Plasma Alkylresorcinols Reflect Gluten Intake and Distinguish between Gluten-Rich and Gluten-Poor Diets in a Population at Risk of Metabolic

The Journal of Nutrition
Nutrition and Disease

Plasma Alkylresorcinols Reflect Gluten Intake and Distinguish between Gluten-Rich and Gluten-Poor Diets in a Population at Risk of Metabolic Syndrome[1–3]

Mads V Lind,[4,5,9]* Mia L Madsen,[6,9] Jüri J Rumessen,[7] Henrik Vestergaard,[6,8] Rikke J Gøbel,[6] Torben Hansen,[6] Lotte Lauritzen,[4] Oluf B Pedersen,[6] Mette Kristensen,[4] and Alastair B Ross[5]

[4]Department of Nutrition, Exercise, and Sports, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark; [5]Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden; [6]The Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, and [7]QD-Research Unit and Department of Gastroenterology, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark; and [8]Steno Diabetes Center, Gentofte, Denmark

Abstract

Background: Many patients with celiac disease experience difficulties in adherence to a gluten-free diet. Methods for testing compliance to a gluten-free diet are costly and cumbersome. Thus, a simple biomarker of gluten intake is needed in a clinical setting and will be useful for epidemiologic studies investigating wider effects of gluten intake.

Objective: The aim was to evaluate plasma total alkylresorcinol concentrations as a measure of gluten intake.

Methods: In this randomized, controlled, crossover intervention study in 52 Danish adults with features of the metabolic syndrome, we compared 8 wk of a gluten-rich and gluten-poor diet separated by a washout period of ≥6 wk. We measured fasting plasma concentrations of alkylresorcinols to determine if they reflected differences in gluten intake as a secondary outcome of the original study. In addition, we investigated in 118 Danish adults the cross-sectional association between self-reported gluten intake and plasma alkylresorcinols in the same and a similar study at baseline. We used mixed-model ANCOVA for examining treatment effects, a classification tree to determine compliance to the gluten-poor diet, and linear regression models for examining baseline correlation between plasma alkylresorcinol concentrations and gluten intake.

Results: Plasma total alkylresorcinols decreased more during the gluten-poor period (geometric mean: –124.8 nmol/L; 95% CI: –156.5, –93.0 nmol/L) than in the gluten-rich period (geometric mean: –31.8 nmol/L; 95% CI: –63.1, –0.4 nmol/L) ($P < 0.001$). On the basis of the plasma alkylresorcinol profile, we built a classification tree to objectively determine compliance and found an overall participant misclassification error of 3.9%. In the cross-sectional study we found a 5.6% (95% CI: 2.4%, 8.9%) increase in plasma total alkylresorcinols per 1-g increase in reported gluten intake ($P < 0.001$).

Conclusion: We propose the use of plasma alkylresorcinols to monitor compliance to a gluten-free diet as well as to help investigations into the possible effects of gluten in the wider population. This trial was registered at www.clinicaltrials.gov as NCT017119913 and NCT01731366. *J Nutr* 2016;146:1991–8.

Keywords: celiac disease, biomarkers, gluten sensitivity, gluten-related disorders, coeliac disease, gluten intolerance, gluten-free diet

Introduction

Celiac disease (CD) is characterized as a state of increased immunologic responsiveness to ingested gluten, and the only effective therapy for patients with CD is to follow a life-long gluten-free diet in order to avoid the long-term risks associated with CD (1). CD is a multiorgan disease, which may remain asymptomatic or become symptomatic at all ages, with a wide variety of gastrointestinal and extraintestinal manifestations that vary in nature and severity (1, 2). The prevalence of CD in

[1] Supported by the Innovation Fund Denmark grant 11-116163. Some of the intervention products were sponsored by Kohberg, Lantmännen, AXA, Wasa, Urtekram, Finax, and Doves Farm.
[2] Author disclosures: ML Madsen, JJ Rumessen, H Vestergaard, RJ Gøbel, T Hansen, L Lauritzen, OB Pedersen, M Kristensen, and AB Ross, no conflicts of interest. MV Lind is partly supported by an unrestricted grant from Cereal Partners Worldwide a joint venture between Nestlé SA and General Mills Ltd. The study sponsors played no role in the design, methods, data management and analysis, or in the decision to publish.

[3] Supplemental Tables 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[9] These authors contributed equally to this work.
*To whom correspondence should be addressed. E-mail: madslind@nexs.ku.dk.

Manuscript received May 27, 2016. Initial review completed July 6, 2016. Revision accepted August 11, 2016.
First published online September 14, 2016; doi:10.3945/jn.116.236398.

Western populations is ~1% of the general population (3–7), although the prevalence appears to be increasing (8, 9), which may be caused by increased awareness and improved diagnostic procedures. If left untreated, CD is associated with a number of complications, such as chronic diarrhea, abdominal pain, anemia, and growth problems in infancy and childhood, as well as osteoporosis and especially gastrointestinal cancers later in life (2, 10, 11). The only therapy for CD is to follow a strict gluten-free diet.

Gluten refers to a range of complex protein structures found in wheat, rye, and barley (12). Despite improvement in the availability of gluten-free products for patients with CD, adolescents and children in particular experience difficulties in adherence to a strict gluten-free diet (1, 13–15). This is mainly due to the fact that wheat is nearly ubiquitous in the normal Western diet and furthermore that gluten is found in a wide range of products beyond those recognized as cereal products. Difficulties in adherence to a strict gluten-free diet in patients with CD can cause problems in clinical settings and complicate clinical decisions relating to reasons for progress or lack thereof in the treatment of patients with CD. Current methods to test for compliance to a gluten-free diet include self-reported dietary intake, serologic measures of CD-related antibodies, detection of gluten peptides in fecal samples, and histologic measurements (16–18). The first method is subjective, and the latter 3 are costly and clinically extensive and can be uncomfortable for the patient. Thus, an objective and simple biomarker of gluten intake is urgently required for clinical work in patients with CD.

In addition to containing gluten, wheat, rye, and barley also contain alkylresorcinols in the outer layers of the grains, and these are currently used as a biomarker of intake of whole grains of these cereals in epidemiologic and intervention studies (19–23). Alkylresorcinols are long-chain phenolic lipids present as 5 main odd-numbered alkyl-chain homologs ranging from 17 to 25 carbons long, with the distribution of the homologs specifically reflecting the type of cereal (23). These homolog fingerprints can be measured in plasma, making it possible to distinguish between persons who mainly consume wheat or rye and total alkylresorcinol (24, 25). Small amounts of alkylresorcinols are also found in the refined flour of wheat (26), and the plasma of persons who consume only refined wheat still contains measurable amounts of plasma alkylresorcinols (27). It has been proposed that alkylresorcinols could be markers of wheat contamination in gluten-free products, and of gluten-free diets in humans (23, 28). Recently, we discovered the presence of odd- and even-chained alkylresorcinols in quinoa, a gluten-free seed that is often used as part of a gluten-free diet (29). However, the unique homolog composition in quinoa makes it possible to differentiate between alkylresorcinols derived from wheat, rye and barley and those derived from quinoa.

A biomarker of gluten intake would have great potential to improve our understanding of the effectiveness of gluten-free diets in preventing the symptoms of CD and in ensuring compliance to gluten-free diets when testing other therapies for CD both in clinical use and in research. The aim of this study was to determine whether plasma total concentrations of alkylresorcinols and homolog distribution could function as biomarkers of compliance to a gluten-free diet. We measured plasma concentrations of alkylresorcinols in a randomized, crossover, clinical intervention trial, with either a gluten-rich or a gluten-poor diet, and further examined plasma alkylresorcinol concentrations and reported gluten intake in a cross-sectional study to examine their usefulness in an epidemiologic setting. This proof-of-concept study was carried out in a population without CD because feeding gluten to patients with CD will lead to a worsening of their symptoms. The study reports a secondary outcome of the original study.

Methods

This study is a part of the 2 large human intervention studies within the Gut, Grain, and Greens Center (3G) investigating the effects of gluten and whole grains, respectively, on gut microbiota composition and metabolic health (30). A total of 60 individuals were included in the gluten trial, which applied a randomized, controlled, crossover design comprising a gluten-rich (based on refined wheat) and a gluten-poor period of 8 wk, separated by a washout period of ≥6 wk. An additional 60 participants were included in a similar study that compared whole and refined grains, and baseline values from these individuals and the individuals from the gluten intervention study (total n = 120) were used for a cross-sectional correlation analysis of gluten intake and plasma alkylresorcinols. The studies were registered at www.clinicaltrials.gov (NCT017119913 and NCT01731366) and approved by the Municipal Ethical Committee of the Capital Region of Denmark in accordance with the Helsinki Declaration (H-2-2012-064 and H-2-2012-065) and the Data Protection Agency (2012-54-0170 and 2007-54-0269). The study design has been described in detail elsewhere (30).

Participants

The study participants for both studies comprised men and women aged 20–65 y who resided in the Greater Copenhagen area. Participants had to be weight stable and exhibit a metabolic risk profile, because the primary aim of the 3G studies was to investigate gut microbiota in persons at increased risk of metabolic disorders (30). The inclusion criteria were BMI (in kg/m^2) of 25–35 and/or a waist circumference ≥94 cm for men and ≥80 cm for women and ≥1 of the following 4 additional inclusion criteria: fasting plasma glucose of 6.1–6.9 mmol/L, fasting serum HDL cholesterol ≤1.03 mmol/L for men and ≤1.29 mmol/L for women, fasting plasma TGs >1.3 mmol/L, and systolic blood pressure >130 mm Hg or medical treatment of hypertension (30). Pregnant and lactating women were excluded from the study as were individuals with diagnosed chronic gastrointestinal disorders, diabetes, or chronic pancreatitis. Additional exclusion criteria included the following: pharmacologic treatment of dyslipidemia, medically prescribed diet, antibiotic treatment (<3 mo before study initiation) or intake of pre- or probiotic supplements (<1 mo before study initiation), hemoglobin <7.0 mmol/L or blood donation <1 mo before study initiation, participation in other biomedical trials (<1 mo before study initiation), intense physical activity (>10 h/wk), and alcohol consumption >21 units/wk for men and >14 units/wk for women. In addition, individuals were screened for latent CD by measurement of serum IgA and IgG transglutaminase at first examination day and participants were excluded if values exceeded the acceptable maximum (<8 units/mL for IgA and <10 units/mL for IgG) (30).

Dietary registration

Before baseline examination and before examination at the end of each intervention period, participants completed a 4-d precoded dietary registration, developed by the National Food Institute at the Technical University of Denmark. Details about the method and calculation of intakes of food and nutrients have been described elsewhere (31).

Gluten content in food items

All food products containing wheat, barley, and rye were considered to contain gluten. From all food products reported in the food record and food diary, the gluten content of these cereals was calculated as previously described (32). Briefly, the gluten content was estimated on the basis of the amount of protein in gluten-containing cereals according to the Danish Food Composition Databank (33) by multiplication of the protein content by 0.8 for wheat (34, 35), by 0.65 for rye (36), and by 0.50 for barley (37). The amount of gluten-containing cereals in composite foods was estimated from recipe information from the producers or the recipes used in the dietary survey.

Dietary intervention

Intervention. The participants of the gluten intervention study were requested to replace all cereal products from their diet with the provided study products, which were consumed ad libitum. The study products were compatible with a typical Danish diet and comprised a range of cereal products, including breads, pastas, and whole-kernel grains

(**Supplemental Table 1**). All cereals for the gluten-poor period were gluten-free or contained only traces of gluten. The gluten-rich diet was based mainly on refined-wheat products. The gluten-poor and the gluten-rich diets were matched for total dietary fiber content in order to exclude dietary fiber as a potential confounder for the overall study. The aim was to keep the daily gluten consumption to an absolute minimum in the gluten-poor period (<2 g/d) and as high as possible in the control period (>20 g/d).

To ensure a limited intake of gluten from nonstudy foods in both periods, participants were instructed to avoid any flour-containing confectioneries, such as cakes and biscuits and savory snacks. Furthermore, flour-based "fast food" meals as well as ready-to-eat meals, such as pizza and lasagna, were to be avoided. Moreover, participants were asked not to consume other starchy products, such as potatoes, >1 time/wk because they might consume these products instead of the provided study products. A trained dietitian provided participants with instructions on how to incorporate the provided study products in the diet. Study products were provided in sufficient amounts to ensure ad libitum consumption during both intervention periods. Baseline samples from participants of a different part of the same study with the same protocol but different diets were included in a cross-sectional analysis (30).

Compliance and deviations from study protocol. Throughout both intervention periods, the participants were instructed to keep a study diary, in which they registered daily consumption (amount and type) of study products. In addition, they noted any deviations from the dietary instructions in the diary. A trained dietician conducted a follow-up telephone call every second week, focusing on consumption of study products and adherence to the diet. The quantification of deviations from study protocol was calculated as numbers of reported intake of breads or other cereal products not provided by the study.

Food alkylresorcinol analysis

Cereal grains and products were analyzed by using ultra HPLC with fluorescence detection (28). Alkylresorcinols were extracted from samples in duplicate and were identified by comparing retention times with authentic standards.

Plasma LDL-cholesterol, TG, and alkylresorcinol analysis

Blood samples for measurements of plasma LDL-cholesterol and TG concentrations as well as plasma total alkylresorcinols were collected in the morning after an 8- to 10-h overnight fast. Plasma concentrations of plasma total LDL cholesterol and TGs were determined by using an automated, enzymatic colorimetric assay on an ABX Pentra 400 (Horiba ABX). Analytical intraserial variations (CV%) were 3.1% for LDL cholesterol and 3.0% for TGs. Plasma total alkylresorcinols were measured by using a recently published normal-phase LC–tandem MS method (38). Briefly, 100 μL plasma was extracted by using supported liquid extraction (HybridSPE; Supelco, Sigma Aldrich) and with 2 × 800 μL acetone. The resulting extract was evaporated and resuspended in heptane:ethanol solution (95:5 vol:vol). Extracts were run on an LC–tandem MS (LCMS 8030+; Shimadzu Europa GmbH), and odd and even alkylresorcinol homologs from C17 to C26 were measured by using multiple-reaction monitoring. Injection to injection runtime for the method is 3 min. Even-chained alkylresorcinol homologs C20, C22, C24, and C26, which are present in quinoa but not in wheat rye or barley (29), were included in this method. The method had a limit of detection of 1.1–1.8 nmol/L, depending on the homolog. Intrabatch variation for control samples was 3–15%, whereas interbatch variation was 8–18%, with the variation being highest around the limit of detection.

Statistical analysis

Baseline descriptive population data are expressed as means ± SDs or medians (25th, 75th percentiles). Alkylresorcinol concentrations were right-skewed and are thus presented as geometric means with 95% CIs in parentheses. All statistical tests were performed by using the R statistical environment (version 3.1.3).

Mixed-model ANCOVAs with study participant as a random variable and treatment as a covariate were used to examine the effects of treatment on changes in plasma alkylresorcinol concentrations. These analyses were performed on data from participants who completed the trial only. The model was further adjusted for changes in plasma LDL-cholesterol and TG concentrations. Differences in study product consumption during intervention periods were tested by using a paired *t* test. Correlations between plasma alkylresorcinol concentrations and reported alkylresorcinol intake during the whole intervention and control period were tested by using Pearson's correlation analysis.

Potential determinants (age; sex; total, LDL, and HDL cholesterol; TGs; and FFAs) of plasma alkylresorcinol concentrations were analyzed on 3G baseline cohort data by using a multivariate linear regression model. Stepwise model reduction was used based on Akaike's information criterion. Potential determinants were included as covariates in later statistical analyses.

Data were also analyzed as quartiles of plasma alkylresorcinol concentration, which were compared with quartiles of reported gluten intakes in the 3G baseline cohort. The percentages of agreement and misclassification were determined, and participants placed in opposite quartiles were classified as grossly misclassified. Cohen's weighted κ statistic was used to determine the agreement between plasma alkylresorcinol concentration and reported gluten intake.

TABLE 1 Baseline characteristics of the included participants in both the gluten intervention and the cross-sectional parts of the study[1]

	Gluten intervention study			Cross-sectional study		
	All (*n* = 52)	Men (*n* = 22)	Women (*n* = 30)	All (*n* = 118)	Men (*n* = 47)	Women (*n* = 71)
Age,[2] y	50 (22, 66)	52 (26, 66)	48 (22, 65)	49 (20, 66)	49 (25, 66)	48 (20, 66)
Height, cm	173 ± 10	182 ± 6	166 ± 7	173 ± 9	181 ± 6	167 ± 6
Weight, kg	86 ± 14	97 ± 9	78 ± 11	86 ± 13	95 ± 10	80 ± 11
BMI, kg/m^2	28.7 ± 3.6	29.3 ± 2.4	28.3 ± 4.2	28.7 ± 3.5	28.9 ± 2.4	28.6 ± 4.1
Waist circumference, cm	99 ± 10	104 ± 8	96 ± 9	100.1 ± 8.95	103.9 ± 7.5	97.5 ± 8.9
Body fat, %	31.4 ± 7.6	25.1 ± 5.2	36.1 ± 5.4	32.3 ± 8.4	24.3 ± 4.9	37.5 ± 5.6
Systolic blood pressure, mm Hg	128 ± 13	135 ± 12	123 ± 12	128 ± 13	134 ± 12	124 ± 12
Diastolic blood pressure, mm Hg	81 ± 10	84 ± 9	79.4 ± 9.7	81 ± 9	83 ± 9	80 ± 9
Fasting plasma glucose, mmol/L	5.8 ± 0.7	6.0 ± 0.6	5.6 ± 0.7	5.68 ± 0.61	5.56 ± 0.63	5.87 ± 0.51
Fasting plasma TGs, mmol/L	1.38 ± 0.69	1.62 ± 0.89	1.21 ± 0.46	1.33 ± 0.59	1.50 ± 0.72	1.22 ± 0.46
Fasting serum HDL cholesterol, mmol/L	1.27 ± 0.32	1.12 ± 0.30	1.39 ± 0.29	1.30 ± 0.28	1.16 ± 0.27	1.39 ± 0.26
Smokers, *n*	7	2	5	8	3	5
Intake of antihypertensive drugs, *n*	7	2	5	12	2	10

[1] All values are means ± SDs unless otherwise indicated.
[2] Values are means (ranges).

We built a classification tree with the use of the machine learning algorithm in the tree package in R to determine an objective model for compliance to the gluten-poor diet. Plasma total alkylresorcinol concentrations, together with the quinoa alkylresorcinols measured in plasma (C20, C22, C24, and C26), were used to build the classification tree. We used a 10-fold cross-validation method to find the best fit and used it to prune the classification tree. Participants considered misclassified by the model were further evaluated by number of reported deviations from the study protocol to determine whether they reported noncompliance. Wilcoxon's Signed Rank test was used to test differences in the reported number of deviations in the 2 study periods. We used Spearman's correlation analysis to correlate the number of deviations in the 2 study periods with plasma alkylresorcinols.

Results

Baseline characteristics

A total of 52 participants completed the gluten intervention study, and 118 participants were included in the baseline cross-sectional study (**Table 1**). At baseline, the mean gluten intake was 13 ± 3 g/10 MJ and the intake of whole grain was 70 ± 39 g/10 MJ (**Table 2**), which is reflected in the relatively high baseline plasma alkylresorcinol concentrations.

Intervention study

Study products. The participants consumed 284 ± 77 g study products/d with the gluten-poor diet, which was significantly more than with the gluten-rich diet (259 ± 67 g/d; $P = 0.001$); however, there was no difference in energy intake from the study products ($P = 0.26$). The total gluten intake from the study products in the gluten-rich period was 17.3 ± 4.6 g/d compared with only trace amounts for the gluten-poor period ($P < 0.001$) (**Table 3**).

The gluten-containing foods all contained measurable amounts of alkylresorcinols (Supplemental Table 1). Some products unexpectedly contained high amounts, including refined rye-wheat bread and pearled spelt kernels, suggesting that they still contained some portion of the outer parts of the grains. Trace amounts of alkylresorcinols were found in cornflakes and gluten-free bread and buns, possibly via contamination from wheat flour during processing. Alkylresorcinols were also measured in quinoa, including the homologs C20 and C22, which were not present in any of the other foods measured (Supplemental Table 1).

Plasma alkylresorcinol concentration. Plasma total alkylresorcinol concentrations decreased more during the gluten-poor intervention period than during the gluten-rich period ($P < 0.001$; **Table 4**). This was also the case for all odd-chain alkylresorcinol homologs (Table 4). For the C22 alkylresorcinol homologs, we observed an increase in the gluten-poor period compared with the gluten-rich period ($P = 0.032$) (Table 4). Plasma alkylresorcinol concentrations decreased with both intervention diets due to a high baseline whole-grain intake and no whole grains with either of the intervention diets. Although previously suggested to improve the relation between diet and plasma alkylresorcinol concentrations (25), adjustment for LDL-cholesterol and TG concentrations did not affect the results. During the gluten-poor period there was a correlation between plasma alkylresorcinol concentrations and reported alkylresorcinol intake during the whole intervention period ($r = 0.28$, $P = 0.047$), and a similar tendency was seen during the gluten-rich period ($r = 0.24$, $P = 0.08$).

TABLE 2 Baseline dietary intakes of participants in the cross-sectional study[1]

	All (n = 118)	Men (n = 47)	Women (n = 71)
Energy intake, kJ/d	9780 ± 2903	10,905 ± 3447	9036 ± 2205
Protein, g/10 MJ	85 ± 13	88 ± 14	83 ± 12
Fat, g/10 MJ	96 ± 13	97 ± 13	96 ± 13
Carbohydrate, g/10 MJ	262 ± 32	256 ± 32	266 ± 31
Total fiber, g/10 MJ	26 ± 9	24 ± 7	27 ± 9
Whole grains, g/10 MJ	70 ± 39	71 ± 37	69 ± 42
Gluten, g/10 MJ	13 ± 3	13 ± 3	12 ± 3

[1] All values are means ± SDs.

TABLE 3 Study product consumption during the 8-wk gluten-poor and gluten-rich diet crossover intervention periods[1]

	Gluten-poor period	Gluten-rich period	P^2
Study products, g/d	284 ± 77	259 ± 67	0.001
Energy, KJ/d	3305 ± 888	3213 ± 836	0.26
Gluten, g/d	0.0 ± 0.0	17.3 ± 4.6	<0.0001
Total alkylresorcinols, mg/d	1.3 ± 0.7	40.5 ± 12.9	<0.0001

[1] All values are mean ± SDs, $n = 52$.
[2] A paired t test was used for testing differences between the 2 study periods.

Number of deviations from the study protocol. The median number of reported deviations from the study protocol per participant was 3.0 (IQR: 1.0, 7.0) in the gluten-poor period and 5.0 (IQR: 3.0, 7.3) in the gluten-rich period ($P = 0.02$). The median numbers of reported deviations from the study protocol in the last week of the gluten-poor period were 0.0 (IQR: 0.0, 1.0) and 1.0 (IQR: 0.0, 1.0) in the last week of the gluten-rich period per participant ($P = 0.03$). We found no correlation between total and last-week reported number of deviations and plasma alkylresorcinols in either period (Spearman's ρ: −0.06 to 0.09, $P = 0.53$–0.87).

Classification tree. We used the classification tree methodology to determine if plasma alkylresorcinols could be an objective measure of compliance to a gluten-poor diet (**Figure 1**). Overall misclassification error was 3.9%. The cutoff for plasma total alkylresorcinol concentrations between the gluten-poor and gluten-rich periods was 27.0 nmol/L. A second decision was based on the quinoa alkylresorcinol homolog C22, in which concentrations >4.6 nmol predicted a gluten-poor diet (**Supplemental Table 2**). The classification tree methodology was able to predict the likelihood of noncompliance with the median number of reported deviations from the study protocol to be higher in the predicted "noncompliant group" (6.0; IQR: 5.0, 10.5) than in the predicted "compliant group" (3.0; IQR: 1.0, 7.0) and the "high-quinoa group" (2.0; IQR: 1.25, 5.75) (Supplemental Table 2).

Cross-sectional study

Agreement between plasma alkylresorcinol concentration and reported gluten intake. The baseline plasma alkylresorcinol concentrations in the cross-sectional study were 136 nmol/L (95% CI: 119, 156 nmol/L) for all participants, 160 nmol/L (95% CI: 127, 201 nmol/L) in men, and 122 nmol/L (95% CI: 104, 144 nmol/L) in women (**Table 5**). In the 3G baseline cohort of 118 participants, classification of participants into the same or adjacent quartiles of gluten intake and plasma alkylresorcinol concentrations ranged from 89.7% (quartile 3) to 58.6% (quartile 4)

TABLE 4 Absolute plasma alkylresorcinol concentrations and changes during the 8-wk gluten-poor and gluten-rich diet crossover intervention periods[1]

	Gluten-rich diet			Gluten-poor diet				
	Week 0	Week 8	Change (Δ)	Week 0	Week 8	Change (Δ)	*P* value[2]	*P* value[3]
Alkylresorcinol homolog, nmol/L								
C17	10.6 (8.76, 12.7)	6.99 (5.79, 8.43)	−3.56 (−5.72, −1.40)	10.9 (8.77, 13.4)	0.777 (0.544, 1.04)	−10.2 (−12.1, −8.22)	<0.001	<0.001
C19	31.7 (25.4, 39.5)	23.1 (18.7, 28.6)	−8.32 (−15.1, −1.51)	33.9 (26.7, 42.9)	2.21 (1.64, 2.91)	−32.2 (−39.2, −25.1)	<0.001	<0.001
C20	0.167 (0.074, 0.268)	0.144 (0.078, 0.215)	−0.0226 (−0.204, 0.158)	0.267 (0.139, 0.409)	0.532 (0.259, 0.865)	0.266 (0.0430, 0.488)	0.022	0.024
C21	45.9 (35.6, 59.2)	36.8 (29.7, 45.6)	−9.22 (−21.2, 2.76)	51.2 (38.9, 67.2)	4.83 (3.52, 6.51)	−46.8 (−59.0, −34.6)	0.002	<0.001
C22	0.269 (0.149, 0.401)	0.211 (0.125, 0.304)	−0.0581 (−0.293, 0.177)	0.410 (0.213, 0.638)	0.756 (0.378, 1.24)	0.346 (0.0410, 0.651)	0.032	0.033
C23	20.5 (15.7, 26.7)	15.6 (12.7, 19.3)	−4.99 (−10.2, 0.171)	22.1 (16.9, 28.9)	2.43 (1.72, 3.32)	−19.9 (−25.0, −14.7)	<0.001	<0.001
C24	0.348 (0.212, 0.500)	0.248 (0.149, 0.356)	−0.0998 (−0.338, 0.138)	0.459 (0.246, 0.707)	0.572 (0.284, 0.926)	0.116 (−0.164, 0.397)	0.18	0.19
C25	12.9 (9.74, 17.0)	9.62 (7.62, 12.1)	−3.75 (−7.00, −0.502)	12.5 (9.13, 17.1)	1.25 (0.856, 1.72)	−11.8 (−14.9, −8.74)	<0.001	<0.001
C26	0.0819 (0.0318, 0.135)	0.0913 (0.0454, 0.139)	0.00933 (−0.0967, 0.115)	0.106 (0.0224, 0.196)	0.191 (0.0680, 0.329)	0.0859 (−0.0273, 0.199)	0.27	0.28
Total odd, nmol/L	127 (100, 160)	94.9 (77.7, 116)	−31.7 (−62.2, −1.22)	135 (105, 174)	11.2 (8.38, 14.9)	−125 (−155, −93.6)	<0.001	<0.001
Total even, nmol/L	0.726 (0.455, 1.05)	0.622 (0.418, 0.856)	−0.103 (−0.557, 0.350)	1.01 (0.621, 1.50)	1.37 (0.716, 2.26)	0.358 (−0.243, 0.958)	0.05	0.06
Total, nmol/L	127 (101, 161)	95.6 (78.2, 117)	−31.8 (−63.1, −0.434)	138 (108, 176)	13.2 (9.67, 17.8)	−125 (−157, −93.0)	<0.001	<0.001
C17:C21 ratio	0.230 (0.188, 0.281)	0.190 (0.159, 0.226)	−0.0542 (−0.127, 0.0183)	0.238 (0.198, 0.279)	0.246 (0.156, 0.343)	0.00774 (−0.0654, 0.0809)	0.09	0.08

[1] Values are geometric means (95% CIs), $n = 52$.
[2] *P* value for comparison of treatment on changes in plasma alkylresorcinol concentrations by using mixed-model ANCOVA with study participant as a random variable.
[3] Further adjusted for changes in plasma TG and LDL-cholesterol concentrations.

(**Table 6**). Gross misclassification rates were 16.7% for the lower quartile and 17.2% for the upper quartile. Agreement as measured by Cohen's κ was 0.24 (95% CI: 0.07, 0.41), which suggests a fair classification of participants into similar quartiles.

Potential determinants of plasma alkylresorcinol concentrations. In addition to intake of alkylresorcinols, there are several other factors that influence plasma alkylresorcinol concentrations, including plasma lipids and sex (23). In our study, the significant predictors of plasma alkylresorcinol concentrations were gluten intake and plasma concentrations of LDL cholesterol and TGs (**Supplemental Table 3**). The estimated effect size for gluten intake was a 5.6% (95% CI: 2.4%, 8.9%) increase in plasma total alkylresorcinols per 1-g increase in gluten intake ($P < 0.001$) and 4.5% ($P = 0.007$) without adjustment for LDL cholesterol and TGs.

Discussion

Compliance to prescribed diets in both clinical and intervention study settings is critical for determining successful outcomes, and problems with commonly used recall-based measures of compliance are known, underlying the importance of finding objective biomarkers—in this case for gluten intake. Here we tested whether plasma alkylresorcinols, which were previously successfully used as biomarkers of whole-grain intake in observation and intervention trials, also have a potential to assess compliance to a gluten-poor diet compared with a refined-grain–based gluten-rich diet.

We found that a gluten-poor diet period led to lower plasma alkylresorcinol concentrations compared with a period of a gluten-rich diet. Furthermore, we found that plasma alkylresorcinol concentrations and homolog fingerprints could be used to determine compliant and noncompliant participants, which might potentially be used in helping to determine compliance to a gluten-free diet in patients with CD. With the use of a classification tree it was possible to establish easy-to-use cutoff concentrations for compliance and noncompliance. Classification trees in medicine have been used successfully to guide medical decisions (39), and in this case they could be used in the clinic to improve dietary advice by targeting patients who have difficulty in complying with a gluten-free diet. The present cutoffs determined here cannot be extrapolated to clinical use because they were established in a healthy population who still had access to gluten-containing foods. Further studies are needed to determine and test optimal cutoff concentrations in clinically relevant settings and patient groups.

The gluten intake at baseline in the present study was comparable to the intake of the average Danish population of 10.4 ± 4.4 g/d. In the gluten intervention study, the gluten intake in the gluten-rich period was slightly above the 90th percentile of the Danish population (16.2 g/d) (40).

We recently discovered alkylresorcinols in quinoa (29), a popular gluten-free food for persons suffering from CD. This could be a potential confounding factor when using alkylresorcinols as biomarkers of gluten intake. However, quinoa alkylresorcinols include even-numbered alkyl-chains, 18–26 carbons long, which are not detectable in wheat, rye, or barley, making it possible to distinguish between alkylresorcinols derived from wheat, rye, and barley and quinoa. In this study, participants with high quinoa intakes were found to have high amounts of the even-chained homologs, especially C22. The decision-tree methodology

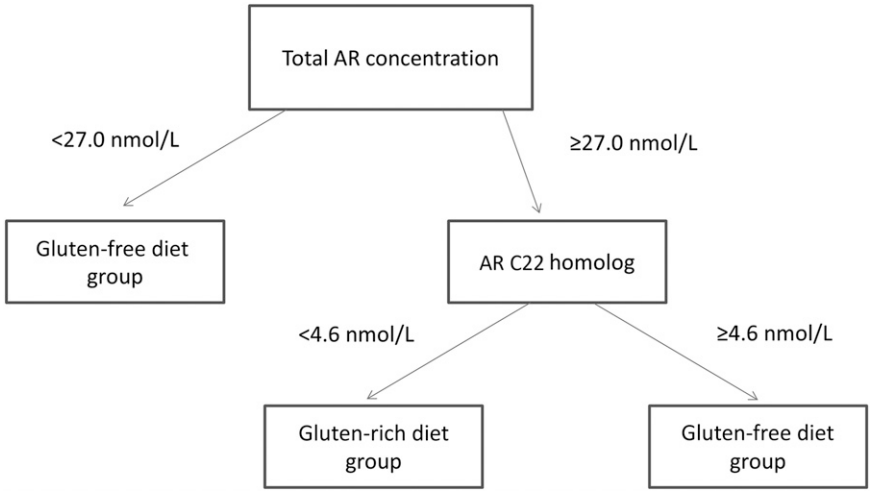

FIGURE 1 Decision tree after cross-validation and pruning based on the plasma AR concentrations of study participants in the crossover intervention study. The first decision is based on whether the plasma total AR concentration is ≥27.0 or <27.0 nmol/L. For concentrations above this level, a second decision based on whether the AR C22 homolog is ≥4.6 or <4.6 nmol/L is used to classify participants into groups. The AR C22 homolog is unique to quinoa and, in cases of higher AR concentrations, is indicative of quinoa intake, a common gluten-free grain substitute. AR, alkylresorcinol.

was found to be useful for accounting for quinoa as a confounder for plasma alkylresorcinols as a compliance biomarker.

A number of studies have shown a strong association between whole-grain wheat and rye intake with plasma alkylresorcinols in a variety of populations (21, 22, 27, 41). These studies also highlight some difficulties in using this marker—mainly the high interindividual variability and the relatively short half-life. Because plasma alkylresorcinols have a half-life of 5 h (42), they only indicate regular intake of alkylresorcinols, and generally 1 wk is sufficient to return to baseline concentrations after a cereal-based intervention (27). This means that alkylresorcinol measurements are unlikely to detect occasional intakes of gluten-containing foods. The high rate of interindividual variation is also problematic for setting a clear cutoff value for "consumed gluten" and "did not consume gluten," mainly from the perspective that very low concentrations may not necessarily indicate a gluten-free diet. However, the presence of cereal alkylresorcinols in a plasma or serum sample is a clear indicator of consumption of gluten-containing cereals, with the exception of quinoa noted above, making plasma alkylresorcinols a fairly sensitive and specific biomarker to test for compliance to a gluten-free diet. Differences in measured alkylresorcinol content of gluten-free foods (<1 μg/g) compared with gluten-containing foods (1–28 μg/g) mean that gluten-free foods used in this study would contribute minimally to plasma alkylresorcinol concentrations (**Supplemental Table 4**).

In intervention studies with a gluten-free diet a simple biomarker of gluten intake, such as plasma alkylresorcinols, is useful to monitor compliance. However, individuals without CD who participate in studies that investigate the possible effects of gluten are probably not aware of gluten found in foods that are not considered typical cereal products (e.g., vegetarian protein and ketchup). This might also have been the case in this study, because the values are quite high compared with a study in patients with CD (43), and this could be problematic for studying other outcomes related to a gluten-poor diet. However, it is not ethical to perform a comparable study in patients with CD because this would include gluten ingestion and likely harm the participants. The findings in the present randomized controlled trial are not directly transferable to a clinical setting for patients with CD. Many more factors need to be addressed before plasma alkylresorcinols can be accurately judged to be biomarkers of gluten-free diets, including dose-response studies with vital gluten (semipurified gluten used as a food ingredient) and establishing the relation between existing markers of gluten exposure and plasma alkylresorcinols. Studies are also required to establish variations in plasma alkylresorcinols in patients with CD and how variations relate to the status of CD.

We also found that plasma alkylresorcinols might be useful in an epidemiologic setting, because we showed a significant association between reported gluten intake and plasma alkylresorcinol concentrations. We also found that plasma alkylresorcinols

TABLE 5 Baseline plasma alkylresorcinol concentrations in participants in the cross-sectional study[1]

	All (n = 117)	Men (n = 46)	Women (n = 71)
Alkylresorcinols, nmol/L			
C17	11.3 (10.0, 12.9)	11.5 (9.06, 14.6)	11.2 (9.68, 13.0)
C19	33.1 (28.9, 38.0)	38.6 (30.7, 48.6)	30.0 (25.4, 35.5)
C20	0.164 (0.105, 0.225)	0.212 (0.102, 0.334)	0.133 (0.0684, 0.203)
C21	50.1 (43.4, 57.8)	60.4 (47.7, 76.6)	44.3 (37.0, 53.0)
C22	0.259 (0.179, 0.345)	0.235 (0.113, 0.370)	0.276 (0.169, 0.392)
C23	22.2 (19.1, 25.8)	26.5 (20.4, 34.3)	19.8 (16.6, 23.7)
C24	0.338 (0.252, 0.431)	0.350 (0.236, 0.474)	0.321 (0.187, 0.470)
C25	14.1 (12.0, 16.6)	16.7 (12.5, 22.3)	12.6 (10.4, 15.3)
C26	0.0599 (0.0304, 0.0903)	0.0678 (0.0161, 0.122)	0.0548 (0.0189, 0.0920)
Total, nmol/L	136 (119, 156)	160 (127, 201)	122 (104, 144)
C17:C21 ratio	0.227 (0.203, 0.252)	0.190 (0.158, 0.230)	0.253 (0.223, 0.288)

[1] All values are geometric means (95% CIs).

TABLE 6 Classification of participants into the correct quartile of gluten intake on the basis of plasma alkylresorcinol concentration[1]

	Quartile				
	1 (n = 30)	2 (n = 29)	3 (n = 29)	4 (n = 29)	κ^2
Total alkylresorcinols, nmol/L	55.8 (48.5, 64.1)	109 (102, 115)	162 (155, 169)	344 (289, 411)	
Total gluten intake, g/d	5.7 (5.2, 6.3)	8.7 (8.5, 9.0)	11 (11, 12)	16 (14, 17)	0.24 (0.070, 0.41)
Same or adjacent quartile, %	73.3	75.9	89.7	58.6	
Gross misclassification, %	16.7	—	—	17.2	

[1] Values are geometric means (95% CIs), n = 117. Cross-sectional analysis based on self-reported gluten intake from a 4-d FFQ.
[2] Cohen's weighted κ statistic with lower and upper confidence boundaries.

performed moderately well for classifying participants into correct quartiles of gluten intake on the basis of a Cohen's κ of 0.24, thus overall indicating the usefulness of plasma alkylresorcinol concentrations in a free-living population. However, in the top quartile, there seemed to be less agreement between the 2 determinants of gluten intake, and there were some participants who were grossly misclassified between the 2 methods. This might be due to the high-plasma-alkylresorcinol group being overrepresented by participants who consumed large amounts of whole grains, which would contribute relatively more to plasma alkylresorcinol concentrations per gram of gluten intake than refined grain, leading to a nonlinear relation between plasma alkylresorcinol concentrations and gluten intake. The fact that we found an association, despite this possibility, is a strong indicator of the potential of plasma concentrations of alkylresorcinols to help estimate gluten intake in observational settings. The use of methods combining plasma alkylresorcinols and food diaries, such as Howe's sum of ranks (19), might be valuable in future epidemiologic studies of gluten intake and health (44). Furthermore, we tested possible noncereal predictors of plasma alkylresorcinols and found that LDL cholesterol and TGs were associated with plasma alkylresorcinols. This is in line with other studies, although whether this is an independent determinant is debated (23).

Of the methods used to date to test for compliance to a gluten-free diet, the serologic test of CD-related antibodies is considered to be optimal due to its objectivity and minimal discomfort for the patient compared with histologic examination of biopsy samples. However, this method has been shown to be insensitive in detecting slight (<0.5 g gluten/d) dietary transgressions (45). Plasma alkylresorcinols have the potential to be used as a simple test in the clinic, able to detect small deviations from a gluten-free diet sufficient to cause clinical symptoms and reduce quality of life in some patients with CD. In cases in which patients seem to adhere to a strict gluten-free diet but still have clinical complaints, the presence of measurable plasma alkylresorcinol concentrations could indicate minor or inadvertent intake of gluten (e.g., from contamination of foods or from foods beyond those easily recognized as containing gluten).

In conclusion, new methods are needed to monitor compliance to a gluten-free diet in patients with CD, as well as to help investigations into the possible effects of gluten on the wider population. Compliance is a major issue for these types of dietary intervention studies, especially because gluten is found in a wide range of products beyond those that would be considered to be cereals. Alkylresorcinols are strongly related to gluten-containing ingredients, and therefore show great promise as biomarkers of compliance to a gluten-free diet. The present study found that it was possible, by using a decision-tree strategy, to predict which participants were likely to be noncompliant. Further work is needed to confirm the sensitivity, selectivity, and predictive values of plasma alkylresorcinols for gluten in the diet, especially in patients with CD.

Acknowledgments

MVL and ABR conceived of the study idea; HV, TH, LL, OBP, and MK designed the 3G study; MVL, MLM, RJG, and MK carried out the 3G studies; LL performed the randomization; MVL and ABR performed the alkylresorcinol measurements; MVL, MLM, and ABR wrote the manuscript; and MVL, MLM, JJR, HV, RJG, TH, LL, OBP, MK, and ABR provided input. All authors read and approved the final version of the manuscript.

References

1. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Pivor M. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American society for pediatric gastroenterology, hepatology and nutrition. J Pediatr Gastroenterol Nutr 2005;40:1–19.
2. AGA Institute. AGA institute medical position statement on the diagnosis and management of celiac disease. Gastroenterology 2006;131:1977–80.
3. Johnston SD, Watson RG, McMillan SA, Sloan J, Love AH. Prevalence of coeliac disease in Northern Ireland. Lancet 1997;350:1370.
4. Sanders DS, Patel D, Stephenson TJ, Ward AM, McCloskey EV, Hadjivassiliou M, Lobo AJ. A primary care cross-sectional study of undiagnosed adult coeliac disease. Eur J Gastroenterol Hepatol 2003;15:407–13.
5. West J, Logan RF, Hill PG, Lloyd A, Lewis S, Hubbard R, Reader R, Holmes GK, Khaw KT. Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. Gut 2003;52:960–5.
6. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: A large multicenter study. Arch Intern Med 2003;163:286–92.
7. Dubé C, Rostom A, Sy R, Cranney A, Saloojee N, Garritty C, Sampson M, Zhang L, Yazdi F, Mamaladze V. The prevalence of celiac disease in average-risk and at-risk western European populations: a systematic review. Gastroenterology 2005;128:S57–67.
8. West J, Fleming KM, Tata LJ, Card TR, Crooks CJ. Incidence and prevalence of celiac disease and dermatitis herpetiformis in the UK over two decades: population-based study. Am J Gastroenterol 2014;109:757–68.
9. Rewers M. Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease? Gastroenterology 2005;128:S47–51.
10. Mäki M, Collin P. Coeliac disease. Lancet 1997;349:1755–9.
11. Holmes GK, Prior P, Lane MR, Pope D, Allan RN. Malignancy in coeliac disease—effect of a gluten free diet. Gut 1989;30:333–8.
12. Blomfeldt TO, Kuktaite R, Johansson E, Hedenqvist MS. Mechanical properties and network structure of wheat gluten foams. Biomacromolecules 2011;12:1707–15.
13. Mayer M, Greco L, Troncone R, Auricchio S, Marsh MN. Compliance of adolescents with coeliac disease with a gluten free diet. Gut 1991;32:881–5.

14. Hall NJ, Rubin G, Charnock A. Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. Aliment Pharmacol Ther 2009;30:315–30.
15. Jadresin O, Misak Z, Kolacek S, Sonicki Z, Zizic V. Compliance with gluten-free diet in children with coeliac disease. J Pediatr Gastroenterol Nutr 2008;47:344–8.
16. Sugai E, Nachman F, Vaquez H, Gonzalez A, Andrenacci P, Czech A, Niveloni S, Mazure R, Smecuol E, Cabanne A, et al. Dynamics of celiac disease-specific serology after initiation of a gluten-free diet and use in the assessment of compliance with treatment. Dig Liver Dis 2010;42:352–8.
17. Schyum AC, Rumessen JJ. Serological testing for celiac disease in adults. United European Gastroenterol J 2013;1:319–25.
18. Comino I, Real A, Vivas S, Angel Siglez M, Caminero A, Nistal E, Casqueiro J, Rodriguez-Herrera A, Cebolla A, Sousa C. Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces. Am J Clin Nutr 2012;95:670–7.
19. Knudsen MD, Kyro C, Olsen A, Dragsted LO, Skeie G, Lund E, Aman P, Nilsson LM, Bueno-de-Mesquita HB, Tjonneland A, et al. Self-reported whole-grain intake and plasma alkylresorcinol concentrations in combination in relation to the incidence of colorectal cancer. Am J Epidemiol 2014;179:1188–96.
20. Andersson A, Marklund M, Diana M, Landberg R. Plasma alkylresorcinol concentrations correlate with whole grain wheat and rye intake and show moderate reproducibility over a 2- to 3-month period in free-living Swedish adults. J Nutr 2011;141:1712–8.
21. Ross AB, Bourgeois A, Macharia HN, Kochhar S, Jebb SA, Brownlee IA, Seal CJ. Plasma alkylresorcinols as a biomarker of whole-grain food consumption in a large population: results from the WHOLEheart intervention study. Am J Clin Nutr 2012;95:204–11.
22. Kyro C, Olsen A, Landberg R, Skeie G, Loft S, Aman P, Leenders M, Dik VK, Siersema PD, Pischon T, et al. Plasma alkylresorcinols, biomarkers of whole-grain wheat and rye intake, and incidence of colorectal cancer. J Natl Cancer Inst 2014;106:djt352.
23. Ross AB. Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake. J Nutr Metab 2012;2012:462967.
24. Kristensen M, Toubro S, Jensen MG, Ross AB, Riboldi G, Petronio M, Bugel S, Tetens I, Astrup A. 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 2012;142:710–6.
25. Linko-Parvinen AM, Landberg R, Tikkarren MJ, Adlercreutz H, Penalvo JL. Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. J Nutr 2007;137:1137–42.
26. Ross AB, Kochhar S. Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC−coularray-based electrochemical detection. J Agric Food Chem 2009;57:5187–93.
27. Ross AB, Bruce SJ, Blondel-Lubrano A, Oguey-Araymon S, Beaumont M, Bourgeois A, Nielsen-Moennoz C, Vigo M, Fay L, Kochhar S, et al. 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 2011;105:1492–502.
28. Ross AB. Analysis of alkylresorcinols in cereal grains and products using ultrahigh-pressure liquid chromatography with fluorescence, ultraviolet, and CoulArray electrochemical detection. J Agric Food Chem 2012;60:8954–62.
29. Ross A, Savolainen O. A new biomarker for quinoa intake (1044.25). FASEB J 2014;28(Suppl 1):1044.25 (abstr).
30. Ibrügger S, Gøbel RJ, Vestergaard H, Licht TR, Frøkiær H, Linneberg A, Hansen T, Gupta R, Pedersen O, Kristensen M, et al. Two randomized cross-over trials assessing the impact of dietary gluten or wholegrain on the gut microbiome and host metabolic health. J Clin Trials 2014;4:1–9.
31. Biltoft-Jensen A, Matthiessen J, Rasmussen LB, Fagt S, Groth MV, Hels O. Validation of the Danish 7-day pre-coded food diary among adults: energy intake v. energy expenditure and recording length. Br J Nutr 2009;102:1838–46.
32. Hoppe C, Trolle E, Gondolf UH, Husby S. Gluten intake in 6–36-month-old Danish infants and children based on a national survey. J Nutr Sci 2013;2:e7.
33. Saxholt E, Christensen A, Møller A, Hartkopp H, Hess Ygil K, Hels O. Danish food composition databank, revision 7. Copenhagen (Denmark): Department of Nutrition, National Food Institute, Technical University of Denmark; 2008.
34. van Overbeek FM, Uil-Dieterman IG, Mol IW, Köhler-Brands L, Heymans HS, Mulder CJ. The daily gluten intake in relatives of patients with coeliac disease compared with that of the general Dutch population. Eur J Gastroenterol Hepatol 1997;9:1097–9.
35. Wieser H, Seilmeier W, Belitz HD. [Comparative investigations of partial amino acid sequences of prolamines and glutelins from cereals. II. Fractionation of glutelins.] Z Lebensm Unters Forsch 1980;171:430–6 (in German).
36. Gellrich C, Schieberle P, Weiser H. Biochemical characterization and quantification of the storage protein (secalin) types in rye flour. Cereal Chem 2003;80:102–9.
37. Ellis HJ, Freedman A, Ciclitira P. Detection and estimation of the barley prolamin content of beer and malt to assess their suitability for patients with celiac-disease. Clin Chim Acta 1990;189:123–30.
38. Ross AB, Svelander C, Savolainen OI, Lind MV, Kirwan JP, Breton I, Godin J, Sandberg A. A high throughput method for LC-MS/MS determination of plasma alkylresorcinols, biomarkers of whole grain wheat and rye intake. Anal Biochem 2016;499:1–7.
39. Podgorelec V, Kokol P, Stiglic B, Rozman I. Decision trees: an overview and their use in medicine. J Med Syst 2002;26:445–63.
40. Hoppe C, Gøbel R, Kristensen M, Lind MV, Matthiessen J, Christensen T, Trolle E, Fagt S, Madsen M, Husby S. Intake and sources of gluten in 20- to 75-year-old Danish adults: a national dietary survey. Eur J Nutr 2015 Oct 6 (Epub ahead of print; DOI: 10.1007/s00394-015-1062-3).
41. Landberg R, Kamal-Eldin A, Andersson A, Vessby B, Aman P. Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. Am J Clin Nutr 2008;87:832–8.
42. Landberg R, Linko A, Kamal-Eldin A, Vessby B, Adlercreutz H, Aman P. Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. J Nutr 2006;136:2760–5.
43. Choung RS, Murray JA, Marietta E, Van Dyke CT, Brantner TL, Sa Ross AB. 1767 alkylresorcinols, indicator for gluten consumption in celiac disease patients. Gastroenterology 2015;148(Suppl 1):S327 No. 1767 (abstr).
44. Freedman LS, Kipnis V, Schatzkin A, Tasevska N, Potischman N. Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? Epidemiol Perspect Innov 2010;7:2.
45. Troncone R, Mayer M, Spagnuolo F, Maiuri L, Greco L. Endomysial antibodies as unreliable markers for slight dietary transgressions in adolescents with celiac disease. J Pediatr Gastroenterol Nutr 1995;21:69–72.