Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records

Rikard Landberg, Afaf Kamal-Eldin, Agneta Andersson, Bengt Vessby, and Per Åman

ABSTRACT

Background: Alkylresorcinols (ARs), phenolic lipids exclusively present in the outer parts of wheat and rye grains, have been proposed as specific dietary biomarkers of whole-grain wheat and rye intake.

Objective: The objective was to validate plasma ARs as a biomarker of whole-grain wheat and rye intakes by studying the correlation between their plasma concentration and intake calculated from food records.

Design: In a randomized crossover study, 22 women and 8 men were given a defined amount of either whole-grain or refined-cereal-grain products to be included in their habitual diets for two 6-wk periods. Blood samples were collected and food intakes were recorded before and after each intervention period.

Results: Plasma AR concentrations were significantly higher after the whole-grain diet period than after the refined-grain period (P < 0.001) and were well correlated with average daily AR intake estimated by self-reported weighed food records (Spearman’s r = 0.58, P < 0.001).

Conclusion: Plasma AR concentrations are correlated with intake assessed by food records, which suggests that ARs are selective nutritional biomarkers for the intake of whole-grain wheat and rye.


INTRODUCTION

Epidemiologic studies have shown a relatively strong inverse correlation between whole grain intake and risk of heart disease (1, 2). More recently, reduced risks of type 2 diabetes and certain cancers have also been found (2). A major problem in nutritional epidemiology is the relatively low accuracy in measuring the intake of foods and nutrients (3), which might weaken the diet-disease association. The assessment of whole grain intake is further complicated by the fact that consumers may have difficulties identifying products containing whole grain among other products. Using a biomarker of whole grain intake would overcome some of the problems associated with dietary assessment methods and could be used to validate other methods (4, 5). A group of phenolic lipids, alkylresorcinols (ARs), seem to fit many of the general criteria for biomarkers of whole-grain wheat and rye intake (6–8).

ARs, 1,3-dihydroxy-5-alkylbenzene homologues with alkyl side chains in the range of 17–25 carbon atoms (Figure 1), are mainly present in wheat (≈400 µg/g), rye (≈700 µg/g), and barley (≈40 µg/g) among foods commonly consumed by humans (9, 10). AR homologues are exclusively found in the outer parts of these cereals, with high concentrations in bran fractions (up to 4000 µg/g), whereas refined flours contain no ARs or only trace amounts, but the variation in content is large within and between species. The homologue profile is rather consistent within species but different between species, which results in a C17:0/C21:0 ratio of ≈1.0 in rye, 0.1 in wheat, and 0.01 in durum wheat (11, 12). The source of grain used in a food product can be identified by the C17:0/C21:0 ratio. The measured AR content and C17:0/C21:0 ratio are strongly correlated with the calculated content, and the C17:0/C21:0 ratio is based on lists of ingredients for products generally available on the Swedish market (11).

The physiologic functions of AR in plants are to great extent unknown, but various bioactivities in different models including animals have been demonstrated (13). AR are absorbed (40–60%) from the small intestine (14, 15), transported via the lymphatic system (16), and carried in lipoproteins and erythrocytes in blood (16, 17). In humans, the terminal AR half-life is rather short, about 5 h for all homologues, suggesting that plasma AR might reflect short- to medium-term intake (6). Plasma AR concentration has been found to respond to differences in intake (7, 16) and to correlate to the amount of rye bread intake (7). The plasma C17:0/C21:0 ratio was found to be ≈0.1 in subjects consuming a wheat-bread diet and ≈0.6–0.9 in subjects consuming a rye-bread diet (7, 16). Until now, no study has attempted to correlate calculated AR intakes with plasma AR concentrations, which is a crucial step in the validation of plasma AR as a marker of whole grain intake.

The aim of this study was to investigate the relation between plasma AR concentration and AR intake calculated from 3-d self-reported weighed food records in a controlled whole grain crossover intervention study.

SUBJECTS AND METHODS

Subjects and study design

A total of 30 subjects (22 postmenopausal women and 8 men) with a mean (±SD) age of 59 ± 5 y and a body mass index (in...
kg/m²) of 28.3 ± 2.0 were included. The present study forms part of an intervention study primarily designed for the investigation of whole-grain foods in the context of insulin sensitivity and inflammation (18). All volunteers were provided with written and oral information about the study before it began. The study was performed as a randomized, nonblind, crossover dietary intervention trial with treatments during 2 consecutive 6-wk periods separated by a 6–8 wk washout period. It was approved by the local ethics committee in the Uppsala region of Sweden.

Diets

Subjects were instructed to adhere to their habitual diet during both treatment periods, except for cereal products, which were provided. During treatment periods, subjects were instructed to replace their habitual cereal foods with a defined amount of several whole-grain or refined-grain products to eat every day. These products included soft bread, crisp bread, pasta, rice, and muesli and were described in detail elsewhere (18). A whole-grain product in this study is defined as a product containing all parts of the naked cereal kernel and 50% whole grain on a dry matter basis. The advised amount of refined grain or whole grain intake during the treatment was 112 g/d, which corresponded to 50% whole grain on a dry weight basis (18). All volunteers were provided with written and oral information about the study before it began. The study was performed as a randomized, nonblind, crossover dietary intervention trial with treatments during 2 consecutive 6-wk periods separated by a 6–8 wk washout period. It was approved by the local ethics committee in the Uppsala region of Sweden.

Alkylresorcinol intake

The average daily AR intake for the whole-grain and refined-grain periods was estimated for all subjects on the basis of the analyzed content in products used in the 2 different diet periods and on the amount of product stated in self-reported, 3-d weighed food records. At baseline periods when subjects ate their habitual diet, reported cereal products that were not analyzed specifically for the study were matched to values of similar products analyzed previously (11, 12, 14). Intake estimates derived from the 3-d weighed food records were compared with those reported in diaries used as a compliance check for the intervention. In diaries, subjects ticked off the intake of a fixed amount of a specific product category during each intervention day. In cases in which more than one product was available within each product category, an average total AR content for each product category was calculated and used (Table 1) (18).

Dietary fiber intake

The dietary fiber contributions from intervention diets were 18 g (whole grain) and 6 g (refined grain), respectively. Total dietary fiber intake and cereal dietary fiber intake from wheat and rye for the 2 intervention periods and for the baseline periods were estimated from the 3-d weighed food records and from dietary fiber contents listed in the database of the Swedish National Food Administration (PC-kost 1/2000). Dietary fiber fractions in test products were analyzed and found to be similar to database values (18). Total dietary fiber intake and cereal fiber intake from wheat and rye were correlated with both the calculated total AR intake and the total plasma AR concentration.

Samples and analytic methods

Blood samples were collected in the morning after the subjects had fasted overnight and before and after the two 6-wk intervention periods. Blood drawn from an antecubital vein into heparin-coated vacuum tubes was immediately centrifuged in a Hettich Rotina 48R centrifuge (Bach, Germany) (2000 g for 10 min at 4 °C) to separate plasma and erythrocytes. Plasma samples were portioned into 2-mL cryotubes and stored at −80 °C until analyzed.

Total AR content and relative homologue composition of food products used during the whole-grain and refined-grain diet periods were analyzed by gas chromatography (GC). Briefly, samples were milled and extracted with a hot 1-propanol:water mixture (3:1, by vol) and analyzed with GC without further purification. Samples were analyzed in triplicate on a fresh batch, and the intraassay precision expressed as CV was <5% and the interassay precision was <8%.

Plasma samples were analyzed for total AR concentration and relative AR homologue composition according to a method slightly modified from Linko et al (20). The AR C20:0 (Reasechem Life Sciences, Burgdorf, Switzerland), which does not exist naturally, was used as an internal standard. Briefly, plasma (0.5 mL) was mixed with the internal standard (45 ng) and incubated with water (0.5 mL) at 37 °C overnight. Samples were extracted with diethyl ether (3 mL × 3), evaporated to dryness, and dissolved in 0.5 mL methanol. The ARs present were separated from nonpolar lipids on a diethyl-amino-ethyl (DEAE)-Sephadex A-25 ion exchange gel (Amersham Biotech, Uppsala, Sweden) in base form dissolved in methanol and packed in Pasteur pipettes to a final height of 2 cm. Columns were washed with 6 mL MeOH to elute neutral compounds, and then ARs were eluted by the addition of 0.1 mol acetic acid/L in 6 mL MeOH. Eluted ARs were evaporated to dryness, silylated with 0.2 mL pyridine:hexamethyldisilazane:trimethylchlorosilane 9:3:1 (vol:vol:vol), and analyzed with a Finnigan Trace GC Ultra Gas chromatograph coupled to a Finnigan Trace DSQ mass detector (Thermo Fischer Scientific, Waltham, MA). The column and temperature program were the same as for the quantitative analysis performed on a GC-FID system described elsewhere (9).
TABLE 1
Total alkylresorcinol (AR) content and relative homologue composition in products used during the whole-grain and refined-grain dietary periods

<table>
<thead>
<tr>
<th>Diet period and product</th>
<th>Total AR content μg/g FW</th>
<th>Relative homologue composition %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C17:0</td>
<td>C19:0</td>
</tr>
<tr>
<td>Whole grain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crisp bread, rye (n = 1)</td>
<td>776</td>
<td>23</td>
</tr>
<tr>
<td>Crisp bread, oats (n = 1)</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td>Sourdough bread, rye and wheat (n = 1)</td>
<td>250</td>
<td>16</td>
</tr>
<tr>
<td>Soft bread, rye and wheat (n = 1)</td>
<td>256</td>
<td>17</td>
</tr>
<tr>
<td>Whole-grain flour, wheat (n = 1)</td>
<td>595</td>
<td>4</td>
</tr>
<tr>
<td>Pasta, wheat (n = 3)</td>
<td>194 ± 16</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>Müesli, rye and oats (n = 3)</td>
<td>161 ± 39</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>Refined grain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crisp bread, wheat (n = 1)</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>Crisp bread, oats and rye (n = 1)</td>
<td>121</td>
<td>19</td>
</tr>
<tr>
<td>Soft bread, wheat (n = 2)</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>Refined flour, wheat (n = 1)</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Pasta, wheat (n = 3)</td>
<td>8 ± 14</td>
<td>ND</td>
</tr>
<tr>
<td>Müesli, wheat and corn (n = 3)</td>
<td>—</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 All samples were analyzed in triplicate. The analytic precision indicated by the CV was <5% for all samples. ND, not detected (limit of detection ≈5 μg/g), FW, fresh weight.

2 The main cereal ingredients of each product are indicated; n is the number of products in each category. Pasta is a composite of macaroni, fusilli, and spaghetti. Müesli is a composite of 3 different products, and refined soft bread is a composite of 2 breads.

3 ARs were only detectable in fusilli.

Statistical analysis

Differences in AR intakes, total plasma AR concentrations, and C17:0/C21:0 ratios between periods were evaluated by an analysis of variance model appropriate for crossover design experiments. Treatment sequence, subjects nested within sequence, period, and treatment were included as factors in the model. The residuals from the model were examined for normality, and if the residuals were extremely skewed (Shapiro-Wilk’s W < 0.95) the variables were transformed to their natural logarithms before analyses. This was the case for total plasma AR concentration, plasma C17:0/C21:0 ratios, and average daily AR intakes. The statistical analysis was conducted in 26–28 subjects, because of the lack of sample in some cases. One individual was excluded from all statistical analysis because of an extreme fast- ing plasma AR concentration (>800 nmol/L). Carryover effects were evaluated by comparing the treatment sequences with patient nested within treatment as an error term. These tests were 2-sided and were performed at 10% significant level. No carryover effects were found. The tests for treatment effect were 2-sided, and P values < 5% were considered significant. All P values were Bonferroni corrected for multiplicity. In addition to an evaluation of treatment effects, correlation analysis was performed by using data reported during the baseline and treatment periods, separately and together. The plasma AR concentration was correlated with total AR, cereal, and total dietary fiber (excluding oats and rice) intakes reported during the intervention periods and baseline periods, individually and together. In correlation analysis, only P values for correlations where data from all periods were included were Bonferroni corrected. Plasma AR concentrations, adjusted for total lipids [plasma AR/(total cholesterol + triacylglycerols)], were also tested as suggested by Linko-Parvinen et al (16). In addition, dietary fiber intake was correlated to AR intake. All correlation coefficients reported are Spearman’s rank correlation coefficients, unless otherwise stated. Pearson’s correlation coefficients were also calculated, which were similar (data not shown). A general linear model (GLM), with subject as a factor, was applied to take repeated measurements of each subject into account when the data from all periods was used together.

The validity of plasma AR concentration as a reflector of AR intake was tested in terms of its ability to accurately rank individuals into quartiles according to their AR intake. Therefore, quartile agreement between total plasma AR concentration and the intake estimated from 3-d records of intervention and baseline periods was calculated, as described by Bhakta et al (5). Agreement between plasma AR concentration and weighed food records was evaluated by dividing the intake and plasma AR concentrations of treatment and baseline periods into quartiles...
and then calculating the percentage of observations classified into the correct or the correct \( \pm \) nearest adjacent quartiles for each period. Additionally, an average of quartile agreement estimated within each treatment and baseline period, separately, was calculated. All statistical analyses were performed with SAS (version 9.1; SAS, Chicago, IL) and Minitab 14.0 (Minitab Inc, State College, PA) software.

RESULTS

Test foods and intake of AR and dietary fiber

The total AR contents in the products provided were in the range of not detected to 776 \( \mu g/g \) fresh weight (Table 1), and the relative homologue composition was comparable with data published previously for similar types of products. The C17:0 to C21:0 ratio was in the range 0.02–1.0, which reflected the source of whole grain used in the products (11).

Compliance during the study was good; 30 individuals completed the intervention and 28 completed diaries and 3-d food records. The 3-d weighed food records and the diary checklist showed good adherence to the dietary advice (18). The calculated records. The 3-d weighed food records and the diary checklist of whole grain used in the products (11).

The relative homologue composition calculated from the advised whole-grain diet was \( \approx 13\% \) (C17:0), 28\% (C19:0), 36\% (C21:0), 15\% (C23:0), and 8\% (C25:0). For the advised refined-grain period, it was \( \approx 10\% \) (C17:0), 30\% (C19:0), 30\% (C21:0), 26\% (C23:0), and 4\% (C25:0). Hence, the average C17:0/C21:0 ratios were 0.4 and 0.3 for the whole-grain and refined-grain intervention periods, respectively.

There was no difference in total energy intake between the 2 intervention periods or compared with baseline. As expected, total dietary fiber intake differed significantly (\( P < 0.001 \)) between the whole-grain period (30 \( \pm \) 4.7 g) and the refined-grain period (18.3 \( \pm \) 7.4 g). Additionally, total fiber intake was lower (\( P < 0.05 \)) during the refined-grain period than at baseline (21.4 \( \pm \) 9.1 g) and higher (\( P < 0.001 \)) during the whole-grain period than at baseline (22.1 \( \pm \) 8.0). Cereal fiber corresponded to 64.7 \( \pm \) 10.5\% and 18.3 \( \pm \) 7.4\% of total fiber during the whole-grain and refined-grain periods and to 45.7 \( \pm \) 14.0\% and 47.6 \( \pm \) 14.2\% during the respective baseline periods. Total dietary fiber and cereal fiber of wheat and rye intakes were both well correlated with calculated AR intake [Pearson \( r = 0.64 \) (\( P < 0.01 \)) and Pearson \( r = 0.81 \) (\( P < 0.01 \)), respectively]. Detailed information about the test foods was published elsewhere (18).

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline (^1)</th>
<th>Treatment (^2)</th>
<th>Baseline (^3)</th>
<th>Treatment (^4)</th>
<th>Baseline (^5)</th>
<th>Treatment (^6)</th>
<th>AR homologue ratio (C17:0/C21:0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total AR intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from diaries</td>
<td>mg/d</td>
<td>mg/d</td>
<td>mg/d</td>
<td>mg/d</td>
<td>mg/d</td>
<td>mg/d</td>
<td></td>
</tr>
<tr>
<td>Whole grain</td>
<td>63.9 ( \pm ) 11.0(^7)</td>
<td>36.1 ( \pm ) 19.0</td>
<td>65.0 ( \pm ) 12.2(^8)</td>
<td>102 ( \pm ) 70</td>
<td>202 ( \pm ) 107(^9)</td>
<td>0.37 ( \pm ) 0.23</td>
<td>0.30 ( \pm ) 0.23</td>
</tr>
<tr>
<td>(53.6–111.7)</td>
<td>(12.8–89.9)</td>
<td>(43.1–98.8)</td>
<td>(21–251)</td>
<td>(45–499)</td>
<td>(0.04–0.85)</td>
<td>(0.05–0.87)</td>
<td></td>
</tr>
<tr>
<td>Refined grain</td>
<td>6.5 ( \pm ) 0.5(^7)</td>
<td>44.0 ( \pm ) 31.6</td>
<td>6.8 ( \pm ) 1.9(^8)</td>
<td>104 ( \pm ) 77</td>
<td>59 ( \pm ) 57(^9)</td>
<td>0.32 ( \pm ) 0.20</td>
<td>0.34 ( \pm ) 0.23</td>
</tr>
<tr>
<td>(5.4–7.8)</td>
<td>(4.0–136.9)</td>
<td>(1.7–11.0)</td>
<td>(23–340)</td>
<td>(9–220)</td>
<td>(0.02–0.80)</td>
<td>(0.09–0.96)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) All values are \( \bar{x} \pm \) SD; range in parentheses. Plasma AR concentrations are single measurements from 28 individuals.

\(^2\) No significant carryover effects were found for any of the studied variables at the level (\( P < 0.10 \)) tested by ANOVA appropriate for crossover designs.

\(^3\) No time-by-treatment interaction was found at the level (\( P < 0.05 \)) tested.

\(^4\) Intake during baseline periods was estimated from pooled 3-d dietary records and by matching reported products to similar products analyzed previously (10, 18).

\(^5\) No significant difference between treatment periods, \( P < 0.0001 \) (Bonferroni corrected). Data were analyzed by ANOVA appropriate for crossover designs.

\(^6\) Significantly different from corresponding baseline period, \( P < 0.0001 \) (Bonferroni corrected). Data were analyzed by ANOVA appropriate for crossover designs.
TABLE 3
Spearman’s correlation coefficients (r) between alkylresorcinol (AR) intake and plasma total AR concentration during baseline and the whole-grain and refined-grain diet periods (n = 28 per period) and for all periods together (n = 4 × 28)†.

| Diet period                  | r      | P  
|------------------------------|--------|-----
| Baseline before whole grain  | 0.33   | 0.018
| Whole grain                  | 0.25   | 0.210
| Baseline before refined grain| 0.40   | 0.042
| Refined grain                | 0.21   | 0.280
| All periods                  | 0.58   | <0.001

† Intake was estimated from 3-d food records.

0.36 (P < 0.010) and r = 0.52 (P < 0.010) for total and cereal fiber, respectively.

In contrast with what was found previously (16), there was no statistically significant change from baseline in the AR C17:0/C21:0 ratio after the whole-grain diet or after the refined-grain period after Bonferroni correction of the P value.

When the estimated AR intake and the plasma AR concentrations for all individuals and all periods were divided into quartiles, ≈51% of the plasma AR concentrations were classified into the correct average daily intake quartile and 87% were classified into the correct ± the nearest adjacent quartiles. The corresponding averages of agreement determined for each treatment and baseline period separately were ≈30% and 70%. The distribution of plasma AR concentrations in each intake quartile from all periods together is shown in Figure 2.

DISCUSSION
This is the first study to show a correlation between plasma AR concentration and calculated AR intake from self-reported weighed food records. It was found that the C17:0/C21:0 ratio in plasma followed that of intake, in agreement with previous reports (7, 16). The results strongly suggest that plasma AR concentrations reflect intake and can therefore serve as a dietary biomarker of whole-grain wheat and rye intake, as previously suggested (6–8).

Diets and intake
Products consumed during the refined-grain period contained only low levels of AR, except for a mistaken crisp bread, which was found to contain 121 μg/g. This product was included on the basis of labeled ingredients (sifted rye flour) and was not expected to contain the amounts of AR found by analysis. The relative homologue composition of some refined-grain products was not in agreement with what could be expected because of the fact that the measured amounts were very close to the detection limit of the method, which made integration of the peaks difficult, especially those of C23:0 and C25:0 (Table 1).

The average habitual AR intake of subjects during baseline periods was ≈40 mg/d, which is higher than the estimated per capita intake of the Swedish population (≈20 mg/d) (21). The intake of ARs during the whole-grain period was 1.5 times that of the habitual intake. There was good agreement between the calculated AR intake and that reported in both the 3-d weighed food records and the diaries (Table 2). Compared with the whole-grain and refined-grain periods, the precision in food recording was less during the baseline periods, possibly caused by consumption of a wider range of products for which AR contents were estimated and not analyzed.

As expected, the correlation between cereal fiber intake from wheat and rye and AR intake was high, because ARs are located in the bran, which is also the main source of cereal fiber. Because

TABLE 4
Spearman’s correlation coefficients (r) between plasma alkylresorcinol (AR) homologues and the average total AR intake estimated from a weighed 3-d food record completed during the whole-grain, refined-grain, and baseline periods (n = 4 × 28)†.

<table>
<thead>
<tr>
<th>AR</th>
<th>Plasma AR†</th>
<th>Lipid-adjusted plasma AR‡</th>
<th>Lipid-adjusted plasma AR§</th>
</tr>
</thead>
<tbody>
<tr>
<td>C17:0</td>
<td>0.29</td>
<td>0.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C19:0</td>
<td>0.55</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C21:0</td>
<td>0.55</td>
<td>0.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C23:0</td>
<td>0.59</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C25:0</td>
<td>0.60</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total AR</td>
<td>0.58</td>
<td>0.59</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

† All AR intakes and plasma AR concentrations were transformed by natural logarithm.
‡ Correlation was obtained when total plasma AR concentrations were adjusted for total plasma lipids (nmol AR/μmol total cholesterol + μmol triacylglycerols).
§ Adjusted for multiplicity by Bonferroni correction.
cereals were the dominant source of dietary fiber in this study. AR intake was also fairly well correlated with total dietary fiber intake.

Plasma AR in relation to estimated intake

The baseline AR concentration observed was slightly higher than that found in postmenopausal Finnish women (7) and was slightly lower than that reported for 15 healthy young Finnish subjects (16). The individual variation was rather high (21–340 nmol/L), in agreement with 2 Finnish studies (7, 16). This large variation might have been due to differences in intake, absorption, elimination, and demographics or to a combination thereof (22). The plasma AR concentration was significantly higher after the whole-grain period than after the refined-grain period, but the magnitude of the difference was not as large as that observed in the 2 previous studies (7, 16), because of the inclusion of refined ingredients other than sifted wheat flour during the refined-grain diet period in the present study. Another explanation might be the slight differences in analytic determinations at low concentrations or a systematic underreporting of AR intake during the refined-grain period. However, the average plasma concentration after 6 wk of the whole-grain diet (with a rather high intake) was similar to values found after a 1-wk rye bread intervention with a corresponding daily AR intake (16), which suggests that, at the level tested, 1 wk would be sufficient to obtain plasma AR concentrations that reflect intake. This finding is in line with the relatively short half-life of plasma AR, which implies that a steady state level would be established within a few days when ARs are consumed regularly (6).

The lack of a correlation between total AR intake and plasma concentration within each intervention period was probably due to the small variation in intake caused by the study design (Table 3). A good correlation was found when data from the intervention periods were used, and baseline periods together and GLM 3). A good correlation was found when data from the intervention period in the present study. Another explanation might be the slight differences in analytic determinations at low concentrations or a systematic underreporting of AR intake during the refined-grain period. However, the average plasma concentration after 6 wk of the whole-grain diet (with a rather high intake) was similar to values found after a 1-wk rye bread intervention with a corresponding daily AR intake (16), which suggests that, at the level tested, 1 wk would be sufficient to obtain plasma AR concentrations that reflect intake. This finding is in line with the relatively short half-life of plasma AR, which implies that a steady state level would be established within a few days when ARs are consumed regularly (6).

The ability to classify AR intake into quartiles depending on plasma AR concentration was fairly good. About 70% were classified in the correct or the correct ± the nearest adjacent quartiles when repeated measurements in each subject were taken into account, which was about the same as for the other dietary biomarkers studied (5, 24).

Results of the present study and previous studies (6, 7, 16, 17, 23) showed that ARs fulfill many of the criteria set for a specific dietary biomarker. However, before ARs are used for the evaluation of other dietary assessment methods, large-scale, well-controlled calibration studies are needed to evaluate dose-response relations and to explore possible factors other than intake that affect plasma concentrations.

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The authors’ responsibilities were as follows—RL: performed the laboratory and data analysis; RL and AK-E: wrote the paper; AA: coordinated the intervention trial and provided information on nutrient calculations and dietary record data; BV, AK-E, and PA: initiated and designed the study and obtained the funding. All authors critically reviewed the manuscript. None of the authors had a potential or actual conflict of interest. This work has not been published before.

REFERENCES


