Whole-Grain Rye Bread Consumption by Women Correlates with Plasma Alkylresorcinols and Increases Their Concentration Compared with Low-Fiber Wheat Bread¹,²

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ABSTRACT  Whole-grain rye and wheat products are rich in lignans, the precursors of enterolactone (ENL) and alkylresorcinols (ARs), which are phenolic lipids. In this crossover trial, we examined the effect of whole-grain rye bread compared with low-fiber wheat bread on plasma AR levels. Women (n = 39) aged 59 ± 0.94 y (mean ± SEM) were advised to consume rye (214 ± 7.1 g/d) or wheat (178 ± 6.5 g/d) bread (20% of total daily energy intake) in random order for 8 wk. The test periods were separated by an 8-wk washout period. ARs with different homologues and ENL were measured in plasma collected at the beginning (habitual diet) and end of the test bread periods. AR concentrations were higher (P < 0.001) after the rye bread (352 ± 24.7 nmol/L) and habitual diet (97.7 ± 12.1 and 88.3 ± 8.7 nmol/L) periods than after the wheat bread period (36.6 ± 4.2 nmol/L). Plasma AR concentrations were correlated with intake of rye bread (r = 0.34, P = 0.037), and with insoluble fiber from the whole diet during the rye (r = 0.39, P = 0.013) and wheat (r = 0.32, P = 0.047) bread periods. The plasma AR 17:0/21:0 ratio differed after the rye (0.84 ± 0.04) and wheat (0.53 ± 0.08) bread diet periods (P < 0.001). These data strongly suggest that plasma ARs could be used as specific biomarkers for whole-grain rye intake, and their homologue pattern could be used as an indicator of the bread type consumed.  J. Nutr. 135: 580–583, 2005.

KEY WORDS:  • alkylresorcinols  • biomarkers  • rye bread

Many epidemiologic studies have linked the intake of whole-grain cereals to a reduced risk of some diseases, such as obesity, type II diabetes, cardiovascular diseases, and certain types of cancer (1–3). Several authorities have recommended including whole-grain products in the diet as a source of carbohydrates, fiber, and minor dietary components. However, no definitive conclusions regarding their protective role have been made, partly because of the lack of a specific biomarker for whole-grain products (1).

Alkylresorcinols (ARs),⁴ homologues of 1,3-dihydroxy-5-methylbenzene, are phenolic lipids that occur in the human diet almost entirely in the bran fractions of wheat (317–1429 μg/g of dry matter), triticale, and rye (360–3200 μg/g of dry matter) grains (4,5). ARs differ mainly because of their odd-numbered chain lengths, which in wheat and rye are 17–25 carbons long. Previously it was thought that ARs are destroyed during baking (6), but Ross et al. (7) showed that ARs can be extracted from baked breads. They estimated the daily intake of AR to be 85–100 mg. ARs are absorbed by humans (8), and have been quantified in human plasma (9). Plasma ARs are not detected when people consume gluten-free diets (9). ARs possess several biochemical properties that were shown mainly in vitro, such as antimicrobial and anticarcinogenic activities, and an ability to affect cell membrane stability and antioxidative properties, although ARs seem to be rather weak antioxidants per se (5,10–14). ARs were detected in the adipose tissue of rats (15) and quantified in human erythrocyte membranes (16). It was suggested that AR could serve as a specific biomarker for whole-grain rye and wheat consumption (9,17). Such a biomarker would provide a powerful tool for epidemiologic studies. Determining the AR homologue composition in plasma could give an additional tool to distinguish between the dietary grain sources, i.e., wheat, rye, or a mixture of the two.

In addition to AR, whole-grain bread is a fairly good source of plant lignans, from which intestinal bacteria produce enterodiol (END) and enterolactone (ENL), which is the main metabolic end product of lignan metabolism, and can therefore be considered to reflect the total lignan intake (18–20). Properties, production, and absorption of ENL and END have been studied intensively (21,22). Lignans and alkylresorcinols exist mainly in the outer layers of grains, and no ARs were detected in the endosperm (23,24). It was suggested that ENL could serve as a biomarker for whole-grain rye and wheat.
intake (25), and some studies showed correlations between rye bread or fiber intake and plasma ENL (26,27). However, some studies did not find this correlation, and no conclusive statement of the usability of ENL as a biomarker has been made (28–30).

The present study is the first to analyze AR in human plasma after a controlled, 8-wk, crossover intervention study with intake of whole-grain rye or low-fiber wheat bread. Changes in plasma AR levels were compared with those in ENL, and the possible utility of ARs as biomarkers for whole-grain rye and wheat intake was investigated.

SUBJECTS AND METHODS

Subjects. Healthy postmenopausal women (n = 39) took part in the study. They were 59 ± 0.94 y old (means ± SEM) and had a BMI of 26.8 ± 0.5 kg/m². This study was part of a larger study in which the effects of a fiber-rich rye bread on glucose, lipid, and insulin metabolism were examined (31). Postmenopausal women were selected because of an increased susceptibility to diabetes mellitus. The inclusion criteria were elevated serum total cholesterol level (5.0–8.5 mmol/L), a non-HDL-cholesterol level of 3.5–6.5 mmol/L, and a BMI of 20–33 kg/m². The subjects did not have diabetes mellitus or diseases that could affect lipid metabolism or bowel function and they did not use any medication (lipid-lowering medication, laxatives, or corticosteroids) for such conditions. Serum follicle-stimulating hormone levels were used to determine postmenopausal status. The Ethics Committee of Kuopio University Hospital, Kuopio, Finland approved the study.

Study design and diets. The study design (2 × 8 wk randomized crossover intervention with an 8-wk washout period), composition of the test breads, and the dietary advice given to the participants were described previously in more detail (31). All breads were baked in Fazer Bakeries and Vaasan & Vaasan Oy bakeries. High-fiber rye bread (17% of dietary fiber) portions weighed 24.1–28.1 g and low-fiber wheat bread (2.8% of dietary fiber) portions 20.8–25.0 g. One portion of rye bread contained 206 kJ (range 174–234 kJ) and 4.4 g (4.1–4.6 g) of fiber, and a portion of wheat bread 241 kJ (233–249 kJ) and 0.6 g (0.5–0.8 g) of fiber. Minimum bread intake was 4–5 portions of the test bread. No maximum for bread intake was set, but subjects were advised to consume grain-based products as normal and to keep the intake of these products constant. During the bread periods, subjects replaced all bread and grain products in their diet with the test bread so that the energy from bread was at least 20% of the total daily energy intake. They were advised to keep the daily bread intake constant during the test periods. Subjects were allowed to eat 1 additional portion of wheat or rye grain products (sweet pastry or porridge)/d during both bread periods, and rice and pasta according to their normal eating habits. The portion was recommended to be a rye-based product during the rye bread period and a wheat-based product during the wheat bread period, but this was not obligatory. Apart from grain products subjects were instructed to eat normally during the follow-up period. To monitor dietary compliance, subjects recorded their food intake for 4 d during the baseline (2 wk), bread (8 wk), and washout (8 wk) periods to determine total energy intake, and for 1 wk during bread periods to record the intake of an additional grain product portion. Subjects also recorded the daily intake of the test breads, and quantity, quality, and frequency of other grain products consumed. The nutritional composition of test breads was analyzed at VTT Biotechnology.

Plasma alkylresorcinols. Alkylresorcinol concentrations in plasma samples were analyzed by a GC-MS method (9). Synthetic AR 20:0 (20 μL), which does not exist naturally in grains (kindly provided by Prof. K. Wahala, Department of Chemistry, University of Helsinki, Finland) was used as an internal standard. After overnight incubation with water, ARs were extracted with diethyl ether, and the samples were purified with DEAE-OH⁻ ion exchange chromatography and analyzed by GC-MS. Quality control samples and a blank sample were included in every batch. The intra-assay variation was <10% in all series, and interassay variation was 16%. We quantified AR homologues 17:0, 19:0, 21:0, 23:0, and 25:0. Results are expressed as a mean of duplicate analyses, and as the total amount of AR homologues, unless otherwise stated.

Plasma enterolactone. Enterolactone in plasma was measured by a time-resolved fluoroimmunoassay (32,33). Samples were hydrolyzed overnight with sulfatase and β-glucuronidase in acetate buffer. Unconjugated ENL was extracted with diethyl ether. [6,7-H]estradiol-17-glucuronidase was used as an internal standard. The extracts with assay buffer, antiserum in bovine serum albumin (dilution 1:250 000), and europium-labeled ENL derivative (dilution 1:400 000) were added on prewashed goat anti-rabbit IgG microstrips and analyzed with the AutoDELFIA 1235 Automatic Immunoassay System (Wallac Oy). Quality control samples and a blank sample were included in every batch. The intra- and interassay variations were <10% in all series. Results are expressed as a mean of duplicate analyses.

Statistical analysis. Normality of the data was tested with Shapiro-Wilk’s test. The AR and ENL concentrations were right skewed and nonparametric tests were employed. Associations of plasma AR and ENL concentrations with age, BMI, consumption of rye and wheat bread, and intake of fiber during bread periods were tested by Spearman’s ρ correlation coefficient. Differences in AR and ENL concentrations and AR homologue patterns between the diet periods were assessed with Friedman’s test followed by Wilcoxon’s signed rank test. All statistical analyses were performed with SPSS 11.0.1 for Windows. Differences were considered significant at P < 0.05. Results are expressed as means ± SEM.

RESULTS

Dietary compliance during the study was good. Subjects consumed 8 test bread portions/d during the bread periods (Table 1), receiving 24 ± 0.6% (rye bread period) and 28 ± 1.1% (wheat bread period) of their daily energy from the breads. Plasma AR and ENL concentrations were not affected by age or BMI (data not shown). Plasma AR and ENL concentrations at the beginning of the 2 bread periods did not differ. Plasma AR and ENL concentrations increased (P < 0.001) during the rye bread period and decreased during the wheat bread period (P = 0.005) compared with baseline (Table 2).

To eliminate the effect of an additional grain portion and

| TABLE 1 | Daily intake by postmenopausal women of energy, macronutrients, fiber, and test breads during the 8-wk rye and wheat bread periods1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Rye bread period | Wheat bread period |                  |
| Energy, kJ/d    | 7553 ± 235      | 7281 ± 238      |                  |
| Protein, % energy | 18.4 ± 0.4  | 17.3 ± 0.4     |                  |
| Fat, % energy   | 27.9 ± 1.0     | 29.5 ± 1.0     |                  |
| Carbohydrate, % energy | 52.6 ± 1.0  | 51.6 ± 1.2     |                  |
| Diet, g/d       |                  |                  |                  |
| Total fiber     | 47.0 ± 1.4     | 15.2 ± 0.7     |                  |
| Soluble fiber   | 9.3 ± 0.3      | 4.9 ± 0.2      |                  |
| Insoluble fiber | 33.4 ± 1.0     | 6.1 ± 0.2      |                  |
| Bread, g/d      | 214 ± 7.1      | 178 ± 6.5      |                  |
| Test bread, g/d |                  |                  |                  |
| Fiber           | 36.4 ± 1.2     | 4.9 ± 0.2      |                  |
| Soluble fiber   | 6.0 ± 0.2      | 1.6 ± 0.1      |                  |
| Insoluble fiber | 30.4 ± 1.0     | 3.3 ± 0.1      |                  |
| Test bread intake,2 portions/d | 8.3 ± 0.28 | 8.0 ± 0.29 |                  |
| Other grain products,3 portions/d | 0.88 ± 0.04 | 0.81 ± 0.52 |                  |

1 Values are means ± SEM, n = 39.
2 One portion of rye bread weighed 24.1–28.1 g and of wheat bread 20.8–25.0 g.
3 One portion of other grain products was a slice, a piece, or a plateful of the product.
We found a significant increase in plasma AR levels after 8 wk of high-fiber rye bread intake compared with 8 wk of low-fiber wheat bread or 8 wk of habitual diet consumption, and plasma AR correlated with the intake of rye bread. The results indicate that the plasma AR 17:0/21:0 ratio could be used as a marker of rye or wheat intake.

Introducing whole-grain rye into the diet increased plasma AR levels remarkably, as expected. The changes in ENL concentrations after the bread periods were considerably smaller. This is probably due to high intake of ENL precursors from plant products other than rye bread, making it a nonspecific marker for whole grains. However, large amounts of ENL precursors are present in rye grains, explaining the significant increase in ENL concentrations after the rye bread period. The habitual consumption of whole-grain products during the washout and baseline periods increased AR concentrations significantly compared with the low-fiber wheat bread period, suggesting that plasma ARs reflect even moderate whole-grain product intake. Low-fiber wheat bread itself contains only minute amounts of AR; therefore, AR present in plasma after the wheat bread period could be from the optional grain product portion that the subjects were allowed to consume, which could have been low-AR wheat or high-AR rye products. AR could also be stored in the body, mainly in lipid tissues and cell membranes (15), and be liberated into plasma when intake of AR from the diet is low. This hypothesis of AR distribution in the body requires more studies about absorption and storage of AR with different chain lengths.

Because we did not analyze AR in the diet, we could not assess the intestinal absorption of AR from the breads, or perform further kinetic calculations. From the literature data, we estimated that the average intake of AR during the rye bread period in this study was ~200 mg/d; similar intake in earlier studies resulted in 60% absorption (7,8). The individual variation in absorption in the present study was taken into consideration because each subject served as her own control.

Wheat grains contain little AR 17:0, whereas 21:0 is abundant. In contrast, rye grains contain 17:0 and 21:0 in proportions similar to the 17:0/21:0 ratio, which is 1.0 and 0.10 for rye and wheat grains, respectively (7). Because the plasma AR 17:0/21:0 ratio was significantly different after the 2 test bread periods and compared with the habitual diet, it could be used as a marker of dietary grain source. Based on this study a higher plasma ratio reflects a rye-based diet and a lower value a wheat-based diet. The ratios in plasma differed from those of grains, possibly due to a different absorption of the homologues. Dietary grain AR composition in this study also may have differed from that reported in the literature.

The significant correlation between rye bread consumption estimate the true effect of rye bread on plasma AR, we subtracted the plasma AR concentration at the end of the wheat bread period from that at the end of the rye bread period. Consumption of rye bread correlated with plasma AR concentration (r = 0.34, P = 0.037), but not with that of ENL (r = 0.17, P = 0.301), whereas intake of wheat bread was not correlated with either. Insoluble fiber from the whole diet during the rye (r = 0.39, P = 0.013) and wheat (r = 0.32, P = 0.047) bread periods correlated with the plasma AR levels, and soluble fiber from the diet during the rye bread period correlated with plasma ENL (r = 0.34, P = 0.036). During the wheat bread period, dietary soluble fiber intake and plasma ENL were not correlated.

The AR homologue patterns after wheat and rye bread intake differed for all homologues (P < 0.05) except 23:0. The plasma AR pattern was consistent with the grain AR pattern (Table 3). The plasma 17:0/21:0 ratio differed after the rye bread (0.84 ± 0.04) and wheat bread (0.53 ± 0.08) periods (P < 0.001). The ratio differed from baseline, also, after the rye (P = 0.003) and wheat (P < 0.001) bread periods. The ratio did not differ after the baseline (0.74 ± 0.06) and washout periods (0.68 ± 0.05).

**DISCUSSION**

These results strongly support the suggestion that ARs could be used as biomarkers for whole-grain rye intake (17).

### TABLE 2

**Plasma AR and ENL concentrations in postmenopausal women after baseline, washout, and rye and wheat bread periods**

<table>
<thead>
<tr>
<th>Period</th>
<th>AR nmol/L</th>
<th>ENL nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>97.7 ± 12.1b</td>
<td>32.8 ± 4.8b</td>
</tr>
<tr>
<td>Wheat bread period</td>
<td>36.6 ± 4.2a</td>
<td>22.7 ± 4.0a</td>
</tr>
<tr>
<td>Washout period</td>
<td>88.3 ± 8.7b</td>
<td>27.1 ± 3.9b</td>
</tr>
<tr>
<td>Rye bread period</td>
<td>352 ± 24.7c</td>
<td>53.5 ± 10.0c</td>
</tr>
</tbody>
</table>

1 Values are means of duplicate measurements of each sample ± SEM, n = 39 (range excluding outliers > 1.5 interquartile from 25th or 75th percentile). Means in a column without a common letter differ, P < 0.05 (Wilcoxon signed ranks test).

### TABLE 3

**Plasma AR homologue composition and the 17:0/21:0 ratio in postmenopausal women after the rye and wheat bread periods**

<table>
<thead>
<tr>
<th>Sample</th>
<th>17:0</th>
<th>19:0</th>
<th>21:0</th>
<th>23:0</th>
<th>25:0</th>
<th>17:0/21:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye diet</td>
<td>17 ± 0.55</td>
<td>30 ± 0.59</td>
<td>21 ± 0.36</td>
<td>17 ± 0.41</td>
<td>15 ± 0.73</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td>Rye grains2</td>
<td>24 ± 0.10</td>
<td>32 ± 0.90</td>
<td>25 ± 0.09</td>
<td>10 ± 0.09</td>
<td>9 ± 0.09</td>
<td>1.0 ± 0.09</td>
</tr>
<tr>
<td>Wheat diet</td>
<td>13 ± 1.3*</td>
<td>27 ± 0.99*</td>
<td>31 ± 1.6*</td>
<td>18 ± 1.3</td>
<td>10 ± 0.62*</td>
<td>0.53 ± 0.08*</td>
</tr>
<tr>
<td>Wheat grains2</td>
<td>5 ± 3.3</td>
<td>48 ± 3.3</td>
<td>10 ± 3.3</td>
<td>4 ± 3.3</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means of duplicate measurements of each sample ± SEM, n = 39. * Different from plasma concentration after the rye diet period, P < 0.05 (Wilcoxon signed ranks test).
2 Source: Ref. 7.
and plasma AR levels indicate that ARs could serve as biomarkers for whole-grain rye intake. ENL was not correlated with the intake of wheat or rye bread, which is in agreement with previous data (30). The wheat bread in the present study was low-AR bread and the effects of whole-grain wheat bread were not examined, but we assume that the results from consumption of rye bread would apply also to whole-grain wheat bread with a higher AR content. Plasma AR correlated with the insoluble fiber in the whole diet, but not with that in the test bread. Most of the insoluble fiber consumed during the rye bread period, and about half during the whole grain period, came from the test bread. Insoluble fiber from the optional daily grain product could explain the correlation between plasma AR and whole diet insoluble fiber.

We conclude that rye bread in the diet significantly increases plasma AR and ENL, but ENL is too nonspecific to be used as a biomarker for intake of whole-grain rye and wheat products. As was suggested, plasma ENL can reflect the individual intestinal microflora or the intake of a diet containing fruit, vegetables, and whole-grain products. The present data strongly support the suggestion (17) that plasma ARs could serve as specific biomarkers for whole-grain rye intake. More data are needed about their absorption and kinetics in humans, and larger epidemiologic studies should be conducted to confirm their importance as biomarkers and as bioactive compounds in human nutrition.

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LITERATURE CITED