Dose response of whole-grain biomarkers: alkylresorcinols in human plasma and their metabolites in urine in relation to intake[1–3]

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ABSTRACT

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

Objective: The objective was to assess the dose response of plasma ARs and the excretion of 2 recently discovered AR metabolites in 24-h urine samples in relation to AR intake and to establish a pharmacokinetic model for predicting plasma AR concentration.

Design: Sixteen subjects were given rye bran flakes containing 11, 22, or 44 mg total ARs 3 times daily during week-long intervention periods separated by 1-wk washout periods in a nonblinded randomized crossover design. Blood samples were collected at baseline, after the 1-wk run-in period, and after each treatment and washout period. Two 24-h urine samples were collected at baseline and after each treatment period.

Results: Plasma AR concentrations and daily excretion of 2 urinary AR metabolites increased with increasing AR dose ($P < 0.001$). Recovery of urinary metabolites in 24-h samples decreased with increasing doses from ≈90% to ≈45% in the range tested. A one-compartment model with 2 absorption compartments with different lag times and absorption rate constants adequately predicted plasma AR concentrations at the end of each intervention period.

Conclusion: Both plasma AR concentrations and urinary metabolites in 24-h samples showed a dose-response relation to increased AR intake, which strongly supports the hypothesis that ARs and their metabolites may be useful as biomarkers of whole-grain wheat and rye intakes. *Am J Clin Nutr* 2009;89:290–6.

INTRODUCTION

Whole-grain cereal foods are reported to have several positive effects on human health and intake has been linked to a reduced risk of several chronic diseases (1–4). The protection mechanisms involved remain largely unknown, but several components within the dietary fiber complex are believed to interact (5). A major obstacle in nutritional epidemiology, on which most of the protective evidence is based, is the relatively low accuracy of measuring intake of foods and nutrients (6), which could weaken the diet-disease association. Assessing whole-grain intake is further complicated by the fact that consumers may have difficulty in distinguishing between products containing and those not containing whole grains. Furthermore, different definitions of whole grain have been used in different studies (7). The use of a valid biomarker for whole-grain intake would overcome some of the problems associated with dietary assessment methods and could complement and validate these methods (6, 8–10). A group of phenolic lipids, alkylresorcinols (ARs), seems to fit the most important criteria for biomarkers of whole-grain wheat and rye intakes (8–10).

ARs are amphiphilic 1,3-dihydroxy-5-alkylbenzene homologs with odd-numbered alkyl side chains containing from C17:0 to C25:0 that are found in the outer parts of wheat and rye contained in commonly consumed foods (11–13). ARs are absorbed in the small intestine and transported via the lymphatic system to the blood, where the major fraction is found in different lipoproteins and erythrocyte membranes (12, 14, 15). Pharmacokinetic studies of ARs in humans have shown a rather short half-life (≈5 h) for all AR homologs, which suggests that plasma AR concentrations reflect short- to medium-term intakes (16). Recent studies have shown that plasma AR concentrations reflect and rapidly respond to changes in intake (8, 9, 15, 16).

Hepatic cytochrome P450-dependent metabolism followed by urinary excretion of 2 main metabolites, 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA), has been suggested as the main route of AR elimination (10). A method relying on HPLC coupled to a coulometric electrode array detector (HPLC-CEAD) was developed recently to quantify 2 AR metabolites, and it has been suggested that these metabolites in 24-h urine samples could be used as biomarkers of whole-grain wheat and rye intakes (17). No study has yet compared urinary AR metabolite excretion in relation to AR

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intake or plasma AR concentration. AR metabolites in 24-h urine samples may provide a complement to plasma AR as biomarkers of whole-grain intake, especially because the intrasubject variation is generally less for recovery biomarkers than for concentration biomarkers (18, 19).

The present study investigated the responses of plasma AR concentrations and urinary AR metabolite concentrations after 3 different AR intakes and sought to identify the most suitable pharmacokinetic model to describe plasma AR concentrations.

SUBJECTS AND METHODS

Study design and subjects

Seventeen healthy volunteers (8 men and 9 women) were recruited after being provided with written and oral information about the study. The mean (±SD) age of the subjects was 30.6 ± 10.3 y, and the mean body mass index (BMI; in kg/m^2) was 23 ± 3.3. The entire study protocol was approved by the local ethics committee for the Uppsala region (Sweden), and recruitment started in September 2006. The study was conducted as a randomized, nonblinded, 3-way crossover design with 1-wk treatment periods separated by 1-wk washout periods. One week before the study began, a baseline sample (plasma and two 24-h urine samples) was collected and thereafter all participants were asked to avoid intake of whole grains and bran of wheat and rye (run-in). After the run-in, subjects were randomly assigned to receive 1 of the 3 doses of ARs and were instructed to take each dose 3 times a day (at the same time in the morning, afternoon, and evening) during the 1-wk intervention periods to reach a steady state concentration. To enable pharmacokinetic predictions and to monitor compliance, each subject was asked to note the exact time of intake on the datasheet provided. During the entire study, no whole grain or bran products other than the bran flakes provided were allowed. Subjects were asked to separately collect all their urine during days 6 and 7 of the baseline and treatment periods. Fasting blood samples were drawn at the clinic in the morning, at baseline, and after the treatment and washout periods.

Diet

Subjects were instructed to adhere to their habitual diet but to avoid intake of whole grains and bran products of wheat and rye. To facilitate compliance, subjects were provided with a list of refined products to replace their normal cereal intake. Subjects were allowed to consume products other than those on the list if they were confident that such products did not contain any whole grain or bran of wheat and rye. During the treatment periods, subjects were asked to include a portion of a rye bran flake product provided (7.5, 15, or 30 g) into their diet 3 times/d and to record the time of intake and the food that accompanied the intake. If a dose was forgotten, the subjects were instructed to take a double dose the next time. Rye bran flakes in amounts providing a daily intake of 33, 66, and 132 mg ARs were provided in plastic bags labeled with the day and number. The main nutrient composition of the bran flakes was reported previously (16).

Sample treatment

Venous blood samples (20 mL) were drawn into EDTA-coated tubes and immediately centrifuged in a Rotina 48R centrifuge (Hettich-Zentrifugen, Tuttlingen, Germany) for 10 min at 4°C (2000 × *g*) to separate plasma and erythrocytes. Plasma samples were then portioned into 2-mL cryotubes and stored at −80°C until analyzed.

Urine was collected in two 24-h periods at the end of the run-in period and at the end of each treatment period in UriSet 24 containers (Sarstedt, Nümbrecht, Germany). No urine was collected after the washout periods to retain good compliance throughout the study. Subjects were instructed to stabilize the collected urine by adding 9 mL 20% hydrochloric acid, which was supplied together with the UriSet 24 containers. Urine was stored at room temperature during collection and brought to the clinic no later than 2 d after sampling. Total urine volume was measured, and two 30-mL subsamples were stored at −80°C until analyzed.

Analytical methods

Rye bran flakes were analyzed for total AR content and the relative homolog composition with a gas chromatographic (GC) method (20). In brief, bran flake samples were milled and extracted with a hot 1-propanol:water mixture (3:1, by vol) and analyzed by GC without purification or silylation. Samples were analyzed in triplicate and quantified by using methyl behenate (22:0, fatty acid methyl ester; Larodan Fine Chemicals AB, Malmö, Sweden) as internal standard. Results are expressed as a mean of triplicate analyses and reported on a fresh weight basis.

Plasma samples were analyzed according to Linko et al (21), except for a minor change in the quantification procedure. In brief, plasma samples (0.5 mL) were incubated with water (0.5 mL) and internal standard (AR homolog 20:0, 10 ng; ReseaChem LifeScience, Burgdorf, Switzerland) overnight at 37°C. Samples were then extracted with diethyl ether (3 × 3 mL) and the extracts were combined, evaporated to dryness, and dissolved in 0.5 mL methanol. ARs were separated from neutral lipids on a diethyl-amino-ethyl (DEAE)-Sephadex A-25 ion exchange gel (GE Healthcare, Uppsala, Sweden) in free base form and packed into Pasteur pipettes to a final height of 1.5 cm. Columns were washed with methanol (6 mL) to elute neutral compounds, and ARs were eluted with acidified methanol (6 mL, 0.1 mol acetic acid/L in MeOH). Eluted ARs were evaporated to dryness under nitrogen steam, silylated, and analyzed by GC-mass spectrometry (MS) at a single ion monitoring mode with the same instrument conditions as described previously (8). Molecular ions of the AR homologs 17:0–25:0 were monitored, as was the base ion for all AR homologs (*m/z*: 268). Quantification was performed by using multipoint calibration curves included in each batch (n = 6–8, 5–500 pg/mL) of each AR homolog (17:0–25:0) (ReseaChem LifeScience). All homologs were quantified by using the base ion (*m/z*: 268), except for 19:0, for which the molecular ion (*m/z*: 520) was used because of interference of the base ion with an unknown co-eluting peak.

Urinary AR metabolites were analyzed according to protocol A of an HPLC-coulometric electrode array detection method described recently (17). In brief, urine (100 μL) and internal standard (305 ng syringic acid; Sigma-Aldrich, St Louis, MO) were incubated overnight with deconjugation solution (100 μL) containing sodium-acetate buffer (0.1 mol/L, pH 5), β-glucuronidase (0.2 kU/L), and sulfatase (2 kU/L). An aliquot of 50 μL (equal to 25 μL urine) was mixed with methanol (50 μL) and HPLC mobile phase (50 μL, 20% phase B/80% phase A). The

mobile phase composition was 50 mmol/L phosphate buffer, pH 2.3/methanol (90/10, vol:vol; phase A) and 50 mmol/L phosphate buffer, pH 2.3/methanol/acetonitrile (40/40/20, vol:vol:vol; phase B). External multipoint calibration ($n = 8$; 10–670 ng/mL) was used for both DHBA and DHPPA. All samples were analyzed as single samples in one batch, with control samples at 3 different levels included at the start and end of the batch. The within-day precision, expressed as the CV for both metabolites, was <5% at all levels. The between-day variation for the method was ≈10% for both metabolites at all levels (17). As a rough check of compliance with the 24-h urine collection, creatinine in urine was analyzed in all urine samples as single measurements in a Konelab 20 Clinical Chemistry Analyzer (Thermo Electron Corporation, Vantaa, Finland).

Pharmacokinetic modeling

On the basis of the existing concentration-time data on total ARs after a single-dose administration in 6 healthy volunteers (16), a population pharmacokinetic model was built by using nonlinear mixed-effects modeling, in which data from all subjects were modeled simultaneously (22, 23). With a population approach, the mean tendencies in the population (ie, typical values), the variability between subjects, and the residual variability can be described. Log-transformed data were used in modeling. One- and 2-compartment models with zero-order or first-order absorption rate processes were evaluated to fit the data. To explain the 2 peaks observed in the plasma-concentration time profiles (16), absorption was assumed to occur from 2 different compartments, with different lag times, and the relative bioavailability from the 2 compartments was estimated. The 2 absorption compartments could be due to different pools in the food matrix, 2 absorption sites in the gastrointestinal tract, differences in gastric emptying rate, etc. Because ARs were present at low concentrations after the washout periods, a baseline concentration was included in the model. Additive, proportional, and combined additive and proportional residual error models were evaluated. Between-subject variability in the model parameters was modeled assuming a log-normal distribution.

On the basis of the model developed for the single-dose data and the doses and recording times used in the present dose-response study, the total AR concentrations at the time of blood sampling were predicted. The agreement between observed and predicted concentrations was investigated with a Bland-Altman plot on which linear regression was applied (24). NONMEM VI (NONMEM Project Group, University of California, San Francisco, CA) with the first-order conditional estimation method was used for model building and simulation (22, 23). Xpose 4 (http://xpose.sourceforge.net) was used for model diagnostics. Model selection was based on the reduction in NONMEM produced by the objective function value, where a reduction of 6.63 approximately corresponds to $P = 0.01$ when one parameter is added.

Statistics

SAS version 9.1 (SAS Institute Inc, Cary, NC) and Minitab version 14.1 (Minitab Inc, State College, PA) were used for the data analysis. Plasma total AR concentrations and urinary DHBA, DHPPA, and creatinine variables were found to be skewed and heteroscedastic (Shapiro-Wilks test, $P < 0.05$) and were therefore log-transformed before statistical analysis. All P values reported were Bonferroni-corrected for multiplicity. Differences in plasma AR concentrations between doses were tested by using a mixed linear model with period and dose as fixed factors and subject as random factor. Plasma AR concentrations after the washout period and before the dose was administered was included as a covariate. No significant differences due to treatment sequence were observed; thus, this factor was omitted in the final models. Dose was set as a class variable because it gave a lower Akaike information criterion (low values are better) than when set as a continuous variable (25). Plasma lipids (HDL, LDL, and triacylglycerols together and separately) and BMI were tested as covariates but were found to have no effect on the results; therefore, they were omitted in the final model. Two-sided t tests were used as post hoc tests. A paired t test was used to compare urinary AR metabolite excretion between the two 24-h collection periods. Differences in mean daily DHBA and DHPPA excretion and in the percentage of excreted DHBA and DHPPA from ingested ARs (recovery) were tested by using mixed linear models with dose and period as fixed factors and subject as a random factor. (Washout period values were lacking and thus could not be included as covariates.)

RESULTS

All subjects completed the study, except for one, who was excluded because of noncompliance with the advised intake (tick-off list) and unsatisfactory urine collections (>100% within-subject CV in creatinine excretion (26)). Blood sampling was completed by all subjects, and 90% of urine collections were reported as completed. Creatinine excretion was analyzed as a rough estimate of compliance with urine collections, and the mean within-personal variability determined as the CV of 24-h samples ($n = 8$) was 17.5% (7.4–36%). Total AR content in rye bran flakes was 1458 μg/g fresh weight, and the relative homolog composition was 20% (17:0), 30% (19:0), 27% (21:0), 12% (23:0), and 11% (25:0). The 17:0/21:0 ratio was thus ≈0.8.

Plasma AR concentration

The mean baseline (habitual) plasma total AR concentration was ≈117 nmol/L (**Table 1**). A highly significant effect of dose was found for all individual AR homologs and for total AR ($P < 0.001$). The plasma concentration returned to ≈60 ± 35 nmol/L after 1 wk of washout (Table 1). The mean within-subject variation in plasma AR concentration estimated from washout periods was found to be ≈30%, determined as the CV. All subjects responded to increased AR intakes with subsequent increases in plasma AR homolog concentrations. The plasma concentration differed significantly ($P < 0.05$) between all doses for all homologs (Table 1). No statistically significant difference was observed for 17:0/21:0 between doses, but there was a trend for an increased ratio with increasing dose that was not statistically significant after Bonferroni correction (Table 1).

Urinary AR metabolites

No statistically significant differences were observed in metabolite excretion between the two 24-h samples at each dose; therefore, mean DHBA, DHPPA, and total metabolite excretions

TABLE 1
Daily bran flake and alkylresorcinol (AR) intakes, plasma AR concentrations, and urinary AR metabolite concentrations before and after low, medium, and high doses in a randomized crossover design[1]

Variable	Baseline	Run-in	Low dose	Washout 1	Medium dose	Washout 2	High dose
Rye bran flakes							
Daily intake (g)	—	—	22.5	—	45	—	90
Daily total AR intake [mg (μmol)]	—	—	33 (85)	—	66 (170)	—	131 (342)
17:0	—	—	6.6 (19)	—	13.2 (38)	—	26.2 (77)
19:0	—	—	9.9 (27)	—	19.8 (54)	—	39.3 (107)
21:0	—	—	8.9 (22)	—	17.8 (44)	—	35.4 (88)
23:0	—	—	4.0 (9)	—	8.0 (18)	—	15.7 (38)
25:0	—	—	3.6 (8)	—	7.2 (16)	—	14.4 (32)
17:0/21:0 ratio	—	—	0.8	—	0.8	—	0.8
Plasma							
Total AR concentration (nmol/L)[2]	117 ± 69 (46–253)[3]	68 ± 33 (23–157)	148 ± 60[a] (80–268)	60 ± 37 (35–178)	210 ± 81[b] (87–346)	65 ± 33 (18–158)	455 ± 189[c] (184–865)
17:0/21:0 ratio	0.31 ± 0.32 (0.10–0.40)	0.22 ± 0.11 (0.08–0.60)	0.35 ± 0.15[a] (0.10–0.77)	0.23 ± 0.22 (0.03–0.75)	0.45 ± 0.31[a] (0.13–0.81)	0.30 ± 0.4 (0.11–0.54)	0.64 ± 0.52[b] (0.10–1.8)
Urine							
DHBA concentration (μmol/L)[4]	24.1 ± 8 (13.2–39.9)	—	21 ± 7[a] (11.2–39.8)	—	35 ± 11[b] (24–70)	—	47.4 ± 16.2[c] (17.6–87.2)
DHPPA concentration (μmol/L)[4]	27.4 ± 12.1 (5.1–57.2)	—	25.9 ± 6.8[a] (12–40.7)	—	43.5 ± 9.2[b] (28.3–60.5)	—	65.5 ± 16.4[c] (36.5–90.5)
Total metabolite concentration (μmol/L)[4]	51.5 ± 19.4 (18.3–97.1)	—	47 ± 12.2[a] (23.2–68.7)	—	78.8 ± 16.5[b] (53.4–114.1)	—	112.9 ± 30.2[c] (54.1–177.8)
DHPPA/DHBA ratio	1.4 ± 0.2 (1.1–1.8)	—	1.4 ± 0.2[a] (0.6–1.8)	—	1.5 ± 0.3[a] (0.9–2.3)	—	1.3 ± 0.5[a] (0.7–2.3)
DHBA excretion (μmol/24 h)[4]	30 ± 8.3 (16.0–43.5)	—	33.4 ± 6.1 (22.6–43.7)	—	54.0 ± 12.0 (33.0–79.4)	—	78.3 ± 25.5 (18.9–118.3)
DHPPA excretion (μmol/24 h)[4]	34.9 ± 14.0 (12.8–66.0)	—	42.6 ± 10.5 (21.7–62.3)	—	68.8 ± 17.9 (33.6–97.6)	—	110.9 ± 35 (40.1–175.4)
Total metabolite excretion (μmol/24 h)[4]	64.9 ± 20.3 (35.5–109.6)	—	76.0 ± 15.0 (51.76–105.9)	—	122.8 ± 25.4 (82.7–154.0)	—	189.3 ± 57.0 (59.0–293.7)
DHBA recovery (% of ingested total AR)[4]	—	—	39.1 ± 7.2[a] (26.4–51.1)	—	20.3 ± 6.6[b] (8.9–30.1)	—	12.1 ± 3.4[c] (3.8–17.3)
DPPA recovery (% of ingested total AR)[4]	—	—	49.9 ± 12.3[a] (25.4–72.9)	—	32.2 ± 7.4[b] (19.6–43.2)	—	25.8 ± 8.7[c] (12.1–51.3)
Total metabolite recovery (% of ingested total AR)[4]	—	—	89.0 ± 17.6[a] (60.6–124.0)	—	58.9 ± 12.9[b] (41.6–86.3)	—	44.6 ± 14.6[c] (17.9–85.9)

[1] Each dose was given 3 times daily during 1-wk periods. DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-propanoic acid. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni-corrected 2-sided t tests).

[2] Significant differences for all AR homologs were attributable to dose in a mixed linear model with AR value before dose included as a covariate (Bonferroni corrected, $P < 0.05$ for all homologs). No significant effect of treatment sequence was found; therefore, this factor was omitted in the final models.

[3] Mean ± SD; range in parentheses (all such values).

[4] Significant differences were attributable to dose (Bonferroni corrected, $P < 0.05$) in a mixed linear model. No significant effect of treatment sequence was found; therefore, this factor was omitted in the final models.

were calculated for each dose. DHBA and DHPPA concentrations in urine samples for all periods were in the ranges of 11–87 and 5–91 μmol/L, respectively, whereas daily excretions from all periods were 18.8–118.2 and 21.7–175.4 μmol/d, respectively (Table 1). All individuals except one responded with an increase in DHBA excretion to increased AR intakes. For DHPPA, excretion increased in all individuals as AR intakes increased. The excretion of both urinary metabolites and their sum increased significantly with dose increases ($P < 0.001$) (Table 1). Significant differences ($P < 0.01$) between the 3 doses were found for both metabolites and for their sum. Recovery of both DHBA and DHPPA decreased significantly with increased AR intake, with mean total metabolite recoveries of 88 ± 18%, 59 ± 12%, and 45 ± 15% for daily AR intakes of 33, 66, and 131 mg, respectively (Table 1). Both DHBA and DHPPA and their sum differed significantly ($P < 0.020$) between all 3 doses. As expected, plasma AR concentrations increased as metabolite excretion increased (**Figure 1**).

Pharmacokinetic modeling

A one-compartment model with 2 absorption compartments with respective lag times of 0.3 and 2.5 h adequately described the single dose data reported elsewhere (16) (**Figure 2** and **Figure 3**). First-order absorption rates were superior to zero-infusion rates. Between-subject variability was not supported by the data in any of the parameters. A proportional model described the residual error. The estimated parameters and their uncertainties are presented in **Table 2**. The calculated half-life was 4.0 h [calculated as ln 2 × (apparent distribution

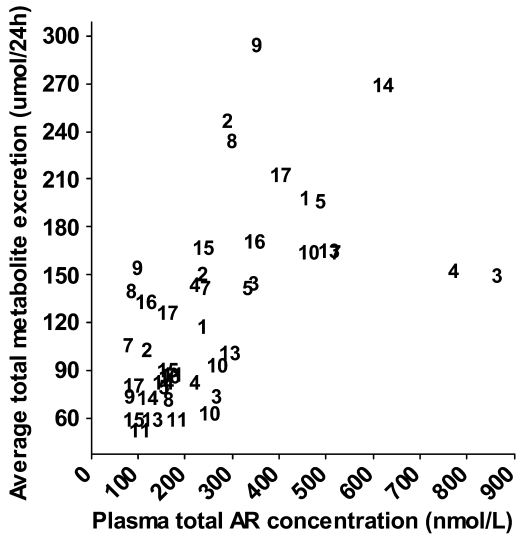

FIGURE 1. Scatter plot of plasma total alkylresorcinol (AR) concentrations after an overnight fast and mean 24-h urinary total metabolite excretion for 16 subjects after 3 different doses ($n = 16 \times 3$). Each subject is indicated by a number.

volume)/(apparent clearance)]. The model was used to predict plasma AR concentrations at the end of each dose period in the present study. Agreement between predicted and observed plasma total AR concentrations was assessed in a Bland-Altman plot. The assessment showed that plasma total AR concentration was overestimated by the model at higher values and underestimated at lower values (**Figure 4**).

DISCUSSION

In this study, plasma AR concentration and urinary AR metabolites were investigated after 3 doses, and predicted plasma AR concentrations were compared with observed data. Both plasma AR concentration and metabolite excretion in 24-h urine samples increased with increasing AR intakes—a fundamental prerequisite for a useful biomarker (27).

Plasma AR concentration

Plasma AR concentrations varied between 19 and 865 nmol/L for the 3 doses tested (33, 66, and 131 mg total ARs/d). The mean

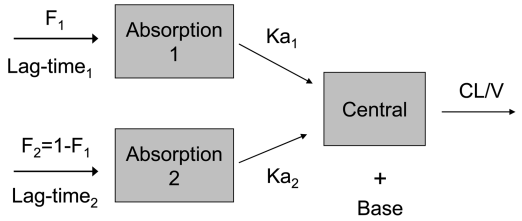

FIGURE 2. Pharmacokinetic model of alkylresorcinols. F_1, relative proportion of the dose absorbed from the first absorption compartment; F_2, relative proportion of the dose absorbed from the second absorption compartment; lag-time$_1$, lag time for start of absorption from first absorption compartment; lag-time$_2$, lag time for start of absorption from second absorption compartment; absorption 1, first absorption compartment; absorption 2, second absorption compartment; Ka_1, absorption rate constant from first absorption compartment; Ka_2, absorption rate constant from second absorption compartment; Central, central compartment; Base, baseline alkylresorcinol concentration (nmol/L); CL/V, clearance/volume of distribution [from apparent clearance $\times$ (apparent distribution volume^{-1})].

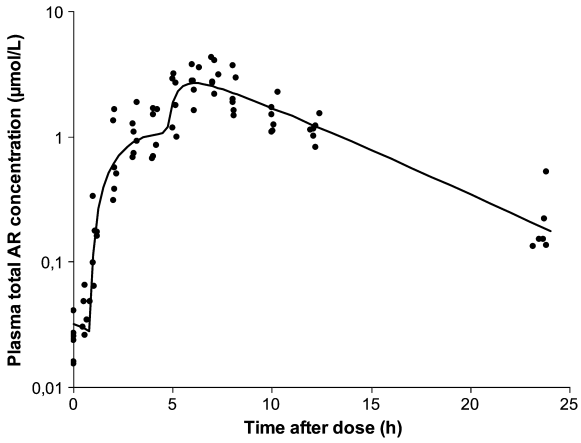

FIGURE 3. Observed concentration-time profile after a single dose of 485 μmol alkylresorcinols (AR) and predicted model fit (solid line). Original data from Landberg et al (16).

baseline total AR concentration (117 nmol/L) was similar to previously reported values (8, 9, 15). Variation in plasma AR concentrations between subjects was considerable, especially at the highest dose. The area under the plasma concentration-time curve and the maximum plasma concentration are usually used as measures of exposure for the assessment of dose proportionality (28). However, here we used single fasting samples after multiple doses at presumed steady state conditions, because such samples are used most commonly in epidemiologic studies. Despite this, a clear dose-response relation was found, probably because of low within-subject variation in AR concentrations, which implies good compliance. Because a statistical model using AR intake as a class rather than as a continuous variable fitted the data better (lower Akaike information criterion value), a linear dose-plasma concentration relation could not be shown, although the relation appeared rather linear for most individuals.

The plasma 17:0/21:0 ratio was in the range 0.06–1.6 throughout the study, and the large between-subject differences observed may have been due to subject-dependent differences in

TABLE 2
Pharmacokinetic parameter estimates and their relative SEs (RSE) determined from the single-dose data

Parameter	Parameter definition	Estimate (RSE%)
Base (nmol/L)	Total alkylresorcinol concentration at baseline	32 (10)
CL/F (L/h)	Apparent clearance	20 (8)
V/F (L)	Apparent distribution volume	114 (13)
F_1 (%)	Relative proportion of the dose that is absorbed from the first absorption compartment	52 (15)
Ka_1 (h^{-1})	Absorption rate constant from first absorption compartment	0.3 (27)
Ka_2 (h^{-1})	Absorption rate constant from second absorption compartment	1.8 (46)
Lag time$_1$ (h)	Lag-time for start of absorption from first absorption compartment	0.9 (5.6)
Lag time$_2$ (h)	Lag-time for start of absorption from second absorption compartment	4.7 (3.5)
Prop error (%)	Proportional residual error	42 (11)

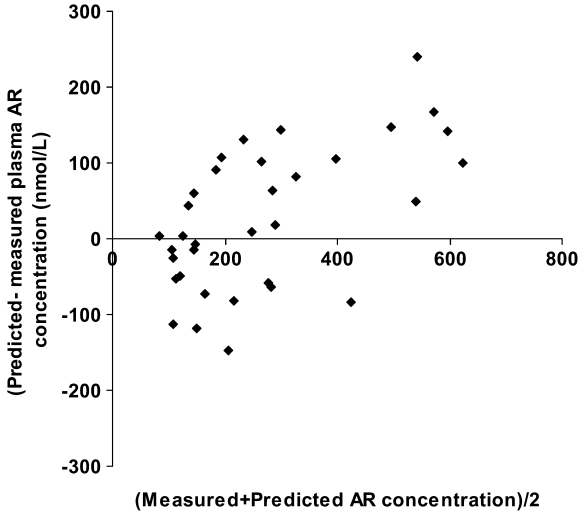

FIGURE 4. Bland-Altman plot of agreement between predicted and measured plasma total alkylresorcinol (AR) concentrations. Prediction error of plasma AR concentration increased proportionally with increasing AR concentration ($P < 0.001$) when linear regression was applied to data.

absorption and/or elimination of individual AR homologs. The 17:0/21:0 ratio could also have differed because of the time window between previous doses and sampling, because the relative proportion of 17:0 has been shown to decrease and that of 23:0 and 25:0 to increase in plasma over time after a high bolus dose of AR (16). Factors affecting AR homolog distribution need further study before the homolog ratio can be used to determine cereal intake type.

Pharmacokinetic modeling

The one-compartment model with 2 absorption compartments used for predictions of plasma AR concentrations was previously shown to have a biphasic nature (16). The prediction error (difference between simulated and observed values) was rather large, with systematic overestimation by the model at high AR concentrations (Figure 4). This may have been because the model was built on only 6 subjects with considerable between-subject variability. The half-life estimated by the model (4.0 h) was somewhat shorter than that estimated by the non-compartmental method (≈4.8 h) (16). It should be noted that the plasma AR concentration never reaches zero, possibly because of liberation from pools with a slow turnover rate, eg, adipose tissue (data not shown). The discrepancy between predicted and observed plasma AR concentrations calls for a better understanding of AR absorption, distribution, and elimination and factors affecting between-person variability. Once established, a good model can be used to predict plasma AR concentrations resulting from a certain intake pattern.

Predicted plasma AR concentrations showed large fluctuations at steady state, despite regular intake 3 times/d (data not shown), which suggests that fasting samples should be used whenever possible to reflect intake or that the number of nonfasting samples should be increased to avoid attenuation of relations between intake and plasma concentration (29). The number of fasting and nonfasting samples needed to accurately reflect AR intake in population-based studies needs to be determined (30).

Urinary AR metabolites and creatinine

Creatinine excretion in all 24-h urine samples ($n = 8$) was measured to check compliance, although creatinine excretion is subject to considerable variation due to different factors, including diet (26, 31). The mean within-subject variation in creatinine excretion was only used to exclude one subject, for whom the CV was >100%.

No significant difference in metabolite excretion occurred between the two 24-h urine samples for each dose, but within-subject variation was large for some individuals. Large within-subject variation in 24-h AR metabolite excretion may have been due to variation in the completeness of urine samples, different elimination routes, or incomplete recovery of a given dose within 24 h. Studies of AR metabolite excretion kinetics are needed to determine the exact cause of this variation.

Metabolite recovery (mean daily metabolite excretion/intake) for different doses decreased with increasing dose, possibly because of decreased absorption (32) or a change in the elimination route at high doses. Because the increase in plasma AR concentration was similar to that for urinary metabolite excretion, differences in absorption appear unlikely. A change in the elimination route after hepatic metabolism, from urinary excretion to biliary excretion, is known for tocopherols (33, 34), which share several features of metabolism with ARs (10, 17, 35). It is also possible that a 24-h collection period is too short to completely recover higher doses. Urinary recovery at different doses needs further evaluation in kinetic studies with different single doses to determine whether 24 h is sufficient to recover the entire dose.

Biomarker classification and comparison of analyzed markers

Dietary biomarkers are traditionally divided into 3 classes (recovery, concentration, and replacement), with different abilities to reflect, replace, and/or adjust dietary intake assessed by other methods (27). A fourth class, prediction biomarkers, falls between recovery and concentration biomarkers. Prediction biomarkers showed a much higher correlation with intake than did concentration biomarkers and are time-related and sensitive to intake in a dose-dependent manner, but the recovery of the ingested dose was too low to be grouped as recovery biomarkers (19). The plasma AR concentration is considered a concentration biomarker, with a correlation with intake of 0.58 (8), whereas urinary metabolites can be either recovery or prediction biomarkers. Recovery at high intakes (45–60%) may be too low to classify the urinary AR metabolites as recovery markers (urinary potassium and nitrogen, which are classified as recovery markers, show ≈80% recovery; 27).

In this study, 24-h urinary excretion of the 2 major AR metabolites DHBA and DHPPA (≈90% recovery at low dose) showed a more linear dose-response relation than did plasma AR concentration. However, plasma AR concentration provided more information on the source of ingested whole grain, although caution is needed because the ratio seems to be affected by factors other than intake (8, 16). Moreover, plasma samples are usually more readily available than are 24-h urine samples. Total urinary metabolite excretion increased with increasing plasma AR concentrations, with no apparent intercept, strongly suggesting that the 2 metabolites are only derived from ARs. Both plasma

AR concentrations and AR metabolite excretion in 24-h urine samples appear to be promising biomarkers of whole-grain intake. In our efforts to date to evaluate ARs as biomarkers of whole-grain wheat and rye intake, we assessed AR pharmacokinetics in humans and compared estimated intakes with plasma concentrations (8, 16). In the present study, we tested the dose response in the range expected for Nordic countries for plasma AR concentration and, for the first time, for 2 main AR metabolites in urine in 24-h samples. All studies were carried out under intervention conditions using fasting samples. Studies to determine reproducibility (reliability) of plasma ARs and urinary metabolites in fasting and nonfasting random samples from different populations are highly warranted as are studies in which biomarkers are compared with dietary assessment methods commonly used in epidemiologic investigations.

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