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Published in:
Journal of Nutrition

Link to article, DOI:
10.3945/jn.115.222620

Publication date:
2016

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Validation of Reported Whole-Grain Intake from a Web-Based Dietary Record against Plasma Alkylresorcinol Concentrations in 8- to 11-Year-Olds Participating in a Randomized Controlled Trial1–3

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Abstract

Background: Whole-grain (WG) intake is important for human health, but accurate intake estimation is challenging. Use of a biomarker for WG intake provides a possible way to validate dietary assessment methods.

Objective: Our aim was to validate WG intake from 2 diets reported by children, using plasma alkylresorcinol (AR) concentrations, and to investigate the 3-mo reproducibility of AR concentrations and reported WG intake.

Methods: AR concentrations were analyzed in fasting blood plasma samples, and WG intake was estimated in a 7-d web-based diary by 750 participants aged 8–11 y in a 2 school meal 3 mo crossover trial. Reported WG intake and plasma AR concentrations were compared when children ate their usual bread-based lunch (UBL) and when served a hot lunch meal (HLM). Correlations and cross-classification were used to rank subjects according to intake. The intraclass correlation coefficients (ICCs) between subjects’ measurements at baseline and after the UBL were used to assess reproducibility.

Results: Correlations between reported WG wheat + rye intake and plasma AR were 0.40 and 0.37 (P < 0.001) for the UBL and the HLM diets, and 78% and 77% were classified in the same or adjacent quartiles for the UBL and HLM diets, respectively. The ICC over 3 mo was 0.47 (95% CI: 0.38, 0.55) for plasma total ARs and 0.64 (95% CI: 0.58, 0.70) for reported WG intake. Correlations were higher when using the AR C17:0 homolog as a biomarker, reflecting rye intake instead of plasma total ARs [UBL: r = 0.47; HLM: r = 0.43, P < 0.001; ICC = 0.51 (95% CI: 0.43, 0.59)].

Conclusions: Self-reported WG wheat + rye intake among children showed moderate correlations with plasma AR concentrations. Substantial intraindividual variation was found in WG intake and plasma AR concentrations. The AR homolog C17:0 may be used as a biomarker for WG intake when the WG intake primarily comes from rye as in the present study. This trial was registered at clinicaltrials.gov as NCT01457794. J Nutr 2016;146:377–83.

Keywords: dietary assessment, 7-d diet record, schoolchildren, validity, biomarker

Introduction

Regular consumption of whole grain (WG) as part of a healthy diet has consistently been associated with reduced risk of heart disease, type 2 diabetes, and some cancers (1–4). Food and health authorities in Denmark, Sweden, and Norway recommend the population consume ≥75 g WG/10 MJ (2400 kcal) daily (5). In the United States three 16-g WG servings are

1 Supported by Swedish Research Council (VR) – Medicine (to RL). The OPUS (Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet) study was supported by the Nordea Foundation, grant 02-2010-0399. Food products were provided in kind for the study. Eggs were supplied by Danæg A/S; dairy products were supplied by Lantmännens ÅS; grains were supplied by Skærtorft Mølle ÅS; potatoes were supplied by Kartoffelpartnerskabet; salt was supplied by AkzoNobel Danmark; fresh herbs were supplied by Gloria Mund; and poultry was supplied by Rose Poultry ÅS.

2 Author disclosures: A Biltoft-Jensen, CT Damsgaard, EW Andersen, KH Ygil, R Andersen, M Ege, T Christensen, AV Thorsen, I Tetens, H Wu, and R Landberg, no conflicts of interest. The funders and food sponsors had no role in the design, analysis, or writing of this article.

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

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recommended daily (total 48 g) on the basis of a 1600- to 2000-kcal diet (6). Other countries such as the United Kingdom and Germany do not have a quantitative recommendation but recommend the population choose WG varieties as often as possible (5).

WG intake in children varies by country depending on the food culture and is lower in English-speaking countries such as United Kingdom, United States, and Ireland (9–19 g) (7–9) and higher in north European countries such as Germany and Denmark (20–58 g/d) (10, 11) where children eat WG rye bread as a staple food and in Denmark especially for lunch (12).

In many countries WG intake is not routinely measured in national dietary surveys, and particular methodological challenges are associated with accurate estimation. WG in foods can be invisible to the consumer and may cause an inability to account for consumption of products that contain less WGs and thereby to report intake correctly (13). Because no analytical tests to measure WG content of foods exist, estimation depends on up-to-date information from manufacturers gathered in WG databases.

In the OPUS (Optimal well-being, development and health for Danish children through a healthy New Nordic Diet) School Meal Study, 8- to 11-y-old Danish children were served hot lunch meals (HLMs) and snacks at school instead of their usual bread-based lunches (UBLs) of WG rye bread. The HLMs and snacks included some WG products among other food items (8, 9). Dietary intake during the school meal study was measured by a self-administered and intuitive Web-based Dietary Assessment Software for Children (WebDASC) that was developed for the purpose (10).

The WebDASC has previously been validated for total energy and fruit/vegetable intake against energy expenditure and plasma carotenoid concentrations, respectively (14, 15). Because WG from different sources is considered a highly nutrient-dense food component of relevance to human health and intake estimation is challenging, it is relevant to validate estimated WG intake against a biomarker that does not have measurement errors that are correlated to self-reported intakes. Alkylresorcinols (ARs) are phenolic lipids, specifically 1,3-dihydroxy-5-alkylbenzene homologs with an odd-numbered alkyl side-chain that typically ranges from 17 to 25 carbon atoms, and plasma ARs are suggested as suitable concentration biomarkers for short- to medium-term WG wheat + rye intake (16). Among commonly consumed foods, ARs are found almost exclusively in the bran of wheat and rye, whereas smaller amounts are found in barley, and only trace amounts are found in refined grain (17). The main AR homologs in wheat and rye are C17:0, C19:0, C21:0, C23:0, and C25:0. The ratio of C17:0 to C21:0 (C17:0/C21:0) is ~0.1 in wheat and 1.0 in rye, and this ratio is suggested to be an indicator for whether a cereal product contains wheat, rye, or a mixture (18, 19). Former validation studies in adults have shown that plasma AR concentrations increase proportionally with AR and WG intake under both controlled (20, 21) and free-living conditions (17, 22–24). However, data from studies that used AR as an independent biomarker of WG intake in children are lacking.

The aim of the present study was to validate WG intake from 2 diets reported by 8- to 11-y-old schoolchildren in a 7-d web-based diary, with the use of plasma AR concentrations. A secondary aim was to investigate the 3-mo reproducibility of AR concentrations and reported WG intake.

Methods

Study design. The OPUS School Meal Study was a cluster-randomized controlled unblinded crossover study. In two 3-mo periods during the 2011–2012 school year, 834 children from third and fourth grades in 9 municipal schools received HLMs and their UBLs in random order. The HLMs contained lower amounts of WG than the UBLs because potatoes and root vegetables were mainly served as the carbohydrate part of the meal, and cereal products such as wheat bread, pearl barley, spelled, and rye bread were served in smaller amounts. The UBLs typically consisted of Danish rye bread with a high WG content (40%) with various toppings, such as sliced meat products, chocolate spread, and liver paste (23). Randomization was performed so that either third- or fourth-grade pupils at each school had the HLM in the first study period, whereas the other group had the HLM in the second study period. The study design and recruitment to the OPUS School Meal Study were described in detail previously (26). The study was conducted according to the guidelines in the Declaration of Helsinki, all procedures involving human subjects were approved by the Regional Research Ethics Committee (H–1–2010–124), and the trial was registered at clinicaltrials.gov as NCT01457794. Written informed consent was obtained from custody holders of all participating children.

Background interview and recording of dietary intake. The whole diet of the children was recorded at baseline and at the end of the 3-mo UBL and HML conditions. The diet recording took place the week before the anthropometric measurements and blood sampling. At baseline, ≥1 parent or custody holder together with each child underwent an in-person interview either at the school or at home by a trained interviewer, including verbal, hands-on, and written instructions in using the dietary assessment tool.

Participants recorded their diet in WebDASC after the final eating occasion on each day for 7 consecutive days. WebDASC was designed for 8- to 11-y-old children, using an age-appropriate interface. An animated armadillo guided respondents through 6 daily eating occasions (breakfast, morning snack, lunch, afternoon snack, dinner, and evening snack). For the diet records, a database of 1300 food items was available, including 265 generic bread and cereal products, either through category browsing or free text search, aided by a spellcheck application. It was also possible to type in foods not otherwise found through category browsing or a text search. The amount consumed was estimated by selecting the portion size from 4 different digital images among 320 photo series. WebDASC included internal checks for frequently forgotten foods (spreads, sugar, sauces, dressings, snacks, candy, and beverages). A food meter and game was included to create motivation. The WebDASC was tested in the target group several times during development (27).

For participants to be included in the analyses, the WebDASC had to be completed for ≥4 d. The intake of cereal foods and WG was calculated for each individual with the use of the software system GIES (version 1.000 d 2010–02–26) developed at the National Food Institute, Technical University of Denmark, and the Danish Food Composition Databank (version 7; Søborg, Denmark; 02–03–2009).

WG intake estimation. WG was defined as the whole kernel of grain/cereal (germ, endosperm, and bran); the whole kernel can be ground, broken, or similar, but the components must, for the respective cereals, be included in the same proportions as in the intact whole kernel. Cereals were defined as wheat, spelled, rye, oats, barley, corn, rice, millet, sorghum, and other sorghum species (28).

The participants did not report the specific brands of the food eaten. Therefore, general market data on specific breads and cereals (n = 709 specific products, covering 90% of the market) purchased by Danish consumers in combination with the WG content in these specific breads and cereals was used to calculate intake. The information about WG
content in bread and cereal products was obtained from manufacturers and millers. A weighting factor was constructed on the basis of market share of the different brands and WG content of the products within a generic food category such as dark rye bread, which was then multiplied by the reported intake of dark rye bread. The same procedure was applied to the recorded intake of the 265 bread and cereal and/or cereal-containing products identified in the WebDASC food list. The intake of WG rye, wheat, and oat was calculated.

**Anthropometric measurements, blood sampling, and analysis of AR concentrations.** Overnight fasting blood samples and weight and height measurements were collected in a mobile laboratory that was placed outside the school during the week after the dietary reporting, at baseline and at the end of the UBL and HLM diets. The anthropometric measurements were described previously (26). The prevalence of underweight, overweight, and obesity was based on age- and sex-specific cutoffs, defined by centiles passing through a BMI (in kg/m²) of 18.5, 25, and 30 at 18 y (29, 30). Furthermore, the ratio of energy intake to estimated basal metabolic rate was calculated (31).

A venous blood sample was drawn from the antecubital vein. AR was measured in plasma (0.2 mL) by gas chromatography-mass spectrometry as described by Wierzbicka et al. (32). All samples from 1 child were analyzed in the same batch and the intra-assay and interassay CVs for total AR were 6.0% and 15.6%, respectively.

**Statistical analysis**

WG intake and plasma AR concentrations were compared between the UBL and HLM diets with the use of hierarchical mixed models. Children were nested in classes, and the classes were nested in schools. This structure in the data resulted in 3 random effects: a child effect, a class effect, and a school effect. The fixed effects were sex, grade, household education, diet (UBL or HLM), and study period (order of the UBLs and HLMs). The model fit was checked by residual plots and QQ plots, and, if necessary, the outcome was transformed with Box-Cox transformations. The estimated HLM effects were back-transformed to the original scale and can be viewed as covariate-adjusted differences in medians which correspond well to the descriptive statistics for skewed data (33).

The difference in WG intake by meal type between UBL and HLM was investigated with linear mixed models, adjusting for sex, grade, BMI, and household education, and taking the design into account with random effect of school, class, and child.

Spearman ρ was calculated for the associations between plasma AR and total WG, WG wheat or rye, and WG wheat + rye intake. WG wheat + rye intake was grouped into quartiles (separately for UBL and HLM) and similarly for total AR in plasma and cross-tabulation for total WG intake, and AR in plasma was presented to study the agreement between quartiles. Statistics of agreement between reported WG wheat + rye and plasma AR concentrations were calculated for both diets.

Finally, the reproducibility of total AR and AR homologs C17:0 and C21:0, C17:0/C21:0, and total WG intake over 3 mo was investigated by calculating the intraclass correlation coefficient (ICC). ICC was calculated from the group of children who started with UBLs to avoid any impact of HLMs. AR concentrations determined after the UBLs were used together with the baseline observation to calculate the ICC on the basis of the linear mixed models with 3 random effects. An approximate CI for the estimate of ICC was calculated with the method described by Hankinson et al. (34) but with a logit instead of an inverse transformation. SAS version 9.3 (SAS Institute) was used for all statistical analyses. Statistical significance was established at P < 0.05.

**Results**

**Baseline characteristics.** Respondents (n = 750) with valid diet reports were included in the present study, and their characteristics are shown in Table 1. Approximately one-half of the children were from households with ≥15–16 y of education, and 13% were overweight or obese. Complete WG intake data were available from 704 and 700 children during the UBL and HLM diets, and among those children AR data were available from 593 and 591 during the UBL and HLM diets, respectively. The mean ± SD reported energy intakes were 7.5 ± 1.9 and 7.4 ± 2.0 MJ during the UBL and HLM diets, respectively, and the ratios of energy intake to basal metabolic rate were 1.47 ± 0.37 and 1.46 ± 0.38, respectively.

**WG intake.** Oats accounted for only ~10% of reported intake during both diets. In general, intake of WG wheat and oats did not differ between the UBL and HLM diets, but reported total intake of WG, WG rye, WG products, and refined grain was significantly higher during the UBL diet than during the HLM diet (P < 0.001) (Table 2). The mean reported WG intake only changed for lunch and morning snack, the meals that were subject for the HLM intervention (Supplemental Table 1).

**AR concentrations.** The crude total AR mean concentration was 54 ± 47 nmol/L for the USB diet and 48 ± 46 nmol/L for the HLM diet (P < 0.0001) (results not shown). The adjusted concentrations of total AR, C21:0, but not C17:0, were higher during the UBL diet than during the HLM diet, whereas the AR ratio C17:0/ C21:0 was significantly higher during the HLM diet, although the mean difference was small (0.02; 95% CI: 0.00, 0.03) (Table 2).

**Comparing WG intake and AR concentrations in plasma.** Spearman ρ between estimated WG wheat + rye intake and plasma AR showed correlations of 0.40 and 0.37 for the UBL and HLM diets, respectively (P < 0.001) (Table 3). Reported WG wheat was not as strongly correlated with AR as reported WG rye and WG wheat + rye. In the present study, the reported WG wheat + rye intake seems to have a higher correlation with plasma AR C17:0 (r = 0.47 and 0.43, P < 0.001) than with total AR (r = 0.40 and 0.37, P < 0.001) for the UBL and HLM diets, respectively.

The cross-classification between reported WG wheat + rye intake and total AR concentrations during both diets is illustrated in Figure 1. During the UBL and HLM diets, 37% and 34%,

### Table 1 Baseline characteristics of children with valid diet reports

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>n</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>385</td>
<td>51</td>
</tr>
<tr>
<td>Girls</td>
<td>365</td>
<td>49</td>
</tr>
<tr>
<td>Age, y</td>
<td>748</td>
<td>10.2 ± 0.6</td>
</tr>
<tr>
<td>Household education highest</td>
<td>749</td>
<td></td>
</tr>
<tr>
<td>No, vocational, or short higher education (≥14 y)</td>
<td>357</td>
<td>49</td>
</tr>
<tr>
<td>Bachelor’s degree or higher (≥15–16 y)</td>
<td>392</td>
<td>51</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>376</td>
<td>17.2 ± 2.4</td>
</tr>
<tr>
<td>Girls</td>
<td>363</td>
<td>17.1 ± 2.5</td>
</tr>
<tr>
<td>Overweight and obese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>376</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Obese</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Girls</td>
<td>363</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Obese</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs or percentages.
2 Categorized according to the standard classifications of Statistics Denmark; that is, the highest level of education achieved by a parent or custody holder in the household.
3 Defined according to the international age- and sex-specific child BMI cutoff points (28, 29).
respectively, were classified in the same quartile, 78% and 77% were classified in the same or adjacent quartile, 18% and 19% were misclassified (2 quartiles apart), and 4% and 4% were misclassified in the opposite quartile during the UBL and HLM diets, respectively. \( \kappa \) Statistics were 0.15 (95% CI: 0.10, 0.21) and 0.11 (95% CI: 0.06, 0.16) for the UBL and HLM diets, respectively.

Reproducibility of WG intake and plasma AR concentrations over 3 mo. The reproducibility over 3 mo was moderate for plasma AR concentrations (ICC = 0.46–0.51) and a little lower (ICC = 0.38) for the AR C17:0/C21:0 ratio of C17:0 to C21:0 (Table 4). The ICC was somewhat higher for the estimated WG intake from the WebDASC. Here, the ICC was 0.64 for total WG, 0.59 for WG rye, 0.38 for WG wheat, and 0.59 for WG wheat + rye.

Discussion

This is the first study, to our knowledge, to compare self-reported intake of WG with plasma AR concentrations in children. The self-reported total WG wheat + rye intake was significantly lower during the HLM diet than during the UBL diet, and plasma AR concentrations reflected this with lower concentrations during the HLMs in response to the lower WG intake. Correlation coefficients and cross-classifications showed that self-reported WG intake could be ranked according to plasma AR concentrations in children 8–11 y of age. Spearman \( p \) values were moderate between reported WG wheat + rye intake and AR concentrations \( (r = 0.40 \) and 0.37) during the 2 diets. Because no other studies have been conducted to test self-reported WG intakes against AR concentrations in children, the following comparisons were made with studies conducted among adults. The fasting plasma AR concentrations found in the present study in children (48–54 nmol/L) were similar to those found in a small intervention study on Danish adults (50 nmol/L) (35).

Ross (19) reported that studies that used food diaries have shown correlation coefficients between 0.32 and 0.52, which is in agreement with the present study. In the present study, estimated WG wheat + rye intake seemed to have higher correlations with C17:0 than with total AR concentrations. This could be because the children normally have a high and consistent intake of WG rye, and this is reflected in the blood by C17:0, which is found in high concentration in rye and low concentration in wheat; therefore, the main homolog reacts to differences between wheat and rye intake (18, 36). As suggested by Anderson et al. (17), AR concentrations can be used as a biomarker in free-living populations with a high and consistent WG intake (37). In a population with high and regular WG rye intake, plasma C17:0 could therefore likely be the best AR homolog to reflect habitual WG intake.

In the present study, the cross-classification between quartiles of WG wheat + rye intake and quartiles of plasma AR concentrations showed that 78% and 77%, respectively, were classified in the correct or adjacent quartile. In a study by Ross et al. (38), a 7-d FFQ was adapted to include questions on the WG products provided in the study to measure WG intake. In that study, the classification of overweight/obese women into the same or adjacent quartiles of WG intake and AR concentrations ranged from 70% to 79%, which is similar to the present study. They also reported \( \kappa \) statistics similar to the present study (0.12 and 0.24 compared with 0.15 and 0.11).

The C17:0/C21:0 in plasma indicates whether AR mostly came from wheat or rye (19). In WG products, WG rye has a

### Table 2

Mean estimated WG intakes and plasma AR concentrations in 8- to 11-y-olds who consumed 2 WG diets

<table>
<thead>
<tr>
<th>Variable</th>
<th>UBL (n = 562)</th>
<th>HLM (n = 700)</th>
<th>Difference, HLM − UBL</th>
<th>P, HLM − UBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary intake, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total WGs</td>
<td>42 (35, 49)</td>
<td>35 (29, 42)</td>
<td>−7 (−9, −5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WG wheat</td>
<td>8 (6, 10)</td>
<td>8 (6, 9)</td>
<td>−0.3 (−0.9, 0.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>WG rye</td>
<td>22 (18, 27)</td>
<td>16 (13, 21)</td>
<td>−6.1 (−7, −5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WG oat</td>
<td>4 (2, 6)</td>
<td>4 (2, 7)</td>
<td>0.7 (−0.1, 1.4)</td>
<td>0.095</td>
</tr>
<tr>
<td>Total cereal</td>
<td>182 (168, 198)</td>
<td>158 (144, 172)</td>
<td>−25 (−30, −21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WG-containing cereal products</td>
<td>79 (67, 93)</td>
<td>61 (50, 73)</td>
<td>−19 (−23, −16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Refined grain cereal products</td>
<td>89 (79, 101)</td>
<td>84 (73, 95)</td>
<td>−6 (−9, −2)</td>
<td>0.001</td>
</tr>
<tr>
<td>ARs (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ARs</td>
<td>36.8 (28.5, 47.6)</td>
<td>32.4 (25.0, 41.9)</td>
<td>−0.9 (−0.9, −0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C17:0</td>
<td>3.1 (2.2, 4.2)</td>
<td>2.9 (2.1, 4.0)</td>
<td>−0.9 (−1.0, −0.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>C21:0</td>
<td>14.0 (10.8, 18.2)</td>
<td>11.8 (9.1, 15.4)</td>
<td>−0.8 (−0.9, −0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C17:0/C21:0</td>
<td>0.24 (0.19, 0.30)</td>
<td>0.26 (0.21, 0.32)</td>
<td>0.02 (0.0, 0.03)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 Values are means (95% CIs) adjusted for sex, grade, household education, and BMI and taking the design into account with random effects of school, class, and child, with the use of linear mixed models. UBL: n = 704 for dietary data, n = 562 for AR data; HLM: n = 700 for dietary data, n = 562 for AR data. AR, alkylresorcinol; C17:0/C21:0, ratio of C17:0 to C21:0; HLM, hot lunch meal; UBL, usual bread-based lunch; WG, whole grain.

2 The sums of WG rye, wheat, and oat do not add up to the total WG because of different effects of the covariates on the different WGs.

3 Not all children with available AR data had available background data. Therefore, n may vary from other tables.

### Table 3

Spearman correlations between reported WG intakes and plasma total alkylresorcinol concentrations in 8- to 11-y-olds who consumed 2 WG diets

<table>
<thead>
<tr>
<th>WG type</th>
<th>UBL (n = 562)</th>
<th>HLM (n = 700)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WGs</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>WG wheat</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>WG rye</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>WG wheat + rye</td>
<td>0.40</td>
<td>0.37</td>
</tr>
<tr>
<td>WG-containing cereal products</td>
<td>0.37</td>
<td>0.35</td>
</tr>
</tbody>
</table>

1 All correlations P < 0.001. HLM, hot lunch meal; UBL, usual bread-based lunch; WG, whole grain.
ratio of 1.0 and WG wheat 0.1. In the present study, the ratio was 0.24 after the UBL diet and 0.26 after the HLM diet. It was suggested that ratios >0.15 are indicative of rye intake (19), suggesting that the children ate more rye than wheat during both diets, which is supported by the self-reported intakes. The ratio also suggests that they had relatively more WG rye in their diet during the HLM diet; however, this is not supported by the estimated rye intakes, which showed that the proportion of rye was lower during the HLM diet. One reason for the higher ratio during the HLM diet, despite lower reported rye intake, could be because of higher intake of WG rye at dinner and/or evening snack during the HLM diet. The impact of rye bread-based meals eaten late during the day will have more relative contribution to the fasting plasma AR concentrations because of a relatively short half-life (~5 h) (39). This could have misclassified some children as high consumers. Another reason could be the intake of cereal products with a high extraction rate but not classified as WGS, such as sifted rye products that contain some ARs. During the HLM diet, children were served pearled barley, breads, and cereal bars that contained sifted rye flour that may have contributed to a higher C17:0/C21:0 during the HLM diet. It could also be because of a higher intake of wheat bran products during the UBL diet. Moreover, other yet unexplored determinants than WG wheat and rye may affect the plasma AR C17:0/C21:0.

Reproducibility of plasma ARs and reported WG intake over 3 mo. The reproducibility of AR concentrations in the present study was moderate (ICC = 0.46–0.51). This is similar to what Landberg et al. (40, 41) found under free-living conditions (ICC = 0.42–0.48 and 0.54) among adults. A similar ICC of 0.48 was also found in the study by Ross et al. (38), in an overweight and obese study sample from the United Kingdom. The moderate ICC for the AR concentrations is also reflected in the ICC for the AR C17:0/C21:0, which was slightly lower. Substantial within-subject variation and between-subject variation because of differences in absorption, bioavailability, and elimination make it difficult to classify an individual’s WG wheat + rye intake with great precision on the basis of determination of ARs in a single blood sample (19). However, plasma AR concentrations can probably be used to distinguish between low- and high-WG consumers with sufficient precision in populations with a regular and frequent WG wheat and rye intake (38). This was verified in recent epidemiologic studies in which plasma ARs were successfully used as a proxy of WG intake in relation to colorectal cancer and body weight (42, 43). The modest reproducibility of plasma AR concentration is partly because ARs reflect short- to medium-term intake and because of substantial day-to-day variation in WG intake as evidenced by the observed reproducibility of WG intake (ICC = 0.60). This can both be because of real differences in WG intake and to the differences in reporting accuracy. The second dietary reporting took place 3 mo after and the third reporting 6 mo after the instructions in use of WebDASC were given, and this might have contributed to poorer reporting accuracy. In addition, the reporting may also be influenced by study fatigue.

One of the strengths of the present study was the large sample size of children and the repeated measurements, making it possible to investigate the relative validity of different reported amounts of WG intake during 2 different diet types by the same population with the use of plasma AR concentrations. Furthermore, a WG database, made for the purpose of this study, that contained the WG content of products available on the market at the time of the school meal study was used to estimate intake. A study weakness is that ARs are not biomarkers for WG oats, but oats only accounted for 10% of the daily reported WG intake during the UBL diet and 11% during the HLM diet. Moreover, the reported intake of WG oats did not differ between the UBL and HLM diets. Plasma AR concentrations may to some degree be determined by factors other than intake, and the magnitude of the importance of such factors may differ between individuals.

### TABLE 4 Intraclass correlation coefficients and 95% CIs for plasma ARs and reported total WG intakes in 8- to 11-y-olds between baseline observations and the subpopulation of children who had the bread-based lunch during the first diet period

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
<th>Intraclass correlation coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ARs</td>
<td>332</td>
<td>0.47 (0.38, 0.55)</td>
</tr>
<tr>
<td>C17:0</td>
<td>332</td>
<td>0.51 (0.43, 0.59)</td>
</tr>
<tr>
<td>C21:0</td>
<td>332</td>
<td>0.46 (0.37, 0.54)</td>
</tr>
<tr>
<td>C17:0/C21:0</td>
<td>332</td>
<td>0.38 (0.29, 0.48)</td>
</tr>
<tr>
<td>Total WGs</td>
<td>418</td>
<td>0.64 (0.58, 0.70)</td>
</tr>
<tr>
<td>WG rye</td>
<td>418</td>
<td>0.59 (0.52, 0.65)</td>
</tr>
<tr>
<td>WG wheat</td>
<td>418</td>
<td>0.38 (0.30, 0.47)</td>
</tr>
<tr>
<td>WG wheat + rye</td>
<td>418</td>
<td>0.59 (0.52, 0.65)</td>
</tr>
</tbody>
</table>

1 Calculated from the random effects model (on transformed outcomes). AR, alkylrecorcinol; C17:0/C21:0, ratio of C17:0 to C21:0; WG, whole grain.
Conclusion. Self-reported WG wheat + rye intakes among children showed similar relative validity in relation to plasma AR concentrations as reported for adults. The reproducibility of plasma ARs and estimated WG intake was moderate over a 3-mo period, suggesting that both instruments may reflect short- to medium-term intake and that observed associations with outcome may be substantially attenuated. ARs might perform better as a biomarker for reported WG intake when using the homolog representative of the usual type of WG consumed in the study population, compared with using plasma total ARs, and this should be investigated further.

Acknowledgments
We thank research data manager Karsten Korup, National Food Institute, Technical University of Denmark, for processing the dietary data; Tom Baranowski from the Children's Nutrition Research Center at Baylor College of Medicine, Houston, for lending us their portion size image series; Karthik Balekudru from the Children's Nutrition Research Center at Baylor College of Medicine, Houston, for providing us with their portion size image series; and Karthik Balekudru from the Children's Nutrition Research Center at Baylor College of Medicine, Houston, for providing us with their portion size image series. AB-J, CTD, IT, and RL designed the study and formulated the research questions; RA, ME, and A-VT collected the dietary intake data; KHY estimated the WG content in the collected cereal data; HW undertook the plasma alkylresorcinol analysis and RL supervised it; EWA undertook the statistical analyses; TC undertook the calculations of the dietary intake data; AB-J drafted the manuscript. All authors read and approved the final manuscript.

References

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