Plasma pharmacokinetics of alkylresorcinol metabolites: new candidate biomarkers for whole-grain rye and wheat intake

Paivi P Soderholm, Anja H Koskela, Johan E Lundin, Matti J Tikkanen, and Herman C Adlercreutz

INTRODUCTION

An inverse association has been found between the intake of whole-grain foods and the risk of some chronic diseases, such as cardiovascular disease, diabetes, and certain cancers (1–9). Although many of the mechanisms are still poorly understood, the beneficial effects are suggested to originate from the cereal fiber complex with all its constituents (10–12).

Several limitations are related to studies concerning the health effects of fiber-rich foods. Inaccurate identification of different cereal-fiber constituents as well as incorrect estimation and misreporting of food intake limit the usability of dietary recording methods (13–15). These problems could be solved by using a biomarker as an objective measure of whole-grain intake (11, 16–18). So far, the best candidate biomarkers for whole-grain rye and wheat intakes are alkylresorcinols (19–23)—the dietary copassengers in the bran fraction of these 2 cereals (24–26). Alkylresorcinols are phenolic lipids (1,3-dihydroxy-5-alkylbenzene homologs) that have a variety of bioactivities in vitro, such as antimicrobial, antioxidative, and antimutagenic activities and interactions with some proteins (25, 27). Alkylresorcinols are stable during food processing (28) and, according to Ross et al (27), they are proposed to metabolize similarly to tocopherols (vitamin E) after ingestion. The 2 end products of alkylresorcinol metabolism—3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA)—were first identified in urine by Ross et al (29), whereas the quantification method for these metabolites in plasma was described by Koskela et al (30). Urinary DHBA and DHPPA have been studied to some extent and have been proposed to serve as biomarkers for whole-grain rye and wheat intakes. First, urine samples are less frequently available in epidemiologic studies. Second, concerning the plasma intact alkylresorcinols, Landberg et al (32) reported a half-life ($t_{1/2}$) of 5 h, which appears relatively short given that blood samples are usually taken after overnight fasting. To our knowledge, no reports concerning alkylresorcinol metabolites in plasma have been published, except our recent quantification method for DHBA and DHPPA (33). In that study, a significant correlation ($P < 0.001$) between these metabolites and plasma intact alkylresorcinols was reported. In our present study, we determined the plasma pharmacokinetics of DHBA and DHPPA in healthy subjects after a single dose of
high-fiber rye bread to evaluate the suitability of these plasma metabolites as biomarkers for whole-grain rye and/or wheat intakes.

**SUBJECTS AND METHODS**

**Instrumentation**

We used an HPLC system from ESA Biosciences Inc (Chelmsford, MA) that was equipped with a model 540 autosampler, 2 model 580 solvent pumps, and a model 5600 coulometric electrode array detector with 8 electrode pairs.

**Chemicals and materials**

Acetonitrile and methanol were obtained from Rathburn Chemicals Ltd (Walkenburg, United Kingdom). Ortho-phosphoric acid was purchased from Riedel-de Hae¨n (Seelze, Germany). Acetic acid, diethylether, potassium dihydrogenphosphate, and sodium acetate were purchased from Merck GmbH (Darmstadt, Germany). ß-Glucuronidase was obtained from Roche Diagnostics GmbH (Mannheim, Germany) and sulfatase from Sigma-Aldrich Co (St Louis, MO). DHBA was obtained from Aldrich (Steinheim, Germany), DHPPA from IsoSep AB (Tollinge, Sweden), and syringic acid from Sigma-Aldrich Co (St Louis, MO). High-fiber rye bread (9.4 g fiber/100 g) was specially baked for our studies (Fazer Bakeries, Lahti, Finland).

**Subjects and study design**

Fifteen healthy volunteers (8 women and 7 men) were recruited for this study. Their mean (±SD) age was 24.3 ± 5.3 y (range: 20–39 y): 25.9 ± 6.4 y for women and 23.1 ± 3.6 y for men. The mean weight of the subjects was 68.3 ± 10.9 kg (range: 52–90 kg): 59.5 ± 4.4 kg for women and 78.5 ± 5.4 kg for men. Body mass index (in kg/m²) was 22.1 ± 2.0 (range: 18.8–26.1): 21.0 ± 1.8 for women and 23.4 ± 1.4 for men. The volunteers were given written and oral information about the study in advance, and they completed an eligibility questionnaire and attended a screening blood test. As exclusion criteria, we specified low hemoglobin values (<125 g/L for women and 135 g/L for men) and the use of antibiotics within the past 3 mo. Informed consent was obtained from all participants. This study was approved by the Ethics Committee at the Helsinki University Central Hospital, Helsinki, Finland.

The participants were asked to follow a low-alkylresorcinol diet by avoiding whole-grain rye and wheat products for 2 d before the study. After the subjects fasted overnight, baseline blood samples were collected. Thereafter, the participants ingested a single dose (198 g) of high-fiber rye bread (18.6 g fiber) containing 45 mg alkylresorcinols plus 21 g butter to ensure the absorption of the highly lipophilic alkylresorcinols. Butter was preferred to vegetable oil because of the concern that the long-chain triacylglycerols might slow the absorption process of alkylresorcinols similarly to that of vitamin E (34). The participants were allowed 25 min for the ingestion of the rye bread and butter. Standardized meals and drinks were served throughout the study, and the participants were advised to avoid consuming any other foods.

Blood samples were collected 3, 4, 5, 6, 7, 8, 10, 12, 14, and 16 h after the rye bread and butter were consumed, and the last sample was drawn at 25 h the next morning after an overnight fast. For the blood sampling, a catheter was placed in the cubital vein to avoid numerous needle punctures. All blood samples were drawn into EDTA-coated tubes by a trained laboratory technician.

**Sample treatment**

After the blood samples were drawn, they were cooled to room temperature and centrifuged for 10 min to separate plasma and erythrocytes. Plasma was collected into 4-mL tubes and stored at −20°C until analyzed.

**Analytic method**

DHBA and DHPPA were analyzed as reported previously (33). Briefly, plasma with the internal standard (syringic acid) was incubated overnight at 37°C, pH 5, with the hydrolytic enzymes ß-glucuronidase and sulfatase to convert the conjugates into free alkylresorcinol metabolites. Thereafter, the sample was acidified by using acetic acid to reach a pH of ≈3 and extracted with diethylether by vigorous shaking. The organic phase was collected, and the procedure was repeated 3 times. The combined organic phases were then evaporated and reconstituted with methanol (50 μL) and HPLC mobile phase (100 μL). The sample was filtered through a Gelman GHP 0.2-μm filter and analyzed with an HPLC-coulometric electrode array detector.

**Analysis of rye bread**

Rye bread was analyzed for alkylresorcinols before this study by using the method of Ross et al (35).

**Pharmacokinetic analysis of the alkylresorcinol metabolites DHBA and DHPPA in plasma**

The maximum plasma concentration of DHBA and DHPPA from baseline (0 h) to 25 h after rye bread intake is defined as $C_{\text{max}}$, and $t_{\text{max}}$ is the time at which $C_{\text{max}}$ was reached. The half-life ($t_{1/2}$) is defined as the time at which the metabolite plasma concentration has decreased to half of $C_{\text{max}}$. All of the pharmacokinetic parameters were calculated by using STATA software (version 10.0; StataCorp, College Station, TX).

**Statistical analysis**

Data on DHBA and DHPPA concentrations are presented as means ± SEMs. The pharmacokinetic parameters $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, and area under the curve (AUC) for DHBA and DHPPA were statistically analyzed by using a paired $t$ test to clarify whether there are significant differences between these 2 metabolites. Also, the groups of women and men were compared by using an unpaired $t$ test to discover potential differences in the pharmacokinetic parameters between sexes. $P$ values <0.05 were considered significant.

**RESULTS**

The mean plasma DHBA and DHPPA concentrations at each time point for the whole group ($n = 15$) are presented in Figure 1, and the pharmacokinetic data are presented in Table 1.
All subjects consumed the dose of rye bread and butter within 25 min. The dose provided 45 mg alkylresorcinols. This is a relatively high amount as a single dose, but yet is achievable as a daily intake of alkylresorcinols in a habitual diet among whole-grain consumers. The mean baseline (0 h) plasma concentrations for DHBA and DHPPA were low (33.2 ± 4.7 and 35.5 ± 5.9 nmol/L, respectively), which indicated that the participants had followed the instructions to avoid eating whole-grain rye and wheat products before entering the study. The corresponding values at the last time point (25 h) were 103.7 ± 9.5 and 95.4 ± 10.0 nmol/L for DHBA and DHPPA, respectively, which were significantly higher than at 0 h (P < 0.0001 for both metabolites), which indicated that significant amounts of the metabolites were still retained in the circulation.

Significant differences (P < 0.0001) in C_max and AUC were found between the 2 metabolites. C_max (n = 15) varied from 316 to 888 nmol/L (570.3 ± 45.7 nmol/L) for DHBA and from 231 to 593 nmol/L (350.5 ± 29.7 nmol/L) for DHPPA. The mean AUC values were 6631 ± 389 and 4269 ± 244 nmol · h/L for DHBA and DHPPA, respectively. The mean t_max was 6.1 ± 0.5 h for DHBA and 6.4 ± 0.7 h for DHPPA. Eleven participants achieved t_max at 5.0 h, but 4 (2 women and 2 men) achieved t_max between 7 and 10 h, which suggested individual differences in metabolism and/or gastric emptying. The t_1/2 for DHPPA was 16.3 ± 1.8 h, which was significantly (P < 0.0002) longer than the t_1/2 for DHBA (10.1 ± 0.8 h).

The AUC for both metabolites was significantly greater in women than in men (P = 0.03 for DHBA and P = 0.01 for DHPPA). However, when corrected for body weight (59.5 kg for women and 78.5 kg for men), the difference was no longer significant (P = 0.80 and P = 0.91, respectively), which suggested that the difference was dependent on body weight and not sex. The C_max and t_1/2 did not differ significantly between women and men.

**DISCUSSION**

Whole-grain foods are considered to be health-promoting components in the human diet. However, lack of a suitable biomarker has been complicating the research regarding the effects of whole-grain cereals on diseases. With a suitable biomarker, objective information about actual intake of whole-grain foods can be achieved, and samples from various biobanks can be analyzed even if no data about the diet are available. Although plasma intact alkylresorcinols have been suggested as biomarkers for the intake of whole-grain rye and wheat, the short t_1/2 limits their usefulness in epidemiologic studies (32). Our objective was to investigate the plasma pharmacokinetics of the alkylresorcinol metabolites DHBA and DHPPA to evaluate their suitability as alternative biomarkers.

To our knowledge, this was the first study to evaluate the plasma pharmacokinetics of DHBA and DHPPA. These metabolites correlate significantly with intact alkylresorcinols (33), which, in turn, correlate significantly with whole-grain rye and wheat intakes (19).

Our findings indicate that the t_1/2 for both DHBA (10.1 h) and DHPPA (16.3 h) in plasma is 2- to 3-fold longer than the t_1/2 for

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>C_max (nmol/L)</th>
<th>t_max (h)</th>
<th>t_1/2 (h)</th>
<th>AUC (nmol · h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHBA</td>
<td></td>
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<tr>
<td>All (n = 15)</td>
<td>570.3 ± 45.7c</td>
<td>6.1 ± 0.5</td>
<td>10.1 ± 0.8b</td>
<td>6631 ± 389c</td>
</tr>
<tr>
<td>Women (n = 8)</td>
<td>625.9 ± 57.5</td>
<td>6.0 ± 0.6</td>
<td>9.6 ± 1.4</td>
<td>7391 ± 441</td>
</tr>
<tr>
<td>Men (n = 7)</td>
<td>506.7 ± 96.2</td>
<td>6.1 ± 0.9</td>
<td>10.8 ± 0.6</td>
<td>5761 ± 510c</td>
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<tr>
<td>DHPPA</td>
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<tr>
<td>All (n = 15)</td>
<td>350.5 ± 29.7c</td>
<td>6.4 ± 0.7</td>
<td>16.3 ± 1.8b</td>
<td>4269 ± 244c</td>
</tr>
<tr>
<td>Women (n = 8)</td>
<td>392.0 ± 43.8</td>
<td>6.1 ± 0.8</td>
<td>17.0 ± 3.0</td>
<td>4794 ± 305</td>
</tr>
<tr>
<td>Men (n = 7)</td>
<td>303.1 ± 33.7</td>
<td>6.7 ± 1.1</td>
<td>15.5 ± 1.8</td>
<td>3668 ± 247c</td>
</tr>
</tbody>
</table>

1 All values are means ± SEMs. C_max, maximum plasma concentration; t_max, time to reach C_max; t_1/2, time at which the concentration has decreased to half of C_max; AUC, area under the concentration curve (0–25 h). Values with different superscript letters are significantly different, P < 0.0002 (paired t test).

2 Significantly different from women, P ≤ 0.03 (unpaired t test). However, after correction for the AUC for body weight, the differences were not significant.
intact alkylresorcinols (5 h), as reported by Landberg et al (32). Accordingly, both alkylresorcinol metabolites are good candidate biomarkers for whole-grain rye and/or wheat intake. DHPPA appeared to be the best indicator because of its longer $t_{1/2}$, at least in this group of individuals.

Our study had some limitations. Although the number of study participants ($n = 15$) was considered sufficient to provide comprehensive pharmacokinetic data, interindividual variation was relatively large. The early time point of ingestion of the test bread (at 0700) may have influenced the rate of gastric emptying, depending on the participants’ habitual timing of breakfast. Ross et al (21) reported large individual variation in intestinal alkylresorcinol absorption (45–71%), and the bioavailability of alkylresorcinols may also vary (32). Metabolic factors could influence the concentrations of DHBA and DHPPA in plasma as well. For example, liver cytochrome P450 factors could influence the concentrations of DHBA and DHPPA in plasma as well. For example, liver cytochrome P450 enzymes are crucially involved in the metabolism of vitamin E, because $\omega$-oxidation of the alkyl side chain appears to be the first and rate-limiting step; thereafter, the alkyl chain is shortened via $\beta$-oxidation (36, 37). This could be a possible metabolic route for alkylresorcinols (27), which have some structural similarities to vitamin E. However, this has not been investigated. Thus, a larger group of individuals would allow a more exact analysis of the mean $t_{\text{max}}$ and $t_{1/2}$ values. In conclusion, DHBA and DHPPA have potential for use as biomarkers, but further research is needed to evaluate the reproducibility of these findings and to assess the feasibility of their use in large epidemiologic studies (4, 38).

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The authors’ responsibilities were as follows—PPS: helped design the study, conducted most of the experimental work, and drafted the manuscript; AHK: helped design the study, performed the laboratory analysis, and contributed to drafting the manuscript; JEL: carried out the pharmacokinetic and statistical analysis; and MTT and HCA: designed the study, supervised the experimental work, and contributed to the drafting of the manuscript. All of the authors helped revise the manuscript. None of the authors had a personal or financial conflict of interest.

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