

Plasma Alkylresorcinol Concentrations Correlate with Whole Grain Wheat and Rye Intake and Show Moderate Reproducibility over a 2- to 3-Month Period in Free-Living Swedish Adults^{1–3}

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Abstract

Plasma alkylresorcinols (AR) are useful as dietary biomarkers of wheat and rye whole grain (WG) during interventions but need to be validated in free-living populations. This study estimated the medium-term reproducibility and relative validity of plasma AR as biomarkers of WG and cereal fiber intake. Seventy-two Swedish adults kept 3-d weighed food records on 2 occasions 2–3 mo apart. Of these men and women, 51 provided a fasting blood sample at the end of each occasion. In addition, 18 participants provided 3 fasting and 3 nonfasting samples for 3 consecutive days on the first and second occasions, respectively. Dietary and blood variables did not differ between the 2 occasions. Nonfasting plasma total AR concentration [210 nmol/L (95% CI: 140, 314)] was higher than fasting [99 nmol/L (95% CI: 72, 137)] ($P < 0.0001$). Mean WG intake was 70 ± 61 g/d (41% from rye) and the intra-class correlation coefficient was 0.44 (95% CI: 0.26, 0.63) for total WG intake and 0.47 (95% CI: 0.27, 0.67) for the fasting plasma total AR concentration, suggesting moderate reproducibility. Fasting plasma total AR moderately correlated with WG rye + wheat ($r_s = 0.53$; $P < 0.001$) and cereal fiber intake ($r_s = 0.32$; $P < 0.05$) when using mean values from both occasions. This suggests that plasma AR concentration in fasting samples can be used as a biomarker of rye + wheat WG intake in free-living populations with a high and consistent WG intake. J. Nutr. 141: 1712–1718, 2011.

Introduction

Prospective cohort studies investigating the association between WG⁶ intake and health outcomes typically rely on self-reported intake data such as 24-h recalls or FFQ (1–3). These methods are prone to systematic and random measurement errors (4,5). A biomarker of WG intake can overcome some of the obstacles and provide a complementary ranking tool of WG intake (6,7), or it can be used in combination with traditional dietary as-

essment, as recently suggested (8). AR, a group of phenolic lipids exclusively occurring in the outer parts of rye and wheat (9), have been suggested as biomarkers for WG and bran intake of rye and wheat (10) and for cereal fiber intake (11). Controlled WG and bran intervention studies in the Nordic countries show that plasma AR concentration proportionally increases with AR and WG intake (6,12–14). Studies in free-living Finnish women (11) showed significant correlations between cereal fiber intake and plasma AR ($r = 0.28$ – 0.43), and in a group of free-living men and women in Switzerland, WG intake estimated from 3DWFR was significantly correlated ($r = 0.57$) with plasma total AR concentration (15). Fasting plasma AR concentration remained stable within individuals (ICC = 0.42) over a 4-mo period in participants from the EPIC-Potsdam cohort, suggesting that it reflects long-term WG intake in Germans reasonably well (16). However, the reproducibility and relative validity of intake biomarkers may vary substantially between cohorts due to differences in intake ranges and regularity of WG consumption (5). In the case of AR, no previous study to our knowledge has evaluated the stability of plasma AR over time (reproducibility) or their correlation to a surrogate measurement of “true” long-term WG intake (relative validity) in a free-living Nordic

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³ Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

⁶ Abbreviations used: AR, alkylresorcinol; ICC, intra-class correlation coefficient; WG, whole grain; 3DWFR, 3-d weighed food record.

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population. This study therefore evaluated the medium-term reproducibility of fasting plasma AR concentrations and the short-term reproducibility of nonfasting plasma AR concentrations and examined the relative validity of fasting plasma AR concentrations as an intake biomarker of different sources of WG, cereal fiber, and AR, estimated by 3DWFR in free-living Swedish adults. Such data can be used to adjust for random measurement errors in the biomarker when studying single AR measurements in fasting plasma samples in relation to disease endpoints and thereby potentially reduce the degree of regression-dilution bias in such epidemiological studies.

Materials and Methods

Participants and study design. We recruited an initial total of 91 participants through advertisements in local newspapers and posters in the university campus area in Uppsala. Inclusion criteria were aged between 20–70 y and had no diagnosed or perceived gastric-intestinal diseases or symptoms. All participants performed a 3DWFR on 2 different occasions, with 2–3 mo between, and were advised to adhere to their usual diet. Thirteen participants dropped out for personal reasons or provided incomplete food records. Six participants were excluded due to unacceptably low reported energy intake, as explained in detail in the section on food records and calculations. In total, 72 participants (55 women and 17 men) completed 2 satisfactory 3DWFR. The mean age of these participants was 42 ± 17 y. Body weight, measured in the fasting state at the study clinic, was 71.2 ± 12.3 kg and BMI was 24.3 ± 3.9 kg/m². Of the 72 participants, 51 donated one single fasting blood sample at the end of each food record period and 18 donated fasting blood samples during 3 consecutive days in the first food recording period and nonfasting samples during 3 consecutive days in the second period. The study protocol was approved by the Regional Ethical Review Board in Uppsala (log. no. 2008:040) and the participants gave their informed consent.

Food records and nutrient calculations. We assessed intake of WG, macronutrients, and AR using the 2 3DWFR (typically including 4–5 weekdays and 1–2 weekend days). We gave all participants written and oral instructions regarding how to keep accurate food records and encouraged them to describe the cereal products consumed in detail, including brand name and type whenever applicable. We gave them all portable weighing scales to be used for all food and beverages consumed. If the weight of a food item was missing in a participant's food record, we entered a standard portion size and/or a portion size equivalent to that participant's previously reported portion size. We calculated nutrient intake using the food database of the Swedish National Food Administration (PC-kost 2008–03–06) and a computerized calculation program (Dietist XP, Kost och Näringsdata). We excluded participants with a food intake level (reported energy intake/calculated basal metabolic rate) ≤ 1.08 because of unacceptable representation of their habitual intake according to the Goldberg cutoff method (17). These calculations concerned reported energy intake representing the sum of all 6 food record days and estimated basal metabolic rate for each participant according to the FAO/WHO/UNU (18) equations, taking gender, body weight, and age into account.

Intake of WG, dietary fiber, and AR. WG was as defined by the American Association of Cereal Chemists (1999): “intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis” (19). According to this definition, bran as an isolated component does not qualify as WG, even when added to foodstuffs such as non-WG bread to increase the fiber content. Intake of WG was separately estimated in g/d from rye, wheat, oats, barley, corn, and rice. We also calculated the sum of ingested WG wheat and WG rye to relate intake of the main sources of AR to plasma concentrations, as described later. There was no threshold for WG content in a product. We constructed a WG database based on the cereal products found in the participants' food records. This comprised a total of 140 different cereal products, including the following food categories:

bread, crisp bread, muesli, breakfast cereals, pasta, rice, grains, millet-based dishes, buns, biscuits, and popcorn. In most cases, WG content was stated on the product or reported by the manufacturer as percentage of total raw weight and we entered these values directly into the food database as g WG/100 g of ready-to-eat product. For crisp bread, the WG content stated on the label was 100%, but we subtracted the moisture content from the product weight to obtain WG/100 g ready-to-eat product. In some cases (<25% of the products), we estimated total WG content based on declared ingredients and moisture contents given by the Swedish food database for similar foodstuffs. The WG content in the different products ranged from 7 to 100% (Table 1). We also entered data on macronutrients, dietary fiber, and bran content declared on the cereal product labels in the database. We based energy value (kJ/g) on standard factors: carbohydrates (excluding fiber) and protein (17 kJ/g), dietary fiber (8 kJ/g), and fat (37 kJ/g). We determined AR homolog content (homologs C17:0–C25:0 and their sum) and the C17:0/C21:0 ratio in reported cereal food products by GC (9) and entered the values in the WG database. In cereals, total AR content typically varies within and between species, but the C17:0/C21:0 ratio is relatively consistent within species and is ~ 1.0 , 0.1, and 0.01 for WG rye, common wheat, and durum wheat, respectively. The ratio can be used as a marker of WG wheat and rye content in cereal foods (20). If food products were not commercially available any longer, we matched these with a corresponding product previously analyzed or used a mean value for the food category.

Laboratory analyses. We analyzed the concentration of individual AR homologs (C17:0–C25:0) and their sum in all individual samples with a GC-MS method (21) and calculated the C17:0/C21:0 ratio to investigate whether it reflected the source of WG intake. We included quality control samples ($n = 5$) in every batch. Inter- and intra-batch variation was <10% for total AR concentration.

Statistical analysis. The data are expressed as means \pm SD unless otherwise stated. We log-transformed skewed variables before statistical analysis to better approximate a normal distribution ($P < 0.05$, Shapiro-Wilks test). We used paired t tests to evaluate differences in relevant dietary intake variables and plasma AR concentrations between occasions at a group level. We calculated Spearman's rank r_s between fiber and AR intake. We also assessed reproducibility, a measure of the intra-person stability in dietary intake or biomarker concentration over time, over a 2- to 3-mo period using the r_s and the ICC. We calculated this for dietary variables, including separate AR homologs and the C17:0/C21:0 ratio. ICC is defined as the inter-participant variance divided by the total variance (i.e. the sum of intra- and inter-participant variance) and it assumes a common mean between replicate measurements. We calculated ICC and intra-participant variance components using a freely available SAS macro written by Dr. Ellen Hertzmark and Professor

TABLE 1 Whole grain (WG) concentration of different cereal product groups included in the database constructed for this study

Cereal product group	Food products in product group, n	WG, g/100 g ready-to-eat product ¹
Crisp bread (rye)	20	87 (64–94)
Crisp bread (wheat)	3	46 (42–52)
WG toast bread	3	25 (19–37)
WG loaf bread	13	20 (7–44)
WG soft bread	27	38 (13–64)
Breakfast cereals classic	5	46 (10–71)
WG breakfast cereals	7	74 (58–98)
Muesli	22	65 (33–100)
Rolled oats	1	100
Rolled oats + fiber	1	85
WG pasta/spaghetti ²	1	55

¹ Values are mean (range).

² Per 100 g dry weight (uncooked).

Donna Spiegelman (22). The macro uses SAS PROC MIXED to estimate the maximum likelihood estimates of intra-participant and inter-participant variance components. We used a method described by Hankinson et al. (23) to calculate 95% CI of the ICC. Fixed factors, such as age and sex, can be adjusted for before computing ICC, but in order to facilitate comparisons with previous studies (with no adjustment), we did not use this option. We estimated the relative validity of plasma AR homologs as biomarkers by calculating Spearman's rank r_s between WG [total, barley, oats, corn, rye, wheat, sum of rye + wheat, ratio of rye/(rye + wheat)], fiber (total and cereal fiber), AR intake (total, C17:0–C25:0, and C17:0/C21:0), and plasma AR homolog concentrations (total, C17:0–C25:0, and C17:0/C21:0). In all cases, we used mean values from the 2 3DWFR and mean values of the 2 plasma AR measurements. To investigate how the relative validity of plasma AR concentration as a biomarker of wheat and rye WG intake was affected by time, we calculated r_s for dietary and biomarker measurements conducted during the same and different sampling occasions. In a secondary analysis, we also included bran in the respective WG intakes when evaluating the relative validity of plasma AR as biomarkers, because bran from wheat and rye contains large amounts of AR. For all statistical analyses, we used SAS v. 9.1 (SAS Institute), with all P -values 2-sided and $P < 0.05$ considered significant.

Results

WG, cereal fiber, and AR intake. The reported mean energy and macronutrient intake did not differ between the 2 occasions and reproducibility of the 2 3DWFR, assessed by Spearman's rank r_s and ICC, was good for energy, carbohydrates, protein, and total dietary fiber but modest for fat and cereal fiber intake (Table 2).

Reported mean WG, AR, and cereal fiber intake did not differ significantly between the first and second occasions (Table 2). Mean total WG intake was ~ 70 g/d (range 0–266 g). Rye was the most common source of WG, followed by wheat and oats (Table 2). Among food products, bread was the largest contributor to WG intake (28%), followed by muesli (18%), flour and grain products (mostly rolled oats) (17%), and crisp bread (16%). The reproducibility of WG intake over the 2- to 3-mo period was reasonable, with large day-to-day variations in WG intake (Table 2). Daily total AR intake was in the range of 0.5–124 mg/d and was highly correlated to total WG intake ($r_s = 0.49$ – 0.82 ; $P < 0.0001$, depending on AR homolog and occasion). As expected, correlation coefficients were even stronger between the sum of WG rye + wheat intake and the intake of AR homologs, because these cereals are the main sources of AR in the diet ($r_s = 0.72$ – 0.94 ; $P < 0.0001$). The reproducibility of AR intake was similar to that of WG intake (Table 2). As expected, cereal fiber intake was highly correlated to total WG intake ($r_s = 0.88$; $P < 0.0001$).

Plasma AR concentration and reproducibility. Fasting plasma total AR concentration ranged from 8 to 2450 nmol/L and the concentrations of specific AR homologs or the ratio between AR C17:0 and C21:0 did not differ between the 2 occasions (Table 3). As expected, C19:0 and C21:0 were present in the highest concentrations. The medium-term reproducibility of fasting plasma AR homologs was reasonable. It was somewhat higher for the shorter AR homologs (C17:0–21:0) and for the C17:0/C21:0 ratio than for the longer homologs (Table 3). The nonfasting plasma total AR concentration was significantly higher than the fasting plasma AR concentration, but the C17:0/C21:0 ratio did not differ between fasting and nonfasting samples (Table 4). The short-term reproducibility over 3 d, when using a mean value for the 3 d on each occasion ($n = 18$), was good for fasting plasma total AR concentration, but poor under nonfasting conditions. The short-term reproducibility of the

C17:0/C21:0 ratio was good for both fasting and nonfasting samples over the period of 3 d. Also, the reproducibility over the period of 2–3 mo, combining the fasting and nonfasting samples, was good for the C17:0/C21:0 ratio (Table 4). Results for specific AR homologs were similar to total AR (data not shown).

WG and dietary fiber intake in relation to fasting plasma AR concentrations. Total WG intake was not significantly correlated with fasting plasma AR, except for a weak correlation with the plasma AR C23:0 concentration (Table 5). Among specific cereals, WG rye intake was highly correlated with the fasting plasma C17:0 concentration. WG wheat intake was moderately correlated with fasting plasma C21:0, C23:0, and total AR concentrations. As expected, WG as oats, barley, corn, or rice was not correlated with plasma AR homologs (data not shown). Intake of WG rye and the ratio of WG rye:(WG rye + wheat), which may serve as a proxy for the C17:0/C21:0 ratio, were significantly correlated with fasting plasma C17:0/C21:0 ratio, whereas WG wheat was negatively correlated with the fasting plasma C17:0/C21:0 ratio. Mean cereal fiber intake was significantly correlated (around $r_s \sim 0.3$; $P < 0.05$) with specific plasma AR homologs, whereas total dietary fiber showed no correlation with fasting plasma AR concentration (Table 5).

There were correlation coefficients in the range $r = 0.43$ – 0.65 ($P < 0.05$) between the sum of WG rye + wheat intake and fasting plasma AR homolog concentrations from the same occasion (Supplemental Table 1). Correlation coefficients were of the same magnitude between mean WG rye + wheat intake and plasma AR concentration for both occasions. We also tested the inclusion of added bran from rye and wheat, because they are rich sources of AR, when correlating WG rye + wheat intake to plasma AR concentration, but this did not substantially change the results (data not shown). There were only a few significant correlations between WG rye + wheat intake and plasma AR homolog concentrations from different occasions.

Intake of AR and fasting plasma AR relationship. The reported AR homolog intake and fasting plasma AR homolog concentrations assessed from the same occasion (second occasion) were correlated ($r_s = 0.48$ – 0.65 ; $P < 0.0001$) (Fig. 1). The correlations were highest for intake and fasting plasma concentrations of C17:0 and C23:0. The correlations for mean intake and plasma AR concentrations over 2–3 mo were similar. However, AR intake and fasting plasma AR concentrations from the different occasions 2–3 mo apart were in most cases not correlated. The results were similar when we compared dietary data from the first occasion and plasma data from the second occasion (data not shown).

Discussion

The present study investigated the medium-term reproducibility and relative validity of fasting plasma AR as a biomarker of WG and cereal fiber intake in a free-living population. Such studies are essential before implementing the biomarker in cohort studies.

WG, cereal fiber, and AR intake and their reproducibility. The observed mean WG intake in the present study (~ 70 g/d) corresponded to the recommended intake in Sweden and Denmark and was almost twice that recently reported for free-living Danes (24). We expected high WG intakes, because the participants volunteering for this type of study may be more health conscious than the general population. When validating a potential biomarker, it may be an advantage to include health-

TABLE 2 Estimated intakes of macronutrients, whole grain (WG), and alkylresorcinol (AR) homologs by free-living Swedish individuals on 2 separate occasions 2–3 mo apart and the medium-term reproducibility^{1,2}

Variable	First occasion ³	Second occasion ³	Spearman's rank r_s	ICC ⁴ (95% CI)
Macronutrients				
Energy, MJ/d	9.3 ± 2.0	9.4 ± 1.9	0.60***	0.70 (0.57, 0.80)
Carbohydrates, g/d	263 ± 78	260 ± 65	0.73***	0.74 (0.62, 0.83)
Protein, g/d	88 ± 25	85 ± 23	0.71***	0.76 (0.66, 0.85)
Fat, g/d	79 ± 23	83 ± 25	0.39***	0.45 (0.28, 0.63)
Dietary fiber, g/d	30 ± 14	28 ± 12	0.73***	0.78 (0.67, 0.86)
Cereal fiber, g/d	11 ± 12	11 ± 7	0.49***	0.36 (0.19, 0.57)
WG, g/d				
Total WG	72 ± 59	70 ± 45	0.45***	0.44 (0.26, 0.63)
WG rye	29 ± 27	29 ± 29	0.46***	0.43 (0.26, 0.63)
WG wheat	20 ± 23	15 ± 16	0.48***	0.28 (0.10, 0.56)
WG rye + wheat	49 ± 38	44 ± 33	0.45***	0.34 (0.17, 0.57)
WG oats	18 ± 19	18 ± 23	0.60***	0.47 (0.28, 0.67)
WG rice	2 ± 8	4 ± 9	— ⁵	— ⁵
WG barley	3 ± 7	2 ± 6	— ⁵	— ⁵
WG corn	0	2 ± 9	— ⁵	— ⁵
WG rye/(WG rye + wheat), %	62 ± 28	63 ± 31	0.51***	0.35 (0.18, 0.59)
AR, mg/d				
C17:0	6 ± 6	6 ± 6	0.53***	0.40 (0.22, 0.61)
C19:0	12 ± 10	10 ± 9	0.51***	0.45 (0.26, 0.65)
C21:0	10 ± 9	9 ± 7	0.45***	0.48 (0.29, 0.68)
C23:0	4 ± 3	3 ± 3	0.55***	0.54 (0.35, 0.71)
C25:0	2 ± 2	2 ± 2	0.59***	0.61 (0.45, 0.75)
Total AR	36 ± 30	34 ± 28	0.45***	0.46 (0.26, 0.66)
C17:0/C21:0	0.7 ± 0.4	0.8 ± 0.7	0.44***	0.21 (0.07, 0.51)

¹ Values are mean ± SD, $n = 72$. Estimated by 3DWFR. *** $P < 0.001$.

² Reproducibility assessed by Spearman's rank r_s and ICC.

³ No significant difference was observed between occasion 1 and 2 (paired t test based on log-transformed data).

⁴ Intra-class correlation coefficient; defined as inter-participant variance/total variance. Variance components were estimated on log-transformed values from occasion 1 and 2 by a random effect model. Values are point estimates and 95% CI.

⁵ Too few consumers ($n < 10$) reported intake > 0 g/d.

conscious individuals, because they are more likely to provide accurate food records. As in other studies (25), we found good reproducibility for reported intake for most macronutrients except fat and cereal fiber. The modest reproducibility for cereal fiber and WG intake in the present study is probably due to large day-to-day variation in WG intake and to some extent to diffi-

culties in accurately reporting WG intake. Based on food consumption data, calculated total AR intake in the present study was ~ 1.5 times the estimated intake in the Swedish population (26).

Plasma AR concentrations and reproducibility. Fasting plasma AR concentrations were in the upper range of values

TABLE 3 Plasma total alkylresorcinol (AR) concentration and its medium-term reproducibility measured 2–3 mo apart in free-living, fasting Swedish individuals¹

AR homolog	Plasma AR ² concentration nmol/L		Reproducibility ²	
	First occasion ³	Second occasion ³	Spearman's r_s	ICC ⁴
C17:0	5 (4,7)	6 (4, 7)	0.40**	0.53 (0.34, 0.71)
C19:0	20 (16,25)	22 (17, 27)	0.46***	0.52 (0.33, 0.71)
C21:0	27 (21,34)	29 (23, 38)	0.46***	0.51 (0.31, 0.70)
C23:0	11 (8,14)	13 (10, 17)	0.33**	0.42 (0.22, 0.65)
C25:0	9 (7,12)	11 (8, 15)	0.30*	0.38 (0.19, 0.63)
Total	73 (57, 92)	84 (66, 107)	0.38***	0.47 (0.27, 0.67)
C17:0/C21:0	0.19 (0.16, 0.23)	0.19 (0.15, 0.25)	0.65***	0.53 (0.35, 0.72)

¹ Values are geometric mean (95% CI), $n = 51$ (participants that provided a single blood sample and acceptable 3DWFR on both occasions). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

² Reproducibility assessed by Spearman's r_s and ICC.

³ No significant difference was observed between occasion 1 and 2 (paired t test based on log-transformed data).

⁴ Intra-class correlation coefficient; defined as inter-participant variance/total variance. Variance components were estimated on log-transformed values from occasion 1 and 2 by a random effect model. Values are point estimates and 95% CI.

TABLE 4 Plasma total alkylresorcinol (AR) concentration, C17:0/C21:0 ratio, and their short- and medium-term reproducibility for free-living Swedish individuals under fasting and nonfasting conditions¹

	Plasma AR concentration <i>nmol/L</i>		Spearman's r_s ³	Reproducibility ²		
	Fasting participants (first occasion)	Nonfasting participants (second occasion)		Short-term ICC ⁴ (first occasion) ^{5,6}	Short-term ICC (second occasion) ^{6,7}	Medium-term ICC for fasting and nonfasting participants ⁸
Total AR	99 (72, 137)	210*** (140, 314)	0.50*	0.60 (0.36, 0.80)	0.18 (0.03, 0.62)	— ⁹
C17:0/C21:0	0.25 (0.17, 0.36)	0.24 (0.17, 0.34)	0.48*	0.54 (0.35, 0.73)	0.63 (0.39, 0.81)	0.75 (0.51, 0.90)

¹ Values are geometric mean (95% CI), $n = 18$ (participants' mean plasma AR concentration over 3 consecutive days on each occasion for participants that provided 3 blood samples and acceptable 3DWFR on both occasions). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

² Reproducibility assessed by Spearman's r_s and ICC defined as inter-participant variance/total variance. Variance components estimated on log-transformed values by a random effect model.

³ Correlation coefficients calculated using mean values of measurements conducted during 3 consecutive days per occasion.

⁴ Intra-class correlation coefficient.

⁵ Based on total AR concentration determined in blood samples donated on 3 consecutive days by 18 fasting individuals.

⁶ ICC calculated over 3 consecutive days.

⁷ Based on total AR concentration determined in blood samples donated on 3 consecutive days by 18 nonfasting individuals (the same individuals as donated blood on the first occasion).

⁸ Medium-term ICC calculated using participants' mean value ($n = 3$) within each occasion.

⁹ Not applicable as ICC assumes the same mean value and this assumption is violated as plasma AR concentrations are significantly lower during fasting conditions (occasion 1) compared with nonfasting conditions (second occasion).

reported for other free-living populations (11,16). Comparison is hampered by the fact that most studies report arithmetic mean values rather than median or geometric means. For fasting samples, the medium-term reproducibility was comparable to that in a recent study in a German population (16) and to that observed for several other biomarkers used in epidemiological investigations, such plasma enterolactone (27) and plasma fatty acid concentrations (28). As expected, the short-term reproducibility (3 d) was excellent for fasting samples, but poor for nonfasting plasma AR concentrations. This relates to the rapid response in plasma AR concentration upon AR intake and rapid elimination of AR (12,29). Similarly to Montonen et al. (16), we observed slightly higher AR concentrations for men compared with women (data not shown) and the reproducibility (ICC) was slightly higher for longer AR homologs when men were excluded from the analysis. This cannot be explained by differences in regularity of WG intake between men and women, because total WG and WG rye + wheat intakes showed lower reproducibility for women only than for both men and women (data not shown). Instead, it may suggest that women have a larger body pool of AR, e.g., stored in the adipose tissue (30), which may result in more stable fasting plasma AR concentrations than for men.

WG, dietary fiber, and AR intake in relation to fasting plasma AR concentrations. The good correlation between

WG rye intake and fasting plasma AR C17:0 and the weaker correlations for WG wheat intake are probably because rye is richer in AR and was the main contributor of AR-containing WG intake for the participants in the study. The AR C17:0 homolog is typically high in rye and low in wheat. The best correlation for wheat was with C21:0, which is the most abundant AR homolog in this cereal. As expected, there were no correlations between WG barley, oats, corn, or rice and AR intake or plasma AR due to very little or no presence of AR in these cereals or to low intake (31).

The correlations between WG rye + wheat intake and plasma AR homologs were strongest when comparing intake and plasma concentration from the same occasion, because both the 3DWFR and the plasma AR concentration reflect intake over the same period. The underlying true correlation coefficients are probably substantially higher, because we observed substantial random intra-participant variation in the biomarker and the 3DWFR. Due to large day-to-day variation in WG intake, as indicated by modest reproducibility and the relatively short half-life of plasma AR (12,29), there were no significant correlations between WG rye + wheat intake and fasting plasma AR concentrations when comparing intake and plasma AR concentration from different occasions. However, there were significant correlations between plasma AR concentrations from either of the 2 occasions and mean intake from both occasions, showing that a

TABLE 5 Spearman rank r_s between intake of whole grain (WG; total, rye, wheat, rye + wheat, and the ratio of rye:rye + wheat), cereal, and total dietary fiber and alkylresorcinol (AR) homolog concentrations in plasma samples from fasting individuals¹

Plasma AR concentration, <i>nmol/L</i>	WG intake, <i>g/d</i>					Dietary fiber intake, <i>g/d</i>	
	Total	Rye	Wheat	Rye + wheat	Rye/ (rye + wheat)	Cereal	Total
C17:0	0.08	0.73*	−0.22	0.37**	0.50***	0.29*	−0.01
C19:0	0.15	0.29*	0.23	0.41**	0.01	0.28*	0.11
C21:0	0.25	0.19	0.47***	0.55*	−0.24	0.32*	0.23
C23:0	0.29*	0.31**	0.43**	0.61*	0.16	0.35**	0.18
C25:0	0.23	0.45**	0.27	0.56*	0.05	0.31*	0.12
Total AR	0.24	0.28*	0.41**	0.53***	−0.14	0.32*	0.16
C17:0/C21:0 ratio	−0.16	0.49***	−0.59***	−0.12	0.64***	—	—

¹ Correlation coefficients were calculated from the mean of 2 3DWFR and mean plasma AR concentrations in samples provided 2–3 mo apart, $n = 51$ (participants with plasma samples and acceptable 3 DWFR for both periods). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

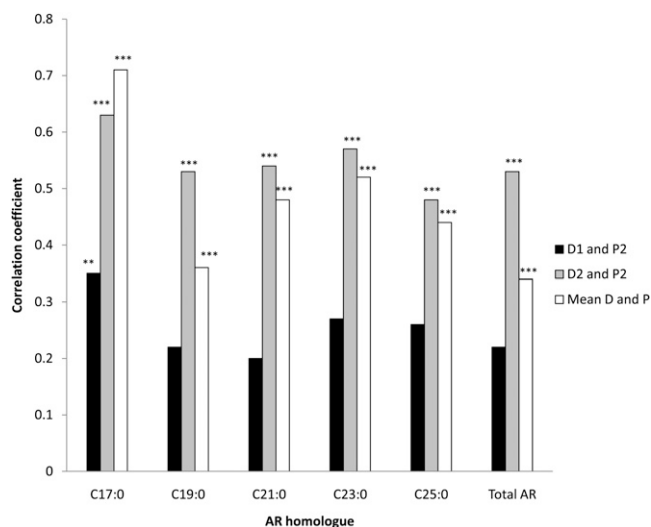


FIGURE 1 Correlation (r_s) between alkylresorcinol homolog intake (D) and plasma fasting alkylresorcinol (AR) homolog concentrations (P) from different occasions (D1 and P2), the same occasion (D2-P2), and the mean from both occasions (Mean D and P), $n = 51$ (participants with plasma samples and acceptable 3-d weighed food record for both periods). *** $P < 0.001$, ** $P < 0.01$.

single measurement of fasting plasma AR can be used to reasonably reflect mean intake of WG rye + wheat over a 2- to 3-mo period. The correlation coefficients observed in the present study were similar to those reported for a group of Swiss men and women, where the correlation between log-transformed WG intake estimated from 3DWF and log-transformed fasting plasma AR concentration was 0.57 (15). That study did not specify the proportions of different sources of WG, but the ratio of AR 17:0/21:0, 0.17 (0.14), indicates that wheat was the most common source.

The definition of WG in the present study did not include added bran. However, bran is particularly high in AR, with ~4–8 times the AR content in WG flour (20,32). AR are therefore markers of bran for these cereals, but because WG contains all parts of the cereal kernel, including the bran, and because WG is generally consumed to a greater extent than bran, AR has been suggested as a biomarker of WG rye and wheat intake (6,10). As a secondary analysis, we compared correlations between plasma AR concentrations and WG + added bran intake from the different cereals, but the results did not differ substantially from those of WG only (data not shown). This is probably due to the fact that the participants consumed rye + wheat bran to a very limited extent (1.1 ± 2.8 g/d).

Recent studies propose plasma AR and their metabolites as biomarkers of cereal fiber intake (11,30). As expected, the intakes of cereal fiber, WG rye + wheat, and AR were highly inter-correlated in this study. However, the correlation with plasma AR concentrations was weaker for cereal fiber intake compared with WG rye + wheat intake and with that previously observed (11). This is probably due to a relatively large contribution of oats to the cereal fiber intake in the present study. AR homologs could probably work well as biomarkers of WG rye + wheat and probably also of cereal fiber intake if the main cereal sources are rye and/or wheat, which is typically the case in Nordic populations (24,26,33).

The AR 17:0/21:0 intake ratio in the present study was high due to overall high intake of WG rye products. Human intervention studies with only WG and bran of rye typically report plasma C17:0/C21:0 ratios in the range of 0.35–0.83, with a

tendency for higher ratios with higher and more frequent intake (12–14,34). However, the plasma AR C17:0/C21:0 ratio was low in the present study despite the major AR source being WG rye. More rapid clearance of C17:0 and proportionally greater incorporation of longer homologs such as C21:0 into the body pool (29,34) may explain the unexpectedly low C17:0/C21:0 ratio in our study with WG from different cereals and substantial day-to-day variation in intake. Until the impact of different determinants on the C17:0/C21:0 ratio is investigated in detail, this ratio should be interpreted with caution, particularly in participants under free-living conditions.

In conclusion, fasting plasma AR concentrations showed moderate medium-term reproducibility and were relatively well correlated with self-reported WG rye + wheat intake. Our results support the proposed use of fasting plasma AR as a biomarker of WG intake in populations where WG rye + wheat intake, and especially rye intake, is high and relatively consistent. Future studies should focus on evaluating AR in populations where intake is mainly from wheat.

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