conjugated dienes was monitored by absorbance at 234 nm at 37°C over 6 h using a UV1601 spectrophotometer (Shimadzu) equipped with a 6-position automated sample changer. The results of the LDL oxidation are expressed as lag time (defined as the intercept at the abscissa in the diene-time plot) (41).

**Pharmacokinetics and statistics.** All results are reported as means ± SEM. The maximum plasma concentration (Cmax) and the time to reach Cmax (Tmax) were determined. The area under the curve (AUC) of plasma free plus conjugated AV concentration vs. time concentration (0–10 h) was calculated using the linear trapezoidal integration (42). The elimination half-life (T1/2) was calculated as 0.693/K, with K (terminal elimination rate constant) being the slope derived from the least square linear regression of the log plasma AV concentration vs. time curve (43). The bioavailability of AV was estimated as the ratio of the AUC of plasma AV concentration to dose. The influence of AEM consumption on measured biomarkers was expressed as the percentage of change from the respective 0-h value (baseline) at each visit. The AUC for the percentage of change for each biomarker vs. time curve (0-10 h) was estimated using the same rule for plasma AV. All percentages of change and AUC data were normalized by a log₁₀ conversion before statistical analysis. The differences in AUC, Tₘax, and bioavailability of AV between the 2 AEM doses were examined using a paired t test; the same approach was used to test differences in MDA, GSH, GSSG, and GSH/GSSG between the vehicle and 1-g AEM dose. These analytes were not measured in the 0.5-g AEM portion of the experiment because there were no effects when subjects consumed the 1.0-g AEM dose. Pharmacokinetic parameters, GPx, and LDL lag time were analyzed by 1-way ANOVA and the post hoc Tukey-Kramer honestly significant difference (HSD) test. Differences with P ≤ 0.05 were considered significant. The JMP IN 4 statistical software package (SAS Institute) was used to perform all statistical analyses.

**Results**

**Pharmacokinetics of oat AV.** After consumption of skim milk (control) or 0.5 or 1.0 g of AEM in milk, the total content of free
plus conjugated AV-A, AV-B, and AV-C after glucuronidase/sulfatase hydrolysis were determined by HPLC-ECD. Three major AV present in the AEM were detected in plasma after administration, with no AV apparent at baseline (Fig. 2B). Although earlier time points were not assessed to determine the first appearance of AV in plasma, AV-A, AV-B, and AV-C were detected 15 min after AEM consumption at both doses (Fig. 3).

Key pharmacokinetic parameters are summarized in Table 1. At 1.0 g AEM, the ranges of C\text{max} for AV-A, AV-B, and AV-C were 166.7 to 1002.2, 49.3 to 153.5, and 29.6 to 328.1 nmol/L, and at 0.5 g AEM the ranges were 50.2 to 226.3, 5.0 to 31.4, and 10.5 to 112.5 nmol/L, respectively. The C\text{max} of the AV-A, AV-B, and AV-C among the participants after consuming 1.0 g AEM were 231, 627, and 115% larger than after they consumed the respective AV. Among the participants after consuming 1.0 g AEM, AV-A, AV-B, and AV-C were detected 15 min after AEM consumption at both doses (Fig. 3). Consistent with AV-A having the highest C\text{max}, its AUC was 2-fold larger than that of AV-C following the 1.0 g dose and 7-fold larger than that of AV-B following the 0.5 g dose (P ≤ 0.05). The bioavailabilities of the AV were compared using the ratio of AUC to the amount of individual AV in each AEM dose. The AV-A bioavailability was 4-fold greater than that of AV-B following intake of 0.5 g AEM (P ≤ 0.05), but did not differ significantly after the 1.0 g dose. The bioavailabilities of AV-A and AV-B were 0.9-fold and 3.4-fold larger, respectively, after the 1.0 g than after the 0.5 g AEM dose (P ≤ 0.05). Based on a plasma volume of 40 mL/kg body weight in humans (44), plasma total AV contents at T\text{max} were 0.30 ± 0.07, 0.03 ± 0.01, 0.11 ± 0.01 μmol for AV-A, AV-B, and AV-C after the 0.5 g doses, and 1.01 ± 0.34, 0.27 ± 0.05, and 0.24 ± 0.13 μmol after the 1.0 g dose, respectively. The ratios of the total plasma content at T\text{max} to the respective oral doses were 0.39 ± 0.08 0.06 ± 0.01, and 0.20 ± 0.07% for AV-A, AV-B, and AV-C after the 0.5 g dose; and 0.65 ± 0.20, 0.24 ± 0.04, and 0.21 ± 0.11% after the 1.0 g dose, respectively.

**FIGURE 3** Plasma concentrations of AV-A (A), AV-B (B), and AV-C (C) in older adults for 10 h after acute intake of 0.5 and 1.0 g AEM. Values are mean ± SEM, n = 6.

**Biomarkers of antioxidant capacity and lipid peroxidation.** Baseline plasma GSH and GSSG concentrations and the GSH/GSSG ratio were 1.02 ± 0.10 and 0.06 ± 0.01 μmol/L and 18.1 ± 2.7, respectively. After consumption of 1.0 g AEM, plasma GSH increased 21% from baseline at 15 min (P ≤ 0.005) and then decreased toward the original concentration (Fig. 4). The AUC of the GSH time course was increased by the 1.0 g AEM dose (P ≤ 0.05) (Table 3). Plasma GSSG and GSH/GSSG were not affected 10 h after AEM intake (data not shown), consistent with the AUC results. Plasma GPx activity was 138.8 ± 8.4 U/L at 0 h and increased 30–35% after each of the 3 treatments, suggesting a postprandial response of this antioxidant enzyme. The GPx AUC after the 3 treatments did not differ. Plasma MDA was 1.50 ± 0.14 μmol/L before AEM consumption and neither its concentration nor AUC were affected by the treatments. At 0 h, the lag time of LDL oxidation was 50.1 ± 1.6 min and was not extended by AEM intake. Similarly, the AUC of LDL oxidation lag time was not affected by the treatments. After the in vitro addition of 6 μmol/L α-tocopherol prior to Cu\textsuperscript{2+} induced oxidation, the lag time of LDL oxidation at 0 h was extended from 50.1 ± 1.6 to 112.9 ± 4.4 min. Consistent with a T\text{max} at 3 h after AEM consumption, the lag times with added α-tocopherol after intake of 0.5 and 1.0 g AEM were 125.4 ± 13 and 116.8 ± 9.5% of that at 0 h, respectively, compared with 98.1 ± 3.8% after the skim milk vehicle (P ≤ 0.1); there was no difference in the LDL lag time between the 2 AEM doses (Fig. 5).

To reduce the influence of the variation in the baseline values, the percentage of change from 0 h was used to determine the change obtained with in vitro addition of α-tocopherol.

**Discussion**

Among the monocot cereal grains, avenanthramides are unique to oats. Our results demonstrate the bioavailability and antioxidant actions of these naturally occurring phytochemicals in humans. At doses of 0.5 and 1.0 g AEM containing 187.5 and 374 μmol total AV, respectively, we found that these compounds...
are bioavailable and increase some biomarkers of antioxidant capacity without apparent adverse side effects. In a previous AV pharmacokinetic study using hamsters, we observed a T_max of AV-A and AV-B at 40 min, with these compounds essentially eliminated by 120 min (30). In contrast, the T_max for AV in humans was 1.5 to 2.3 h, indicating that absorption and metabolism of AV are species dependent. The C_max in hamsters was 40 and 30 nmol/L for AV-A and AV-B, respectively, following oral doses of 0.63 and 0.49 μmol. Adjusting for differences in body weight and treatment doses by using the formula C_max × body weight/oral dose, bioavailability of AV-A and AV-B was 18- and 5-fold greater in humans than in hamsters, respectively. These findings are possibly a result of species’ innate differences in detoxification pathways (45,46).

AV have similar structures to the synthetic drug Tranilast (N-[3’,4’-dimethoxycinnamoyl]anthranilic acid) (Fig. 1), an anti-allergic medication used in Japan for over 20 y to treat allergic rhinitis, bronchial asthma, and to prevent keloid formation after skin injury (47,48). Thus, the pharmacokinetic profiles of these compounds are expected to be comparable. Indeed, the T_1/2 of AV at 1.5 to 4.3 h in this study was similar to the 4-h value reported for a 200-mg oral dose of Tranilast taken with water by 2 subjects (49). In another study that tested 80 mg of Tranilast in 22 young men, Lan et al. (50) observed a longer T_1/2 for 9.10 ± 0.98 h. However, when the bioavailability of these compounds were compared using the ratio of AUC to dose, AV were ≥10,000% less bioavailable than Tranilast (34,50). Although the mechanism for this disparity in bioavailability remains to be explored, the difference might be attributed to the difference in chemical structure, the competition for absorption from other constituents present in the AEM, the difference in clearance, and/or a binding to milk proteins in our beverage vehicle. Nonetheless, the bioavailabilities of AV are comparable to other to dietary polyphenols, e.g., flavonoids, when similar amounts are consumed (51).

Despite their structural similarity, the T_max of phenols can vary markedly. We have previously shown that p-coumaric and sinapic acids containing a hydroxycinnamoyl functional group had a different plasma concentration time profile in hamsters fed an oat extract; we also found different T_max of catechin and epicatechin in hamsters fed an almond skin extract (30,33). The AV investigated in this study have the same basic structure with differences only in the hydroxycinnamic acid moiety with R3 substitutions in AV-A, AV-B, and AV-C of H, OCH3, and OH, respectively (see Fig. 1). AV plasma C_max were simply dependent upon the administered dose. In contrast, T_max and T_1/2 were dependent on AV structure, with the OCH3 group in the hydroxycinnamoyl moiety of AV-B possessing a shorter T_max and longer T_1/2 than that of AV-A, which lacks this substitution and is thus more hydrophilic. The elimination AV from plasma followed first-order kinetics, further suggesting the dependence of T_max and T_1/2 on the specific AV structure. Although plasma AV were higher after the 1.0 g than the 0.5 g dose, adjusting for dose with the use of the AUC to oral dose ratio revealed that the larger dose had a disproportionately greater bioavailability. This might be due to an insufficient capacity of the phase II metabolism toward AV; e.g., less AV-A and AV-B may be conjugated through glucuronidation and sulfation for urinary and/or biliary excretion. However, it is not clear why AV-C did not show a similar differentiation between doses. The smaller ratio of AUC to oral dose of AV-B than AV-A at the 0.5 g dose also suggests a dependence of bioavailability on AV structure, especially when the ortho position of the hydroxycinnamoyl moiety is substituted with an OCH3 group. The mechanism by which this disparity occurs requires further research.

### TABLE 1  Pharmacokinetics of avenanthramides in older adults after acute intake of AV-enriched mixture

<table>
<thead>
<tr>
<th>AV oral dose</th>
<th>AUC</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 g</td>
<td>1.0 g</td>
<td>0.5 g</td>
</tr>
<tr>
<td>AV-A</td>
<td>71.0</td>
<td>154.0</td>
</tr>
<tr>
<td>AV-B</td>
<td>55.6</td>
<td>109.0</td>
</tr>
<tr>
<td>AV-C</td>
<td>555</td>
<td>111.0</td>
</tr>
</tbody>
</table>

1. Values are mean ± SEM, n = 6. Means in column with superscripts without a common letter differ, P ≤ 0.05 (Tukey’s HSD test; AUC data were log10 transformed). Means in the same category and row differ, ^P ≤ 0.05 and *P ≤ 0.001 (paired t test after AUC were log10 transformed).

### TABLE 2  Bioavailability of AV in older adults after acute intake of AV-enriched mixture

<table>
<thead>
<tr>
<th>AV oral dose</th>
<th>AUC</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 g</td>
<td>1.0 g</td>
<td>0.5 g</td>
</tr>
<tr>
<td>AV-A</td>
<td>71.0</td>
<td>154.0</td>
</tr>
<tr>
<td>AV-B</td>
<td>55.6</td>
<td>109.0</td>
</tr>
<tr>
<td>AV-C</td>
<td>555</td>
<td>111.0</td>
</tr>
</tbody>
</table>

1. Values are mean ± SEM, n = 6. Means in column with superscripts without a common letter differ, P ≤ 0.05 (Tukey’s HSD test; AUC data were log10 transformed).
The bioactivity of AV has been shown in vitro and in animal models. For example, AV modulates antioxidant defense enzymes in rats (27) and may contribute to the increased resistance of LDL to oxidation in hamsters (30). The antioxidant activity of AV is dependent upon their structure, with AV-C being more potent than AV-A and AV-B in AEM (25). We found AV and other constituents in AEM can affect antioxidant status in humans by increasing plasma GSH, although we did not determine whether this change resulted from an increase in hepatic release or synthesis, enhanced recycling, or some other mechanism. Interestingly, many flavonoids also increase plasma GSH concentration (52,53). However, the effect of AV and other constituents in AEM on other thiol/disulfide systems, e.g., thioredoxin and cysteine, remains to be tested. In this study, AV and other AEM constituents did not affect other measures of antioxidant capacity or lipid peroxidation, i.e., GPx, MDA, and LDL resistance to oxidation. However, providing α-tocopherol in vitro during the ex vivo assessment of LDL oxidizability, we found that vitamin E unmasked an AEM effect on LDL, particularly about the Tmax. Thus, AV and other AEM constituents in AEM on other thiol/disulfide systems, e.g., thioredoxin and cysteine, remains to be tested. In this study, AV and other AEM constituents did not affect other measures of antioxidant capacity or lipid peroxidation, i.e., GPx, MDA, and LDL resistance to oxidation. However, providing α-tocopherol in vitro during the ex vivo assessment of LDL oxidizability, we found that vitamin E unmasked an AEM effect on LDL, particularly about the Tmax. Thus, AV and other AEM constituents had an impact on the resistance of LDL to oxidation that was apparent in this situation probably only when interaction between in vitro added vitamin E and endogenous antioxidants in LDL occurred during the assay. Using the same approach in vitro, we showed a similar synergy between oat bran phenols and vitamin C and between almond skin phenols and vitamin E (30,33). The mechanism underlying this synergistic relation is still under investigation.

In considering the potential mechanisms of AV action, it is worthwhile noting that both AV and Tranilast have been reported to block the expression of vascular endothelial cell adhesion molecules and cytokines via inhibition of nuclear factor-κB (NF-κB) and its transcriptional coactivator (29,54), suggesting potential antiatherogenic activity. Interestingly, Tranilast has been tested in clinical trials for the prevention of restenosis and found to block the proliferation and deposition of vascular matrix fibroids and the migration of aortic smooth muscle cells into the vessel intima following arterial injury (48,55,56).

In summary, this randomized, crossover clinical trial showed that three AV, derived from oats, were bioavailable in humans with AV-A being the most bioavailable. The T1/2 and Tmax of AV were comparable, albeit with slight differences that depended upon dose and structure. AV and other AEM constituents after a single oral administration enhance some antioxidant defenses in vivo via increasing GSH status and acting in synergy with other antioxidants such as vitamin E. However, the antioxidant effect of chronic AV consumption in amounts relevant to current dietary intakes remains to be explored. As bioavailable and bioactive phytochemicals, AV and other AEM constituents may contribute to the health benefits associated with the consumption of oats. Further research on the metabolism of AV and on the bioavailability of related phenols from oats and other whole grains is warranted.

Acknowledgment
We thank Helen Rasmussen, RD, for her assistance in constructing the guidelines for the low flavonoid diet and counseling the subject volunteers.

Literature Cited


