Simultaneous Pharmacokinetic Modeling of Alkylresorcinols and Their Main Metabolites Indicates Dual Absorption Mechanisms and Enterohepatic Elimination in Humans\textsuperscript{1–3}

Matti Marklund,\textsuperscript{4} Eric A. Strömberg,\textsuperscript{5} Helle N. Lærke,\textsuperscript{6} Knud E. Bach Knudsen,\textsuperscript{6} Afaf Kamal-Eldin,\textsuperscript{7} Andrew C. Hooker,\textsuperscript{5} and Rikard Landberg\textsuperscript{4,9}

\textsuperscript{4}Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, and \textsuperscript{3}Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden; \textsuperscript{5}Department of Animal Science, Aarhus University, Tjele, Denmark; \textsuperscript{6}Department of Food Science, College of Food and Agriculture, United Arab Emirates University, Al Ain, United Arab Emirates; \textsuperscript{7}Department of Food Science, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden; and \textsuperscript{8}Nutritional Epidemiology Unit, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Abstract

Background: Alkylresorcinols have proven to be useful biomarkers of whole-grain wheat and rye intake in many nutritional studies. To improve their utility, more knowledge regarding the fate of alkylresorcinols and their metabolites after consumption is needed.

Objective: The objective of this study was to develop a combined pharmacokinetic model for plasma concentrations of alkylresorcinols and their 2 major metabolites, 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA).

Methods: The model was established by using plasma samples collected from 3 women and 2 men after a single dose (120 g) of rye bran and validated against fasting plasma concentrations from 8 women and 7 men with controlled rye bran intake (23, 45, or 90 g/d). Alkylresorcinols in the lymph and plasma of a pig fed a single alkylresorcinol dose (1.3 mmol) were quantified to assess absorption. Human ileostomal effluent and pig bile after high and low alkylresorcinol doses were analyzed to evaluate biliary alkylresorcinol metabolite excretion.

Results: The model contained 2 absorption compartments: 1 that transferred alkylresorcinols directly to the systematic circulation and 1 in which a proportion of absorbed alkylresorcinols was metabolized before reaching the systemic circulation. Plasma concentrations of alkylresorcinols and their metabolites depended on absorption and formation, respectively, and the mean \( \pm \) SEM terminal elimination half-life of alkylresorcinols (1.9 \( \pm \) 0.59 h), DHPPA (1.5 \( \pm \) 0.26 h), and DHBA (1.3 \( \pm \) 0.22 h) did not differ. The model accurately predicted alkylresorcinol and DHBA concentrations after repeated alkylresorcinol intake but DHPPA concentration was overpredicted, possibly because of poorly modeled enterohepatic circulation. During the 8 h following administration, <2% of the alkylresorcinol dose was recovered in the lymph. DHPPA was identified in both human ileostomal effluent and pig bile, indicating availability of DHPPA for absorption and enterohepatic circulation.

Conclusions: Intact alkylresorcinols have advantages over DHBA and DHPPA as plasma biomarkers for whole-grain wheat and rye intake because of lower susceptibility to factors other than alkylresorcinol intake.

Introduction

Alkylresorcinols are phenolic lipids abundantly present in the bran of wheat and rye and can be used as biomarkers for intake of whole-grain products made from these cereals (1–4). As biomarkers, alkylresorcinols could potentially aid investigations of the relation between consumption and health (5). The fate of alkylresorcinols after consumption was assessed in several studies in humans, pigs, and rats (6–9). In humans, about 60% of alkylresorcinols ingested are apparently absorbed from the small intestine, and the proposed route of absorption is via the lymphatic pathway (10,11). Elimination of alkylresorcinols from plasma is rapid and was suggested to comprise extensive hepatic metabolism similar to that of tocopherols (12). Recent in vitro studies have confirmed several steps of the hypothesized metabolic pathway of alkylresorcinols, which is initiated by \( \omega \)-oxidation (13).
enables subsequent shortening of the hydrocarbon chain by β-oxidation and the formation of 2 major metabolites, 3,5-dihydroxybenzoic acid (DHBA) and 3-[3,5-dihydroxyphenyl]-propanoic acid (DHPPA). These phenolic acids have been quantified in plasma and urine (15–17), but, to our knowledge, no study has reported their presence in bile or feces. As a result of phase II metabolism, DHBA and DHPPA are not only found as free aglycones but also as different conjugates (e.g., glucuronides and sulfonates) in plasma and urine (17,18). Similarly to alkylresorcinols, DHBA and DHPPA have been proposed as potential biomarkers for intake of whole-grain wheat and rye (15). Two other alkylresorcinol metabolites, 3,5-DHBA glycin (a conjugate of DHBA) and 3-[3,5-dihydroxyphenyl]-pentanoic acid, have recently been identified in human urine samples after consumption of whole grains and in mice fed alkylresorcinol homologs C19:0 and C21:0, with recovery corresponding to about 3% of the alkylresorcinol dose ingested (19).

The pharmacokinetics of alkylresorcinols in humans has been assessed previously, and a compartmental model describing alkylresorcinol absorption and elimination has been presented and validated (8,20). The human model includes 2 separate absorption compartments and a central compartment from which alkylresorcinols disappear by first-order kinetics. Plasma concentrations and urinary excretion rates of alkylresorcinol metabolites have previously been monitored in humans following a single dose of alkylresorcinol-containing rye bread (21,22). However, the reported half-life of alkylresorcinol metabolites might not represent the terminal half-life, because elimination could be limited by rate of formation, as has been observed in rats (9).

In a previous rat study, we simultaneously assessed the pharmacokinetics of alkylresorcinols and their metabolites (9). A pharmacokinetic model describing alkylresorcinols and their metabolites in human plasma could help us understand the fate of alkylresorcinols after intake and provide information on how alkylresorcinols, together with their metabolites, can be used to improve estimates of whole-grain and bran-food intake from wheat and rye.

The primary aim of the present study was to build and validate a single pharmacokinetic model for alkylresorcinols and their main metabolites in humans. To assess alkylresorcinol absorption and biliary excretion and potential enterohepatic circulation (EC) of alkylresorcinol metabolites, alkylresorcinols were quantified in the plasma and lymph of a pig given a single dose of purified alkylresorcinols, whereas DHBA and DHPPA were analyzed in ileostomy effluent from humans and bile from pigs.

Materials and Methods

Materials. Alkylresorcinols were purchased from ReseaChem Life Science. DHBA, syringic acid, and type-H1 β-glucuronidase (with additional sulfatase activity) were obtained from Sigma-Aldrich. DHPPA was purchased from Isosep. Methanol, acetic acid, and diethyl ether were obtained from Merck, and quick silylation mixture was prepared as previously described (23).

Samples. A combined pharmacokinetic model for alkylresorcinols and their metabolites was established by quantifying plasma concentrations of DHBA and DHPPA in plasma samples from a previous study, where repeated blood samples were drawn from 6 healthy volunteers (3 women and 3 men) after ingestion of a single dose of rye bran (8). However, 1 man was excluded because of insufficient plasma volumes. The combined model was validated by using data on plasma concentrations of DHBA and DHPPA taken from a randomized crossover study of healthy volunteers assessing the alkylresorcinol dose-response relation (20). For 1 of the 15 participants (8 women and 7 men) with measured plasma alkylresorcinol concentrations, alkylresorcinol metabolite concentrations could not be measured at any dose because of insufficient plasma volume; hence, only 14 participants with sufficient sample volume at ≥1 dose levels were included for alkylresorcinol metabolite analysis. At the lowest dose, alkylresorcinol was quantified in all participants (n = 15) and alkylresorcinol metabolites in 14 individuals, whereas at the 2 highest doses, plasma volumes were only sufficient for alkylresorcinol and alkylresorcinol metabolite quantification in 14 and 13 participants, respectively. Total DHBA and DHPPA content were determined in ileostomy effluents from 4 randomly selected individuals who participated in a previously published feeding trial (24). The subjects consumed an alkylresorcinol-rich (147 mg/portion) high-fiber diet (HFD) or a low-fiber diet (LFD) with alkylresorcinol content below the analytic detection level at the time of the study. The protocols for the human studies were approved by the local ethical committee of Uppsala County or the ethical committee of Umeå University Hospital (8,20,24).

One pig, surgically modified to collect lymph and blood as previously described (25), was fed a single dose of 1.3 mmol purified alkylresorcinols isolated from rye, administered together with an alkylresorcinol-free basal feed (25). Blood samples from the vena cava were taken hourly at 0–8, 16, and 24 h following administration, whereas lymph was sampled continuously from the trunci jejunalis for 30 min prior to administration, at hourly intervals for 0–8 h after administration, and again for 30 min at 16 h after administration. Pig bile from a previous trial (26) was analyzed for free and conjugated alkylresorcinols, DHBA, and DHPPA. Bile was collected from the gall bladder of pigs euthanized with an overdose of pentobarbital sodium followed by exsanguination 3 h after a meal consisting of a refined wheat bread diet (n = 8) containing 0.01 g alkylresorcinols, or a whole-grain rye bread diet (n = 8) containing 1.45 g alkylresorcinols. The pig experiments were conducted according to protocols approved by the Danish Animal Experiments Inspectorate and complied with the guidelines of the Danish Ministry of Justice concerning animal experimentation and care of animals under study (25,26).

Analysis of alkylresorcinol metabolites in human plasma. Human plasma samples, previously analyzed for alkylresorcinol concentration, were thawed at room temperature after storage at −80°C. Fifteen microliters of internal standard solution [containing syringic acid (1 mg/L) and alkylresorcinol C20:0 (1 mg/L) in methanol] and 200 μL deconjugation solution (0.1 mol/L sodium acetate buffer, pH 5; containing 7 μL β-glucuronidase and additional sulfatase activity) were added to 200 μL plasma. After incubation overnight at 37°C, 130 μL concentrated acetic acid was added to the samples. The alkylresorcinol metabolites and internal standard were extracted 3 times with 5 mL diethyl ether, and the extracts were pooled after evaporation under a stream of nitrogen. Solid-phase extraction (SPE) and silylation were performed as previously described (23), and the samples were then transferred to GC vials for analysis by GC-MS, as also previously described (9). Samples from the kinetic study were analyzed in duplicate and quality control samples (n = 4) were included in each batch. In total, 6 batches were analyzed and the within- and between-batch CV was found to be <15% for both metabolites. Samples for which the difference between duplicates was >15% of the mean were reanalyzed. Alkylresorcinol metabolites in plasma samples from the dose-response study were determined in single samples analyzed in 8 batches. Quality control samples (n = 4) were included in each batch, and the within- and between-batch CV was found to be 12% and 15%, respectively (for both metabolites).

Analysis of alkylresorcinol metabolites in ileostomal effluent. An existing GC-MS method for quantification of DHBA and DHPPA in urine (16) was modified to determine the content of alkylresorcinol metabolites in the effluent collected, which was previously analyzed for alkylresorcinol content (24). To determine the sum of free and conjugated metabolites, 300 mL syringic acid (in methanol, 9 mg/mL) was added to 0.15 g frozen and dry ileostomal effluent (stored at −70°C), and the samples were dried to remove methanol and then incubated overnight at 37°C with 2 mL deconjugation solution as described above.

10 Abbreviations used: DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-[3,5-dihydroxyphenyl]-propanoic acid; EC, enterohepatic circulation; HFD, high-fiber diet; LFD, low-fiber diet; SPE, solid-phase extraction.
Prior to extraction, samples were acidified with 50 μL concentrated hydrochloric acid. Samples were extracted with 6 mL diethyl ether, evaporated, and reconstituted in 2 mL methanol. After being mixed on a vortex briefly, samples were sonicated for 5 min, filtered, and evaporated to dryness. This was repeated twice, after which samples were purified by SPE as previously described (23). Samples purified by SPE were evaporated to complete dryness under a stream of nitrogen, dissolved in 200 μL quick silylation mixture, and incubated for 1 h at 60°C. Samples were analyzed by GC-MS as previously described (16). Recovery of the ingested dose as DHBA, DHP, and their sum was calculated by dividing the metabolite content in 24-h ileostomal content by the daily alkylresorcinol intake. Similarly, recovery of ileal-digested alkylresorcinols as ileostomal metabolites was calculated by dividing the metabolite content by the amount of ileal-digested alkylresorcinols (defined as the difference between the ingested amount and the amount of intact alkylresorcinols quantified in 24-h effluent).

Analysis of alkylresorcinols in pig lymph and plasma. Plasma and lymph samples were analyzed for alkylresorcinols by a modified GC-MS method (27). For plasma, 2 protocols differing only in sample volume (0.2 mL or 0.5 mL) were tested. Similarly, 3 different volumes (5 μL, 50 μL, and 100 μL) of each lymph sample were analyzed for alkylresorcinols. After the addition of 1 ng internal standard C20:0, plasma and lymph samples were incubated overnight with 0.5 mL H2O at 37°C. Extractions, purification, silylation, and GC-MS analysis were performed as previously described (23). All samples were analyzed in duplicate. The CV of a human plasma sample analyzed for quality control (n = 3) was ≤ 17% for the different alkylresorcinol homologs. No differences in alkylresorcinol concentration due to sample volume were observed in pig lymph or plasma samples. Therefore, all available replicates of plasma (n = 1–4) and lymph (n = 2–6) were used to calculate the mean concentration for each time point.

Analysis of intact alkylresorcinols and their metabolites in pig bile. Bile samples (1.2 mL) were mixed with internal standards (25 ng C20:0 and 290 ng syringic acid) and 3 mL deconjugation solution (as described above), or 3 mL 0.1 mol/L sodium acetate (pH 5.0) was added. Samples were incubated overnight at 37°C. Extraction, purification, derivatization, and analysis were performed according to a previously published protocol for quantification of alkylresorcinol metabolites in human urine (16). Samples were analyzed in duplicate in 1 batch.

Pharmacokinetic calculations and modeling. Log-transformed plasma concentrations were used for pharmacokinetic modeling. Initially, a previously presented 1-compartment population pharmacokinetic model for plasma alkylresorcinols (20) was used for development of an alkylresorcinol model including the 2 metabolites DHBA and DHP. Several different model structures were evaluated, including models with simple and multiple absorption compartments, models where metabolite formation explicitly came from absorbed alkylresorcinols (in a central compartment) or metabolite formation in a precentral compartment, and elimination pathways where fractions of eliminated alkylresorcinols returned as metabolites in plasma. Additive, proportional, and residual error models were evaluated, as well as combinations thereof. Distribution volumes of the alkylresorcinol metabolites DHBA and DHP were estimated by allometric scaling (28) of estimates determined in a previously published study using rats (9). As previously described (9), model selection was based on objective function values, visual inspection of goodness-of-fit plots, and reasonability of parameter estimates. The selected model was validated with data from plasma samples collected from subjects after 1-wk consumption of alkylresorcinols at 3 different doses (20). Validation of the model’s ability to predict concentrations of alkylresorcinols, DHBA, and DHP was performed separately for the 3 different compounds by using Bland-Altman plots (29). Pharmacokinetic models were constructed and validated by using NONMEM M7 (ICON) executed through Perl-speaks-NONMEM (30), and model diagnostics were performed with Xpose 4 (31).

Statistical analysis. Values are expressed as means ± SEMs unless otherwise stated. Skewed variables were transformed (natural logarithm) before statistical analysis. The AUC (0–24 h) was calculated by the trapezoidal method. Paired t tests were performed to investigate differences in AUC, maximum plasma concentration, and the time taken to reach maximum concentrations between alkylresorcinols, DHBA, and DHP. Differences in plasma concentrations between doses were tested by using mixed linear models with subject ID as random effect, whereas dose and occasion were fixed effects. Differences between the individual doses were further tested by using paired t tests. Paired t tests were also used to test differences in DHPA concentration and proportion of conjugates in bile. All statistical analyses were performed by using Stata 11.2 for Windows (StataCorp), and P < 0.05 was considered significant.

Results

Alkylresorcinol metabolites in human plasma. The time-concentration profile of alkylresorcinols in plasma following a single dose of rye bran has previously been described in detail (8). After the 1-wk run-in period prior to the single dose of rye bran, small concentrations (<60 nmol/L) of DHBA and DHP were detected in plasma. One subject reported unintentional alkylresorcinol intake during the run-in period, and the baseline concentrations of that participant were 80 nmol/L (DHBA) and 43 nmol/L (DHP). The AUC (0–24 h) of DHBA (8.2 ± 1.7 μmol · h · L−1) was higher than the AUC of DHP (6.9 ± 1.8 μmol · h · L−1) (P < 0.05), but still much smaller than the AUC of alkylresorcinols (28 ± 6.0 μmol · h · L−1) (P < 0.001). The plasma concentration-time curves of alkylresorcinol metabolites exhibited different patterns from alkylresorcinols (Fig. 1). For alkylresorcinols, 2 distinct peak concentrations were observed in all subjects, and the second peak concentration was almost 3-fold higher than the first (8). The concentration curves of DHBA and DHP differed between subjects, although in general a dominant peak concentration at 4–6 h, accompanied by a shoulder or a second smaller peak, was observed. The maximum concentration of DHPA (660 ± 77 nmol/L) was higher (P < 0.01) than that of DHPA (485 ± 68 nmol/L). However, DHPA was more abundant in plasma samples taken 24 h after consumption (P < 0.05).

Alkylresorcinol concentrations in samples used for model validation have previously been described in detail (20). In these
Table 2

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Low dose</th>
<th>Medium dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assigned daily alkylresorcinol intake, µmol/d</td>
<td>85</td>
<td>170</td>
<td>342</td>
</tr>
<tr>
<td>Plasma alkylresorcinols, nmol/l</td>
<td>142 (115, 176)</td>
<td>201 (158, 259)</td>
<td>421 (354, 501)</td>
</tr>
<tr>
<td>Plasma DHPPA, nmol/l</td>
<td>67 (68, 77)</td>
<td>122 (98, 152)</td>
<td>214 (182, 253)</td>
</tr>
<tr>
<td>Plasma DHBA, nmol/l</td>
<td>69 (68, 82)</td>
<td>134 (94, 190)</td>
<td>214 (188, 271)</td>
</tr>
</tbody>
</table>

1 Values are geometric means (95% CI), n = 13–15 (see Samples). Values within columns with different superscript letters differ significantly, P < 0.05. DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-propanoic acid.

2 Alkylresorcinol intake and plasma alkylresorcinol concentrations were previously reported (20).

plasma samples, the concentration of DHBA and DHPPA increased significantly (P < 0.001) with increasing doses (Table 1). At the medium dose (2 × low dose), the concentrations of DHBA and DHPPA increased by 88% (95% CI: 36%, 161%) and 85% (95% CI: 43%, 138%), respectively, from the concentration at the low dose, whereas at the high dose (4 × low dose) the metabolite concentrations were ~3-fold those of the low dose.

Pharmacokinetic modeling. The model best describing plasma concentrations of alkylresorcinols, DHPPA, and DHBA after a single dose of rye bran contained 2 absorption compartments (Supplemental Fig. 1, Fig. 2). In this model, the baseline concentrations of total alkylresorcinols, DHPPA, and DHBA after a 1-wk wash-out period were 24 ± 4, 40 ± 6, and 33 ± 12 nmol/L, respectively (Table 2). Absorption was initiated through the first absorption compartment 22 ± 3 min after consumption and was completed (>95%) 18 h later. Alkylresorcinols absorbed through this first absorption compartment were transferred to a precentral compartment (Fig. 2, Table 2), from which the alkylresorcinols reached the systemic circulation, or as DHPPA and DHBA. Another absorption started 4.6 ± 0.2 h after consumption and was completed (>95%) 3 h later. From this second absorption compartment, alkylresorcinols were directly transferred to the systemic circulation, and this absorption was more rapid than the transfer of alkylresorcinols from the first absorption compartment to the central compartment. Approximately 50% of alkylresorcinols in plasma were absorbed via the second absorption compartment, whereas approximately one-third of all alkylresorcinols absorbed from the gastrointestinal tract reached the systemic circulation only after transformation to DHBA or DHPPA.

Alkylresorcinols eliminated from the central compartment were transferred to a 10-unit series of transformation compartments. The mean transit time of the transformation compartments was 3.3 ± 2.1 h, after which DHPPA and DHBA were released to the systemic circulation. Unlike metabolite transfer from the precentral compartment, the transfer rate constant of DHPPA from the transformation compartments was higher than that of DHBA. The terminal elimination half-life of alkylresorcinols, DHPPA, and DHBA was 1.9 ± 0.6, 1.5 ± 0.2, and 1.3 ± 0.2 h, respectively.

The model predictions of alkylresorcinol concentration following 1-wk alkylresorcinol consumption were comparable to predictions generated by the original model (20) (Supplemental Fig. 2A). The model satisfyingly predicted DHBA concentrations (Supplemental Fig. 2B) but overpredicted DHPPA concentrations (Supplemental Fig. 2C).

Alkylresorcinol metabolites in human ileostomal effluent. During the HFD, daily ileostomal excretion of DHBA and DHPPA was 23 ± 6.6 µmol/d and 91 ± 7.7 µmol/d, respectively. Ileostomal recovery of ingested alkylresorcinols, expressed as alkylresorcinol metabolites during the HFD, was 3–6% and 15–22% for DHBA and DHPPA, respectively. Daily excretion of DHPPA during the LFD was 8.5 ± 7.7 µmol/d, whereas DHBA was only detectable, in small amounts, in 1 of the collections analyzed. In contrast to previously reported results (from GC–flame ionization detection analysis), the ileostomal collection bags from the LFD contained small amounts of intact alkylresorcinols with an alkylresorcinol homolog profile typical for wheat when analyzed by GC-MS in the present study. The alkylresorcinol content and composition in ileostomal effluent after the HFD have been described previously (24).

Alkylresorcinols and their metabolites in pigs. Baseline alkylresorcinol concentrations in lymph and plasma were 5.2 ± 2.4 µmol/L and 56 ± 13 nmol/L, respectively. Maximum alkylresorcinol concentration (511 ± 73 nmol/L) was reached after 5 h, and a second peak (265 ± 37 nmol/L) appeared ~10 h after the first peak (Supplemental Fig. 3). The greatest rate of
lymphatic alkylresorcinol absorption (6.00 ± 0.75 μmol/h) was measured after 8 h, and at 16 h after dose administration the rate had decreased to 0.47 ± 0.05 μmol/h (Supplemental Fig. 3).

The biliary concentration of DHPPA in pigs after the wheat diet [18 nmol/L (95% CI: 14, 23)] was 15% (P < 0.001) of the concentration after the rye diet [117 nmol/L (95% CI: 78, 175)] (Supplemental Fig. 4), whereas the alkylresorcinol content of the wheat diet was 1% that of the rye diet. The proportion of conjugated DHPPA was also higher (P < 0.01) after rye consumption, when only 21% (95% CI: 9%, 51%) of DHPPA was present as free aglycone, whereas the proportion of free DHPPA after the wheat-bread diet was 80% (95% CI: 59%, 108%). Analytic complications caused by coelution of interfering compounds prevented reliable quantification of biliary DHBA. Alkylresorcinol homologs C19:0 and C21:0 were tentatively identified in bile at low intensities, although only after incubation with deconjugating enzymes.

Discussion

This is the first study to present a pharmacokinetic model that simultaneously describes human plasma concentrations of alkylresorcinols and their 2 major metabolites, DHBA and DHPPA, after a single dose of alkylresorcinols. It is also the first study to demonstrate the presence of alkylresorcinol metabolites in human ileal effluent and in pig bile, suggesting that alkylresorcinol metabolites are formed in the intestine and/or excreted in bile.

Although participants abstained from consuming whole-grain wheat and rye for 1 wk prior to the single dose of rye bran, small concentrations of alkylresorcinols, DHBA, and DHPPA were measured in their fasting plasma samples. As previously suggested, these concentrations most likely arise from consumption of refined cereal products containing minor amounts of alkylresorcinols, accidental whole-grain consumption, or liberation of alkylresorcinols from stored body pools.

The plasma concentration-time profiles in this study differed from those reported in previous animal studies (6,9). Consequently, the model developed for humans displayed a different structure from that previously developed for rats. This is most likely due to study differences regarding designs and species. For example, in the present study the pharmacokinetics of DHBA and DHPPA was estimated after ingestion of rye bran, whereas in the rat study they were estimated after oral administration of pure alkylresorcinols in corn oil, which can make parameter estimates less reliable. The participants in the present study consumed rye bran containing not only a mixture of alkylresorcinol homologs, but also comparatively lower amounts of alkenylresorcinols (8).

The 2 peaks in the alkylresorcinol concentration-time curve and the 2 absorption compartments in the model developed

\[
\text{\textbf{TABLE 2}} \quad \text{Pharmacokinetic parameter estimates (β) and RSE of alkylresorcinols and their metabolites estimated from plasma concentrations of 3 women and 2 men consuming a single dose of 120 g rye bran (containing 495 μmol alkylresorcinols)}^{1}
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alkylresorcinols</th>
<th>DHPPA</th>
<th>DHBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>RSE</td>
<td>β</td>
<td>RSE</td>
</tr>
<tr>
<td>C₀, nmol/L</td>
<td>24</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>F₁</td>
<td>70</td>
<td>8.0</td>
<td>—</td>
</tr>
<tr>
<td>t¹/₂a₁</td>
<td>0.36</td>
<td>14</td>
<td>—</td>
</tr>
<tr>
<td>t¹/₂a₂</td>
<td>4.6</td>
<td>4.8</td>
<td>—</td>
</tr>
<tr>
<td>k₁a₁, h⁻¹</td>
<td>0.16</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>k₁a₂, h⁻¹</td>
<td>1.1</td>
<td>54</td>
<td>—</td>
</tr>
<tr>
<td>t₁/₂a₁, h</td>
<td>4.4</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>t₁/₂a₂, h</td>
<td>0.62</td>
<td>54</td>
<td>—</td>
</tr>
<tr>
<td>k₁o₁, h⁻¹</td>
<td>0.36</td>
<td>27</td>
<td>0.14</td>
</tr>
<tr>
<td>CL/F, L/h</td>
<td>11</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>V/F, L</td>
<td>30</td>
<td>25</td>
<td>39²</td>
</tr>
<tr>
<td>k₂, h⁻¹</td>
<td>0.37</td>
<td>31</td>
<td>0.47</td>
</tr>
<tr>
<td>t₁/₂k₂, h</td>
<td>1.9</td>
<td>31</td>
<td>1.5</td>
</tr>
<tr>
<td>MTT, h</td>
<td>3.3</td>
<td>63</td>
<td>—</td>
</tr>
<tr>
<td>k₁o₂, h⁻¹</td>
<td>—</td>
<td>0.016</td>
<td>64</td>
</tr>
<tr>
<td>E₁, %</td>
<td>41</td>
<td>13</td>
<td>27</td>
</tr>
</tbody>
</table>

1 C₀, baseline concentration; CL/F, apparent clearance; DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3,6-dihydroxyphenylpropanoic acid; E₁, proportional residual error; F₁, proportion of total absorption absorbed through first compartment; k₁a₁, absorption rate constant from first compartment; k₁a₂, absorption rate constant from second compartment; k₂, elimination rate constant; k₁o₁, rate constant from postabsorption compartment to central compartment; k₁o₂, rate constant of the alkylresorcinol metabolites from transformation compartments; t¹/₂a₁, lag time of the absorption from first compartment; t¹/₂a₂, lag time of the absorption from second compartment; MTT, mean transit time in transformation compartments; RSE, relative standard error; t₁/₂a₁, absorption half-life from first compartment; t₁/₂a₂, absorption half-life from second compartment; t₁/₂k₂, elimination half-life; V/F, apparent volume of distribution.

2 Calculated by allometric scaling of distribution volumes estimated in rats (9).
Absorption from the second absorption compartment could represent lymphatic absorption due to the later initiation and the absence of apparent metabolism. The earlier onset of the first absorption pathway and the presence of a precentral compartment in which a large fraction of absorbed alkylresorci-nols is apparently metabolized before reaching the site of measurement could indicate absorption through the portal vein and first-pass metabolism (Fig. 3). Portal vein absorption has been observed for other compounds that are predominantly lymphatically absorbed, including cholesterol and fat-soluble vitamins (32). In the present study, the model developed indicated that such first-pass metabolism was rather extensive, with at least one-third of all alkylresorcinols absorbed being metabolized to DHBA and DHPPA prior to entering the systemic circulation. However, the model could have underestimated first-pass metabolism because some alkylresorcinol metabolites might have been excreted to the intestine and thus never reached the systemic circulation (Fig. 3). Previously, we suggested that pharmacokinetic differences between alkylresorcinol homologs observed in rats could partly be attributed to diverse absorption routes (9). The suggested alkylresorcinol absorption via the lymphatic system is supported by findings of similar alkylresorcinol concentrations in portal venous and arterial blood of pigs fed rye bread (6,7). However, although the alkylresorcinols taken up by the lymph may be underestimated because of incomplete lymph collection, the scarceness of alkylresorcinols in pig lymph analyzed in the present study (<2% of ingested dose) raises questions regarding the proposed pathway, and further research is needed to investigate the mechanisms of alkylresorcinol absorption.

The elimination half-life of alkylresorcinols in the model developed here was shorter than that in the previously published model (20). However, except for the few hours during which the second absorption route was active, the elimination of alkylresorcinols in the present study was limited by the rates of absorption and transfer from the pre-central compartment, while in the previous study absorption was not rate-limiting. Similarly, concentrations of alkylresorcinol metabolites were determined by their formation rate, as observed previously in rats (9). Similar elimination rates of alkylresorcinols and their metabolites contradict previous findings suggesting that plasma alkylresorcinol metabolites are advantageous as biomarkers because of slower elimination compared with intact alkylresorcinols (21,22). The half-life values reported in those studies were calculated based on the apparent elimination rates of the metabolites, and the possibility of rate-limited formation was not taken into account. Although alkylresorcinol metabolites may have a longer residence time in plasma than intact alkylresorcinols, their concentrations are determined by factors other than alkylresorcinol intake. However, urinary alkylresorcinol metabolites could act as useful biomarkers of whole-grain wheat and rye intake because of the cumulative nature of urine collections. This was verified in recent studies in which the relative validity of plasma alkylresorcinols and urinary alkylresorcinol metabolites was similar (33,34).

Previously, the ileal digestibility of intact alkylresorcinols was determined to be about 60%. In the present study, 40% of ileal-digested alkylresorcinols were recovered as alkylresorcinol metabolites in the ileal effluent. Similarly, DHBA and DHPPA were tentatively identified in small intestinal effluent and in feces of pigs fed a rye diet, and both metabolites appeared to be conjugated to some extent (data not shown). The presence of alkylresorcinol metabolites in ileal effluent suggests that alkylresorcinols are metabolized in the small intestine and/or that alkylresorcinol metabolites are excreted in the bile (Fig. 3). The latter is supported by the finding of DHPPA in bile from pigs after both rye- and wheat-based diets. The inverse relation between alkylresorcinol dose and urinary recovery of alkylresorcinol metabolites has been hypothetically attributed to a dose-dependent increase in biliary alkylresorcinol metabolite excretion (20). In fact, biliary concentration of DHPPA in pigs increased with alkylresorcinol dose, as did the proportion of DHPPA excreted as conjugates. Although alkylresorcinols in the small intestine and feces of pigs did not appear to be conjugated, conjugated alkylresorcinols were tentatively identified in pig bile, indicating a novel, yet minor, pathway of alkylresorcinol elimination. However, it is possible that alkylresorcinol metabolites in the collection bags of ileostomy patients were to some extent the result of microbiologic degradation. A recent metabolomics study proposed that phenolic compounds identified in urine after rye consumption were potential products of hepatic alkylresorcinol metabolism and gut microbiota transformation (35), but the potential role of the microbiota on alkylresorcinol metabolism needs to be confirmed. Further studies are necessary to elucidate the extent of gut wall elimination and biliary excretion of alkylresorcinols and their metabolites in humans.

The presence of DHPPA in human ileal effluent and pig bile indicates that DHPPA excreted in bile and/or formed in the intestine is available for absorption (Fig. 3). Furthermore, the existence of multiple local maxima in plasma DHPPA concentration observed for some subjects in the present study is comparable to urinary excretion rates previously reported (22) and provides additional indication of EC. The extended duration of eliminated plasma alkylresorcinols returning to plasma as metabolites from the transformation compartments further supports this hypothesis, especially because DHPPA was liberated to a higher extent from the transformation compartments than DHBA, unlike the release from the precentral compartment. However, the design of the present study prevented optimal modeling of such circulation, which could instead have interfered with the estimation of DHPPA clearance. Hence, the actual elimination kinetics of DHPPA might be faster than represented here. More rapid elimination compared with DHBA could explain why DHPPA is the major alkylresorcinol metabolite in urine (20,34) and ileal effluent, despite DHBA being predominant in plasma. Poorly modeled absorption and EC of DHPPA could also have contributed to overprediction in the validation dataset.

In conclusion, a combined pharmacokinetic model of alkylresorcinols and their main metabolites in human plasma was constructed from single-dose data and validated with data from a feeding trial. The model indicated 2 separate absorption pathways, one of which included first-pass metabolism. Elimination rates of alkylresorcinols and their major metabolites were comparable, and plasma concentrations of alkylresorcinols and their metabolites were not determined by their elimination, but by the rate of absorption and formation, respectively. The model achieved good validity in predicting plasma concentrations of alkylresorcinols and DHBA after repeated alkylresorcinol intake, whereas DHPPA concentrations were overpredicted, possibly because of poorly modeled EC. Ileal effluent contained extensive amounts of alkylresorcinol metabolites, predominantly DHPPA, indicating the importance of enterohepatic alkylresorcinol metabolite elimination. This was further supported by the presence of DHPPA, mainly in conjugated form, in bile from pigs. More research is needed to evaluate and quantify the role of intestinal metabolism in the pharmacokinetics of alkylresorcinols and their metabolites.
Acknowledgments
The authors thank Maria Karlsson and Sanaz Hassanazadeh for analysis of plasma alkylresorcinol metabolites and Juana Seira Ibáñez for her work with the ileostomy samples. M.M., E.A.S., and R.L. designed the research; H.N.L. conducted the experiments; K.E.B.K. provided the pigs’ facilities; R.L. was responsible for the chemical analyses; M.M. performed the statistical analysis; E.A.S. performed the pharmacokinetic modeling under the supervision of A.C.H.; M.M., E.A.S., A.K.-E., and R.L. interpreted the data; and M.M. wrote the paper and had primary responsibility for the final content. All authors read and approved the final manuscript.

References
Modeling of alkylresorcinols and metabolites 7 of 7