Short Communication

Plasma alkylresorcinol metabolites as potential biomarkers of whole-grain wheat and rye cereal fibre intakes in women

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It has been demonstrated that intact plasma alkylresorcinols (AR) and urinary AR metabolites could be used as biomarkers of whole-grain intake. Thereafter, we developed the method for the plasma AR metabolites, which is more convenient and requires less sample pretreatment than the analysis of intact plasma AR. The aim of the present study is to evaluate whether AR metabolites measured in plasma, in the same population, could also be considered as useful biomarkers of cereal fibre. Fifty-six women were recruited in a cross-sectional and observational study. Dietary intake (5-d record) and plasma AR metabolites (3,5-dihydroxybenzoic acid, DHBA; 3-(3,5-dihydroxyphenyl)-1-propanoic acid, DHPPA) were measured. The relationship between plasma AR metabolites and cereal fibre intake was examined using partial correlation and stepwise regression. Cereal fibre intake correlated significantly with plasma DHBA (r 0·411; $P=0·002$) and DHPPA (r 0·463; $P=0·000$) even after adjustment for BMI and age. Thus, plasma AR metabolites correlate with cereal fibre intake as noted with plasma intact AR and urinary AR metabolites. We observed that plasma DHPPA was the independent predictor of cereal fibre intake, explaining 18 % of the variance (adjusted r^2 0·176; $P=0·002$). In epidemiological screening, it might be easier to obtain and to collect plasma than urine samples. In addition, the plasma AR metabolites half-life seems longer than those of intact plasma AR, and their measurements are more convenient, and faster. Thus, sum of plasma AR metabolites and more specifically plasma DHPPA seems to be good and specific biomarkers of cereal fibre intake.

Biomarkers: Alkylresorcinol metabolites: Cereal fibre: Rye: Nutrition

Increasing attention has been paid to the role of nutrition in health promotion and the prevention of diseases. The intake of whole-grain cereals has been linked to the reduced risk of some diseases such as CVD, type 2 diabetes, obesity and certain cancers[1]. One possible mechanism to explain this protective health effect is that whole grains slow the digestion, tend to decrease the glycaemic index and could improve insulin sensitivity[2]. In postmenopausal women, the risk of mortality was inversely associated with the whole grains but not with the refined grains[3]. Thus, different compounds in the dietary fibre complex are believed to play important roles[4]. However, partly due to the lack of specific biomarker for whole-grain products, no definitive conclusions regarding their protective role have been made. It is recognised that enterolactone is regarded to be a biomarker of total fibre intake and reflects a healthy lifestyle[5]. In addition, the main problem in nutritional epidemiologic data is the poor accuracy in measuring the intake of foods and nutrients[6]. There are some inherent weaknesses of FFQ[4,7]. The assessment of whole-grain intake is further complicated by the fact that consumers may have difficulties to identify products containing whole grain. To support epidemiological results, it would be very important to have a specific biomarker of cereal fibre[8].

Alkylresorcinols (AR), homologues of the 1,3-dihydroxy-5-alkylbenzene with odd-numbered alkyl side chains containing from C15 : 0 to C25 : 0, are phenolic lipids that occur in the human diet. They are abundant in the outer layers of rye, wheat and triticale grains, found in low amounts in barley, maize and peas, and absent in highly refined white flour and in most other foods used for human nutrition[9,10]. In grains, AR exist mainly with alkyl chain lengths C15 : 0–C25 : 0[11]. It has been demonstrated that AR are stable during food processing[12]. Ross *et al.*[13] reported in the human ileostomy study that AR absorption was approximately 60 %. The pig and human ileostomy studies suggest that AR are absorbed in the upper intestine via the lymphatic system and carried in intact form in lipoproteins and erythrocyte membranes in blood[14,15]. In human subjects, AR have been quantified in intact form in plasma[16] and as metabolites (3,5-dihydroxybenzoic acid, DHBA; 3-(3,5-dihydroxyphenyl)-1-propanoic acid, DHPPA) in urine[17,18]. In interventional studies[19,20], intact plasma AR and, in an observational study[21], urinary

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Abbreviations: AR, alkylresorcinols; DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid.
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DHPPA have been proposed to be useful biomarkers for whole-grain wheat and rye intakes and indicators of the cereal type consumed. Alkylresorcinol metabolites are possibly formed via β-oxidation of intact plasma AR[9].

We have recently published an article showing that intact AR measured in plasma and the AR metabolites measured in urine could be used as biomarkers of whole-grain intake[22]. Thereafter, we developed the method for the plasma AR metabolites[23]. The aim of the present study is to evaluate whether AR metabolites measured in plasma in the same population[22] could also be considered as useful biomarkers of whole-grain rye and wheat cereal fibre. This question is relevant because: (1) in epidemiological studies, the fasting blood collection is easier and more often available than the collection of urine; and (2) the analysis of plasma AR metabolites is more convenient and requires less sample pretreatment than the analysis of intact plasma AR.

Subjects and methods

Subjects

Fifty-six women living in the Helsinki area were recruited. Subjects with a history of cancer or any major diseases, or using drugs like oral contraceptives, hormone replacement therapy or antibiotics were excluded. The subjects included also vegetarians (*n* 20) to obtain a broad range of cereal intake. All subjects agreed to consume the diet as before the recruitment during the study. Age, weight, height, BMI (kg/m^2), age at menarche, type of diet, number of children, menopausal status, smoking status and physical activity level were recorded during the screening visit by questionnaire and published in details previously (Table 1)[22]. All subjects gave their written informed consent and were initially interviewed by a doctor who explained the study. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the ethics committee of the Helsinki University Central Hospital.

Data collection

All subjects were studied for 5 d during the same year on two occasions with 6 months between collections. The 5-d food record was initiated 2 d before the 3-d plasma collection.

Table 1. Anthropometric characteristics

(Mean values and standard deviations)

Variables	Mean	SD
Age (years)	46	13
BMI (kg/m^2)	22·5	2·7
Plasma DHBA (nmol/l)	94	82
Plasma DHPPA (nmol/l)	90	59
Plasma DHBA + DHPPA (nmol/l)	185	135
Cereal fibre intake (g/d)	10·02	3·05
Total fibre intake (g/d)	19·84	6·27

More details for anthropometric characteristics are in Aubertin-Leheudre *et al.* [22].
DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid.

The food records included at least one weekend day. The blood samples were taken every morning in our laboratory during three consecutive days. The heparin plasma samples were collected from fasting subjects. After the 3-d collection, the samples were pooled. Sodium azide (0·1 %) and ascorbic acid (0·1 %) were added, and the samples were frozen at −20°C. The premenopausal women collected the samples during the mid-follicular phase of the menstrual cycles (days 5(3)–7(9)).

Dietary intake

All subjects agreed to maintain their habitual diet throughout the whole study. Each subject was provided with a letter balance and was instructed on how to complete the dietary records. A 3-d dietary record has been demonstrated to be valid for estimating dietary intakes in adults without cognitive impairments[24]. Dietary analyses were completed by a nutritionist using the tables of Southgate for the fibre data (total, cereal, vegetables and fruit and berry)[25]. For some typical Finnish food (e.g. rye products), we used the fibre data given by the manufacturers. The vegetable fibre values include also the legume fibre values.

Plasma alkylresorcinol metabolites

The two plasma AR metabolites, DHBA and DHPPA, were analysed by HPLC with coulometric electrode array detection (ESA Biosciences, Chelmsford, MA, USA) as described by Koskela *et al.* [23].

Briefly, to 100 μl plasma, syringic acid was added as the internal standard. The enzymatic hydrolysis was carried out overnight at 37°C. The sample was acidified to reach pH about 3, and thereafter extracted thrice with diethyl ether. Separation of diethyl ether and water phase was done by freezing. The combined organic phases were evaporated to dryness. The sample was reconstituted in 50 μl methanol, and 100 μl HPLC mobile phase was added. The sample was filtered through Gelman GHP 0·2 μm filter and analysed with HPLC–coulometric electrode array detection[23].

The intra-assay CV for DHBA was 4·2 % and for DHPPA 3·8 %. The inter-assay CV for DHBA was 7·4 % and for DHPPA 10·7 %. This method is considered as accurate, specific and reproducible[23].

Statistical analysis

Normality of distribution was determined using the Skewness test. All AR and diet variables were log transformed for statistical analysis. First, we used Pearson correlation test to examine the relationship between plasma AR metabolites and dietary fibre intake (total, cereal, vegetables or/and fruit and berry). We performed partial correlations between plasma AR metabolites and dietary fibre intake using BMI and age as covariables, which could be confounding variables. Finally, stepwise regression analysis was used to determine the independent predictors of cereal fibre intake. The variables plasma AR (C17:0, C21:0 and sum of intact AR), plasma DHBA, plasma DHPPA, sum of plasma AR metabolites, weight, BMI, age and enterolactone were entered in this order in the model. *P* values of ≤0·05 were considered

statistically significant. Analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

Cereal fibre intake correlated significantly with plasma DHBA (r 0·411; $P=0·002$) and DHPPA (r 0·463; $P=0·000$; Table 2) even after adjustment for BMI and age. No significant association was detected between plasma AR metabolites and vegetable or berry/fruit fibre intake (Table 2).

Because our sample included omnivores and vegetarians, we evaluated whether the present results were influenced by the type of diet. We observed a significant correlation between plasma DHPPA and cereal fibre intake in both vegetarians (r 0·514; $P=0·019$) and omnivores (r 0·404; $P=0·038$), respectively, even after adjustment for BMI and age. Thus, the type of diet does not seem to influence the relationship between the plasma DHPPA level and the cereal fibre intake.

Finally, as a means of examining a greater depth of the relationship between cereal fibre intake and alkylresorcinols, we performed a stepwise linear regression analysis with plasma AR (C17 : 0, C21 : 0 and sum of intact AR), plasma DHBA, plasma DHPPA, sum of plasma AR metabolites, weight, BMI, age and enterolactone in the model. We observed an absence of intercorrelation between residuals (Durbin–Watson statistic = 1·8), showing that the model presented no outliers (leverage = 0·005; Cook's distance = 0·001), no problem of multicollinearity between variables (variance inflation factor < 10; tolerance = 1) and that the residuals were normally distributed. Thus, the model respected the postulates of a stepwise linear regression. We observed that plasma DHPPA was the independent predictor of cereal fibre intake, explaining 18 % of the variance (adjusted r^2 0·176; $P=0·001$; unstandardised coefficients: $\beta = 0·24$; standardised coefficients: $\beta = 0·43$).

Discussion

The aim of the present study was to evaluate whether plasma AR metabolites could be used as biomarkers of whole-grain rye and wheat cereal fibre intakes in women.

We observed that rye/wheat cereal fibre intake in women during their habitual diet correlates significantly with plasma AR metabolites but not with plasma enterolactone ($P=0·995$) even after adjustment for BMI and age, which could be confounding variables (Table 2). This result agrees with other recent studies[19,26] showing the non-specificity of enterolactone as biomarker of whole-grain intake. Furthermore, the present results are not influenced by the diet status

of our subjects because, both in omnivores and vegetarians, rye/wheat cereal fibre intake correlates significantly with plasma DHPPA (r 0·404 and 0·514, respectively) even after adjustment for BMI and age. This indicates that plasma AR metabolites are good biomarkers of rye/wheat cereal fibre intake in Finnish women. At present, there are no accepted biomarkers of whole-grain cereal fibre intake. We demonstrated earlier that plasma intact AR and urinary AR metabolites could be used as biomarkers[27]. In addition, we showed that plasma AR metabolites correlate with intact plasma AR and urinary AR metabolites[23]. Thus, intact plasma AR and AR metabolites (in urine and plasma) could be used as biomarkers in free-living population independently of the type of diet.

Because of some commonly known weaknesses of FFQ in epidemiological studies[4,7], the precise estimation of the intake of various food components is difficult. The use of a quantifiable biomarker could confirm and strengthen the conclusions in such studies and correct for the measurement errors[6,7].

The lack of significant correlation between plasma AR metabolites and the other fibres confirmed the specificity of plasma AR metabolites as biomarkers for cereal rye and wheat fibre intakes. The present results are not influenced by age or BMI, which are known as confounding variables[28]. Studies have reported that with ageing, people tend to change their dietary habits by increasing the amount of whole-grain cereal intake[28] causing the differences in consumption of AR. Moreover, we have previously shown that in free-living women or after a dietary intervention, the plasma intact AR concentration and urinary AR metabolites appeared to be useful biomarkers of whole-grain cereal intake[19,20,27]. In the present study, we found that a higher coefficient of correlation between cereal fibre intake and plasma DHPPA (r 0·463) than with plasma AR C17 : 0 (r 0·368), plasma AR C21 : 0 (r 0·416), urinary DHPPA (r 0·408) or urinary DHBA (r 0·372)[22]. In addition, we carried out a stepwise regression with plasma AR (C17 : 0, C21 : 0 and sum of intact AR), plasma DHBA, plasma DHPPA, sum of plasma AR metabolites, weight, BMI, age and enterolactone in the model. Plasma DHPPA was found to be the only independent predictor of cereal fibre intake in this population.

The present study has some limitations. It has been carried out in Finnish women who are known to consume a high daily amount of cereal fibre rich in AR, mainly from whole-grain rye and wheat[28]. Finland and Denmark have the highest intake of AR compared with other European countries, the estimated average daily intake of AR in free-living population being about 11 mg/d in United Kingdom and about 40 mg/d in Finland[28,29]. Moreover, the coefficients of correlation are

Table 2. Partial correlation between fibre intake and plasma alkylresorcinol metabolites with BMI and age as covariables (n 56)

	Plasma DHPPA (nmol/l)	Plasma DHBA (nmol/l)	Plasma DHPPA + DHBA (nmol/l)
Cereal fibre intake (g/d)	0·463***	0·411**	0·423**
Vegetable fibre intake (g/d)	0·024	− 0·028	0·011
Fruit and berry fibre intakes (g/d)	0·117	− 0·059	0·010
Total fibre intake (g/d)	0·376**	0·280*	0·312*

DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid.
r (coefficient of correlation) obtained using log values.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$.

significant, but the r-values are clinically moderate ($r < 0.750$). However, these values are in agreement with the values expected in epidemiological studies and in free-living populations[30].

To our knowledge, this is the first study demonstrating that plasma AR metabolites (DHBA, DHPPA and sum of plasma AR metabolites) correlate significantly with whole-grain rye/wheat cereal fibre intake as noted with plasma intact AR and urinary AR metabolites. Cereal fibre intake correlated highly with plasma DHPPA, and plasma DHPPA is the only independent predictor of cereal fibre intake in this population. We believe that in epidemiological screening, it might be easier to obtain and collect fasting blood samples than urine samples. In addition, according to the present preliminary kinetic results, it seems that the half-life of plasma AR metabolites (mean approximately 12 h; data not shown) is longer than the half-life of intact plasma AR (mean approximately 5 h[31]). Finally, the method for analysing the plasma AR metabolites is more convenient, faster and requires less sample pretreatment compared with the method for plasma intact AR. Thus, sum of plasma AR metabolites and more specifically plasma DHPPA could better reflect the amount of AR intake than the intact plasma AR.

In conclusion, sum of plasma AR metabolites and more specifically plasma DHPPA seems to be good and specific biomarkers of whole-grain rye and wheat cereal fibre intakes. Further research in larger free-living populations in other countries is needed to generalise and confirm the present findings.

Acknowledgements

References

1. Slavin J (2004) Whole grains and human health. Nutr Res Rev **17**, 99–110.
2. Pereira MA, Jacobs DR Jr, Pins JJ, et al. (2002) Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. Am J Clin Nutr **75**, 848–855.
3. Jacobs DR, Pereira MA & Meyer KA (2000) Fiber from whole grains, but not refined grains, is inversely associated with all-cause mortality in older women: the Iowa women's health study. J Am Coll Nutr **19**, 326S–330S.
4. Slavin J (2003) Why whole grains are protective: biological mechanisms. Proc Nutr Soc **62**, 129–134.
5. Adlercreutz H (2002) Phyto-oestrogens and cancer. Lancet Oncol **3**, 364–373.
6. Kaaks RJ (1997) Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. Am J Clin Nutr **65**, 1232S–1239S.
7. Kaaks R, Ferrari P, Ciampi A, et al. (2002) Uses and limitations of statistical accounting for random error correlations, in the validation of dietary questionnaire assessments. Public Health Nutr **5**, 969–976.
8. Ocke MC & Kaaks RJ (1997) Biochemical markers as additional measurements in dietary validity studies: application of the method of triads with examples from the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr **65**, 1240S–1245S.
9. Ross AB, Kamal-Eldin A & Aman P (2004) Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. Nutr Rev **62**, 81–95.
10. Ross AB, Shepherd MJ, Schupphaus M, et al. (2003) Alkylresorcinols in cereals and cereal products. J Agric Food Chem **51**, 4111–4118.
11. Ross AB, Kamal-Eldin A & Aman P (2001) Gas chromatographic analysis of alkylresorcinols in rye (Secale cereale L) grains. J Sci Food Agric **81**, 1405–1411.
12. Chen Y, Ross AB, Aman P, et al. (2004) Alkylresorcinols as markers of whole grain wheat and rye in cereal products. J Agric Food Chem **52**, 8242–8246.
13. Ross AB, Kamal-Eldin A, Lundin EA, et al. (2003) Cereal alkylresorcinols are absorbed by humans. J Nutr **133**, 2222–2224.
14. Linko AM & Adlercreutz H (2005) Whole-grain rye and wheat alkylresorcinols are incorporated into human erythrocyte membranes. Br J Nutr **93**, 11–13.
15. Linko-Parvinen AM, Landberg R, Tikkanen MJ, et al. (2007) Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. J Nutr **137**, 1137–1142.
16. Linko AM, Parikka K, Wahala K, et al. (2002) Gas chromatographic–mass spectrometric method for the determination of alkylresorcinols in human plasma. Anal Biochem **308**, 307–313.
17. Koskela A, Linko-Parvinen AM, Hiisivuori P, et al. (2007) Quantification of alkylresorcinol metabolites in urine by HPLC with coulometric electrode array detection. Clin Chem **53**, 1380–1383.
18. Ross AB, Aman P & Kamal-Eldin A (2004) Identification of cereal alkylresorcinol metabolites in human urine-potential biomarkers of wholegrain wheat and rye intake. J Chromatogr B Analyt Technol Biomed Life Sci **809**, 125–130.
19. Linko AM, Juntunen KS, Mykkanen HM, et al. (2005) Whole-grain rye bread consumption by women correlates with plasma alkylresorcinols and increases their concentration compared with low-fiber wheat bread. J Nutr **135**, 580–583.
20. Landberg R, Kamal-Eldin A, Andersson A, et al. (2008) Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. Am J Clin Nutr **87**, 832–838.
21. Guyman LA, Adlercreutz H, Koskela A, et al. (2008) Urinary 3-(3,5-dihydroxyphenyl)-1-propanoic acid, an alkylresorcinol metabolite, is a potential biomarker of whole-grain intake in a U.S. population. J Nutr **138**, 1957–1962.
22. Aubertin-Leheudre M, Koskela A, Marjamaa A, et al. (2008) Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in Finnish women. Cancer Epidemiol Biomarkers Prev **17**, 2244–2248.
23. Koskela A, Samaletdin A, Aubertin-Leheudre M, et al. (2008) Quantification of alkylresorcinol metabolites in plasma by high-performance liquid chromatography with coulometric electrode array detection. J Agric Food Chem **56**, 7678–7681.
24. Luhrmann PM, Herbert BM, Gaster C, et al. (1999) Validation of a self-administered 3-day estimated dietary record for use in the elderly. Eur J Nutr **38**, 235–240.
25. Paul AA & Southgate DA (1979) McCance and Widdowson's 'The composition of foods': dietary fibre in egg, meat and fish dishes. J Hum Nutr **33**, 335–336.

26. Horner NK, Kristal AR, Prunty J, et al. (2002) Dietary determinants of plasma enterolactone. Cancer Epidemiol Biomarkers Prev 11, 121–126.

27. Aubertin-Leheudre M, Koskela A, Marjamaa A, et al. (2008) Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in Finnish women. Cancer Epidemiol Biomarkers Prev 17, 2244–2248.

28. Ross AB, Becker W, Chen Y, et al. (2005) Intake of alkylresorcinols from wheat and rye in the United Kingdom and Sweden. Br J Nutr 94, 496–499.

29. Ross AB, Shepherd MJ, Bach Knudsen KE, et al. (2003) Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. Br J Nutr 90, 787–794.

30. van Dam RM & Hu FB (2008) Are alkylresorcinols accurate biomarkers for whole grain intake? Am J Clin Nutr 87, 797–798.

31. Landberg R, Linko AM, Kamal-Eldin A, et al. (2006) Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. J Nutr 136, 2760–2765.