Human Plasma Kinetics and Relative Bioavailability of Alkylresorcinols after Intake of Rye Bran

Rikard Landberg, Anna-Maria Linko, Afaf Kamal-Eldin, Bengt Vessby, Herman Adlercreutz, and Per Aman

Abstract

Alkylresorcinols (ARs) are phenolic lipids present in whole grain and bran of wheat and rye. Chemically, they comprise 1,3-dihydroxy-5-alkylbenzene homologs with odd-numbered, mainly saturated hydrocarbon side chains in the range of 17–25 carbon atoms. ARs are evaluated both for physiological effects and for their potential use as biomarkers of whole-grain wheat and rye intake. In this study, plasma kinetics and relative bioavailability of ARs in humans were investigated after a single intake of rye bran 120 g (190 mg ARs). The shapes of plasma concentration time curves were similar in the subjects (n = 6) with 2 peaks at −2.8 and 6.5 h and maximum concentrations (mean ± SEM) of 1253 ± 125 and 3365 ± 309 nmoL/L, respectively. The relative bioavailability of different homologs increased with increasing length of the AR side chain (r = 0.97, P < 0.001), indicating differences in metabolism. The apparent half-lives were rather short, ~5 h for all homologs, which suggests that the AR concentration in plasma could be used as a short- to medium-term biomarker of regular intake of whole-grain wheat and rye.

Introduction

The regular consumption of whole-grain foods has been associated with positive health effects compared with the intake of foods based on refined cereals. Epidemiological evidence suggests a decreased risk of cardiovascular disease, diabetes, obesity, and some types of cancer (1–4). The mechanisms have not been clarified, but different compounds within the dietary fiber complex are believed to play important roles (5).

A common problem in epidemiological studies investigating the effect of whole grain on disease is the difficulty of making accurate estimates of food intake. For example, it is hard for the consumers to recognize whole-grain products among other cereal products. Moreover, there is some inherent weaknesses of food frequency questionnaires, insofar as they depend on memory and accurate reporting (5,6). The use of a biomarker has the potential to objectively measure the intake and may provide improved correlations between intake of whole grain and the reduced risk of certain diseases (6). A biomarker for whole-grain intake might also be useful when samples from biobanks are used, because information on whole-grain intake is generally missing.

Alkylresorcinols (ARs) is a group of phenolic lipids found in substantial amounts in wheat and rye kernels (Fig. 1). They have been shown to be specific markers for whole-grain and bran fractions of wheat and rye because they are only found in the outer parts of the kernel and not in the white flour or in any other commonly consumed foods. They are also present in barley, but at very low concentrations (7). Alkylresorcinols in wheat and rye comprise 1,3-dihydroxy-5-alkylbenzene homologs with odd-numbered alkyl side chains from C17:0 to C25:0 carbon atoms (C15:0 is only present at low levels in rye). The homolog profile is rather specific for different cereals and the C17:0 to C21:0 ratio (1.0 for rye, 0.1 in common wheat, and ~0.01 for durum wheat) may therefore be used to determine the source of whole grain in food products (8–10). Methods for the analysis of alkylresorcinols in cereal products, plasma, and erythrocytes have been developed in recent years (7,11–13). Because ARs are absorbed by humans and could be quantitatively measured in human samples, they may have the potential to serve as biomarkers for whole-grain intake of wheat and rye.

To evaluate the potential use of ARs as long- or short-term biomarkers for whole-grain intake, knowledge about their absorption, distribution, and elimination kinetics in humans is needed. The mean AR extent of absorption was estimated to be ~60% in pig and human ileostomy models (14,15). Ross et al. (14) fed rats with labeled ARs to study the recovery in tissues, feces, urine, and blood and found 31% of the radioactivity in urine, 61% in feces, and very small amounts in tissues. Recently,

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ARs were shown to be incorporated into human erythrocyte membranes (11) and in adipose tissue of rats (16). Two potential urinary metabolites of ARs were isolated in humans by Ross et al. (17) after consuming a rye diet. The kinetics of ARs after a habitual rye and wheat diet, and after a single intake of rye bread, was assessed using pigs as a model (18). There was a rapid increase in total plasma AR concentration after a single intake with a peak concentration of 650 nmol/L after 3–4 h. The apparent half-life was rather short, ~4 h.

Information on kinetic parameters, such as peak plasma concentration, elimination half-life, and bioavailability, are important both for the evaluation of bioactivity of ARs in vivo and for the establishment of ARs as biomarkers for whole-grain intake of wheat and rye. In this study, we assessed the plasma kinetics and relative bioavailability (defined as AUC0–100 h/Intake) of homologs normalized to C17:0 of individual AR homologs in the plasma of healthy subjects after a single intake of rye bran.

Materials and Methods

Subjects and experimental design. The protocol for the study was approved by the local ethical committee in the Uppsala region, Sweden. Six healthy volunteers (3 men and 3 women, body weight 74 ± 3.9 kg, age 26 ± 1.1 y) were assigned after being provided with oral and written information about the study. One week before the study, all participants were asked to avoid any food containing whole grain or bran of wheat and rye. After fasting overnight, subjects were given a single portion of rye. After fasting overnight, subjects were given a single portion of rye bran meal intake. Subjects were asked to avoid food and water between consuming the rye bran meal and the lunch. After the lunch, all subjects consumed their meal within 25 min and were monitored until everyone had completed their intake. A standardized lunch (sausage and mashed potatoes) was served 4 h after the rye bran meal intake. Subjects were asked to avoid food and water between consuming the rye bran meal and the lunch. After the lunch, all dietary restrictions were relieved.

Sample treatment. Immediately after collection, blood samples were centrifuged in a Hettich Rotina 48R centrifuge (Germany) (2000 × g for 10 min at 4°C) to separate plasma and erythrocytes. Plasma samples were then portioned into 2-mL cryo tubes and stored at −80°C until analysis.

Analytical methods. Total AR content and relative homolog composition of rye bran flakes was determined with a gas chromatographic method previously described (7). Briefly, samples were milled and extracted with a hot 1-propanol:water mixture (3:1, v/v) and analyzed by GC without purification or silylation. Samples were analyzed in triplicate and quantified using methyl behenate (C22:0, fatty acid methyl ester, Larodan Fine Chemicals AB) as an internal standard. Results are expressed as the mean of the triplicates and reported on a fresh weight basis (Table 1).

Plasma samples were analyzed according to the method by Linko et al. (12). Briefly, plasma (0.5 mL) and internal standard (45 μg) were incubated with water (0.5 mL) at 37°C overnight. As an internal standard, an AR that does not naturally exist was used (C20:0). Samples were then extracted with diethyl ether, and extracts were evaporated completely and redissolved in methanol (0.5 mL). ARs were separated from nonpolar lipids on a diethyl-amino-ethyl (DEAE)-Sephadex A-25 ion exchange gel (Amersham Biosciences) in a free base form, packed in Pasteur pipettes to a final height of 1.5 cm. Eluted ARs were silylated and analyzed by GC-MS. The molecular ions for ARs [m/z 492 (C17:0), 520 (C19:0), 534 (C20:0), 548 (C21:0), 576 (C23:0), and 604 (C25:0)] were used as a conformation ion. Standard curves for quantification were prepared by diluting a known amount of a syntetic mixture of ARs C17:0-C23:0 and relating the area ratio of homolog and internal standard to known concentrations. Due to the lack of a reference compound, homolog C25:0 was quantified using the curve of C23:0. All plasma samples were analyzed in duplicates, and samples outside the range of the calibration curves were diluted and reanalyzed.

Pharmacokinetics. The absorption phase was evaluated visually and by the method of residuals to determine if absorption followed first-order kinetics (20). A one-compartment model was used, since visual inspection of log-transformed plasma concentration curves showed no clear distribution phase (Fig. 2). All pharmacokinetic parameters were calculated manually using Microsoft Excel, version 2003. The maximal concentration (Cmax), time to reach maximal concentration (tmax), total area under the plasma concentration time curve (AUC0–t), and (apparent) terminal elimination half-life (t1/2) were determined from plasma concentration time courses.

The area under the plasma concentration time curve from time zero to the last-measured concentration at 24 h (AUC0–24) was calculated using the linear trapezoidal formula (20). To determine the area total under the plasma concentration time curve (AUC0–∞), the last measured plasma concentration was divided by the elimination rate constant (kel). The elimination rate constant (kel) was estimated from the 3–5 last points on the terminal slope of the plasma concentration time curves after logarithmic transformation and application of linear regression (20). The number of data points included was chosen so that the R2-value was as good as possible. Terminal elimination half-life (t1/2) was calculated as

### Table 1

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>g/100 g Fresh weight</th>
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<tbody>
<tr>
<td>Water</td>
<td>5.4 ± 0.1</td>
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<tr>
<td>Carbohydrates2</td>
<td>53.4</td>
</tr>
<tr>
<td>Ash</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Fat</td>
<td>5.9 ± 0.5</td>
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<tr>
<td>Protein, N × 6.25</td>
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<tr>
<td>Dietary fiber</td>
<td>20 ± 2.0</td>
</tr>
<tr>
<td>Energy, kJ</td>
<td>1298</td>
</tr>
<tr>
<td>Total alkylresorcinol ingested, μmol</td>
<td>495 (100)</td>
</tr>
<tr>
<td>C17:0</td>
<td>138 (29)</td>
</tr>
<tr>
<td>C19:0</td>
<td>175 (35)</td>
</tr>
<tr>
<td>C21:0</td>
<td>109 (22)</td>
</tr>
<tr>
<td>C23:0</td>
<td>44 (8)</td>
</tr>
<tr>
<td>C25:0</td>
<td>30 (6)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 3. All analysis except alkylresorcinols was performed by AnalyCen AB.
2 Calculated.

3 Relative proportions are within parentheses.

### Figure 1

Structures of alkylresorcinols (ARs) commonly found in cereals.
The relative bioavailability of different AR homologs was determined by normalizing the AUC$_0$-$t$ corrected for intake (AUC$_0$-$t$/intake) to that of C17:0.

Statistics. To determine if there was a relative increase or decrease of individual homologs over time, statistical significance of positive or negative slopes were evaluated by applying linear regression with the relative homolog composition as the dependent variable over the time interval 1–8 h after intake. Differences in t$_{1/2}$ and t$_{\text{max}}$ values for different AR homologs were evaluated by 1-way ANOVA and Tukey’s pairwise comparison. To evaluate the effect of different AR homologs on the relative bioavailability, linear regression was applied on intake adjusted AUC$_0$-$t$ for the different homologs after log transformation. Differences were considered significant at $P$, 0.05. All statistical calculations were performed in Minitab, version 14. Results are reported as means ± SEM unless otherwise stated.

Results

Small peaks eluting before AR homologs were tentatively identified as monounsaturated AR homologs (alkenylresorcinols), because those showed both the base ion (m/z 268) and the masses of the corresponding AR molecular ions minus 2 (Fig. 3). These have been reported previously in rye bran (21), but not in plasma samples.

Subjects consumed the entire meal within 25 min, except subject 4, who left 15 g. The meal contained 190 mg ARs (495 µmol), and the relative homolog composition was similar to what normally is present in rye foods (Table 1) (7). Very small amounts of ARs were found in plasma samples (total AR <25 nmol/L), after the first week run-in period, when no whole-grain or bran intake of wheat and rye were allowed. The baseline sample for subject 6 was ~40 nmol/L and that person reported an unintentional intake of some bread containing sifted rye flour containing small amount of ARs, during the run-in period.

Absorption. After the intake, absorption of ARs started almost immediately and 2 peak concentrations were found for all homologs in all subjects (Fig. 2, Fig. 4). The first peak occurred after 2.4–3.4 h (t$_{\text{max},1}$), and was 1253 ± 123 nmol/L (C$_{\text{max},1}$) (Table 2). The second peak concentration (C$_{\text{max},2}$) was reached after ~6.5 h (t$_{\text{max},2}$), and was 2.6-fold the first peak (Fig. 2, Table 2). Evaluation of the absorption phase visually and using the method of residuals (data not shown) (21) suggested that absorption was not a simple first-order process. Therefore, no absorption half-life was calculated. The estimated AUC$_{24}$ did not exceed 10% of AUC$_{t}$ for any of the homologs, except for C25:0 in subject 6, showing that the time points chosen covered >90% of the exposure (Table 2).

Relative homolog composition. The relative homolog composition in plasma was different from that of the rye bran flakes and varied over time (Table 1 and Fig. 5). There was a significant increase in the relative proportions of homologs C23:0 and

Figure 2 Total AR concentrations (A) and log-transformed concentrations (B) in human plasma (n = 6) after a single intake of rye bran flakes containing 190 mg ARs.

Figure 3 Typical chromatogram of a human plasma sample (m/z = 268) after a single intake of rye bran flakes containing 190 mg ARs. Small peaks eluting right before C19:0-C25:0 were tentatively identified as alkenylresorcinols.

Figure 4 Individual AR homolog composition in human plasma after a single intake of rye bran containing 190 mg ARs. Values are means ± SEM, n = 6.
Discussion

To our knowledge, this is the first study to assess the plasma kinetics and relative bioavailability of dietary ARs in healthy human volunteers (n = 6) after a single intake of rye bran. Such information is needed for the evaluation of plasma ARs as biomarkers for the intake of whole grain in wheat and rye.

The variation in t_{max} and t_{1/2} among subjects was rather small and the shape of the plasma concentration time curves was similar among subjects and among homologs (Table 2, Fig. 2, Fig. 4). Differences among subjects likely were minimal due to unity in body weight and age. The variations in C_{max} and AUC_{0-\infty} were much larger, as expected, because those parameters are highly affected by interindividual differences in absorption (22). The amount of ARs given in this study [190 mg (495 μmol)] was much higher than the typical daily intakes by Swedes (~20 mg/d) (24). However, this intake is possible to achieve by consuming bran products and rye bread several times a day. Compared with pigs, the subjects in this study had much higher C_{max} and AUC_{0-\infty}, despite lower intakes (18). Physiological differences in body composition, hepatic blood flow, enzymes, etc. between humans and pigs makes it difficult to perform direct quantitative comparisons in pharmacokinetic parameters (24).

Despite a 1-wk run-in period, all subjects had low concentrations of ARs in their plasma. Contamination of the refined cereal products consumed during the run-in period with ARs or intake by mistake are possible explanations. It might also be due to liberation of ARs from compartments with a longer turnover rate, like erythrocyte membranes or adipose tissue, as previously suggested (11).

Absorption. Contrary to what was found in pigs (18), ARs showed 2 maximum plasma concentrations in this study. Double peaks were found for all individual homologs (Fig. 4). There are several explanations for 2-peak absorption profiles in the literature. A common suggestion is enterohapatic circulation,
which often is manifested by 2 peaks or by 1 peak with a “shoulder” and a prolonged apparent elimination half-life (25). However, in this study, the first peak was much smaller than the second and the apparent half-life was rather short (~5 h). Gastric emptying, which sometimes is the rate-limiting step in the transfer of drugs from stomach to the duodenum, can, under some conditions, result in 2 absorption peaks (26). The second peak could also be due to increased digestibility of ARs induced by the food served at lunch (4 h after the intake). Absorption of carotenoids, for example, is increased when they are administered with food (27). Absorption windows, separated by a region of relatively low absorption, have also been suggested as an explanation for 2 and multiple peaks (22). However, alterations in absorption kinetics by different routes of administration or types of food formulations may provide an explanation for the double-peak phenomenon observed in this study.

Relative homolog composition. The C17:0 to C21:0 ratio is useful to determine the source of whole-grain and bran cereal products; a value of ~1.0 indicates rye; 0.1 common wheat, and 0.01 durum wheat (8,10). The C17:0 to C21:0 ratio in blood is proposed to indicate the source of ingested whole-grain foods (9,11). Linko et al. (18) showed that AR homolog composition in plasma among pigs was relatively consistent over time after a single intake and that it reflected the composition in the diet fairly well (18). In the present study, the relative homolog composition changed over time and was different from that of rye bran (Fig. 5, Table 1). Before deciding how to interpret any AR homolog ratios (e.g., C17:0 to C21:0), studies are warranted that compare AR homolog profiles after intake of whole-grain wheat and rye.

Relative bioavailability. The difference in relative bioavailability of the AR homologs may be due to differences in absorption kinetics, or disposition kinetics, or both. Ross et al. (15) reported the fraction absorbed to be 45–71% as determined by disappearance from the ileostomy effluent in humans. Despite the large difference in the total AR fraction absorbed among individuals, there were no major differences in the absorption of different AR homologs. This suggests that the difference in bioavailability and the change in relative homolog composition found in the present study are due to differences in elimination between homologs or to a combination of differences in elimination and absorption rates rather than to differences in the extent of absorption. Hepatic metabolism, followed by urinary excretion, is probably the predominant route of AR elimination (14) and 2 metabolites have been identified in urine (17). ARs could also be excreted into bile, either intact or in a metabolized form. Differences in the metabolism of AR homologs are, however, a more likely explanation for the difference in relative bioavailability observed, than difference in eventual bile clearance. The possibility of AR clearance to bile has to be further investigated when methods for analysis of that matrix become available.

Apparent elimination half-life. The terminal elimination half-life was rather short (~5 h for all AR homologs), which suggests that ARs are eliminated rapidly from the central plasma compartment. The time points used for calculation of the terminal half-life were few (3–5), which might weaken the deductions about the absolute terminal half-life. However, the decay curves of all subjects fit well to a linear regression, after log transformation ($R^2 > 0.990, P < 0.001$). A short half-life suggests that the time of consumption will greatly affect the nonsteady-state AR concentration in plasma. At an individual level, it might be difficult to determine whether a difference in plasma concentration is due to time of intake or dietary differences, unless the individual consumes whole grain regularly. In pigs fed an AR-rich diet 3 times/d for 1 wk, there was an increase in baseline AR concentration indicating accumulation (18). However, compartments other than plasma, with slower turnover rates, might give a more stable, long-term reflection of intake and provide a more suitable site of measurement (28), especially when intake is more irregular. Linko et al. (11) showed that the AR concentration in erythrocyte membranes was more stable than in corresponding plasma samples. Others have suggested adipose tissue as a suitable compartment for long-term reflection of the intake of vitamin E, carotenoids, and fatty acids (29,30). Intake-level-dependent studies, where both plasma and erythrocyte concentrations are correlated with intake, are needed before deciding which matrix is most suitable to reflect AR intake.

To our knowledge, this was the first human study in which plasma kinetics and relative bioavailability of alkylresorcinols were assessed after a single intake of rye bran. The relative AR homolog composition changed over time and needs further investigation before arriving at a final conclusion as to how homolog ratios should be interpreted. Meanwhile, we recommend that all AR homologs are measured. The relative bioavailability was highly dependent on the length of the AR-side chain. A rather short terminal half-life was found for all homologs, suggesting that plasma ARs give a short- to medium-term reflection of regular whole-grain intake.

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Literature Cited


