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SHORT COMMUNICATION

Reliability of fasting plasma alkylresorcinol metabolites concentrations measured 4 months apart

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Alkylresorcinols (AR) have been suggested as specific dietary biomarkers of whole-grain wheat and rye intake. AR are metabolised to 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA), which have longer apparent half-lives and were recently proposed to better reflect long-term whole-grain consumption than the intact AR. The objective of this study was to analyse the reliability—expressed by the intraclass correlation coefficient (ICC)—of AR metabolite concentrations among 100 participants from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study who provided two fasting plasma samples 4 months apart. DHBA and DHPPA concentrations were not significantly different between the first and second measurement over the 4-month period ($P > 0.05$). The ICC was 0.32 (95% confidence interval (CI) = 0.13–0.49) for DHBA and 0.37 (95%CI = 0.19–0.53) for DHPPA. These results suggest that AR metabolites cannot be considered to be better biomarkers of whole-grain wheat and rye intake than the intact AR in fasting plasma (ICC = 0.42).

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INTRODUCTION

Assessment of whole-grain consumption in epidemiological studies is usually based on self-reports. Besides measurement errors, the lack of standardised definitions of whole-grain foods challenge the accuracy of assessment. Alkylresorcinols (AR) are phenolic lipids present almost exclusively in the bran of rye and wheat, and have been suggested as promising candidate biomarkers of whole-grain consumption.[1,2]

To gain reliable risk estimates with a single blood measurement, as is usually obtained in epidemiological studies, the within-person variance over time should be small compared with between-person variance.[3] The intraclass correlation coefficient (ICC), defined as the ratio of between-person variance and the sum of between- and within-person variance, is an established indicator of reliability taking both components into account.[3]

We previously observed moderate reliability in plasma of total and individual AR concentrations over time (ICC = 0.42),[4] which could be due to the short half-life of AR (~5 h).[5] AR are metabolised in humans via oxidation.[1] The metabolites, 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA) have longer half-lives (~11–16 h) and may better reflect long-term whole-grain consumption.[6]

The aim of this study was to assess the reliability (as expressed by the ICC) of concentrations of AR metabolites, DHBA and DHPPA, in human fasting plasma over a 4-month period in a population-based sample, which we previously used to assess the reliability of AR.[4]

SUBJECTS AND METHODS

This study was based on the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study,[7,8] as described previously.[4] From EPIC-Potsdam, 407 persons were invited to participate in a validation study of physical activity assessment, which included repeated blood sampling. These persons were randomly selected among participants <64 years, without history of heart disease and impaired mobility, and with systolic and diastolic blood pressure levels <180 and <110 mm Hg, respectively, at the time of invitation. Among participants with repeated blood sampling at least 4 months apart, we randomly selected 100 individuals. The first and second blood sample was drawn between October 2007 and March 2008, and between February and July 2008, respectively. Participants provided informed consent, and the study was approved by the Ethics Committee of the state of Brandenburg (Germany). Blood drawing was conducted with EDTA as anticoagulant. Plasma was stored at −80 °C until analysis.

Plasma DHBA and DHPPA concentrations were analysed with a modified GC–MS method, described in the Supplement. The within-day coefficient of variation for two quality control samples included in every batch was from 5% to 6% for DHBA and 6% to 8% for DHPPA, respectively. Between-day CVs were from 11% to 13% and from 4% to 6%, respectively.

Log-transformed AR metabolite concentrations were used and geometric means and 95% confidence intervals (CIs) are presented. Student's paired *t*-test was used to compare biomarker concentrations measured 4 months apart and unpaired *t*-test to compare concentrations between men and women. ICCs with 95% CIs were estimated with a random effect model[3] and compared between sexes using an unpaired *t*-test with standard errors based on bootstrap sampling with 1000 replications for each sex.[9]

RESULTS

Mean age of participants was 56.1 years. Men were slightly older, had a higher BMI and a larger waist circumference than women.[4]

Geometric mean concentrations of DHBA and DHPPA at baseline were 39.7 nmol/l and 50.6 nmol/l, respectively (Table 1). Higher values were observed for men than for women ($P < 0.05$). No significant differences in levels of AR metabolites were

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Table 1. Geometric means and ICCs for the AR metabolites DHBA and DHPPA over 4 months

	1st measurement Mean (95% CI)	*2nd measurement Mean (95% CI)*	P-*value for difference*[a]	*ICC*[b] *(95% CI)*
DHBA				
All	39.7 (34.7–45.3)	38.6 (33.8–44.2)	0.74	0.32 (0.13 − 0.49)
Men	47.6 (41.7–54.4)	46.1 (40.3–52.8)	0.79	0.23 (− 0.05–0.48)
Women	32.2 (28.2–36.8)	31.7 (27.7–36.2)	0.88	0.27 (− 0.01–0.51)
P-value for difference[c]	0.003	0.006		0.07
DHPPA				
All	50.6 (44.1–58.1)	48.6 (42.1–56.1)	0.62	0.37 (0.19–0.53)
Men	59.2 (51.6–67.9)	59.0 (51.1–68.2)	0.98	0.33 (0.06–0.56)
Women	42.1 (36.7–48.3)	38.9 (33.7–44.9)	0.51	0.30 (0.02–0.53)
P-value for difference[c]	0.01	0.004		0.002
Total metabolites				
All	91.8 (80.6–105)	88.8 (77.6–102)	0.67	0.34 (0.16–0.50)
Men	109 (95.9–124)	108 (94.0–123)	0.89	0.26 (− 0.02–0.50)
Women	75.1 (66.0–85.6)	71.5 (62.5–81.8)	0.67	0.27 (− 0.01–0.51)
P-value for difference[c]	0.004	0.003		0.84

Abbreviations: CI, confidence interval; DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid; ICC, intraclass correlation coefficient. [a]A paired *t*-test (based on log-transformed values) was used to compare geometric means of plasma AR concentrations between first and second measurements. [b]ICC defined as between-subject variance/total variance. Variance components were estimated on log-transformed values. [c]An unpaired *t*-test (based on log-transformed values) was used to compare geometric means of plasma AR concentrations between men and women. Bootstrap method was used to compare ICC values between men and women.

observed between the two measurements. The ICCs (95% CI) were 0.34 (0.16–0.50), 0.32 (0.13–0.49) and 0.37 (0.19–0.53) for the sum of the two metabolites, DHBA and DHPPA, respectively.

CONCLUSION

Reliability of fasting plasma DHBA and DHPPA concentrations was poor over a 4-month period, suggesting that these metabolites may have limited use as biomarkers of long-term whole-grain intake in epidemiological studies relying on a single blood measurement. This is in line with a recent study showing low reliability in spot urine.[10]

Higher AR metabolite concentrations observed for men versus women are probably due to higher intact AR concentrations in men, as observed previously among these participants.[4] This may partly be explained by higher intake of AR containing foods or by lower clearance of intact AR among men versus women.

Because of their longer half-lives, DHBA and DHPPA were expected to have higher reliability than intact AR. However, their 4-month reliability was poorer than observed previously for total AR (ICC = 0.42).[4] Plasma concentration of intact AR may depend on intake, extent and rate of absorption and disposition, whereas concentrations of metabolites may additionally depend on extent and rate of formation.[11] Also, in contrast to intact AR, their metabolites may undergo enterohepatic circulation.[5,6] These differences may have caused lower reliability of AR metabolites compared with intact AR.

Information on whole-grain intake was not available at time of blood collection. Thus, it was not possible to investigate whether variation in concentrations of AR and metabolites over time may be owing to seasonal differences in intake. Because AR metabolite concentrations are expected to depend on time since food intake, we restricted our study to fasting samples. We expect reliability to be lower when metabolites are measured under random blood sampling conditions.

Assuming a true relative risk between total AR metabolite concentration and disease of 0.5, an ICC of 0.34 would lead to observed relative risks ($RR_{observed\ exp} = ((\ln RR_{true}) \times r_{intraclass})$) of 0.79, indicating severe attenuation of the risk estimate if relying on a single blood measurement.[12] ICCs can only be used to correct attenuation of disease risks if they are adjusted for the same factors as the disease model. We computed unadjusted ICCs, which may have a higher between-subject variation than adjusted ICCs, and thus may artificially inflate the strength of the ICC and consequently underestimate the level of attenuation of the observed risk.

In summary, average concentrations of the AR metabolites DHBA and DHPPA did not significantly change over a 4-months period but showed poor reliability. These results should be taken into account when selecting biomarkers for assessment of whole-grain intake in large-scale epidemiological studies on diet–disease relationships.

CONFLICT OF INTEREST

The authors declare not conflict of interest.

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Supplementary Information accompanies the paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)