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Plasma alkylresorcinols, biomarkers of whole-grain intake, are not associated with progression of coronary artery atherosclerosis in postmenopausal women with coronary artery disease

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Abstract

Objective: The objective of the present study was to examine the relationship between plasma alkyresorcinol (AR) concentrations, which are biomarkers of whole-grain intake, and atherosclerotic progression over 3 years in postmenopausal women with coronary artery disease.

Design: Plasma AR concentrations were measured by a validated GC–MS method in fasting plasma samples. Atherosclerosis progression was assessed using change in mean minimal coronary artery diameter (MCAD) and percentage diameter stenosis (%ST), based on mean proximal vessel diameter across up to ten coronary segments. Dietary intake was estimated using a 126-item interviewer-administered FFQ.

Setting: A prospective study of postmenopausal women participating in the Estrogen Replacement and Atherosclerosis trial.

Subjects: For the analysis of plasma AR concentrations and atherosclerotic progression, plasma samples and follow-up data on angiography were available for 182 women.

Results: Mean whole-grain intake was 9·6 (SE 0·6) servings per week. After multivariate adjustment, no significant associations were observed between plasma AR concentrations and change in mean MCAD or progression of %ST. Plasma AR concentrations were significantly correlated with dietary whole grains ($r = 0{\cdot}35$, $P < 0{\cdot}001$), cereal fibre ($r = 0{\cdot}33$, $P < 0{\cdot}001$), bran ($r = 0{\cdot}15$, $P = 0{\cdot}05$), total fibre ($r = 0{\cdot}22$, $P = 0{\cdot}003$) and legume fibre ($r = 0{\cdot}15$, $P = 0{\cdot}04$), but not refined grains, fruit fibre or vegetable fibre.

Conclusions: Plasma AR concentrations were not significantly associated with coronary artery progression over a 3-year period in postmenopausal women with coronary artery disease. A moderate association was observed between plasma AR concentrations and dietary whole grains and cereal fibre, suggesting it may be a useful biomarker in observational studies.

Keywords
Alkylresorcinols
Whole grains
Biomarkers
Coronary artery disease

Higher intake of whole grains has been linked to lower risk of several chronic diseases including obesity, type 2 diabetes and CVD[1–4]. As with other dietary exposures, capturing self-reported whole-grain intake is subject to measurement error[5]. For instance, study participants may inaccurately recall or report intake of whole-grain foods, and questionnaires may not be optimized to accurately capture frequently consumed foods containing whole grains. In addition, the inability of individuals to distinguish whole- from refined-grain foods, for example on the basis of colour, may result in unintentional misreporting of whole-grain intake[6]. The incorporation of independent biomarkers of exposure can be used to confirm diet–disease associations based solely on self-reported intakes[7].

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Plasma alkyresorcinols (AR), 1,3-dihydroxyl-5-n-alkylbenzene derivatives, have been used as a biomarker for whole-grain intake[8–10]. In cereal grains, these compounds are found in the cuticula between the outer testa and inner pericarp layer[11]. AR are phenolic lipids abundant in whole wheat and rye grains as homologues with odd-numbered hydrocarbon side chains. They also occur to a lesser extent in barley[12]. During the refining of grains, the outer AR-containing bran layer is discarded; dramatically decreasing AR concentrations[13]. AR are rapidly absorbed and metabolized by man[14,15]. The half-life of AR in plasma has been estimated at 5 h[16]. Although they have a short half-life, single plasma AR measures are thought to reflect long-term whole-grain intake in populations with regular and frequent whole-grain wheat and rye intake[17].

To date, few observational studies have used plasma AR as independent biomarkers of whole-grain intake to confirm observed associations between intake and disease outcomes[18–20]. The main advantage of using an independent biomarker of food intake to determine diet–disease associations in observational studies is that they are independent of subjective errors in reporting dietary intake[21]. In a sample of postmenopausal women in the Estrogen Replacement and Atherosclerosis trial, we previously reported that progression of coronary atherosclerosis over 3 years was moderately lower in those women who reported consuming higher intakes of cereal fibre and whole-grain foods[22]. Using blood samples from this cohort, the objective of the present study was to determine whether a single determination of plasma AR concentrations was associated with progression of coronary atherosclerosis during the same time period. In addition, we examined whether there was an association between plasma AR concentrations and estimates of dietary intake derived from self-reported FFQ.

Experimental methods

Participants

The present study is an ancillary analysis using data from the Estrogen Replacement and Atherosclerosis trial, a randomized, double-blind, placebo-controlled trial conducted in postmenopausal women with coronary stenosis[23]. Briefly, 309 postmenopausal women were enrolled from six US sites (one in Alabama; one in Connecticut; and four in North Carolina). Women were eligible if they were postmenopausal, were not currently receiving hormone replacement therapy, and had one or more epicardial coronary stenoses ≥30 % of the luminal diameter. Women were randomly assigned to receive conjugated equine oestrogen (0·625 mg), conjugated equine oestrogen (0·625 mg) plus medroxyprogesterone acetate (2·5 mg) or placebo. Neither treatment had a significant effect on the progression of coronary atherosclerosis[23].

At baseline (1995–1996), women completed questionnaires about their health status, medical history, physical activity and cardiovascular risk factors, as described elsewhere[23]. Of the 309 women enrolled in the trial, baseline blood samples for the determination of plasma AR were available for 215 participants. For analyses of plasma AR with progression of coronary artery disease (CAD), we excluded women missing the follow-up angiographic measure (n 33), for a sample size of 182 women. For correlations of plasma AR and dietary intake, we excluded women with missing or invalid baseline dietary data (n 25) for a sample size of 190 women. In order to maximize power, we did not restrict the data set for this analysis to women who also had data on CAD progression, as this would have reduced our sample size to 168 women.

The Institutional Review Board at Tufts Medical Center approved the present ancillary study, which utilized previously collected data from the Estrogen Replacement and Atherosclerosis trial.

Assessment of dietary intake

Habitual dietary and nutrient intake during the previous year was assessed prior to treatment at baseline with an interviewer-administered, 126-item FFQ as described elsewhere[24]. Dietary information was considered valid if reported energy intake was ≥2·51 MJ/d (600 kcal/d) and <6·74 MJ/d (4000 kcal/d). The FFQ included questions regarding the consumption of whole-grain foods such as cooked and cold breakfast cereals, dark bread, brown rice, popcorn and other grains (e.g. bulgur, kasha and couscous), as well as brand and type of cold breakfast cereal usually eaten. Breakfast cereal intake was subdivided into whole and refined grain as previously reported[2,25]. In addition, the contribution of total dietary fibre was calculated for food sources (e.g. fibre from cereal). Prior data using the FFQ indicated that daily intakes of cold and hot breakfast cereals estimated from FFQ and diet records were highly correlated ($r \geq 0{\cdot}70$)[26,27]. The correlation coefficient for other sources of whole grains ranged between 0·37 for dark bread and 0·79 for popcorn[27].

Blood alkylresorcinol measures

The homologues of AR analysed included C17:0, C19:0, C21:0, C23:0 and C25:0; and the sum of these was used to determine the total AR concentrations. Baseline samples obtained prior to treatment were analysed by a GC–MS method in single ion monitoring mode as described previously[28]. Samples were analysed randomly in ten batches along with four quality control samples per batch. Intra- and inter-assay CV were <15 % for total AR.

Covariate assessment

Potential confounding factors included age (years, continuous), race (white *v.* other), BMI (kg/m^2, continuous), oestrogen treatment arm (placebo *v.* oestrogen/oestrogen + progesterone), study site (four categories), follow-up time

(years, continuous), current smoking status (yes *v.* no), physical activity (MET/week, continuous; where MET is metabolic-equivalent task-hours), educational level (three categories), lipid-lowering medication (yes *v.* no), coronary artery bypass grafting (CABG; yes *v.* no), percutaneous transluminal coronary angioplasty (PTCA; yes *v.* no) and systolic blood pressure (mmHg, continuous).

Outcome measures

As previously described[22,23,29], quantitative coronary angiography was determined using standardized methods to measure the luminal diameters of up to ten proximal epicardial segments (mean: 9·6 segments per women) and the degree of stenosis as a percentage of the reference diameter at baseline and after 3·2 (SD 0·6) years in 248 postmenopausal women. Mean follow-up time in the present sample (*n* 182) was 3·1 (SD 0·7) years. Review and analyses of the paired films were performed by using a previously validated system of cine projectors (SME-3500; Sony, Park Ridge, NJ, USA) and software (QCAPlus; Sanders Data Systems, Palo Alto, CA, USA), as previously described[23]. Operators were blinded to the temporal sequence of the films.

Statistical analyses

Normality of continuous variables was checked; to reduce skewness, a natural logarithmic transformation was applied to plasma AR. Age-adjusted means or age-adjusted geometric means were calculated for lifestyle and dietary characteristics; dietary characteristics were also adjusted for total energy intake. The associations of plasma AR with changes in mean minimal coronary artery diameter (MCAD) and mean percentage diameter stenosis (%ST) were tested using mixed-model ANCOVA. The potential confounders controlled for in our basic model included age, oestrogen treatment arm, race, BMI, clinic, follow-up time and the baseline outcome measure. Additional models were further adjusted for current smoking, physical activity, education levels, lipid-lowering medication use, CABG, PTCA and systolic blood pressure. In a sensitivity analysis, we excluded segments of the coronary artery totally occluded at baseline (defined as a baseline MCAD $< 0{\cdot}1$ mm or baseline stenosis ≥ 99 %, *n* 31 segments in twenty-six individuals). To confirm that plasma AR are a biomarker of whole-grain intake in this population, partial Spearman rank-order correlation coefficients were calculated for plasma AR and whole- and refined-grain intakes, as well as various sources of fibre, adjusted for total energy intake. Further adjustment of correlations for age and BMI did not affect the correlations (data not shown).

Values are reported as means with their standard errors, unless otherwise noted. The significance level was set as a two-tailed *P* value $<0{\cdot}05$. Statistical analyses were conducted using the statistical software package SAS version 9·3.

Results

The age-adjusted baseline participant characteristics are presented in Table 1. The mean age was 65·0 (SE 0·5) years and mean BMI was 29·6 (SE 0·4) kg/m^2. In this sample, 85 % were Caucasian, 24 % were taking anti-hypertensive medication, 29 % were classified as having diabetes, and 33 % were taking lipid-lowering medication. Mean baseline whole-grain intake in the subset of women with dietary data (*n* 190) was 9·6 (SE 0·6 servings) per week, in contrast with refined-grain intake of 16·0 (SE 0·6) servings per week. Geometric mean plasma AR concentration was 18·0 nmol/l. The distribution of plasma AR was skewed, and the 25th, 50th and 75th percentiles of plasma AR were 10·8, 16·1 and 25·3 nmol/l, respectively.

Estimated progression of coronary atherosclerosis per 50 % increment in total plasma AR concentrations is shown in Table 2. No significant association was observed for change in MCAD or %ST ($P = 0{\cdot}61$ and $P = 0{\cdot}59$, respectively) after adjustment for age, oestrogen treatment arm, race,

Table 1 Baseline characteristics* of the study participants: postmenopausal women (*n* 190), Estrogen Replacement and Atherosclerosis trial, USA

	Mean	SE
General characteristics		
Age (years)	65·0	0·5
Race (% white)	85	3
BMI (kg/m^2)	29·6	0·4
Education (% high school or more)	60	4
Current cigarette smoker (%)	19	3
Anti-hypertensive medication (%)	24	3
Diabetes (%)	29	3
Lipid-lowering medication (%)	33	4
Physical activity level (MET/week)	1·13	0·01
Dietary characteristics†		
Energy (kJ/d)	6929	188
Energy (kcal/d)	1656	45
Protein (g/d)	68·7	1·1
Protein (%E)	16·8	0·3
Saturated fat (g/d)	16·5	0·4
Saturated fat (%E)	9·1	0·2
Monounsaturated fat (g/d)	17·9	0·4
Monounsaturated fat (%E)	9·8	0·2
Polyunsaturated fat (g/d)	10·4	0·2
Polyunsaturated fat (%E)	5·7	0·1
Carbohydrate (g/d)	241·6	2·7
Carbohydrate (%E)	58·1	0·6
Alcohol (g/d)	0·8	0·2
Dietary fibre (g/d)	20·9	0·5
Cereal fibre (g/d)	5·5	0·2
Fruit (servings/week)	14·0	0·7
Vegetables (servings/week)	26·7	0·9
Whole grains (servings/week)	9·6	0·6
Refined grains (servings/week)	16·0	0·6
Bran (g/d)	1·3	0·1
Biomarker		
Plasma AR (nmol/l)‡	18·0	1·0

MET, metabolic-equivalent task-hours; %E, percentage of energy intake; AR, alkylresorcinols.
*Data are presented as means or percentages with their standard errors; all characteristics, except age, are age-adjusted.
†Foods and nutrient characteristics, except energy, are also adjusted for energy.
‡Geometric mean with its standard error.

Table 2 Estimated progression of coronary atherosclerosis, as change in MCAD or %ST, per 50 % increment in total plasma AR among postmenopausal women (*n* 182), Estrogen Replacement and Atherosclerosis trial, USA

	Effect on outcome per 50 % increment in total plasma AR					
	Change in mean MCAD			Change in mean %ST		
	Estimate	95 % CI	*P* value	Estimate	95 % CI	*P* value
Model 1*	−0·0035	−0·0171, 0·0101	0·61	0·14	−0·36, 0·64	0·59
Model 2†	−0·0021	−0·0161, 0·0118	0·76	0·09	−0·42, 0·61	0·73

MCAD, minimum coronary artery diameter; %ST, percentage diameter stenosis; AR, alkylresorcinols.
*Adjusted for age, race, BMI, treatment group, follow-up years, clinic and the corresponding baseline measure.
†Adjusted as for model 1, plus current smoking, physical activity and education level.

Table 3 Partial Spearman correlations (*r*) and *P* value between energy-adjusted grain and fibre intakes and plasma AR among postmenopausal women (*n* 190), Estrogen Replacement and Atherosclerosis trial, USA

	Total AR	*P* value
Refined grains (servings/week)	−0·06	0·45
Whole grains (servings/week)	0·35	<0·001
Total fibre (g/d)	0·22	0·003
Bran (g/d)	0·15	0·05
Cereal fibre (g/d)	0·33	<0·001
Cruciferous fibre (g/d)	0·03	0·68
Fruit fibre (g/d)	0·07	0·35
Vegetable fibre (g/d)	0·08	0·27
Legume fibre (g/d)	0·15	0·04

AR, alkylresorcinols.

BMI, clinic, follow-up time and the respective baseline outcome measure (model 1). Further adjustments for lifestyle factors (model 2) did not change these observations. In addition, further adjustment for lipid-lowering medication, CABG, PTCA or systolic blood pressure also did not change these observations. Using the median cut point for plasma AR (16·1 nmol/l), no significant difference in change in mean MCAD was observed in those with higher plasma AR (median 25·3 nmol/l) compared with lower plasma AR concentrations (median 10·8 nmol/l; −0·06 (SE 0·02) *v.* −0·08 (SE 0·02 mm), *P* trend = 0·43) in any model (data not shown). Using the median cut point, plasma AR concentrations were not significantly associated with mean progression of %ST (2·31 (SE 0·82) *v.* 3·63 (SE 0·82) mm in high and low plasma AR categories, respectively, *P* trend = 0·14). Sensitivity analyses excluding occluded segments did not substantively alter any results.

A significant correlation was observed between plasma AR concentrations and weekly servings of whole grains ($r = 0·35$, $P < 0·001$) and cereal fibre ($r = 0·33$, $P < 0·001$). A weaker correlation was observed with total fibre ($r = 0·22$, $P = 0·003$), total bran ($r = 0·15$, $P = 0·05$) and legume fibre ($r = 0·15$, $P = 0·04$; Table 3). In contrast, there was no significant correlation between AR and weekly servings of refined grains, fruit fibre or vegetable fibre. Further adjustment for age and BMI did not substantively alter our results (data not shown).

Discussion

In nutritional epidemiology, a common application of plasma biomarker concentrations is to verify estimation of diet–disease risk associations[30]. In this cohort of postmenopausal women with CAD, plasma AR concentrations, a biomarker of whole-grain intake, were not significantly associated with lesion progression during a 3-year period. These findings are in contrast to prior work that reported higher intakes of whole-grain foods and cereal fibre were moderately associated with less progression of coronary atherosclerosis in the same sample of women[22]. Of note, the association with whole grains in the above-mentioned study[22] was slightly attenuated after adjustment for other aspects of diet, suggesting perhaps that dietary patterns associated with whole-grain intake, rather than whole-grain intake *per se*, were associated with less atherosclerotic progression. In contrast, whole-grain intake was inversely associated with several markers of atherosclerosis progression, independent of a healthy dietary pattern, in a multiethnic cohort of middle-aged adults[31]. The reason for these discrepancies is not clear. We cannot rule out the possibility in the current study that misclassification of exposure attenuated the association between plasma AR concentrations and the progression of coronary atherosclerosis.

Observational studies in free-living adults have only recently begun to include measures of plasma AR concentrations as independent biomarkers for assessing whole-grain exposure[18–20,32]. In a small sample of 241 older community-dwelling adults, plasma AR concentrations were inversely associated with BMI[20], confirming a previously observed inverse relationship between whole-grain intake and BMI[33]. In a nested case–control study[19], higher plasma total AR concentrations were negatively associated with incidences of distal colon cancer but not with overall colorectal cancer, proximal colon cancer or rectal cancer[32]. In this sample of postmenopausal women, the median plasma AR was low (16 nmol/l), reflective of the low self-reported daily whole-grain intake (average intake ≈1·4 serving/d), and the range was relatively narrow[22]. The narrow range may have limited our ability to detect an association between AR and atherosclerotic progression, were there to be one. The data

reflect whole-grain intakes in a subgroup of women and document that these intakes are low relative to both recommendations at the time the samples were collected and current recommendations. This median plasma AR concentration observed is similar to that reported for participants in the intervention arm of a metabolic study who were provided with a control diet devoid of whole grains, and that reported for older men and women living in the USA[20,34]. In comparison to European cohorts, these AR concentrations are low, confirming low intake of whole-grain wheat[10,12,35,36].

Although the correlations between the single fasting plasma AR concentrations and self-reported intake of whole grains and cereal fibre were moderate ($r = 0{\cdot}33$–$0{\cdot}35$), the weaker or non-significant associations between plasma AR and the other dietary fibres confirms the specificity of AR as a biomarker of whole grains. The magnitude of this correlation is fairly consistent with other studies that have correlated whole-grain intake estimated from FFQ with plasma AR concentrations[9,20,37,38]. Factors that can affect the relationship between whole-grain intake estimated with an FFQ and plasma AR concentrations may include intra-individual variation in AR concentrations inherent to a short-half life, irregular and low whole-grain intake, and inter-individual AR variation in AR metabolism as recently demonstrated by monitoring urinary AR metabolite excretion[39]. Because of these potential limitations, multiple measures of fasting AR may be required to capture an individual's representative whole-grain intake.

The current work has some limitations. Plasma AR is only reflective of wheat and rye; thus, intakes of popcorn and brown rice, which are also whole grains, particularly in the USA, are not reflected by this biomarker. Further, data on whole-grain intake at follow-up was not available for this analysis and diet may have changed over this time period. While we adjusted for interim procedures such as CABG and PTCA, we did not have information on and, thus, did not adjust for potential changes in usage of medications. In addition, it is possible that sample handling or duration of storage for the blood samples (approx. 20 years) may have influenced the AR concentrations. However, inherent to the molecular structure of AR and on the basis of an ongoing stability study (R Landberg, personal communication), this is unlikely. It is possible that given the short half-life of the intact AR (approx. 5 h), plasma AR concentrations are suitable as a biomarker only in populations with stable and frequent whole-wheat (or whole-rye) intake, where these biomarkers would be useful in distinguishing non-consumers from high consumers[9,15].

In contrast to our *a priori* hypothesis, plasma AR concentrations were not significantly associated with atherosclerotic lesion progression over a 3-year period in postmenopausal women with CAD. Further studies using this biomarker should be undertaken in populations where there is a wider range of whole-grain intake and in conjunction with traditional dietary assessment methods that capture long-term intake.

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References

1. McKeown NM, Hruby A, Saltzman E *et al.* (2012) Weighing in on whole grains: a review of evidence linking whole grains to body weight. *Cereal Foods World* **57**, 20–27.
2. Jacobs DR Jr, Meyer KA, Kushi LH *et al.* (1998) Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Women's Health Study. *Am J Clin Nutr* **68**, 248–257.
3. Ye EQ, Chacko SA, Chou EL *et al.* (2012) Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *J Nutr* **142**, 1304–1313.
4. Mellen PB, Walsh TF & Herrington DM (2008) Whole grain intake and cardiovascular disease: a meta-analysis. *Nutr Metab Cardiovasc Dis* **18**, 283–290.
5. Keogh RH & White IR (2014) A toolkit for measurement error correction, with a focus on nutritional epidemiology. *Stat Med* **33**, 2137–2155.
6. Mozaffarian RS, Lee RM, Kennedy MA *et al.* (2013) Identifying whole grain foods: a comparison of different approaches for selecting more healthful whole grain products. *Public Health Nutr* **16**, 2255–2264.
7. Freedman LS, Kipnis V, Schatzkin A *et al.* (2010) Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? *Epidemiol Perspect Innov* **7**, 2.
8. Kristensen M, Toubro S, Jensen MG *et al.* (2012) Whole grain compared with refined wheat decreases the percentage of body fat following a 12-week, energy-restricted dietary intervention in postmenopausal women. *J Nutr* **142**, 710–716.
9. Ross AB, Bourgeois A, Macharia HN *et al.* (2012) Plasma alkylresorcinols as a biomarker of whole-grain food consumption in a large population: results from the WHOLEheart Intervention Study. *Am J Clin Nutr* **95**, 204–211.

10. Magnusdottir OK, Landberg R, Gunnarsdottir I *et al.* (2013) Plasma alkylresorcinols reflect important whole-grain components of a healthy Nordic diet. *J Nutr* **143**, 1383–1390.
11. 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.
12. 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.
13. Ross AB & Kochhar S (2009) Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC–coularray-based electrochemical detection. *J Agric Food Chem* **57**, 5187–5193.
14. Ross AB, Kamal-Eldin A, Lundin EA *et al.* (2003) Cereal alkylresorcinols are absorbed by humans. *J Nutr* **133**, 2222–2224.
15. 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.
16. 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.
17. Landberg R, Aman P, Hallmans G *et al.* (2013) Long-term reproducibility of plasma alkylresorcinols as biomarkers of whole-grain wheat and rye intake within Northern Sweden Health and Disease Study Cohort. *Eur J Clin Nutr* **67**, 259–263.
18. Olsen A, Landberg R, Aman P *et al.* (2010) Plasma levels of alkylresorcinols and incidence of endometrial cancer. *Eur J Cancer Prev* **19**, 73–77.
19. Kyro C, Olsen A, Landberg R *et al.* (2014) Plasma alkylresorcinols, biomarkers of whole-grain wheat and rye intake, and incidence of colorectal cancer. *J Natl Cancer Inst* **106**, djt352.
20. Ma J, Ross AB, Shea MK *et al.* (2012) Plasma alkylresorcinols, biomarkers of whole-grain intake, are related to lower BMI in older adults. *J Nutr* **142**, 1859–1864.
21. Kipnis V, Subar AF, Midthune D *et al.* (2003) Structure of dietary measurement error: results of the OPEN biomarker study. *Am J Epidemiol* **158**, 14–21.
22. Erkkila AT, Herrington DM, Mozaffarian D *et al.* (2005) Cereal fiber and whole-grain intake are associated with reduced progression of coronary-artery atherosclerosis in postmenopausal women with coronary artery disease. *Am Heart J* **150**, 94–101.
23. Herrington DM, Reboussin DM, Brosnihan KB *et al.* (2000) Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. *N Engl J Med* **343**, 522–529.
24. Rimm EB, Giovannucci EL, Stampfer MJ *et al.* (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* **135**, 1114–1126.
25. Liu S, Stampfer MJ, Hu FB *et al.* (1999) Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* **70**, 412–419.
26. Hu FB, Rimm E, Smith-Warner SA *et al.* (1999) Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* **69**, 243–249.
27. Feskanich D, Rimm EB, Giovannucci EL *et al.* (1993) Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc* **93**, 790–796.
28. Landberg R, Man P & Kamal-Eldin A (2009) A rapid gas chromatography–mass spectrometry method for quantification of alkylresorcinols in human plasma. *Anal Biochem* **385**, 7–12.
29. Imamura F, Jacques PF, Herrington DM *et al.* (2009) Adherence to 2005 Dietary Guidelines for Americans is associated with a reduced progression of coronary artery atherosclerosis in women with established coronary artery disease. *Am J Clin Nutr* **90**, 193–201.
30. Van Dam RM & Hunter D (2013) Biochemical indicators of dietary intake. In *Nutritional Epidemiology*, 3rd ed., pp. 150–201 [W Willett, editor]. New York: Oxford University Press.
31. Mellen PB, Liese AD, Tooze JA *et al.* (2007) Whole-grain intake and carotid artery atherosclerosis in a multiethnic cohort: the Insulin Resistance Atherosclerosis Study. *Am J Clin Nutr* **85**, 1495–1502.
32. Knudsen MD, Kyro C, Olsen A *et al.* (2014) Self-reported whole-grain intake and plasma alkylresorcinol concentrations in combination in relation to the incidence of colorectal cancer. *Am J Epidemiol* **179**, 1188–1196.
33. McKeown NM, Yoshida M, Shea MK *et al.* (2009) Whole-grain intake and cereal fiber are associated with lower abdominal adiposity in older adults. *J Nutr* **139**, 1950–1955.
34. Ross AB, Bruce SJ, Blondel-Lubrano A *et al.* (2011) A whole-grain cereal-rich diet increases plasma betaine, and tends to decrease total and LDL-cholesterol compared with a refined-grain diet in healthy subjects. *Br J Nutr* **105**, 1492–1502.
35. 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.
36. 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.
37. Landberg R, Kamal-Eldin A, Aman P *et al.* (2011) Determinants of plasma alkylresorcinol concentration in Danish post-menopausal women. *Eur J Clin Nutr* **65**, 94–101.
38. Kyro C, Olsen A, Bueno-de-Mesquita HB *et al.* (2014) Plasma alkylresorcinol concentrations, biomarkers of whole-grain wheat and rye intake, in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Br J Nutr* **111**, 1881–1890.
39. Landberg R, Townsend MK, Neelakantan N *et al.* (2012) Alkylresorcinol metabolite concentrations in spot urine samples correlated with whole grain and cereal fiber intake but showed low to modest reproducibility over one to three years in US women. *J Nutr* **142**, 872–877.