**Plasma Alkylresorcinols and Urinary Alkylresorcinol Metabolites as Biomarkers of Cereal Fiber Intake in Finnish Women**

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**Abstract**

Alkylresorcinols (AR) could be good biomarkers of consumption of fiber-rich cereal products. The aim of this study was to examine the relationship between plasma ARs or urinary AR metabolites and cereal fiber intake in women consuming their habitual diet. Twenty-five postmenopausal and 31 premenopausal women were recruited. The subjects included also vegetarians \( (n = 20) \) to obtain a broad range of cereal intake. Dietary intake, plasma ARs, and urinary AR metabolites \( [3,5\text{-dihydroxybenzoic acid and } 3\text{-}(3,5\text{-dihydroxyphenyl})\text{-1-propanoic acid}] \) were measured. Pearson's and Partial correlation tests were done between dietary fiber intake and plasma ARs or urinary AR metabolites. Cereal fiber intake correlated significantly with plasma AR C17:0 \((r = 0.387)\), AR C19:0 \((r = 0.350)\), AR C21:0 \((r = 0.428)\), AR C23:0 \((r = 0.409)\), AR C25:0 \((r = 0.283)\), and total AR \((r = 0.406)\) and with urinary AR metabolites DHBA \((r = 0.359)\) and DHPPA \((r = 0.402)\) even after adjustment for body mass index and age, which could be confounding variables. This is the first study to show a significant correlation between plasma ARs or urinary AR metabolites and cereal fiber intake during consumption of a habitual diet. These results indicate that assay of plasma ARs or urinary AR metabolites may be used as biomarkers in epidemiologic studies in free-living populations to evaluate the role of cereal fiber intake in various diseases.

**Introduction**

Alkylresorcinols (AR) are a group of phenolic lipids abundant in the outer fiber layers of rye and wheat grains and absent in highly refined white flour and in most other foods \((1, 2)\). Very small amounts are found in some other cereals. In grains, they exist mainly with alkyl chain lengths C15:0-C25:0 \((3)\). It has been proposed that ARs could function as biomarkers of human whole grain intake \((1, 4)\). In human subjects, ARs have been detected in intact form in plasma \((4)\) and as metabolites \([3,5\text{-dihydroxybenzoic acid (DHBA) and } 3\text{-}(3,5\text{-dihydroxyphenyl})\text{-1-propanoic acid (DHPPA)}]\) in urine \((5, 6)\) and plasma. \(^1\) Alkylresorcinols are stable during food processing \((7)\). Finland and Denmark have the highest intake of ARs compared with other European countries \((8)\). Studies have reported that the differences in consumption of ARs are due to age but not to body mass index \((BMI)\) because older people change their dietary habits, increasing the amount of whole grain cereal intake \((8)\). It has been estimated that the average daily intake of ARs in free-living population is around 11 mg/day in United Kingdom and around 40 mg/day in Finland \((9, 10)\), and that 60% of this amount is absorbed \((9)\).

The regular consumption of whole grain foods has been associated with many positive health effects including a reduction in the risk of cardiovascular disease, diabetes, obesity, and certain types of cancer \((11)\). In fact, whole grains slow the digestion, tend to have a low glycaemic index, and could improve insulin sensitivity \((12)\). The total mortality of postmenopausal women was negatively associated with whole grain intake and positively with refined grain intake \((13)\). The mechanisms have not been clarified, but different compounds in the dietary fiber complex are believed to play important roles \((14)\).

It has been suggested that plasma AR concentration could be a useful biomarker of whole grain wheat and rye intake and an indicator of the bread type consumed \((15, 16)\). It has been shown that in rye and wheat cereals, the quantitatively most important ARs are C19:0 \((31-32\%\text{ versus } 29-35\%)\) and C21:0 \((22-25\%\text{ versus } 46-51\%)\). The amount of AR C17:0 is close to AR C21:0 in rye cereals \((2)\). The AR C17:0/C21:0 ratio seems to be a good indicator of whether a flour or cereal product contains whole-grain wheat or rye or a combination of these two cereals \((7)\), and

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this is reflected in the plasma values (15). The AR C17:0/C21:0 ratio is around 0.1 in wheat and 1.0 in rye (7).

In some studies, plasma or urinary enterolactone have been found to be a relatively good biomarker of cereal fiber intake (17), but today, enterolactone is regarded to be a better biomarker of total fiber intake and in general reflects a healthy lifestyle (17). We may conclude that there are no accepted markers of whole grain cereal intake but ARs seem to be good candidates for this role.

The aim of this study was to examine (a) the relationship between plasma intact ARs and urinary AR metabolites and (b) the relationship between plasma ARs or urinary AR metabolites and cereal fiber intake.

Materials and Methods

Subjects. Twenty-five postmenopausal and 31 premenopausal women living in the Helsinki area were recruited. Subjects with a history of cancer or any major diseases, or using drugs such as oral contraceptives, hormone replacement therapy, or antibiotics were excluded. The subjects included also vegetarians (n = 20) to obtain a broad range of cereal intake. The vegetarians could be vegans (without animal products; n = 1), lacto-vegetarians (which included milk product; n = 11) or lacto-ovo-vegetarians (which included milk product and eggs in their diet; n = 8). To be included as a regular vegetarian, women must have been on this diet for at least 1 y (mean, 14 y). All subjects agreed to consume during the study the diet as before the recruitment. Age, weight, height, BMI (kg/m²), age at menarche, type of diet, number of children, menopausal status, smoking status, and physical activity level were recorded during the screening visit by questionnaire. All subjects gave their informed consent and were initially recruited. Age, weight, height, BMI (kg/m²), age at menarche, type of diet, number of children, menopausal status, smoking status, and physical activity level were recorded during the screening visit by questionnaire. All subjects gave their informed consent and were initially interviewed by a doctor who explained the study. The

Table 1. Descriptive data

<table>
<thead>
<tr>
<th>Variables</th>
<th>All subjects (n = 56)</th>
<th>Vegetarians (n = 20)</th>
<th>Omnivores (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46 ± 13</td>
<td>45 ± 12</td>
<td>47 ± 13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60 ± 9</td>
<td>58 ± 9</td>
<td>62 ± 9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 2.7</td>
<td>21.5 ± 2.3</td>
<td>23.1 ± 2.8</td>
</tr>
<tr>
<td>Age of menarche (y)</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Number of children</td>
<td>1.8 ± 1.4</td>
<td>1.5 ± 1.7</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>Menopausal status (%)</td>
<td>43</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>Nonphysically active (%)</td>
<td>42</td>
<td>37</td>
<td>46</td>
</tr>
<tr>
<td>Omnivore diet (%)</td>
<td>87</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>Urinary DHBA by HPLC (μmol/24 h)</td>
<td>26.8 ± 15.6</td>
<td>32.8 ± 21.6</td>
<td>24.2 ± 15.6</td>
</tr>
<tr>
<td>Urinary DHPPA by HPLC (μmol/24 h)</td>
<td>40.2 ± 27.6</td>
<td>54.1 ± 39.9</td>
<td>34.4 ± 17.8</td>
</tr>
<tr>
<td>Plasma AR C17:0 by GC/MS (nmol/L)</td>
<td>10.5 ± 5.7</td>
<td>9.2 ± 4.3</td>
<td>10.9 ± 6.1</td>
</tr>
<tr>
<td>Plasma AR C19:0 by GC/MS (nmol/L)</td>
<td>19.3 ± 12.2</td>
<td>17.6 ± 8.8</td>
<td>20.1 ± 13.4</td>
</tr>
<tr>
<td>Plasma AR C21:0 by GC/MS (nmol/L)</td>
<td>17.3 ± 9.5</td>
<td>16.9 ± 8.7</td>
<td>17.5 ± 9.8</td>
</tr>
<tr>
<td>Plasma AR C23:0 by GC/MS (nmol/L)</td>
<td>11.3 ± 6.0</td>
<td>10.3 ± 4.6</td>
<td>11.7 ± 6.5</td>
</tr>
<tr>
<td>Plasma AR C25:0 by GC/MS (nmol/L)</td>
<td>7.1 ± 3.9</td>
<td>6.9 ± 3.2</td>
<td>7.2 ± 4.2</td>
</tr>
<tr>
<td>Plasma AR C17:0/21:0 ratio (nmol/L)</td>
<td>0.62 ± 0.22</td>
<td>0.59 ± 0.18</td>
<td>0.64 ± 0.23</td>
</tr>
<tr>
<td>Total plasma ARs (C17:0-C25:0; nmol/L)</td>
<td>65.5 ± 34.7</td>
<td>60.9 ± 26.6</td>
<td>67.3 ± 37.5</td>
</tr>
<tr>
<td>Cereal fiber intake (g/d)</td>
<td>10.0 ± 3.0</td>
<td>11.0 ± 3.6</td>
<td>9.5 ± 2.6</td>
</tr>
<tr>
<td>Berry fiber intake (g/d)</td>
<td>3.41 ± 3.80</td>
<td>4.88 ± 5.29</td>
<td>2.59 ± 2.36</td>
</tr>
<tr>
<td>Legume fiber intake (g/d)</td>
<td>0.35 ± 0.67</td>
<td>0.62 ± 1.08</td>
<td>0.20 ± 0.12</td>
</tr>
<tr>
<td>Vegetable fiber intake (g/d)</td>
<td>4.14 ± 2.26</td>
<td>5.51 ± 2.73</td>
<td>3.36 ± 1.52</td>
</tr>
<tr>
<td>Total fiber intake (g/d)</td>
<td>19.8 ± 6.3</td>
<td>24.0 ± 7.3</td>
<td>17.5 ± 4.2</td>
</tr>
</tbody>
</table>

NOTE: Means ± SD or %.

Abbreviations: HPLC, high performance liquid chromatography; GC/MS, gas chromatography-mass spectroscopy.
and redissolved in 300 µL of methanol and further purified using DEAE-Sephadex ion exchange chromatography (in 0.5 x 1.5-cm columns) in the free base form (19). After the samples were transferred to the columns, neutral steroids were eluted with 6 mL of methanol and discarded. ARs were eluted with 6 mL 0.1 mol/L acetic acid in methanol. The AR fraction was evaporated to dryness and derivatized with 100 µL of silylating reagent [pyridine:hexamethyldisilazanetrtrimethylchlorosiliane 9:3:1 (v:v:v)] for gas chromatography-mass spectroscopy analysis (4).

**Urine Analysis.** The 72-h urine samples were collected in plastic bottles containing 1 g of ascorbic acid per liter volume. During collection, the bottles were stored as cool as possible and brought to the laboratory every morning and stored at +4°C. After the 3-d collection, the 24-h urine samples were pooled, volume was measured, and after addition of 0.1% of sodium azide to counteract bacterial activity. All samples were stored at −20°C and analyzed for AR metabolites using the published protocol A (5). To 100 µL of urine, we added 422 ng of internal standard Syringic Acid in 10 µL of methanol. The sample was hydrolyzed overnight at 37°C with equal volume (100 µL) of hydrolysis solution containing 0.1 mol/L Na-acetate buffer (pH 5), 0.2 U/mL β-glucuronidase, and 2 U/mL sulfatase. After incubation, 50 µL aliquot (equal to 25 µL of urine) were removed and 50 µL of methanol, and 650 µL high performance liquid chromatography mobile phase 20% B/A were added to the sample, and the sample was analyzed for the two AR metabolites, DHPPA and DHBA, by high performance liquid chromatography with coulometric electrode array detection (ESA Biosciences).

**Statistical Analysis.** Normality of distribution was determined using the Skewness test. All AR and diet variables were log transformed for statistical analysis. First, we used Pearson’s correlation test to examine the relation between urinary AR metabolites and plasma AR levels and between dietary fiber intake and plasma ARs or urinary AR metabolites. We calculated Partial correlations between individual ARs and cereal fiber intake using BMI and age as covariates because they could be confounding variables. Furthermore, we calculated partial correlation between total plasma AR sand cereal fiber intake using BMI and age as covariates based on diet status (vegetarians or omnivores). Finally, stepwise regression analysis was used to determine the independent predictors of cereal fiber intake. P values of ≤0.05 were considered statistically significant. Analyses were done using SPSS 15.0 program.

**Results**

We observed that all ARs correlated significantly with plasma AR C17:0 (r = 0.387; P = 0.004), AR C19:0 (r = 0.350; P = 0.010), AR C21:0 (r = 0.428; P = 0.001), AR C23:0 (r = 0.409; P = 0.002), AR C25:0 (r = 0.283; P = 0.040), and total ARs (r = 0.406; P = 0.003), and with urinary AR metabolites DHBA (r = 0.359; P = 0.008) and DHPPA (r = 0.402; P = 0.003; Fig. 1) even after adjustment for BMI and age.

We observed a significant correlation between total plasma ARs and cereal fiber intake both in vegetarians (r = 0.548; P = 0.018) and in omnivores (r = 0.372; P = 0.033) even after adjustment for BMI and age.

Finally, a stepwise linear regression analysis was done with plasma ARs (17, 19, 21, 23, 25, and total), DHBA, DHPPA, BMI, age, and enterolactone in the model. We observed that AR C21:0 and DHPPA were both independent predictors of cereal fiber intake, explaining 26% of the variance (adjusted r² = 0.264; P = 0.001).

**Discussion**

The aim of this study was to examine (i) the relationship between plasma ARs and urinary AR metabolites and (ii) the relationship between plasma ARs or urinary AR metabolites and cereal fiber intake. We observed that cereal fiber intake during a habitual diet correlates significantly with all plasma ARs and urinary AR metabolites in women even after adjustment for BMI and age, which could be confounding variables (Fig. 1). Furthermore, our results are not influenced by the diet status of our subjects. This indicates that ARs are good biomarkers of cereal fiber intake. This result is important because at present, there are no accepted biomarkers of whole grain cereal product intake (20). Because lignans are abundant in whole grain products and correlations were found with high rye bread intake (21, 22), the main metabolite enterolactone was originally suggested to be a biomarker of whole grain intake. However, it is derived from several dietary sources (23), and in regions with low whole grain intake, no consistent correlation between enterolactone and whole grain cereal intake has been reported. Thus, enterolactone seems not suitable as a specific biomarker of whole grain intake (24).

Epidemiologic studies suggest that a regular consumption of whole grain foods is associated with a decreased risk of cardiovascular disease and some types of cancer (25, 26). The main problem in epidemiologic studies is to estimate precisely the intake of various food components. There are some inherent weaknesses of food frequency questionnaires (14, 27). The use of a biomarker, which can be quantified, could confirm and strengthen the conclusions made in such studies (27).

The lack of significant correlation between plasma ARs and the other fibers confirmed the specificity of ARs as biomarker for cereal rye and wheat fiber intake. Our results are also not influenced by age or BMI, which are known as confounding variables (10). Moreover, in
Figure 1. Pearson’s correlation between cereal fiber intake and plasma ARs/urinary AR metabolites.
previous studies, it has been reported that ARs are present in cereal grains, specifically C19:0 and C21:0 in rye, in wheat, in triticale, and in barley, and C17:0 in rye (2, 7). It has been also shown that rye (average AR content, 734 μg/g) and wheat cereals (average AR content, 583 μg/g) contain the highest amount of ARs (2).

We have shown that after a dietary intervention in women, the plasma total AR concentration seemed to be a useful biomarker of whole grain cereal intake (15, 16). We have shown that after a dietary intervention in women, the plasma total AR concentration seemed to be a useful biomarker of whole grain cereal intake (15, 16). In the present study, the plasma AR C17:0/21:0 ratio is a useful biomarker of whole grain cereal intake (15, 16). We have shown that after a dietary intervention in women, the plasma total AR concentration seemed to be a useful biomarker of whole grain cereal intake (15, 16).

In the present study, the plasma AR C17:0/21:0 ratio is 0.62 ± 0.22 nmol/L, which indicates that women consumed both rye and wheat whole grain cereals (7). We also found that plasma AR C21:0 had the highest correlation with cereal fiber intake (r = 0.428; P = 0.001; Fig. 1), which is in agreement with a content of both rye and wheat in the diet (2, 7). The AR C21:0 was found to be an independent predictor of cereal fiber intake (r = 0.410; P = 0.002) in this population.

Our study had several limitations. First, our study has been carried out in Finnish women who are known to consume a high daily amount of cereal fiber and ARs (10). Moreover, the coefficients of correlation are significant but the r values are clinically moderate (r < 0.750). Hence, further research, in larger free-living populations in other countries is needed to be able to generalize and confirm our findings.

In conclusion, this is the first study to show a significant correlation between plasma ARs and urinary AR metabolites. This result is important because it confirmed that urinary AR metabolites are derived from plasma ARs as suggested by Ross et al. (6). It also indicates that the urinary AR metabolites (5) may be used as biomarkers in epidemiologic studies on cereal fiber intake and disease in free-living populations.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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