Pharmacokinetics of alkylresorcinol metabolites in human urine

Päivi P. Söderholm[1,2,3]*, Johan E. Lundin[4], Anja H. Koskela[5], Matti J. Tikkanen[1,3] and
Herman C. Adlercreutz[1,5]

[1]*Folkhälsan Research Centre, Helsinki, Finland*
[2]*Institute of Clinical Medicine, University of Helsinki, Finland*
[3]*Department of Medicine, Division of Cardiology, Helsinki University Central Hospital, Finland*
[4]*Institute for Molecular Medicine Finland, Helsinki, Finland*
[5]*Division of Clinical Chemistry, University of Helsinki, Finland*

Abstract

Wholegrain cereals are reported to promote beneficial health effects. Wholegrain wheat and rye are almost exclusive sources of alkyl-resorcinols, and intact alkylresorcinols together with their plasma and urinary metabolites, 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA) and 3,5-dihydroxybenzoic acid (DHBA), have been proposed as biomarkers of the intake of these foods in humans. The pharmacokinetics of alkylresorcinols and their metabolites in plasma have been determined but not that of the urinary metabolites. We aimed to characterise the urinary pharmacokinetics of alkylresorcinol metabolites in humans to evaluate their potential as biomarkers of wholegrain wheat and rye. A group of fifteen volunteers followed a low-alkylresorcinol diet for 2 d before ingesting a single dose of rye bread, containing 100 mg alkylresorcinols. Urine was collected between baseline (0 h) and 25 h after administration. Thereafter alkylresorcinol metabolites were quantified by HPLC with coulometric electrode array detection. Maximum excretion rates were observed at 5–6 h for both metabolites, DHPPA being predominant over DHBA and also possessing a greater area under the curve$_{0-25\,h}$. Total urinary recovery between 0 and 25 h yielded 43 % of ingested alkylresorcinols, and at 25 h significant amounts of metabolites were still retained in the body, suggesting that even a spot urine sample may be sufficient to indicate whether or not wholegrain wheat or rye is a daily dietary component. These results support the use of urinary DHPPA and DHBA as biomarkers of wholegrain wheat and rye and enable new potential for studying the association between wholegrain intake and diseases, even in the absence of dietary data.

Key words: Rye fibre: Pharmacokinetics: Alkylresorcinol metabolites: Biomarkers

Increased consumption of wholegrain foods has been associated with reduced risk of several chronic diseases such as CVD, type 2 diabetes and certain cancers[1–7]. Beneficial health effects are suggested to originate from several constituents of whole grains, including fibre, fermentable carbohydrates, antioxidants, minerals and phytochemicals[8]. Among these phytochemicals, alkylresorcinols are almost exclusively found in rye and wheat, being located in the intermediate layer, between the testa and pericarp[9] and therefore they have been suggested to serve as objective measures for the intake (biomarkers) of these two cereals[10]. Biomarkers enable the evaluation of intake of certain foods even if there are problems concerning the identification and documentation of these food items[11–14]; alkylresorcinols or their metabolites may fill this role for wholegrain wheat and rye. According to Ross *et al.*[15] Finns have the highest alkylresorcinol intake

among several populations studied and we have previously shown that in Finnish women, the urinary alkylresorcinol metabolites reflect cereal fibre (wheat + rye) intake[16]. Alkylresorcinols (1,3-dihydroxy-5-alkylbenzene homologues) are members of a large group of phenolic lipids, which, in rye and wheat, occur mainly bound to saturated odd-numbered hydrocarbon side-chains[17,18]. In addition to having potential as biomarkers, they eventually possess a large variety of bioactivities *in vitro*, such as antimicrobial, antioxidative and antimutagenic activities, as well as inter-action with some proteins[17,18]. Alkylresorcinols are reported to be stable during food processing and baking[10], and after extremely high intake, minute amounts of intact, albeit conju-gated, alkylresorcinols have been identified in urine[19]. However, typically after ingestion, these lipophilic compounds are converted to water-soluble metabolites in excretable form,

similar to tocopherols (vitamin E)[17,19,20]. The two urinary alkyresorcinol metabolites 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA) and 3,5-dihydroxybenzoic acid (DHBA) were first described by Ross et al.[19]; these too have been proposed to serve as biomarkers for wholegrain rye and wheat intake[21,22] like their intact precursors, plasma alkyl-resorcinols[23,24], and the more recently determined plasma metabolites DHPPA and DHBA[20,25]. In order to evaluate the suitability of these compounds to serve as biomarkers, pharmacokinetic data are helpful. The kinetics of plasma alkylresorcinols[26] and plasma DHBA and DHPPA[20] have been described, but to our knowledge the kinetics of urinary DHBA and DHPPA have not been determined previously. In our present study we aimed to determine the pharmacokinetics of urinary DHBA and DHPPA in healthy subjects after a single dose of rye bread.

Subjects and methods

Chemicals and materials

Acetonitrile and methanol were obtained from Rathburn Chemicals Ltd (Walkenburg, Scotland, UK). Ortho-phosphoric acid was purchased from Riedel-de Haën (Seelze, Germany). Acetic acid, potassium dihydrogenphosphate and sodium acetate were purchased from Merck GmbH (Darmstadt, Germany). β-Glucuronidase was obtained from Roche Diagnostics GmbH (Mannheim, Germany), and sulfatase and syringic acid from Sigma-Aldrich Co. (St Louis, MO, USA). DHBA was obtained from Aldrich (Steinheim, Germany) and DHPPA from IsoSep AB (Tollinge, Sweden). The sourdough-processed, yeast-leavened rye bread, consisting of (by dry weight) wholegrain rye (68·3 %), added rye bran (8·3 %), refined wheat flour (20 %), barley and rye malt extract, mineral salt, magnesium sulfate, potassium chloride and yeast (altogether 3·3 %), was specially baked for our studies by Fazer Bakeries (Lahti, Finland). For nutrient content, see Table 1.

Subjects and study design

The basic characteristics of the study participants are shown in Table 2. A total of fifteen healthy volunteers participated in the study after completing an eligibility questionnaire and attending a screening blood test.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all

Table 1. Nutrient content of the rye bread* per 100 g fresh weight and per test dose

Nutrient	Per 100 g	Per test dose (198 g)
Energy (kJ)	800	1584
Protein (g)	6·5	12·9
Carbohydrates (g)	36	71·3
Fat (g)	1·3	2·6
Fibre (g)	9·4	18·6
Alkylresorcinols (mg)	50·5	100
Na (g)	0·3	0·6

* Fazer Bakeries, Lahti, Finland.

Table 2. Basic characteristics of the participants (Mean values and standard deviations)

Variable	Mean	SD
n	15	
Females	8	
Males	7	
Age (years)	24·3	5·3
Weight (kg)	68·3	10·9
BMI (kg/m^2)	22·1	2·0

procedures involving human subjects were approved by the Ethics Committee at the Helsinki University Central Hospital, Helsinki, Finland. Written informed consent was obtained from all subjects.

The participants were asked to avoid wholegrain rye and wheat products for 2 d before the study to minimise alkyl-resorcinol intake. After overnight fasting, the baseline urine sample was collected. Thereafter the participants ingested a single dose (198 g) of rye bread (Table 1) containing 100 mg (258 μmol) alkylresorcinols together with 21 g butter. Fat was considered an important supplement to ensure the absorption of the highly lipophilic alkylresorcinols. However, vegetable margarine was not served for the concern of long-chain TAG potentially retarding the intestinal absorption of alkylresorcinols similarly to that of vitamin E[27]. The participants were allowed 25 min for the consumption of the rye bread and butter. Standardised meals and drinks not containing wholegrain cereals were served throughout the study (ham and potato casserole, chicken soup, refined wheat bread, cucumber, lettuce, yogurt, coffee, tea, milk, water), and the participants were advised to avoid consuming any other foods.

After the ingestion of rye bread and butter, all urine was collected for the following 25 h at twelve time points 3, 4, 5, 6, 7, 8, 10, 12, 14 and 16 h. During the night-time (between 16 and 24 h) participants collected the urine in a container. The collection was completed on the following morning at 25 h.

Analytical methods

Rye bread was analysed for alkylresorcinols using the method of Ross et al.[10]. The volume of the urine sample was measured and 10 ml of each sample was stored at −20°C until the analysis of DHBA and DHPPA by the method of Koskela et al.[28]. In detail, the procedure was as follows: a 25 μl urine sample was hydrolysed overnight at 37°C with an equal volume (25 μl) of hydrolysis solution (0·1 mM-sodium acetate buffer (pH 5), β-glucuronidase (0·2 U/ml) and sulfatase (2 U/ml)). After incubation, 318·5 ng of the internal standard syringic acid was added in 50 μl methanol and 650 μl HPLC mobile phase (20 % phase B−80 % phase A; for compositions, see below) and 10 μl was analysed with HPLC (ESA Biosciences, Inc., Chelmsford, MA, USA) equipped with a model 540 autosampler, two model 580 solvent pumps, and a model 5600 coulometric electrode array detector (CEAD) with eight electrode pairs. The analytes were

separated using mobile phases consisting of 50 mM-phosphate buffer (pH 2·3)–methanol (90:10, v/v) (phase A) and 50 mM-phosphate buffer (pH 2·3)–methanol–acetonitrile (40:40:20, by vol.) (phase B), with a 25-min linear gradient from 0 to 100% phase B, thereafter 12 min 100% phase B, and the column was re-equilibrated with 0% B for 15 min, total flow was 0·3 ml/min. The analytical column was an Inertsil ODS-3 (GL Sciences Inc., Tokyo, Japan), 3×150 mm with particle size 3 μm, connected to a Quick Release RP-18 (Upchurch Scientific Inc., Oak Harbor, WA, USA) 3×10 mm guard column. DHBA was quantified at 670 mV, DHPPA at 570 mV and syringic acid at 380 mV.

Pharmacokinetic analysis

The highest excretion rate of DHBA and DHPPA between baseline (0 h) and 25 h after the rye bread intake is defined as the maximum excretion rate (ER_{max}; μmol/h), with t_{max} being the time at which ER_{max} is reached. The half-life ($t_{1/2}$) is defined as the time at which the urinary excretion rate has decreased to half of ER_{max}. The area under the curve (AUC) as well as other pharmacokinetic parameters were calculated using STATA software (version 10.0; StataCorp, College Station, TX, USA).

Statistical analysis

Data on DHPPA and DHBA excretion rates are presented as means and standard deviations. The pharmacokinetic parameters ER_{max}, t_{max}, $t_{1/2}$ and AUC for DHPPA and DHBA were statistically analysed using unpaired t tests (STATA 10.0; StataCorp LP, College Station, TX, USA) to clarify whether there are significant differences between these two metabolites. The groups of females and males were compared using unpaired t tests to determine potential differences in the pharmacokinetic parameters between the sexes. $P < 0.05$ was considered significant.

Results

All the participants consumed the test dose within the given 25 min; thus all had an intake of 100 mg rye alkylresorcinols, of which the relative homologue composition was C15:0 (1%), C17:0 (27%), C19:0 (28%), C21:0 (22%), C23:0 (12%) and C25:0 (10%).

The mean urine DHPPA and DHBA excretion rates at each time point for the whole group (n 15) are presented in Fig. 1, and the pharmacokinetic data in Table 3. Maximum concentration (μmol/ml urine) values are not used in the present study because of vast variation in urine volumes, both between individuals and also collection time points. Instead, the excretion rates of DHBA and DHPPA for each 1 h are calculated from the samples by first correcting for the volume of the sample and then dividing by the amount of time (in h) that had passed since previous collection time point. Therefore the use of creatinine as a corrector for urinary volume was not needed. The mean baseline (0 h) urinary excretion rates for DHPPA and DHBA were low; 0·737
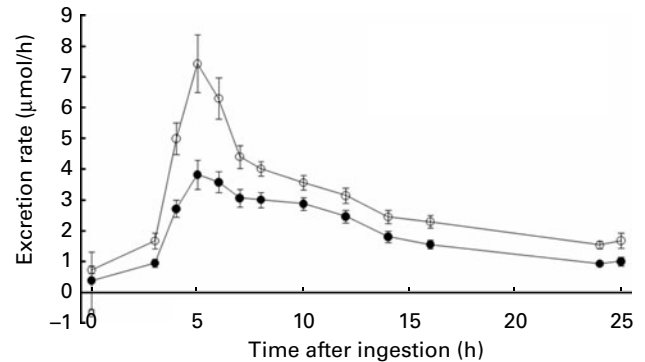

Fig. 1. Urinary pharmacokinetics of alkylresorcinol metabolites between baseline (0 h) and 25 h after consumption of a single dose of rye bread containing 258 μmol alkylresorcinols (n 15). Values are means, with standard errors of the mean represented by vertical bars. The time point of 24 h represents the overnight urine collection between 16 and 24 h; other time points represent spot urine samples. (–○–), 3-(3,5-Dihydroxyphenyl)-1-propanoic acid; (–●–), 3,5-dihydroxybenzoic acid.

(SD 0·46) and 0·386 (SD 0·36) μmol/h, respectively, indicating that participants had avoided alkylresorcinol-containing foods before the study. At 3 h after consuming the bread dose, urinary excretion rates rose rapidly and every participant reached ER_{max} fairly simultaneously for both metabolites (data not shown); thirteen of the fifteen participants reached ER_{max} for DHPPA and DHBA between 5 and 6 h, while one reached ER_{max} at 3 h and another at 10 h. DHPPA was the major metabolite in all participants, reaching higher ER_{max} and AUC than DHBA ($P < 0.0001$ for both). Excretion rates declined gradually after ER_{max} and the mean $t_{1/2}$ for DHPPA and DHBA was reached at 11·9 and 9·9 h, respectively ($P = 0.142$) (n 12). In three participants there were multiple peaks and hence the $t_{1/2}$ could not be determined for them (data not shown). The final (25 h) urinary excretion rates for DHPPA and DHBA (1·68 (SD 0·96) and 1·01 (SD 0·57) μmol/h, respectively) were significantly different from baseline ($P = 0.001$ for both). The mean total urinary recovery of the two metabolites at 25 h was 43·4% calculated from the ingested alkylresorcinols. There were no significant differences between females and males in any of the pharmacokinetic parameters ($P > 0.168$ for all; Table 3).

Discussion

The present study reports the pharmacokinetics of urinary DHPPA and DHBA, which to our knowledge have not been determined before. The present study strengthens the suggestions that these compounds are useful biomarkers for the intake of wholegrain wheat and rye[21,22]. Wholegrain cereals are of increasing interest for their health-promoting effects and the present data might be helpful when analysing existing samples from previous trials or databanks without dietary documentation. The wash-out period needed before the present study as well as the time points for sampling were determined after conducting a pilot study. According to that data we were expecting a very low rate of alkylresorcinol metabolites after 25 h and therefore a 25 h collection period

Table 3. Pharmacokinetic parameters of alkylresorcinol metabolites in human urine collected between baseline (0 h) and 25 h after administration of a single dose of rye bread containing 258 μmol alkylresorcinols

(Mean values and standard deviations)

Metabolite	Baseline concentration (μmol)		ER_{max} (μmol/h)		t_{max} (h)		AUC (μmol/25 h)		$t_{1/2}$ (h)*		Recovery (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DHPPA												
All (n 15)	0·74	0·46	8·15	3·51	5·40	0·63	67·26	16·98	11·89	5·30	26·11	6·59
Females (n 8)	0·69	0·46	8·76	4·02	5·38	0·52	66·63	15·72	13·44	6·67	25·87	6·10
Males (n 7)	0·79	0·50	7·46	3·00	5·43	0·79	67·98	19·57	10·35	3·41	26·39	7·60
DHBA												
All (n 15)	0·39	0·36	4·46	1·72	6·0	1·73	44·65	13·69	9·93	2·61	17·33	5·31
Females (n 8)	0·38	0·39	4·60	2·04	6·0	1·69	43·49	14·35	9·93	2·10	16·88	5·57
Males (n 7)	0·40	0·36	4·31	1·43	6·0	1·91	45·98	13·91	9·93	3·25	17·85	5·40

ER_{max}, maximum excretion rate; t_{max}, time to reach maximum excretion rate; AUC, area under the curve; $t_{1/2}$, half-life; DHPPA, 3-(3,5-dihydroxyphenyl)-1-propanoic acid; DHBA, 3,5-dihydroxybenzoic acid.
* For $t_{1/2}$: all subjects, n 12; females, n 6; males, n 6.

was regarded sufficient, and a 2 d wash-out period before the study was considered appropriate. In the present study we observed very low baseline values, indicating that this wash-out period was successful. After consumption of rye bread the excretion rate for DHPPA and DHBA increased rapidly, reaching maximum values between 5 and 6 h. Conversely, the decrease in the excretion rate was quite slow for both metabolites. The lowest excretion rates were detected during the overnight collection (16–24 h), indicating a slight slow-down of alkylresorcinol metabolism during the sleeping hours. At 25 h the final excretion rates for both metabolites were still more than double compared with baseline, but, on the other hand, the rates during the last 1 h accounted only for about 2·5 % of the $AUC_{0-25 h}$. Despite this, the total recovery of the two alkylresorcinol metabolites in urine at 25 h accounted only for 43 % of the ingested alkylresorcinols, being similar to values reported by Landberg et al.[21]. The low urinary recovery is in concordance with recent findings. Firstly, Ross et al.[23] described the absorption of alkylresorcinols to be about 60 % and in another study on pigs[29] they suggested a decrease in absorption with higher levels of intake. Furthermore, Landberg et al.[21] reported the urinary recovery of alkylresorcinols to decrease with increasing dose from 90 % to approximately 45 %. Thus, one could speculate that a lower dose of alkylresorcinols with a higher rate of absorption as well as urinary recovery would probably give quite similar pharmacokinetic curves as observed in the present study. Second, it is possible that from the absorbed amount of alkylresorcinols, a proportion may still be stored or delayed in the body. This is supported by our recently reported plasma pharmacokinetic data of DHPPA and DHBA which indicated that at the time point of 25 h after consumption of the same rye bread, significant amounts of the alkylresorcinol metabolites remained in the circulation[20]. Besides alkylresorcinols, also alkenylresorcinols with unsaturated hydrocarbon side-chains are known to exist in wholegrain wheat and rye. For the rye bread used in the present study, alkenylresorcinols accounted for about 20 % of the total amount of alkylresorcinols (data not shown). The metabolism of alkenylresorcinols has not been studied yet but if they

too are precursors of DHBA and DHPPA, the urinary recovery of these metabolites as calculated above is overestimated.

Nevertheless, the present pharmacokinetic data suggest that even a single spot urine sample, collected after overnight fasting, potentially can act as a qualitative or semi-quantitative biomarker for the intake of wholegrain wheat or rye during the previous day and indeed distinguish between the consumers and non-consumers of wholegrain wheat and rye. However, to clarify this, a larger set of subjects would be needed for the comparison of the 24 h urine collections with spot urine samples.

The urinary pharmacokinetics of alkylresorcinol metabolites appear to closely reflect those reported for plasma[20]. However, in contrast to the findings in plasma, urinary DHPPA achieved higher concentrations than DHBA, possibly explaining why the opposite[20] was observed in plasma. The formation of DHPPA from alkylresorcinols requires fewer metabolic steps – at least one less – compared with DHBA and it may therefore be excreted more rapidly.

The relatively small number of study participants and the large variation between individuals, which might be explained by differences in intestinal microbiota and absorption as well as the extent of metabolism in the liver and enterohepatic circulation, can be considered as limitations of the present study. Further studies are needed to understand the metabolism and excretion of alkylresorcinols in human subjects.

Taken together, our data suggest that urinary DHPPA and DHBA can be utilised as biomarkers for wholegrain wheat and rye intake, which is particularly helpful in the absence of dietary data. In addition, even a spot urine sample may be used to find out whether or not wholegrain wheat or rye is a daily dietary component.

References

1. Schatzkin A, Park Y, Leitzmann MF, *et al.* (2008) Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. *Gastroenterology* **135**, 1163–1167.
2. Anderson JW (2003) Whole grains protect against atherosclerotic cardiovascular disease. *Proc Nutr Soc* **62**, 135–142.
3. Seal CJ (2006) Whole grains and CVD risk. *Proc Nutr Soc* **65**, 24–34.
4. McKeown NM, Meigs JB, Liu S, *et al.* (2002) Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *Am J Clin Nutr* **76**, 390–398.
5. Montonen J, Knekt P, Jarvinen R, *et al.* (2003) Whole-grain and fiber intake and the incidence of type 2 diabetes. *Am J Clin Nutr* **77**, 622–629.
6. Murtaugh MA, Jacobs DR, Jacob B, *et al.* (2003) Epidemiological support for the protection of whole grains against diabetes. *Proc Nutr Soc* **62**, 143–149.
7. Larsson SC, Giovannucci E, Bergkvist L, *et al.* (2005) Whole grain consumption and risk of colorectal cancer: a population-based cohort of 60 000 women. *Br J Cancer* **92**, 1803–1807.
8. Slavin J (2003) Why whole grains are protective: biological mechanisms. *Proc Nutr Soc* **62**, 129–134.
9. Landberg R, Kamal-Eldin A, Salmenkallio-Marttila M, *et al.* (2008) Localization of alkylresorcinols in wheat, rye and barley kernels. *J Cereal Sci* **48**, 401–406.
10. Ross AB, Shepherd MJ, Schupphaus M, *et al.* (2003) Alkylresorcinols in cereals and cereal products. *J Agric Food Chem* **51**, 4111–4118.
11. Westerterp KR & Goris AHC (2002) Validity of the assessment of dietary intake: problems of misreporting. *Curr Opin Clin Nutr Metab Care* **5**, 489–493.
12. Bingham S, Luben R, Welch A, *et al.* (2008) Associations between dietary methods and biomarkers, and between fruits and vegetables and risk of ischaemic heart disease, in the EPIC Norfolk Cohort Study. *Int J Epidemiol* **37**, 978–987.
13. Jenab M, Slimani N, Bictash M, *et al.* (2009) Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum Genet* **125**, 507–525.
14. Potischman N & Freudenheim JL (2003) Biomarkers of nutritional exposure and nutritional status: an overview. *J Nutr* **133**, 873S–874S.
15. 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.
16. 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.
17. Ross AB, Kamal-Eldin A & Åman P (2004) Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutr Rev* **62**, 81–95.
18. Kozubek A & Tyman JHP (1999) Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chem Rev* **99**, 1–26.
19. Ross AB, Åman 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.
20. Soderholm PP, Koskela AH, Lundin JE, *et al.* (2009) Plasma pharmacokinetics of alkylresorcinol metabolites: new candidate biomarkers for whole-grain rye and wheat intake. *Am J Clin Nutr* **90**, 1167–1171.
21. Landberg R, Aman P, Friberg LE, *et al.* (2009) Dose response of whole-grain biomarkers: alkylresorcinols in human plasma and their metabolites in urine in relation to intake. *Am J Clin Nutr* **89**, 290–296.
22. 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.
23. Ross AB, Kamal-Eldin A, Lundin EA, *et al.* (2003) Cereal alkylresorcinols are absorbed by humans. *J Nutr* **133**, 2222–2224.
24. Linko A-M, 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.
25. 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.
26. Landberg R, Linko A-M, Kamal-Eldin A, *et al.* (2006) Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. *J Nutr* **136**, 2760–2765.
27. Borel P (2003) Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin Chem Lab Med* **41**, 979–994.
28. Koskela A, Linko-Parvinen A-M, Hiisivuori P, *et al.* (2007) Quantification of alkylresorcinol metabolites in urine by HPLC with coulometric electrode array detection. *Clin Chem* **53**, 1380–1383.
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.