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ARTICLE

New alkylresorcinol metabolites in spot urine as biomarkers of whole grain wheat and rye intake in a Swedish middle-aged population

Rikard Landberg[1,2,3] · Roksana Wierzbicka[2] · Lin Shi[1,2] · Sanna Nybacka[4] · Afaf Kamal-Eldin[5] · Bo Hedblad[6] · Anna Karin Lindroos[7] · Anna Winkvist[4] · Heléne Bertéus Forslund[4]

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Abstract

Background/objectives Studies on the health effects of whole grains typically use self-reported intakes which are prone to large measurement errors. Dietary biomarkers that can provide an objective measure of intake are needed. New alkylresorcinol (AR) metabolites (3,5-dihydroxycinnamic acid (DHCA), 2-(3,5-dihydroxybenzamido)acetic acid (DHBA-glycine) and 5-(3,5-dihydroxyphenyl) pentanoic acid (DHPPTA)) in 24 h urine samples have been suggested as biomarkers for whole grain (WG) wheat and rye intake but remain to be evaluated in spot urine samples.

Subjects/methods The reproducibility of the new AR metabolites (DHCA, DHBA-glycine and DHPPTA) was investigated in 4 repeated samples over a period of 2 wk in spot urine from 40 Swedish men and women enroled in the SCAPIS-study, after adjustment of creatinine. Metabolite concentrations were correlated with total whole grain intake estimated during the same period.

Results The medium-term reproducibility determined for DHCA, DHPPTA and DHBA-glycine varied from moderate to excellent (intra-class correlation coefficient = 0.35–0.67). Moreover, DHCA and DHBA-glycine were independently associated with self-reported total WG intake ($\beta = 0.18$, $P = 0.08$ and $\beta = 0.18$, $P = 0.02$, respectively) and all metabolites except for DHPPA were higher among women.

Conclusions This study supports the idea of using AR metabolites in one or several spot urine samples as biomarkers of whole grain intake. These findings need to be confirmed in different populations.

Introduction

A high intake of whole grain (WG) foods has consistently been associated with lowered risk of developing chronic life-style related diseases such as obesity, type 2 diabetes, cardiovascular disease and colorectal cancer in observational studies [1–3]. However, inconsistent results have been obtained in WG intervention studies on important risk factors such as overweight, obesity, insulin sensitivity, inflammation and plasma lipid profiles [3–8]. Health effects of WGs are difficult to study in observational studies due to imprecision in estimated intakes inherent to few questions about WG foods in questionnaires, large variation in WG different foods, self-reporting bias and a strong correlation of WG intake with an overall healthy life-style [2, 9, 10]. In WG intervention studies, lack-of compliance and miss-reporting are important sources of error that may lead to actual effects remaining undetected [2].

✉ Rikard Landberg
Rikard.landberg@chalmers.se

1 Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg SE-412 96, Sweden

2 Department of Molecular Sciences, Swedish University of Agriculture Science, P.O. Box 7051, Uppsala SE-75007, Sweden

3 Nutritional Epidemiology Unit, Institute of Environmental Medicine, Karolinska Institutet, P.O. Box 210, Stockholm SE-171-77, Sweden

4 Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

5 Department of Food Science, Faculty of Food and Agriculture, United Arab Emirates University, P.O. Box 17551, Al-Ain, United Arab Emirates

6 Department of Clinical Sciences in Malmö, Cardiovascular epidemiology, Lund University, Lund, Sweden

7 National Food Agency, Uppsala, Sweden

Using a dietary biomarker has the potential to overcome some of the obstacles related with self-reporting and may provide a complementary tool to judge compliance in dietary interventions [2, 11, 12]. Odd numbered alkylresorcinols (AR), a group of phenolic lipids only present in the bran of wheat and rye among commonly consumed foods have been suggested, evaluated and used as specific biomarkers of WG wheat and rye intake [13–15]. Total plasma AR concentration reflect total WG wheat and rye intake in a dose dependent manner and the ratio of two specific AR homologues (C17:0/C21:0) reflects the relative intake of WG rye to WG wheat intake, since the ratio is always 1.0 in WG rye-based foods, 0.1 in common wheat and 0.01 in durum wheat [14–19]. Plasma AR reflect medium to long-term WG wheat and rye intake in populations with a stable and frequent intake [2, 20], but is less suitable in populations where intake is less frequent due to a short half-life [21, 22]. Urinary excretion of two main AR metabolites, DHBA and DHPPA has been shown similar validity and somewhat higher reproducibility as plasma AR concentrations in free-living subjects with high and frequent intake [23, 24]. As expected, spot urine samples showed lower reproducibility and relative validity compared with 24 h collections [24–26].

Recently, new AR metabolites (DHBA-glycine, DHPPTA, DHCA) were detected in urine from mice and/or humans and their half-lives were suggested to be longer than that of previously identified AR metabolites [27–29]. A quantitative gas chromatographic-mass spectrometric method was developed and validated for these metabolites and their reproducibility and relative validity were estimated in a population of free-living Swedish men and women that have a high and frequent WG intake reported [28, 30]. Results showed that DHPPTA and DHCA determined in single 24 h urine excretions had excellent reproducibility (intra-class correlation coefficient (ICC) = 0.63 for both) and good relative validity ($r = 0.40$–0.65), and thus could be useful as long-term biomarkers of WG wheat and rye intake [30]. However, 24 h urine collections are typically not available in large scale epidemiologic studies but spot urine samples may be more readily available. It is therefore highly relevant to asses if these biomarkers analyzed in spot urine samples could be used as reliable biomarkers, despite the challenge in accurate reflection of absolute excretion in a spot urine sample, even after adjustment of dilution with the use of urinary creatinine concentration [26, 31].

The aim of the present study was to evaluate AR metabolites, particularly DHPPTA, DHCA, DHCA and DHBA-glycine in a spot urine sample as biomarkers of WG intake by assessing reproducibility and correlation with intake in a population of middle-aged Swedish men and women.

Material and methods

Study population

The current study comprise an extensive collection of clinical data, blood and urine samples and dietary data assembled for the diet-sub study which was part of the pilot-study [32] of SCAPIS (Swedish CArdioPulmonary bioImage Study) [33]. SCAPIS is a large prospective, multicenter observational study that combines the use of new imaging technologies, OMICs and epidemiological analysis to improve risk prediction of cardiopulmonary diseases and optimize the ability to study disease mechanisms [33]. For the pilot-study, men and women from Gothenburg aged 50–64 y were invited based on computerized random selection made from six areas, representing two socioeconomic statuses, respectively. In total, 2243 men and women were invited and 1111 gave informed consent and carried out all examinations including clinical measurements, anthropometry, bio-imaging and blood samples were drawn from February–December 2012. A diet sub-study with the aim to validate two dietary assessment methods used in SCAPIS was initiated with the aim to include 100 women and 100 men [32]. Invitation letters were sent to 575 eligible subjects who had finished all examinations in SCAPIS-pilot visits 1 and 2 and who could feasibly be enroled within 5 weeks from pilot-study visit 1. Among the included subjects in the diet sub-study, urine samples for estimation of energy expenditure by double labelled water technique was collected and available from a subset of 40 subjects [32]. Urine and plasma samples as well as dietary and life-style data from these subjects were included in the present study. The study was approved 2012-02-06 by the Gothenburg Regional Ethics Committee (registration number: 1061-11). Informed consent was obtained from all subjects.

Study design and protocol

Participants enroled in the diet sub-study underwent anthropometric examinations and donated blood at visit one of the SCAPIS Pilot study. After completing examinations at both visits of SCAPIS Pilot, participants were invited to attend a 1 h group meeting with a dietician. At this meeting, participants completed a web-based food frequency questionnaire, MiniMeal-Q, which is a short version of Meal-Q [34]. At the meeting, subjects also got instructed on how perform a self-administered web-based 4 day food record (the Riksmaten method), developed by the National Food Agency in Sweden [35]. The food list in the web-based tool included 1909 food items and mixed dishes developed from the Swedish food composition database (The National Food Agency food database, version Riksmaten 2010–2011). The

Table 1 Characteristics of the SCAPIS diet sub-study population

	Total ($n = 40$)	Men ($n = 20$)	Women ($n = 20$)
Age, y	58 ± 5	59 ± 5	58 ± 4
BMI, kg/m2	26.5 ± 3.1	27.3 ± 3.0	25.7 ± 3.11
Smoking (current smoker)	8(20%)	3(15%)	5(25%)
Education (university degree)	13(32.5)	7(35%)	6(30%)
Plasma total AR (nmol/L)[a,b]	43 ± 53	40 ± 47	46 ± 59
Total whole grain intake (g/d)	44 ± 31	45 ± 32	43 ± 31
Creatinine-adjusted spot urine metabolite concentration (μmol/mol)[b]			
DHBA	1667 (1174)	1535 (943)	2510 (1792)[b,c]
DHPPA	1685 (1612)	1903 (1646)	1669 (1597) [b]
DHCA	837 (530)	829 (508)	910 (495)[b]
DHPPTA	226 (234)	135 (100)	336 (319)[b]
DHBA-glycine	733 (718)	552 (312)	997 (501)[b]

[a]Data obtained from Nybacka et al. [37]

[b]Expressed as mean ± SD

[c]Significantly higher creatinine-adjusted metabolite concentration among women than men

database includes WG content of foods based on product ingredient lists. In most cases, such reports are based on the WG definition according to the definition by AACC International as 'the 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'. The following grains are included in the definition: wheat, rye, oats, barley, spelt, maize, millet, rice and sorghum [36]. WG intake in g/day was calculated from reported food intake and WG content of the foods.

In the present study, we only include dietary data from the Riksmaten method, because it was collected during the same time-frame as urine samples. The Riksmaten method has been described in detail elsewhere [35]. Fourteen days after the meeting with the dietician, subjects were invited to estimate total energy intake, corresponding to total energy expenditure at weight-stable conditions, with the double-labelled-water technique. A spot urine sample (30 mL) was collected just before receiving the oral dose of double labelled water and then at days 1, 3, 12 and 14. Subjects were instructed on how to collect the second-voiding of the day, note the exact time and samples were stored in subjects' freezer at home and brought to the clinic at the end of the study.

Sample analysis

Total alkylresorcinols in plasma were analyzed by gas chromatography–mass spectrometry (GC-MS) method and results have been reported before [37]. Alkylresorcinol metabolites (DHBA, DHPPA, DHPPTA, DHBA-glycine and DHCA) were analysed in spot urine samples using a recent GC–MS method [28]. The Limit of detection was 0.3, 0.15, 0.1, 0.1, 0.1 μmol/L for DHBA, DHPPA, DHCA, DHPPTA and DHBA-glycine, respectively. The accuracy was 80–108% for the corresponding molecules. The within and between coefficients of variation of all AR metabolites have been established during the method validation and were both <10% [28]. Creatinine in urine was analyzed enzymatically with an Architect ci8200 (Abbott). The concentrations of AR metabolites in urine are reported as μmol/mmol creatinine.

Statistical analysis

The data are expressed as means and standard deviations, unless otherwise stated. Variables that were non-normally distributed (as determined by Shapiro- Wilks test) were log transformed to improve normality before further analysis. Differences in creatinine-adjusted metabolite concentrations in spot-urine samples between occasions were evaluated by a paired *t*-test. Spearman's rank correlation coefficients were calculated to investigate associations between urinary AR metabolites and with dietary- and anthropometric data as well as with AR in plasma. In addition, multiple linear regression models were used in order to evaluate associations between reported WG intake (independent variable) and log-transformed creatinine adjusted urinary AR metabolites (dependent variable) adjusted for confounding factors. Confounding factors were selected by analyzing Spearman's rank correlation coefficients between urinary AR metabolites and previously suggested confounding factors such as sex, age and body mass index (BMI). Correlation coefficients were $\geq r = 0.1$ for age for some of the metabolites and age was therefore included in the multivariable linear regression analysis. To assess the stability of biomarkers excretion, reproducibility over different days and over a period of 2 weeks was evaluated by calculating the intra class correlation coefficient. ICC can be defined as a ratio of the between participant variance to the total participant variance ICC values and their 95% CI were calculated as described previously [26]. All statistical analyses were performed by SAS v.9.1 (SAS institute) and correlation plots were made in R version 3.4.0 [38] using the R package 'corrplot' [39]. *P*-values < 0.05 were considered statistically significant.

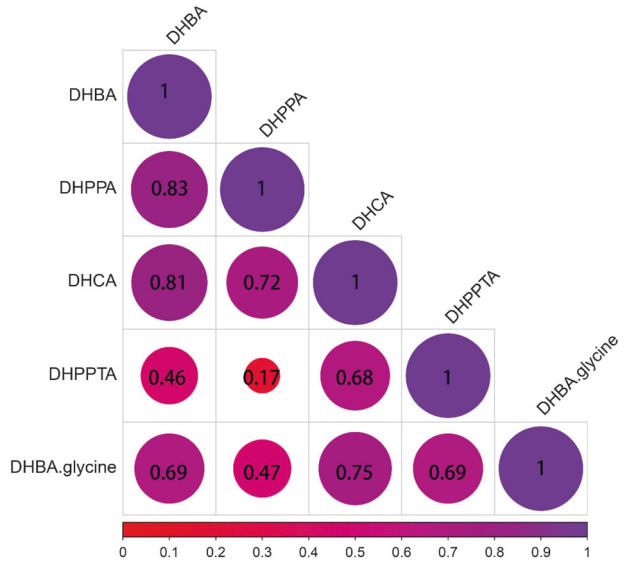

Fig. 1 Spearman's rank correlation coefficients for mean creatinine-adjusted spot urine alkylresrocinol metabolite concentrations from 4 spot urine samples taken at Day 1, 2, 13 and 14 from middle-aged men and women ($n = 40$) from the SCAPIS diet sub-study. All correlation coefficients except for that of DHPPA and DHPPTA, were statistically significant ($P < 0.001$)

Results

In total, 20 men and 20 women were included in the present study. They were of similar age (mean 58 y), had similar education level, WG intake and plasma AR concentrations, but men had slightly higher BMI (Table 1).

The concentration of AR metabolites in urine was highest for DHBA and DHPPA followed by DHCA, DHBA-glycine and DHPPTA (Table 1) and their relative proportion was 33, 33, 16, 14 and 4%, respectively. The concentration of all metabolites, except for DHPPA, were significantly higher for women after adjusting for differences in estimated WG and age (Table 1).

In general, creatinine adjusted AR metabolites in spot urine were modest to highly correlated with each other ($r =$ 0.46–0.83, $P < 0.001$) (Fig. 1), except for DHPPTA which was not correlated with DHPPA ($r = 0.17$, $P > 0.05$) and modestly with DHBA and DHBA-glycine (Fig. 1). The strongest correlation was found for DHCA ($r = 0.68$–0.81). None of the creatinine adjusted AR metabolites were correlated with plasma AR concentrations in this study (data not shown).

The day to day variability of AR metabolites in creatinine adjusted spot urine was substantial and ICCs between Day 1 and 2 were in the similar range as between day 13 and 14, ICC = 0.35–0.67 (Table 2). Notably, it appeared that the reproducibility over 2 week was similar as for day to day variation, i.e., when comparing reproducibility of Day 1–2, 13–14 with that of day 1–13 or 2–14 (Table 2). The reproducibility over 2 weeks, based on mean metabolite concentration for Days 1–2 and 13–14 was in the range ICC = 0.75–0.85 (Table 2).

Total WG intake estimated by the Riksmaten method during 4 days at the same time-period as urine samples were collected, was generally correlated with the mean creatinine-adjusted AR metabolite concentrations from 4 spot urine samples collected at Day 1, 2, 13 and 14: DHBA ($r = 0.49$, $P < 0.05$), DHPPA ($r = 0.38$, $P < 0.05$), DHCA ($r = 0.49$, $P < 0.05$), DHBA-glycine ($r = 0.42$, $P <$ 0.05). No significant correlation was observed between WG intake and DHPPTA concentration.

When further investigating estimated WG intake, age and sex as determinants of creatinine-adjusted AR metabolite concentrations in spot urine, using multivariable linear regression analysis, WG intake was independently associated with higher DHBA, DHCA and DHBA-glycine concentrations, but not with DHPPA or DHPPTA (Table 3). Age was independently associated with DHBA and DHPPA concentrations and all metabolites except for DHPPTA were significantly higher among women compared with men.

Discussion

In this study, we investigated for the first time the feasibility of using recently discovered alkylresorcinol metabolites (DHCA, DHPPTA and DHBA-glycine) in spot urine samples as biomarkers of WG intake among Swedish middle aged men and women. This was accomplished through evaluation of the short to medium-term reproducibility and by investigating the correlation of the intake of WG and creatinine-adjusted AR metabolite concentrations in spot urine samples.

Several studies have evaluated and proposed AR in plasma (C17:0–25:0 and their sum) and two main metabolites (DHBA and DHPPA) plasma and in urine as medium to long-term biomarkers of WG wheat and rye intake based on modest to good reproducibility and relative validity, despite the fact that different WG foods contain different amounts of AR [23–25, 40–43]. However, due to a short half-life (~5 h), application of plasma AR concentrations as biomarkers may be limited to populations with a high and frequent intake [20, 22, 44]. Due to longer half-lives (10 and 12 h, respectively) [45], AR metabolites DHBA and DHPPA in plasma and in 24 h urine excretion were suggested as more long-term biomarkers, but showed approximately similar reproducibility and relative validity as plasma AR [23, 24]. Moreover, DHBA and DHPPA concentrations in creatinine adjusted spot urine which are typically more commonly available, showed as expected weaker correlations with estimated WG intake and lower

Table 2 Intra-class correlation coefficient (ICC) and 95% confidence intervals of creatinine-adjusted alkylresorcinol metabolite concentration in spot-urine, measured on collection Day 1, 2, 13 and 14 in middle-aged Swedish men and women of participants ($n = 40$) in SCAPIS diet sub study

Metabolite	ICC (95% CI)			
	Day 1–2	Day 13–14	Day 1–13	Mean day 1 and 2 vs mean day 2 and 14
DHBA	0.49 (0.27, 0.72)	0.43 (0.21, 0.68)	0.59 (0.38, 0.77)	0.76 (0.61, 0.87)
DHPPA	0.41 (0.19, 0.67)	0.56 (0.35, 0.75)	0.58 (0.37, 0.76)	0.75 (0.58, 0.86)
DHCA	0.35 (0.14, 0.64)	0.45 (0.23, 0.69)	0.49 (0.27, 0.71)	0.75 (0.59, 0.86)
DHPPTA	0.45 (0.23, 0.70)	0.67 (0.49, 0.82)	0.65 (0.44, 0.81)	0.76 (0.60, 0.87)
DHBA-glycine	0.52 (0.31, 0.73)	0.63 (0.43, 0.79)	0.54 (0.32, 0.74)	0.85 (0.75, 0.92)

reproducibility than DHBA and DHPPA in 24 h urinary excretions [24, 26]. The search for more long-term biomarkers of WG intake has therefore continued [46, 47].

Recently, free and conjugated DHPPTA and DHBA-glycine were identified as new AR metabolites in urine from humans ($n = 12$) after feeding WG wheat breads containing 61 mg AR and their half-lives were estimated to 11.1 and 16.6 h, respectively [29]. Moreover, DHCA-sulfate was identified in urine from 20 Finnish women and men consuming WG rye [27]. Wierzbicka et al. [28] developed a GC–MS method to quantify these metabolites in urine samples after enzymatic hydrolysis, and investigated their medium-term reproducibility and relative validity as biomarkers of WG wheat and rye intake, in 24 h urine samples from 69 free-living Swedish men and women [30]. They found that 24 h urine DHBA-glycine, DHPPTA and DHCA excretion were highly correlated with each other and that they good to excellent medium term reproducibility (ICC = 0.59–0.63). Furthermore, DHPPTA and DHCA were well correlated with WG wheat and rye intake ($r = 0.4$–0.58, $P < 0.05$) suggesting that they could serve as medium-term biomarkers in populations with high and frequent WG intake.

In the present study, AR metabolite concentrations were determined from the second void of morning spot urine and adjusted for creatinine concentration. The DHBA and DHPPA concentrations were about double as high compared to what was found in spot urine samples from US women who had about half of the reported WG intake as in the present study [26] and similar range as for in Swedish men and women after taking differences in their intakes into account [30]. The relative abundance of the AR metabolites in our study (DHPPA > DHBA > DHCA > DHBA-glycine > DHPPTA) was also similar to what has been found in two previous studies where wheat [29] and rye [30] were the major sources of provided or reported WG intakes, respectively. The strong correlations between the recent suggested AR metabolites (DHCA, DHBA-glycine and DHPPTA) with established AR metabolites (DHBA and DHPPA) along with their presence in free and/or conjugated forms in urine after controlled WG wheat and rye consumption [27, 29] suggest that they are all AR metabolites. The relative abundance of the different metabolites does not differ after intake of wheat or rye, with different relative AR homologue profiles, which suggests that the AR metabolites are formed similarly, independent of AR precursor. However, variation in bioavailability of AR after ingestion of different WG foods will certainly affect down-stream variation in AR metabolite concentrations to some degree and thus contribute to some miss-classification.

None of the creatinine-adjusted metabolites were correlated with plasma AR concentrations in the present study. This is likely due to small number of subjects included, large fluctuations in single plasma samples and spot urine samples. DHBA, DHPPA, DHPPTA and DHBA-glycine in 24 h urine collections and spot urine have previously been shown to be well correlated with plasma AR concentrations in free-living subjects [48]. However, in these studies, larger samples sizes have been used and plasma and urine samples have typically been collected during the same day whereas plasma was collected 5–7 weeks prior to urine samples in the current study [23, 24, 30].

In the present study, DHCA in urine was strongly correlated with other AR metabolites in urine (Fig. 1) and it has previously been correlated with plasma AR concentrations [30] and found as a urinary sulfate conjugate after controlled rye intake [27]. Moreover, DHCA showed high cross-reactivity in ELISA for DHBA. Based on these findings, it is likely that DHCA is an AR metabolite but biochemically this is not evident, due to the presence of a double bond between carbon 2 and 3 in the propionic acid part of the molecule, which suggests that is must be formed through a different, currently unknown, route than the established pathway for hepatic AR metabolism [49, 50]. Animal feeding studies with pure AR homologues needs to be undertaken in order to confirm that DHCA is an AR metabolite and not just correlated with WG rye and wheat intake. We have also found that DHCA-amide showed high cross-reactivity with ELISA for DHBA, but this metabolite was not correlated with WG wheat and rye intake, nor with plasma AR concentrations [28]. It is yet unclear to what extent this molecule may be derived from DHCA and/or AR [30]. Due to poor correlation with WG intake and to plasma AR concentrations, we did not evaluate its performance as

Table 3 Whole grain (WG) intake, age and sex were evaluated as determinants of creatinine-adjusted alkylresorcinol metabolites in morning spot-urine samples from middle-aged Swedish men and women ($n = 40$) in SCAPIS diet sub study

AR metabolite	WG intake, g/d		Age, y		Sex[a]		R^2
	β	*P*-value	β	*P*-value	β	*P*-value	
DHBA	0.18	0.08	0.035	0.06	0.35	0.034	0.24
DHPPA	0.13	0.26	0.05	0.01	0.15	0.45	0.18
DHCA	0.18	0.02	0.02	0.12	0.28	0.030	0.25
DHPPTA	0.06	0.61	−0.0004	0.81	0.72	<0.001	0.37
DHBA-glycine	0.27	0.002	0.01	0.39	0.48	0.002	0.38

[a]β shown for women when β for men = 0

biomarker in the present study. All metabolites were investigated separately with regards to reproducibility and correlation with intake, due to poorer performance of their total sum, probably inherent to different half-lives and due to differential effects of determinants other than intake.

We found that the day-to-day variation in urinary AR metabolites was similar in magnitude as the variation over a period of 2 weeks (similar ICCs) suggesting that the random-variation in WG intake was similar between days as between weeks. This may suggest that WG intake fluctuates similarly between days as between weeks. No study has compared day-to-day fluctuations in WG intake compared with that between weeks, but intake was shown to fluctuate substantially over a period of 2 months when estimated by 3 day weighed food records (ICC = 0.45) [48].

In our study we found that the reproducibility of AR metabolites in spot urine was in the same magnitude as previously reported for DHBA and DHPPA in 24-h excretions [24] or higher than what has been reported for DHBA and DHPPA in creatinine-adjusted spot urine [24, 26]. Moreover, the reproducibility of the new AR metabolites (DHCA, DHBA-glycine and DHPPTA) in spot urine samples taken two weeks apart in our study, were in the same magnitude or slightly higher than what was recently shown for the same metabolites in two 24-h urine collections taken 2–3 months apart in a population with a wide intake range [30]. This is likely due to the shorter time period between sampling which may lead to less fluctuation.

The reproducibility was excellent when using the mean of AR metabolite determinations in 2 spot urine samples taken on 2 consecutive days (Table 2). This suggests that the mean of the AR determination in two urine spot samples taken at consecutive days may substantially reduce random variation in the biomarker and it seemed that the day-to-day variation in the biomarker concentration was higher than that over longer time (e.g., 2 weeks). Thus, collection of spot urine samples during 2 consecutive days may be a feasible strategy to reduce random variation in the biomarkers without causing substantial additional burden on the participants who can make the two collections within 24 h bring the two tubes back to the investigators.

The fact that only DHBA, DHCA and DHBA-glycine were independently associated with WG intake is probably due to the relatively small sample size and due to the inclusion of non-AR containing grains in reported total WG intake. Other studies have shown modest to good correlations between WG intake from rye and/or wheat with DHBA, DHPPA [24–26] and also recently with DHCA, DHBA-glycine and DHPPTA [29, 30]. Age was found to be independently associated with higher DHBA and DHPPA concentrations but not with other metabolites. All metabolites except for DHPPA were independently associated with sex and were significantly higher for women. Previous studies have shown that AR concentrations in plasma are higher among men compared with women [19, 51]. Marklund et al. [52] suggested this may be due to sex-related differences in AR metabolism as observed for γ-tocopherols [53]. The findings of the present study support that idea and suggests that sex should be taken into account if using urinary AR metabolites as biomarkers. However, sex nor age were found to be significant determinants of spot urine DHPPA in a study of US men and women [25], but this may have been due to a narrow concentration range of DHPPA in the US population as a result of overall low WG wheat and rye intake compared with the present study.

In conclusion, all metabolites investigated showed good reproducibility in single-spot urine samples over a period of 2 weeks and it was further improved when using 2 consecutive spot urine samples. All, except for DHPPA, were correlated with total WG intake, but age and sex were important confounders. Our findings suggest that determination of AR metabolites in a single or duplicate spot urine samples may be useful as biomarkers. However, the long-term reproducibility needs to be investigated in different populations before testing the applicability in large scale epidemiological studies.

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Author contributions SN, AW, AKL, HBF designed and conducted the diet sub-study within SCAPIS Pilot and provided data and samples; BH provided essential databases; RW analyzed alkylresorcinol metabolites in urine samples; LS performed statistical analysis; RL conceived the study together with HBF, supervised sample and statistical analysis and wrote the paper. AKE provided valuable scientific input.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

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