Alkylresorcinols from Whole-Grain Wheat and Rye Are Transported in Human Plasma Lipoproteins1,2

Anna-Maria Linko-Parvinen,3* Rikard Landberg,4 Matti J. Tikkanen,3,5 Herman Adlercreutz,3 and José L. Peñalvo3

Abstract

Alkylresorcinols with alkyl chains C17:0-C25:0 are abundant in whole-grain wheat and rye. Concentrations in human plasma have been suggested to be biomarkers of dietary whole-grain intake. We measured human plasma, erythrocyte, and lipoprotein alkylresorcinol concentrations and alkylresorcinol homolog distribution, and evaluated the use of plasma alkylresorcinol concentration as a dietary biomarker of whole-grain intake compared with serum enterolactone. A total of 15 subjects (8 women) consumed whole-grain wheat or whole-grain rye crisp bread (~100 g/d) in a crossover design for 1 wk. The test bread periods were preceded by 1-wk periods of consuming refined wheat bread. Plasma, erythrocyte, lipoprotein alkylresorcinol, and serum enterolactone concentrations were measured before and after each period, and plasma alkylresorcinols and serum enterolactone were measured after habitual diet intake before and 1 wk after the trial. Plasma alkylresorcinols are transported in lipoproteins with VLDL and HDL being the main carriers. AR concentrations in plasma, erythrocytes, and lipoproteins were increased (P < 0.05) by whole-grain wheat bread and even more so with rye crisp bread, although interindividual variation was high. The alkylresorcinol homolog C17:0 to C21:0 ratio in plasma was higher after the whole-grain rye diet period compared with the whole-grain wheat diet period (P < 0.05). Serum enterolactone concentrations were increased significantly by whole-grain rye intake only in men. This is the first report to show that alkylresorcinols in human plasma are mainly transported in lipoproteins. The plasma alkylresorcinol C17:0 to C21:0 ratio reflects intake of whole-grain wheat and rye, and the plasma total alkylresorcinol concentration appears to be a useful biomarker of whole-grain cereal intake. J. Nutr. 137: 1137–1142, 2007.

Introduction

Increasing attention has been paid to the role of human nutrition in health promotion and the prevention of chronic diseases, such as certain cancers, type II diabetes, and cardiovascular diseases (1,2). Partly due to lack of a reliable biomarker for whole-grain intake, with consequent difficulties in defining dietary intake, the disease preventing mechanism of whole-grains in humans remains to be clarified (3,4).

Alkylresorcinols (AR)6 are phenolic lipids with an odd-numbered alkyl chain attached to position 5 of the 1,3-dihydroxybenzene ring. They are abundant in the outer layers of rye and wheat grains and are absent in highly refined white flour and in other foods used for human nutrition (5,6). In grains they exist mainly with alkyl chain lengths C15:0-C25:0 (7). The AR homolog composition is different in rye and wheat grains, and the AR C17:0 to C21:0 ratio has been proposed to indicate the source of grains in foods (8) and, hypothetically, in human blood samples (6). Preliminary data suggests that AR could function as a biomarker for human whole-grain intake (6). AR are absorbed from the small intestine via the lymphatic system (9,10) and are incorporated into human erythrocyte membranes (11). They have also been detected in the adipose tissue of rats (12). In vitro studies suggest that AR affect the properties and functions of biological membranes (13–16). AR seem to be rather weak antioxidants per se (17), but recent studies have shown that AR can inhibit LDL oxidation in vitro (18) and might provide antioxidant protection when present in biological membranes (19).

Whole grains, in particular rye, and other foods, such as berries, certain fruits and vegetables, and seeds, constitute the main sources of plant lignans in the human diet (20–23). The mammalian lignan enterolactone (ENL) is formed as the main end-product from plant lignans by intestinal microflora (24–26). The consumption of rye

1 Supported by Ida Montin Foundation, Maud Kuistila Memorial Foundation, and Finska Läkarealliansen and Sigrid Juselius Foundation. Vaasan and Vaasan Oy of Finland provided the test breads used in the study.
2 Author disclosures: A.-M. Linko-Parvinen, no conflicts of interest; R. Landberg, no conflicts of interest; M. J. Tikkanen, no conflicts of interest; H. Adlercreutz, no conflicts of interest; and José L. Peñalvo, no conflicts of interest.
3 Abbreviations used: AR, alkylresorcinols; ENL, enterolactone; RD, whole-grain rye bread diet; RF, refined bread diet; WD, whole-grain wheat bread diet.
4 To whom correspondence should be addressed. E-mail: anna.linko@helsinki.fi.
products has been shown to increase serum ENL concentrations and to correlate positively with these concentrations in men and in some studies in women (27–30), but because ERL is derived from several dietary sources, it does not seem to function as a specific marker of whole-grain intake (31–33).

To our knowledge, this is the first study to determine AR concentrations and their homolog distribution in human plasma, erythrocytes, and lipoproteins in a whole-grain feeding trial. The plasma AR and serum ENL concentrations were compared after the consumption of whole-grain rye and wheat breads and after the consumption of the diet based on refined grains.

Subjects and Methods

Subjects and study design. Eight women and 7 men, aged 21–36 years with a BMI of 24.4 ± 0.99 kg/m² (mean ± SEM), participated in the study. The exclusion criteria were diseases or medications affecting bowel function or plasma lipoproteins, antibiotics taken within 3 months before the study, serious chronic illnesses, and alcohol abuse. Hemoglobin had to be >125 g/L for women and 135 g/L for men to avoid anemia due to sample taking. The trial was a 2 x 1 wk randomized crossover study with whole-grain wheat and rye crisp breads as the test breads and with 1-wk refined bread periods, baseline (RF I) and washout (RF II), before the test bread weeks. Subjects were instructed to eat 8 pieces (12.5 g per piece of bread, a total of 100 g/d) of whole-grain wheat (wheat diet, WD) or whole-grain rye crisp bread (rye diet, RD) per day. During RF I and RF II subjects consumed soft refined wheat bread. All breads were provided by Vaasan and Vaasan Oy (Table 1). Subjects were allowed to eat 1 additional portion of white refined cereal product daily, such as pasta, but no bread or any other whole-grain products other than the study breads during the follow-up. Otherwise their dietary habits were not changed. Study participants used food diaries to record their daily intake of breads (as pieces per day) and their consumption of cereal products 1 wk before the study and during the whole trial. They were also asked to record their entire habitual diet for 1 d prior to the study. Blood samples were taken after the habitual diet and before the first refined bread week as well as after the refined-bread and whole-grain test periods and 1 wk after the trial. Plasma, erythrocyte, and lipoprotein AR concentrations, and serum ENL concentrations were analyzed in the samples taken after RF I, RF II, wheat diet (WD), and rye diet (RD). In addition, plasma AR and serum ENL concentrations after habitual diets were analyzed before the study and 1 wk after the study. Hemoglobin, blood cells, and lipoprotein fractions were measured in all samples. The Ethics Committee for the Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland, approved the study. All study participants gave permission to publish the results.

Blood samples. Spot blood samples after overnight fasting were collected into 10 mL EDTA-containing tubes and serum tubes. Plasma and lipoprotein cholesterol and plasma triacylglycerols were measured by Konelab 60i (Thermo Electron). Hemoglobin, erythrocyte mean corpuscular volume, and blood cells were measured from whole blood in EDTA-tubes by Sysmex KX-21 (TOA Medical Electronics). Blood samples were centrifuged at 3500 × g for 10 min at 10°C and plasma and serum were separated and stored at −20°C until analysis.

Isolation of lipoproteins. Lipoproteins [VLDL (density 1.006 kg/L), LDL (1.019–1.063 kg/L), and total HDL (1.063–1.21 kg/L)] were isolated from 3 mL of plasma by sequential ultracentrifugation as previously described (34,35). Lipoprotein fractions (sample volume 2 mL) were purified, and unbound AR and other small-molecular weight compounds, including ERL, that protected the samples from oxidation prior to analysis, were removed by size exclusion gel filtration on Sephadex G-25 (Pharmacia Biotech; column dimensions 1 x 20 cm). PBS (pH 7.4) was used as eluting solvent (36). Samples were stored at −80°C until further analysis.

Analysis of alkylresorcinols and enterolactone. Bread AR were analyzed in triplicate using a gas chromatographic method for cereal products (5). Plasma AR were analyzed by GC-MS as previously reported (37). AR in VLDL, LDL, and HDL fractions were analyzed using the plasma method and modified by extracting 4 times with 4 mL diethyl ether for better analyte recovery. After the 4th extraction, only traces of AR were detectable in the remaining solution, which suggests that the extraction was adequate. Erythrocyte alkylresorcinols were analyzed with a modified method for the analysis of vitamin E (11,38,39). In short, erythrocytes were separated from plasma by centrifugation (3500 × g for 10 min) and washed 3 times with 0.9% NaCl solution. Following the removal of the washing solution, 1 mL of cells was pipetted and combined with 1 mL of 2 mmol/L EDTA. The cells were stored at −80°C until analysis. Prior to analysis, an internal standard AR C20:0 that does not exist naturally in grains (kindly provided by Prof. K. Wahala, Department of Chemistry, University of Helsinki, Finland) was added and proteins were denaturated by adding 5 mL of methanol and shaking for 2 min. AR were extracted 3 times with 7 mL of n-heptane. The combined organic phase was completely evaporated, and the samples were purified with anion exchange chromatography DEAE-Sephadex A-25 (Pharmacia Biotech AB; column dimensions 0.5 x 15 cm) in a free base form and analyzed by the GC-MS (Fisons Instruments) as described earlier (37). All plasma and erythrocyte samples were analyzed in duplicate, but lipoprotein samples were analyzed once due to scarcity of the sample material. A control sample and a blank were included in all assays. Interseries CV was 12% for the plasma method, 11% for erythrocytes, and 20% for lipoproteins. The results are expressed as the total amount of AR homologs (AR C17:0–C25:0), unless otherwise stated.

 Serum ERL concentrations were measured by a time-resolved fluorimunoassay with AutoDELFIA 1235 Automatic Immunoassay System (Wallac Oy) (40,41). Control samples with 3 different concentrations and a blank were included in every batch. Interseries CV was <10%.

Statistical analysis. Normality of the data were tested with Shapiro-Wilk’s test. Spearman’s rho was used to test associations between AR and bread intake and plasma, erythrocyte, and lipoprotein AR concentrations, and to test associations between plasma, erythrocyte, and lipoprotein AR concentrations after different diets. Differences in AR and ENL concentrations after the test periods and between women and men, and AR homolog distribution as well as AR C17:0 to C21:0 ratio between the diets were tested with Friedman’s test followed by Wilcoxon’s signed rank test. We performed all statistical analyses with SSPS 11.0.1. Differences were considered significant at the 95% CI (P < 0.05). The results are expressed as means ± SEM.

Results

Plasma, erythrocyte, and lipoprotein AR concentrations did not differ between women (n = 8) and men (n = 7) so their data were combined (n = 15). Serum ENL concentrations differed between women and men and were analyzed separately. The sequence of the
feeding periods did not affect either variable so the and the data from the 2 RD periods and those from the WD periods were combined.

Dietary compliance throughout the study was good. Bread consumption was 95.5 ± 2.1 g/d (7.6 ± 0.17 pieces/d) during WD and 97.5 ± 0.97 g/d (7.8 ± 0.08 pieces/d) during RD of the recommended 100 g/d (8 pieces/d), and no intake of additional breads was recorded. Calculated intake of AR was 29.4 ± 0.65 mg/d (75.0 ± 1.6 μmol/d) during WD and 67.3 ± 0.67 mg/d (174.6 ± 1.7 μmol/d) during RD. RF diets did not contain AR.

When subjects consumed their habitual diet before the study, plasma AR concentration was 127 ± 33.9 nmol/L (range 7.5–434 nmol/L). Refined bread periods effectively decreased AR concentrations significantly (Fig. 1). The AR homolog pattern in plasma, erythrocytes, and lipoproteins differed significantly between the WD and RD periods (Table 2). However, AR concentrations did not correlate with the AR concentrations in plasma, erythrocytes, or lipoproteins. The plasma AR concentration was greater when the subjects consumed more AR; concentrations were higher after the RD period than after the WD period and both were greater than after the RF periods. To eliminate variations in AR carriers, plasma AR also are expressed relative to plasma cholesterol and triacylglycerols (Table 2).

The AR C17:0 to C21:0 ratio was 0.12 ± 0.002 in whole-grain wheat crisp bread and 1.02 ± 0.01 in whole-grain rye crisp bread. The AR homolog pattern in plasma, erythrocytes, and lipoproteins differed significantly between the WD and RD periods (Fig. 1). After WD and RD, the AR C17:0 to C21:0 ratios in plasma were 0.10 ± 0.01 and 0.60 ± 0.06, respectively, and in erythrocytes 0.06 ± 0.01 and 0.33 ± 0.03, respectively (Fig. 1). These ratios differed after the subjects consumed WD and RD compared with RF I (0.05 ± 0.03) and RF II (0.01 ± 0.01) (P < 0.05). The corresponding ratios in plasma were 0.28 ± 0.05 and 0.52 ± 0.09 after subjects consumed their habitual diets before and 1 wk after the trial, respectively. The ratio of total AR in plasma (nmol/L) to that in erythrocytes (nmol/L packed cells) was 0.53 ± 0.1 after WD and 0.71 ± 0.1 after RD. Erythrocyte mean corpuscular volume and hemoglobin did not change during the study.

AR concentrations measured in the combined lipoprotein fractions were 69 ± 2.5% after WD and 72 ± 3.9% after RD of the corresponding plasma total AR concentrations (Table 2). Plasma AR (nmol/L) and total lipoprotein AR (nmol/L of plasma) were correlated after RD (r² = 0.839, P < 0.05) and after WD (r² = 0.939, P < 0.05). The AR contents of lipoprotein classes did not differ between women and men. VLDL contained 46 ± 2.1%; LDL, 20 ± 1.3%; and HDL, 33 ± 1.5% of the lipoprotein AR (Table 2). Lipoprotein-deficient plasma after ultracentrifugation did not contain detectable amounts of AR. Lipoprotein concentrations did not change during the study (Table 2).

Plasma (nmol/L) and erythrocyte (nmol/L packed cells) AR concentrations were correlated after the RD period (r² = 0.854, P < 0.05) and tended to be correlated after the WD period (r² = 0.421 P = 0.12), but the correlations were stronger when plasma AR were expressed relative to plasma cholesterol and triacylglycerols (r² = 0.925, P < 0.05 after RD and r² = 0.668, P < 0.05 after WD).

Serum ENL concentrations were affected by the intake of RD only in men (Table 3). Serum ENL concentrations after the habitual diet periods were 21.7 ± 4.4 and 19.5 ± 2.7 nmol/L in women and 10.5 ± 2.2 and 11.9 ± 2.3 nmol/L in men. Serum ENL concentrations in men were lower than in women throughout the study, but the differences were significant only during the RF periods. Bread intake did not correlate with serum ENL concentrations. There were 2 men whose ENL concentrations did not increase and 1 female whose ENL concentration decreased after intake of RD, whereas no intake of antibiotics was recorded. Serum ENL and plasma AR concentrations were not correlated.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Plasma cholesterol, mmol/L</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>Plasma TG, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma lipoproteins</td>
<td>4.57 ± 0.23 (3.12–6.22)</td>
<td>0.22 ± 0.05 (0.04–0.63)</td>
<td>2.75 ± 0.19 (1.43–4.07)</td>
<td>1.61 ± 0.12 (0.88–2.87)</td>
<td>1.00 ± 0.12 (0.54–2.15)</td>
</tr>
<tr>
<td>VLDL</td>
<td>4.61 ± 0.25 (3.02–6.61)</td>
<td>0.26 ± 0.04 (0.07–0.58)</td>
<td>2.70 ± 0.18 (1.99–3.85)</td>
<td>1.53 ± 0.14 (1.00–3.01)</td>
<td>1.26 ± 0.19 (0.49–3.40)</td>
</tr>
<tr>
<td>LDL</td>
<td>4.39 ± 0.18 (3.09–5.95)</td>
<td>0.33 ± 0.05 (0.08–0.71)</td>
<td>2.40 ± 0.14 (1.17–3.29)</td>
<td>1.66 ± 0.14 (0.96–3.11)</td>
<td>1.25 ± 0.19 (0.47–3.54)</td>
</tr>
<tr>
<td>HDL</td>
<td>4.53 ± 0.26 (2.57–6.34)</td>
<td>0.34 ± 0.07 (0.08–1.15)</td>
<td>2.65 ± 0.20 (1.09–4.52)</td>
<td>1.87 ± 0.15 (0.94–3.20)</td>
<td>1.04 ± 0.11 (0.59–1.74)</td>
</tr>
<tr>
<td>Plasma TG</td>
<td>2.2 and 11.9</td>
<td>6.0c (11.5–88.3)</td>
<td>24.5c (55.1–389)</td>
<td>14.9c (4.8–232)</td>
<td></td>
</tr>
</tbody>
</table>

1. Values are means ± SEM (range), n = 15. Means in a row with superscripts without a common letter differ, P < 0.05.
2. Triacylglycerols.

**Discussion**

To our knowledge, this is the first study to show that AR are carried by human lipoprotein particles in the circulation. Also, the increased concentrations were increased by the intake of whole-grain products, and more so with whole-grain rye than with whole-grain wheat.

In a previous study we found a significant correlation between an 8-wk intake of whole-grain rye bread and plasma AR concentrations (42). In the present study, after 1 wk of whole-grain consumption, plasma AR concentrations (n = 15) increased significantly with intake of whole-grain wheat crisp and whole-grain rye crisp bread (Table 2). However, AR concentrations did not correlate with individual bread intake, which can be explained by the study’s shorter intervention period. AR concentrations were...
50–100% higher after whole-grain rye intake than after the habitual diet, which agrees with our previous study (42). AR plasma concentrations were significantly higher after consumption of the habitual diet both before and after the feeding study than after the baseline (RF I) or washout (RF II) periods \( (P < 0.05) \), which indicates the habitual intake of whole-grain breads and agrees with dietary food records. AR in plasma, erythrocytes, and in lipoproteins after the AR-free RF I and RF II periods can perhaps be explained by their storage in tissues and their liberation during minimal AR intake. Erythrocyte AR may function as long-term biomarkers of whole-grain intake, insofar as AR appear to be retained in erythrocyte membranes during low whole-grain intake. High extracellular AR concentrations have caused haemolysis in vitro (13,14). Erythrocyte cell size remained the same throughout the present study, indicating that AR incorporation into the cells did not cause swelling or shrinkage of the cells in vivo. In this study, erythrocyte AR concentrations immediately after whole-grain intake were not analyzed, and therefore we cannot draw any conclusions about the possible effects of extreme AR concentrations in vivo on erythrocyte cell size. Human plasma AR concentrations up to 3365 nmol/L have been reached after intake of rye bran (43).

AR homolog composition differed after WD and RD in all measured samples. The plasma AR C17:0 to C21:0 ratio seemed to best distinguish between the 2 test breads. The ratio was significantly higher after RD, compared with WD, although it was lower after 1 wk of RD (0.60) in the present study compared with 8 wk of whole-grain rye bread intake in our previous study (0.84) (42). There are data of increasing relative bioavailability with increasing AR chain length, which may affect homolog distribution in the body (43). A high variation of AR concentrations between subjects after intake of the same amounts of AR was probably caused by individual differences in metabolism or absorption from the intestine. The amount of fiber in the test breads might also affect the absorption. The amount of fat consumed, which was not monitored in the present study, is another possible factor affecting AR absorption.

We found quantifiable amounts of AR in all major lipoprotein fractions. The amount of AR measured in the combined lipoprotein fractions constituted 70–80% of that measured in total plasma. Taking into account the procedural losses during quantitative measurement, this indicates that AR are almost exclusively transported in lipoproteins in plasma. Plasma AR concentrations can be expressed in relation to cholesterol and triacylglycerol concentrations, as recommended for tocopherols (44–46), to adjust for variations in the concentrations of carrier lipoproteins. Although LDL is the main cholesterol carrier in human subjects, VLDL, followed by HDL, are the main carriers of AR. The samples in the present study were taken after an overnight fast, which can affect the AR distribution in the lipoproteins. AR are probably absorbed and transported in chylomicrons via the lymphatic system to the circulation and to the liver and thereafter incorporated into VLDL or into HDL in a similar way as tocopherols. Tocopherols have a similar structure and log \( P \) (octanol-water partition coefficient) to alkylresorcinols (45,47). HDL could also receive AR directly from chylomicrons and VLDL during lipolysis, which could partly explain higher AR concentrations compared with LDL.

Intake of whole-grain cereals, especially rye, has been linked to increased serum ENL concentrations, although no consistent correlation with intake has been reported (27,28,48). In this study, serum ENL concentrations increased significantly during 1 wk of whole-grain rye intake in men but not in women. In some study participants, serum ENL concentrations did not increase at all, although increased AR concentrations indicated dietary compliance. This is likely due to inefficient conversion of plant lignans to enterolactone by intestinal microflora. Minimal changes in serum ENL concentrations during plant lignan intake have also been seen in previous studies (31). Our results indicate that women may consume more plant lignans derived from other sources than whole-grain bread, resulting in greater ENL production. Accordingly, ENL is not a useful biomarker of whole-grain intake, although it may reflect a healthy diet with high intake of lignan-rich foods, or an effective individual gut microflora activity, as previously suggested (27,31–33).

In conclusion, the plasma AR C17:0 to C21:0 ratio reflects the relative contents of whole-grain wheat and rye in the diet; high values, 0.60 in this study, indicating a predominance of rye. The

---

**FIGURE 1** AR homolog (C17:0–C25:0) composition of plasma, erythrocytes, and lipoproteins, and in the test bread after subjects consumed the WD (A) and RD (B) for 1 wk. Values are means ± SEM, \( n = 15 \) except diet, \( n = 3 \). *Different from RD, \( P < 0.05 \).

**TABLE 3** Serum enterolactone (ENL) concentrations in subjects after WD, RD, RF I, and RF II periods

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>RF I</th>
<th>WD</th>
<th>RF II</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ENL, women</td>
<td>8</td>
<td>12.9 ± 1.1 (7.2–18.7)</td>
<td>16.2 ± 3.0 (5.8–41.3)</td>
<td>17.3 ± 2.0 (3.3–27.5)</td>
<td>22.8 ± 3.1 (11.3–38.7)</td>
</tr>
<tr>
<td>Serum ENL, men</td>
<td>7</td>
<td>6.1 ± 1.0* (0.76–10.9)</td>
<td>5.9 ± 1.8† (0.71–21.4)</td>
<td>6.1 ± 1.2† (1.6–14.6)</td>
<td>18.0 ± 3.2* (1.4–35.8)</td>
</tr>
</tbody>
</table>

* Values are means ± SEM (range), \( n = 15 \). Means in a row with superscripts without a common letter differ, \( P < 0.05 \). *Different from women, \( P < 0.05 \).
plasma total AR concentration and the homolog C17:0 to C21:0 ratio could be used as specific dietary biomarkers to determine the amount and the type of whole-grain product intake, although there appears to be a large amount of interindividual variation. Expressing plasma AR relative to plasma cholesterol and triacylglycerols might provide a more accurate estimate of the individual AR status by eliminating the effects in variation of carrier lipoprotein concentrations. To our knowledge, this is the first study to show that AR are transported in human lipoproteins in vivo.

Acknowledgments
We thank Anja Koskela, Adile Samaletdin, Inga Wiik, and Terhi Hakala for excellent technical assistance.

Literature Cited


