# Plasma alkylresorcinols, biomarkers of whole-grain wheat and rye intake, and risk of type 2 diabetes in Scandinavian men and women[1,2]

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## ABSTRACT

**Background:** Studies that use dietary biomarkers to investigate the association between whole-grain intake and the risk of developing type 2 diabetes (T2D) are lacking.

**Objective:** We examined the association between plasma total alkylresorcinols and the alkylresorcinol C17:0-to-C21:0 ratio, biomarkers of whole-grain wheat and rye intake and relative whole-grain rye over whole-grain wheat intake, respectively, and the risk of T2D among Scandinavian men and women.

**Design:** A nested case-control study was established within the Northern Sweden Health and Disease Study and the Danish Diet, Cancer and Health cohort. Alkylresorcinol concentrations and the ratios of C17:0 to C21:0 were determined in plasma samples from 931 case-control pairs. ORs for T2D were calculated for plasma total alkylresorcinol concentration or C17:0-to-C21:0 ratio in quartiles with the use of conditional logistic regression that was adjusted for potential confounders. Additional analyses with whole-grain wheat and rye intake estimated from food-frequency questionnaires (FFQs) as exposures were also performed.

**Results:** The plasma total alkylresorcinol concentration was not associated with T2D risk (OR: 1.34; 95% CI: 0.95, 1.88) for the highest compared with the lowest quartiles in multivariable adjusted models. However, the C17:0-to-C21:0 ratio was associated with a lower diabetes risk (OR: 0.54; 95% CI: 0.37, 0.78). Analyses with whole-grain intake estimated from FFQs yielded similar results.

**Conclusions:** Total whole-grain wheat and rye intake, reflected by alkylresorcinols in plasma, was not associated with a lower risk of T2D in a population with high whole-grain intake. In contrast, the proportion of whole-grain rye to whole-grain wheat intake, indicated by the plasma C17:0-to-C21:0 ratio, was inversely associated with T2D. This suggests that whole-grain intake dominated by rye may be favorable for T2D prevention. *Am J Clin Nutr* 2016;104:88–96.

**Keywords:** type 2 diabetes, whole grains, biomarker, alkylresorcinols, nested case-control studies

## INTRODUCTION

Approximately 382 million adults worldwide have diabetes, and that number is projected to increase to >592 million by 2035 (1). Type 2 diabetes (T2D)[13] is the most prevalent form of diabetes, and important risk factors of T2D include family history of diabetes, age, and excess adiposity (2). Diet is a modifiable risk factor of importance for preventing T2D (3).

Whole-grain food intake has been consistently associated with a lower risk of T2D in different populations (4–6). A 2012 meta-analysis on human intervention studies (7) found beneficial effects of whole grains on several cardiometabolic risk factors such as fasting glucose, blood lipids, and blood pressure. Whole grains are rich in dietary fiber, vitamins, minerals, unsaturated fatty acids, and phytochemicals, all of which may contribute to protective effects (8). Cereals differ in the content and composition of these components, but this difference has typically not been accounted for in observational or randomized controlled studies (4, 9–11).

Rye is the richest source of dietary fiber among whole-grain cereals and contains a large variety of bioactive compounds (12, 13). A few intervention studies have investigated the effects of high-fiber rye foods or diets rich in these foods on cardiometabolic

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[2] Supplemental Tables 1–3 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://ajcn.nutrition.org.

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[13] Abbreviations used: DCH, Diet, Cancer and Health; FFQ, food-frequency questionnaire; MONICA, Monitoring Trends and Determinants in Cardiovascular Disease; NSHDS, Northern Sweden Health and Disease Study; T2D, type 2 diabetes; VIP, Västerbotten intervention program.

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risk factors (14–17). High-fiber rye bread had beneficial long-term effects on $\beta$ cell function (15), insulin sensitivity (18), and inflammation biomarkers (17, 19). These studies suggest that long-term whole-grain rye intake may reduce the risk of T2D, as supported by one epidemiologic study (5).

In most studies, whole-grain intake has been estimated by food-frequency questionnaires (FFQs), which are known to suffer from measurement errors inherent to self-reporting and limitations of whole-grain food composition data (20, 21). The use of plasma alkylresorcinols, phenolic lipids derived from the bran of wheat and rye, as a biomarker for whole-grain wheat and rye intake has been suggested (22–24). In human plasma, alkylresorcinol homologs with 17–25 carbon atoms in the alkyl chain are typically analyzed (25). The C17:0-to-C21:0 ratio in cereals can be used to distinguish the source of whole grains. A ratio close to 0.1 reflects whole-grain wheat, whereas a ratio close to 1.0 reflects whole-grain rye (26). The plasma C17:0-to-C21:0 ratio is to a great extent determined by the intake of whole-grain rye over the sum of whole-grain wheat and rye and has been associated with improved lipid profile and insulin sensitivity in a cross-sectional study of men and women with metabolic syndrome (14, 18).

In this study, we examined the association between plasma total alkylresorcinol concentration as a biomarker of whole-grain wheat and rye intake as well as the plasma alkylresorcinol C17:0-to-C21:0 ratio as an indicator of the proportion of whole-grain rye over whole-grain rye and wheat intake and incident T2D in Scandinavian men and women.

## METHODS

### Study population and data collection

A nested case-control study was established that included participants from the Danish DCH (Diet, Cancer and Health) cohort and the NSHDS (Northern Sweden Health and Disease Study) (27, 28) (**Figure 1**).

#### *DCH cohort*

DCH is a prospective study initialized in the 1990s and has been described in detail elsewhere (28). In brief, participants living in the Copenhagen or Aarhus areas and aged 50–64 y were invited to participate between 1993 and 1997 (baseline). In total, 53,057 of those invited accepted to participate (37% of the women and 34% of the men). Before visiting the study center, the participants completed a validated FFQ (29); at the study center they completed a lifestyle questionnaire and had anthropometric measurements taken, and biological samples, including blood, were also collected (28).

#### *NSHDS cohort*

The NSHDS cohort contains 3 subcohorts: the Västerbotten intervention program (VIP) cohort, MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) cohort, and mammary screening cohort (27), but only participants from the VIP and MONICA cohorts were used for this study. In the

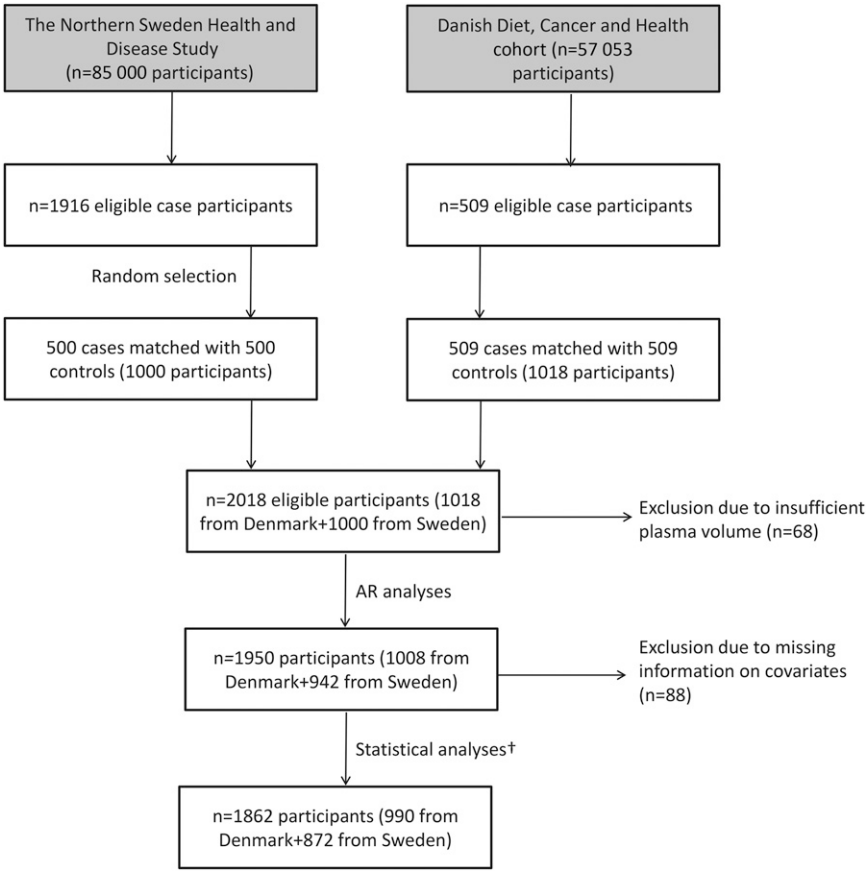


**FIGURE 1** Flowchart of study selection. †Conditional logistic regression analysis. AR, alkylresorcinol.

MONICA cohort, 2000–2500 randomly selected participants aged 25–74 y living in the 2 northern counties in Sweden have been invited to participate in a health survey every 4 or 5 y since 1986. In total, 77% have agreed to participate. In VIP, all residents of Västerbotten County were invited to a similar health survey at their primary health care center when turning ages 30, 40, 50, and 60 y, of whom 59% agreed to participate (30). The recruitment for the VIP cohort started in 1985. Participants included in the case-control study from NSHDS were recruited from 1992 to 2005 (baseline). Blood samples were drawn at baseline and transferred to the Northern Sweden Medical Biobank. A questionnaire was also completed. By 2002, 85,000 participants were enrolled in the NSHDS cohort (27). In NSHDS, 2 different FFQs were used: one with 84 food items and another with 64 items, as described by Johansson et al. (31, 32). For the shorter FFQ, 2 questions were merged into one to facilitate comparability between the 2 FFQs.

This study was conducted in accordance with the Declaration of Helsinki, and all procedures involving human subjects were approved by the regional ethical review board in Uppsala, Sweden. All participants provided written informed consent.

### Case ascertainment and selection

In NSHDS, diabetes cases were identified from the diabetes register in Northern Sweden, and in DCH, diabetes cases were defined from the National Diabetes Registry (33). From DCH, 4148 incident cases were identified between baseline and the end of follow-up. In NSHDS, 1916 incident diabetes cases were identified between baseline and the end of follow-up. The Danish National Diabetes Registry does not distinguish between type 1 and 2 diabetes. We considered all the cases from DCH as T2D because most participants were middle-aged at the time of diagnosis. In the diabetes register in Northern Sweden, T2D cases were ascertained by a diabetes specialist and glutamic acid decarboxylase autoantibody tests.

### Matching

Only participants for whom blood samples were available, who were fasting when the blood was drawn (for >7 h), and had completed the lifestyle and FFQs were eligible for this study. Participants were excluded if they had a diagnosis of diabetes, cancer, or cardiovascular disease before baseline.

In total, 509 cases from DCH participants fulfilled the eligibility criteria, whereas 500 cases among eligible participants from NSHDS were randomly selected. All cases were individually matched (1:1) with controls based on the following criteria: age, sex, cohort (DCH, NSHDS), fasting status (>7 h since last meal), and type of FFQ (NSHDS participants only).

### Whole-grain exposure measurements

#### *Alkylresorcinols in plasma as biomarkers of whole-grain wheat and rye intake*

Alkylresorcinol homologs (C17:0, C19:0, C21:0, C23:0, C25:0, and their sum) were extracted and purified from plasma samples and analyzed by gas chromatography–mass spectrometry as described by Landberg et al. (25) with the following modifications: 150 $\mu$L plasma was used instead of 200 $\mu$L, and trifluoroacetic anhydride was used for derivatization instead of a quick-silylation mixture according to Wierzbicka et al. (34). Matched case-control pairs were analyzed in the same batch, and 4 replicate quality-control samples were included in all batches. The within- and between-batch CVs were both <10%. Total alkylresorcinol concentration was calculated as the sum of homologs C17:0–C25:0 and used in all statistical analyses. The C17:0-to-C21:0 ratio was calculated for each individual and used for statistical analysis. Alkylresorcinols were analyzed in 1950 (975 matched case-control pairs) of 2018 samples because of missing samples and/or insufficient volume of plasma available.

#### *Whole-grain intake derived from FFQs*

The definition of whole grains in this study was according to the definition given by the American Association of Cereal Chemists from 1999: "Whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components—the starchy endosperm, germ and bran—are present in the same relative proportions as they exist in the intact caryopsis" (35). Daily intake of whole grain (g/d) from wheat and rye according to this definition was used for analyses in this study. Intakes were estimated from the FFQ with the use of information on their whole-grain content obtained from a 24-h dietary recall on a subset of the participants (36). There was no limitation set on the minimum content in the products provided that they contained whole-grain wheat or rye (37). The different FFQs used and the whole-grain intake estimation have been described in detail elsewhere (38).

### Statistics

In total, alkylresorcinols were analyzed for 1950 participants (975 matched case-control pairs). Eighty-eight participants (44 matched pairs) were excluded from statistical analysis because of missing covariate information or because of missing matched case/control because only complete case sets can be included in the analyses. In total, 1862 participants (931 matched pairs) were included in the statistical analyses with alkylresorcinols as the exposure measurement.

Conditional logistic regression analysis stratified by case-control pair was used to estimate ORs and CIs of T2D in relation to total alkylresorcinol concentration as well as the C17:0-to-C21:0 ratio. ORs were calculated for sex-specific quartiles of plasma total alkylresorcinol concentration or C17:0-to-C21:0 ratio among control participants or by increments (per 25 nmol/L for alkylresorcinol and per SD for the alkylresorcinol C17:0-to-C21:0 ratio).

Three models were used to investigate the associations between alkylresorcinols and T2D with various adjustments for potential confounders. Model 1 was a crude univariate model that included total alkylresorcinols only. BMI (in kg/m$^2$) was included in model 2, and in model 3 smoking status (former, never, or current smoker), education (primary, technical, secondary, or postsecondary), physical activity (active or inactive), alcohol intake (g/d; continuous), energy intake (kcal/d; continuous), and coffee intake (g/d; continuous) were further adjusted for. Similar models were used for testing the C17:0-to-C21:0 ratio in relation to T2D. These models were also adjusted for total alkylresorcinol concentration to investigate the association

of predominant whole-grain source (rye or wheat) irrespective of the total amount of whole-grain wheat and rye intake. All continuous variables were tested for linearity with the use of linear splines with 3, 4, and 9 kn. The assumption of linearity was verified by visual inspecting the plots. No deviations from linearity were found.

Additional sensitivity analyses were performed for Swedish participants of the population, in which model 3 was further adjusted for plasma total cholesterol and triacylglycerol concentrations as well as for red and processed meat intake and model 4 was further adjusted for fruit, vegetable, and dairy product intake.

In a secondary analysis, whole-grain wheat and rye intake estimated by the FFQ was investigated in relation to T2D. Among 1437 participants with such data, only 711 case-control pairs (1422 participants) of the studied population were included in the analysis because of missing variables for one of the subjects in the matched pair or because of exclusion from subdata sets for which the whole-grain intake variables had not been derived. Three models were used as described previously but with whole-grain wheat, whole-grain rye, or total whole-grain wheat plus rye as the exposure variable instead of total alkylresorcinols. Analyses were conducted for both Swedish and Danish participants and for men and women together or separately. Descriptive and conditional logistic regression analyses were performed with the use of SAS software version 9.3 (SAS Institute Inc.).

## RESULTS

Baseline characteristics of T2D cases ($n$ = 931) and their individually matched controls ($n$ = 931) are shown in **Table 1**. Cases had somewhat higher plasma alkylresorcinol concentrations (43 nmol/L) than controls (37 nmol/L), but the C17:0-to-C21:0 ratio was higher in controls than in cases. Among potential confounding factors, BMI was higher in cases than in controls, and controls were more physically active than cases. Alcohol intake was higher in controls than in cases, whereas coffee intake and smoking status were similar for both cases and controls. Education was also similar except for postsecondary education, which was somewhat more common in controls than in cases.

Overall, no association between plasma total alkylresorcinol concentration and T2D was observed for any of the examined

**TABLE 1**
Characteristics of type 2 diabetes patients and their matched control subjects[1]

| | All ($n$ = 1862) | | Denmark ($n$ = 990) | | Sweden ($n$ = 872) | |
|---|---|---|---|---|---|---|
| | Cases | Controls | Cases | Controls | Cases | Controls |
| Sex, % | | | | | | |
| Men | 62 | 62 | 66 | 66 | 58 | 58 |
| Women | 38 | 38 | 34 | 34 | 42 | 42 |
| Age at recruitment, y | 55 (40–62) | 54 (40–62) | 55 (50–63) | 55 (50–63) | 50 (40–60) | 50 (40–60) |
| BMI, kg/m$^2$ | 29 (23–38) | 26 (21–33) | 29 (23–39) | 26 (21–33) | 29 (24–38) | 25 (21–32) |
| Education, % | | | | | | |
| Primary | 41 | 37 | 41 | 37 | 40 | 37 |
| Technical/professional | 34 | 29 | 38 | 32 | 29 | 25 |
| Secondary | 12 | 14 | 7 | 12 | 18 | 18 |
| >Secondary | 14 | 19 | 14 | 18 | 13 | 20 |
| Energy intake, kcal/d | 1944 (1018–3484) | 1944 (1068–3350) | 2134 (1144–3680) | 2129 (1267–3509) | 1702 (931–3031) | 1758 (986–3118) |
| Smoking status, % | | | | | | |
| Never | 37 | 40 | 25 | 26 | 51 | 55 |
| Former | 26 | 26 | 26 | 26 | 26 | 27 |
| Current | 37 | 34 | 49 | 48 | 23 | 18 |
| Physical activity, % | | | | | | |
| Active | 39 | 45 | 53 | 58 | 22 | 30 |
| Inactive | 61 | 55 | 47 | 42 | 78 | 70 |
| Beverage consumption, g/d | | | | | | |
| Alcohol | 5 (0–69) | 7 (0.01–67) | 14 (0.2–87) | 16 (0.6–86) | 2 (0–11) | 3 (0–14) |
| Coffee | 500 (21–1600) | 500 (21–1600) | 900 (7–1600) | 900 (7–1600) | 375 (54–750) | 375 (54–750) |
| Alkylresorcinol, nmol/L | | | | | | |
| Total | 43 (11–186) | 37 (9–162) | 47 (10–206) | 42 (9–193) | 39 (12–157) | 33 (10–105) |
| C17 | 3 (0.5–15) | 3 (0.5–15) | 3 (0.3–16) | 3 (0.4–17) | 3 (0.7–14) | 3 (0.6–11) |
| C19 | 12 (3–51) | 10 (2–48) | 13 (3–62) | 12 (2–56) | 11 (3–47) | 10 (2–32) |
| C21 | 15 (3–61) | 12 (3-51) | 15 (3-71) | 13 (2-68) | 14 (4-49) | 11 (3-34) |
| C23 | 6 (1–25) | 3 (1–23) | 7 (1–28) | 6 (1–30) | 5 (1–21) | 4 (1–14) |
| C25 | 6 (1–35) | 5 (1–29) | 7 (1–36) | 6 (1–36) | 5 (1–33) | 5 (1–22) |
| C17:0-to-C21:0 ratio | 0.22 (0.06–0.52) | 0.26 (0.06–0.60) | 0.20 (0.05–0.46) | 0.25 (0.05–0.53) | 0.23 (0.07–0.57) | 0.27 (0.09–0.69) |
| Whole-grain intake,[2] g/d | | | | | | |
| Total from FFQ | 38 (7–95) | 40 (8–100) | 29 (6–67) | 32 (7–77) | 66 (16–124) | 63 (24–124) |
| Wheat | 3 (0.08–21) | 3 (0.02–21) | 2 (0.004–9) | 2 (0.04–10) | 9 (1–31) | 12 (1–30) |
| Rye | 24 (4–62) | 25 (4–65) | 21 (3–54) | 21 (4–57) | 38 (7–76) | 36 (11–74) |

[1]Values are medians; 5th, 95th percentiles in parentheses unless otherwise indicated. FFQ, food-frequency questionnaire.
[2]Data from FFQs were available for 711 cases (494 from Denmark and 217 from Sweden) and 711 controls (494 from Denmark and 217 from Sweden).

models across quartiles, but a 25-nmol/L higher total alkylresorcinol concentration was associated with a greater likelihood of developing T2D (OR: 1.04; 95% CI: 1.00, 1.09) (**Table 2**). Separate analyses were also conducted for men and women and for Danish and Swedish participants, respectively. When men and women were analyzed separately, Swedish men in the highest alkylresorcinol quartile had higher odds of T2D than those in the lowest quartile (OR: 4.01; 95% CI: 1.85, 8.66) and those with

**TABLE 2**
Estimated ORs for T2D according to plasma total AR concentrations (in nmol/L) in sex-specific quartiles or per 25 nmol/L among T2D cases and matched controls in a nested case-control study[1]

| | Quartile of plasma total AR concentrations | | | | Continuous, per 25 nmol/L |
|---|---|---|---|---|---|
| | Q1 (referent) | Q2 | Q3 | Q4 | |
| Range/increment | | | | | |
| Women | 0–19 | >19–32 | >32–58 | >58 | |
| Men | 0–23 | >23–41 | >41–70 | >70 | |
| All subjects | | | | | |
| Cases/control subjects, *n* | 186/234 | 222/232 | 238/233 | 285/232 | 931/931 |
| Model 1 | 1 | 1.22 (0.93, 1.60) | 1.32 (1.01, 1.72) | 1.61 (1.23, 2.12) | 1.05 (1.01, 1.09) |
| Model 2 | 1 | 0.91 (0.66, 1.27) | 0.99 (0.72, 1.37) | 1.25 (0.90, 1.74) | 1.04 (1.00, 1.08) |
| Model 3 | 1 | 0.92 (0.66, 1.28) | 1.02 (0.74, 1.42) | 1.34 (0.95, 1.88) | 1.04 (1.00, 1.09) |
| All Swedish subjects | | | | | |
| Cases/control subjects, *n* | 91/119 | 115/121 | 117/120 | 113/76 | 436/436 |
| Model 1 | 1 | 1.27 (0.87, 1.86) | 1.29 (0.88, 1.89) | 2.02 (1.33, 3.05) | 1.17 (1.07, 1.27) |
| Model 2 | 1 | 1.06 (0.64, 1.74) | 1.24 (0.77, 2.00) | 2.15 (1.26, 3.68) | 1.20 (1.08, 1.34) |
| Model 3 | 1 | 1.05 (0.63, 1.76) | 1.31 (0.81, 2.13) | 2.36 (1.35, 4.13) | 1.21 (1.08, 1.35) |
| All Danish subjects | | | | | |
| Cases/control subjects, *n* | 95/115 | 107/111 | 121/113 | 172/156 | 495/495 |
| Model 1 | 1 | 1.18 (0.80, 1.73) | 1.32 (0.91, 1.93) | 1.38 (0.96, 2.00) | 1.03 (0.99, 1.06) |
| Model 2 | 1 | 0.83 (0.53, 1.29) | 0.84 (0.54, 1.30) | 0.91 (0.59, 1.39) | 1.01 (0.97, 1.05) |
| Model 3 | 1 | 0.82 (0.52, 1.29) | 0.82 (0.52, 1.31) | 0.92 (0.59, 1.44) | 1.01 (0.97, 1.06) |
| All men | | | | | |
| Cases/control subjects, *n* | 104/146 | 134/145 | 148/145 | 195/145 | 581/581 |
| Model 1 | 1 | 1.35 (0.95, 1.91) | 1.48 (1.04, 2.11) | 2.02 (1.42, 2.87) | 1.06 (1.02, 1.10) |
| Model 2 | 1 | 1.13 (0.75, 1.73) | 1.13 (0.74, 1.71) | 1.73 (1.14, 2.64) | 1.05 (1.01, 1.10) |
| Model 3 | 1 | 1.15 (0.75, 1.77) | 1.19 (0.77, 1.83) | 1.91 (1.24, 2.96) | 1.06 (1.01, 1.11) |
| Swedish men | | | | | |
| Cases/control subjects, *n* | 45/66 | 66/71 | 67/72 | 75/44 | 253/253 |
| Model 1 | 1 | 1.43 (0.85, 2.40) | 1.35 (0.80, 2.30) | 2.60 (1.49, 4.55) | 1.23 (1.10, 1.37) |
| Model 2 | 1 | 1.64 (0.85, 3.16) | 1.50 (0.76, 2.93) | 3.68 (1.75, 7.71) | 1.30 (1.14, 1.49) |
| Model 3 | 1 | 1.67 (0.85, 3.29) | 1.57 (0.78, 3.12) | 4.01 (1.85, 8.66) | 1.31 (1.14, 1.51) |
| Danish men | | | | | |
| Cases/control subjects, *n* | 59/80 | 68/74 | 81/73 | 120/101 | 328/328 |
| Model 1 | 1 | 1.29 (0.80, 2.09) | 1.57 (0.98, 2.51) | 1.73 (1.09, 2.74) | 1.03 (0.99, 1.07) |
| Model 2 | 1 | 0.92 (0.52, 1.60) | 0.95 (0.55, 1.66) | 1.18 (0.69, 2.01) | 1.02 (0.97, 1.07) |
| Model 3 | 1 | 0.87 (0.49, 1.55) | 0.93 (0.52, 1.67) | 1.21 (0.69, 2.12) | 1.02 (0.97, 1.07) |
| All women | | | | | |
| Cases/control subjects, *n* | 82/88 | 88/87 | 90/88 | 90/87 | 350/350 |
| Model 1 | 1 | 1.08 (0.71, 1.66) | 1.10 (0.72, 1.67) | 1.12 (0.72, 1.74) | 1.02 (0.94, 1.11) |
| Model 2 | 1 | 0.66 (0.38, 1.13) | 0.81 (0.49, 1.34) | 0.71 (0.41, 1.24) | 0.98 (0.89, 1.07) |
| Model 3 | 1 | 0.65 (0.37, 1.12) | 0.82 (0.49, 1.38) | 0.74 (0.42, 1.30) | 0.99 (0.90, 1.09) |
| Swedish women | | | | | |
| Cases/control subjects, *n* | 46/53 | 49/50 | 50/48 | 38/32 | 183/183 |
| Model 1 | 1 | 1.13 (0.63, 2.00) | 1.21 (0.70, 2.10) | 1.40 (0.74, 2.65) | 1.03 (0.89, 1.20) |
| Model 2 | 1 | 0.58 (0.26, 1.30) | 0.99 (0.49, 1.99) | 1.07 (0.46, 2.47) | 1.02 (0.85, 1.22) |
| Model 3 | 1 | 0.50 (0.21, 1.20) | 0.96 (0.45, 2.03) | 1.13 (0.47, 2.74) | 1.01 (0.84, 1.22) |
| Danish women | | | | | |
| Cases/control subjects, *n* | 36/35 | 39/37 | 40/40 | 52/55 | 167/167 |
| Model 1 | 1 | 1.02 (0.54, 1.94) | 0.96 (0.50, 1.81) | 0.91 (0.48, 1.70) | 1.02 (0.93, 1.12) |
| Model 2 | 1 | 0.70 (0.33, 1.46) | 0.63 (0.30, 1.33) | 0.53 (0.25, 1.13) | 0.97 (0.88, 1.08) |
| Model 3 | 1 | 0.71 (0.33, 1.53) | 0.67 (0.30, 1.51) | 0.52 (0.24, 1.14) | 0.98 (0.88, 1.09) |

[1]Calculated with conditional logistic regression. Values are ORs; 95% CIs in parentheses unless otherwise noted. Model 1 was a crude model conditioned on the following matching factors: year of birth, sex, age, fasting status (time since last meal), and country; model 2 was adjusted for BMI (in kg/m$^2$); and model 3 was further adjusted for smoking status, education, physical activity, alcohol intake, coffee intake, and energy intake. AR, alkylresorcinol; Q, quartile; T2D, type 2 diabetes.

25-nmol/L higher total alkylresorcinol concentration (OR: 1.31; 95% CI: 1.14, 1.51). Additional analyses in which models were further adjusted for red and processed meat, fruits, vegetables, and dairy products were also conducted, but estimates did not change much except in subgroups in which the number of participants were few. In these cases, estimates were somewhat attenuated and no longer reached statistical significance, but the overall interpretation remained similar (data not shown). When adjusting for total cholesterol and triacylglycerol concentrations among Swedish participants, associations were attenuated but remained significant (**Supplemental Table 1**).

In contrast, the C17:0-to-C21:0 ratio, a biomarker of the proportion of whole-grain rye to total whole-grain wheat and rye, was consistently inversely associated with T2D overall and in all subgroups (**Table 3**). Adjusting for BMI (model 2) and the other potential confounding factors (model 3) slightly attenuated the association, but it remained statistically significant in the overall population (OR: 0.79; 95% CI: 0.69, 0.89).

The sum of whole-grain wheat and rye and whole-grain rye separately was moderately correlated with plasma alkylresorcinol homologs among all subjects and for Danish and Swedish subjects separately, whereas whole-grain wheat was not (**Supplemental Table 2**).

No statistically significant associations were observed for reported whole-grain wheat, rye (data not shown), or their sum and T2D when comparing the highest with the lowest whole-grain wheat and rye intake (**Supplemental Table 3**). However, a tendency of positive associations was observed among Swedish men (OR: 3.06; 95% CI: 0.85, 11.04). A tendency for an inverse association between whole-grain wheat and rye intake and likelihood of T2D was observed for Danish women (OR: 0.68; 95% CI: 0.50, 1.00).

## DISCUSSION

We investigated the association between plasma total alkylresorcinol concentration as biomarkers of whole-grain wheat and rye intake and the development of T2D in a nested case-control study in Scandinavian men and women. Overall, plasma total alkylresorcinol concentrations were not significantly associated with T2D risk. Among Swedish men, however, higher plasma total alkylresorcinol concentrations were associated with a higher risk of T2D. We also observed that a high C17:0-to-C21:0 ratio in plasma, a biomarker of the relative whole-grain rye intake to the total whole-grain wheat and rye intake, was consistently associated with a lower risk of T2D. Results of no overall association with whole-grain wheat, rye, or their sum were confirmed when reported whole-grain variables were used as the exposure variable.

The lack of an inverse association between plasma total alkylresorcinol concentration and the risk of T2D was surprising in light of results from other studies. Aune et al. (6) found a consistent inverse association for whole-grain intake and T2D risk (32% lower risk per 3 servings of whole grains/d), but the relative risk reduction appeared nonlinear, with a more pronounced relative risk reduction at low intakes. Whole-grain wheat was the main whole-grain source in most studies (7), but in one Finnish study, whole-grain rye was the main source of whole-grain intake and was associated with a lower risk of T2D (5).

In this population, whole-grain intake was high and diverse (37). The lack of an association between plasma total alkylresorcinols and T2D may have resulted from a lack of the increased benefits of whole-grain intake in a population in which the intake in the lowest quartile corresponds to the highest quintile in many of the studies previously conducted (4, 39). The reason behind the observed higher risk of T2D in the highest plasma total alkylresorcinol concentration quartile among Swedish men is not obvious. One reason could be residual confounding by other dietary factors that may increase the risk of T2D that is associated only with whole-grain intake in Swedish men. Another reason could be because of higher concentrations of triacylglycerols, LDL cholesterol, and low HDL cholesterol among this group. Plasma total alkylresorcinol concentrations have been associated with triacylglycerols in several studies (18), but it is not yet clear whether or to what extent triacylglycerols determine plasma alkylresorcinols, although it is known that alkylresorcinols are mainly transported in VLDL particles, which are rich in triacylglycerols (40, 41). Unfortunately, data on blood lipids were available for only 760 Swedish participants of this study. For Swedish men, the association between total alkylresorcinols and T2D was substantially attenuated after adjusting for triacylglycerols and total cholesterol, but a significant association remained (Supplemental Table 1).

On the contrary, the C17:0-to-C21:0 homolog ratio was inversely associated with T2D. This may indicate that the whole-grain intake with a higher proportion of rye may lower T2D risk in a population in which the total whole-grain wheat and rye intake is high. In recent studies conducted in populations with a comparably high habitual whole-grain rye intake, the plasma alkylresorcinol C17:0-to-C21:0 ratio has been associated with higher insulin sensitivity (14) and a beneficial blood lipid profile (18). Other studies have shown that the consumption of high-fiber rye products compared with refined wheat during an 8-wk dietary intervention led to improved $\beta$ cell function (15) and that a low glycemic index diet rich in high-fiber rye instead of a high glycemic index diet based on refined wheat, potatoes, and oats led to the downregulation of proinflammatory gene expression in the adipose tissue (16).

For analyses based on reported whole-grain wheat, rye (data not shown), or wheat and rye intake, no statistically significant associations were observed except for Danish women ($P = 0.05$), but overall findings were in line with those that used alkylresorcinols as an exposure variable.

This study has several strengths. First, plasma total alkylresorcinol concentration was used as an independent, novel biomarker of whole-grain wheat and rye intake (22), and it has recently been used in several nutritional studies to evaluate the impact of whole-grain wheat and rye on human health (18, 42, 43). Alkylresorcinol concentrations in fasting plasma samples have been shown to have a modest-to-good long-term reproducibility (intraclass correlation coefficient of 0.6–0.7 between measurements made 0.1–4 y apart) in a subpopulation from the Swedish part of this study in which whole-grain intake was high and frequent (44). It is therefore likely that plasma alkylresorcinol concentrations determined in a single plasma sample reflect long-term whole-grain intake with adequate precision in this population. All plasma samples included in this study were from participants who fasted overnight (Sweden) and from participants who had gone >7 h since their last meal (Denmark), and case and control subjects were matched on time since last meal. The prospective design with blood samples drawn before diagnosis reduced the likelihood of selection bias because the

**TABLE 3**
Estimated ORs for T2D per AR C17:0-to-C21:0 ratio, a biomarker of whole-grain rye instead of whole-grain wheat intake, in sex-specific quartiles or per SD among T2D cases and matched controls in a nested case-control study[1]

| | Quartile of plasma C17:0-to-C21:0 ratio | | | | Continuous, per SD |
|---|---|---|---|---|---|
| | Q1 (referent) | Q2 | Q3 | Q4 | |
| Range/increment | | | | | |
| Women | 0–0.17 | >0.17–0.27 | >0.27–0.38 | >0.38 | |
| Men | 0–0.16 | >0.16–0.26 | >0.26–0.38 | >0.38 | |
| All subjects | | | | | |
| Cases/control subjects, *n* | 315/234 | 273/231 | 196/232 | 147/234 | 931/931 |
| Model 1 | 1 | 0.83 (0.65, 1.07) | 0.55 (0.42, 0.73) | 0.37 (0.27, 0.50) | 0.71 (0.63, 0.79) |
| Model 2 | 1 | 0.85 (0.63, 1.14) | 0.64 (0.46, 0.87) | 0.53 (0.37, 0.75) | 0.78 (0.69, 0.88) |
| Model 3 | 1 | 0.86 (0.64, 1.16) | 0.66 (0.48, 0.92) | 0.54 (0.37, 0.78) | 0.79 (0.69, 0.89) |
| All Swedish subjects | | | | | |
| Cases/control subjects, *n* | 137/95 | 117/110 | 99/102 | 83/129 | 436/436 |
| Model 1 | 1 | 0.73 (0.50, 1.06) | 0.59 (0.40, 0.89) | 0.37 (0.24, 0.57) | 0.74 (0.64, 0.85) |
| Model 2 | 1 | 0.91 (0.57, 1.46) | 0.82 (0.50, 1.34) | 0.55 (0.32, 0.93) | 0.82 (0.69, 0.97) |
| Model 3 | 1 | 0.93 (0.57, 1.50) | 0.90 (0.54, 1.51) | 0.58 (0.33, 1.00) | 0.84 (0.71, 1.00) |
| All Danish subjects | | | | | |
| Cases/control subjects, *n* | 178/139 | 156/121 | 97/130 | 64/105 | 495/495 |
| Model 1 | 1 | 0.91 (0.65, 1.28) | 0.52 (0.35, 0.75) | 0.37 (0.23, 0.57) | 0.66 (0.56, 0.79) |
| Model 2 | 1 | 0.82 (0.56, 1.20) | 0.53 (0.35, 0.81) | 0.51 (0.31, 0.84) | 0.73 (0.60, 0.88) |
| Model 3 | 1 | 0.82 (0.56, 1.22) | 0.53 (0.34, 0.82) | 0.52 (0.31, 0.87) | 0.73 (0.60, 0.89) |
| All men | | | | | |
| Cases/control subjects, *n* | 201/146 | 171/144 | 115/145 | 94/146 | 581/581 |
| Model 1 | 1 | 0.83 (0.60, 1.13) | 0.52 (0.37, 0.74) | 0.37 (0.25, 0.55) | 0.70 (0.61, 0.80) |
| Model 2 | 1 | 0.70 (0.49, 1.01) | 0.54 (0.37, 0.80) | 0.48 (0.31, 0.76) | 0.74 (0.64, 0.87) |
| Model 3 | 1 | 0.72 (0.49, 1.05) | 0.57 (0.38, 0.86) | 0.50 (0.31, 0.80) | 0.75 (0.64, 0.88) |
| Swedish men | | | | | |
| Cases/control subjects, *n* | 75/53 | 68/65 | 57/59 | 53/76 | 253/253 |
| Model 1 | 1 | 0.76 (0.46, 1.25) | 0.62 (0.37, 1.05) | 0.42 (0.24, 0.73) | 0.77 (0.64, 0.91) |
| Model 2 | 1 | 0.68 (0.37, 1.25) | 0.69 (0.37, 1.30) | 0.48 (0.24, 0.97) | 0.78 (0.63, 0.97) |
| Model 3 | 1 | 0.65 (0.34, 1.24) | 0.72 (0.37, 1.41) | 0.49 (0.23, 1.01) | 0.78 (0.62, 0.98) |
| Danish men | | | | | |
| Cases/control subjects, *n* | 126/93 | 103/79 | 58/86 | 41/70 | 328/328 |
| Model 1 | 1 | 0.86 (0.57, 1.31) | 0.45 (0.28, 0.72) | 0.32 (0.18, 0.56) | 0.62 (0.50, 0.76) |
| Model 2 | 1 | 0.73 (0.46, 1.16) | 0.45 (0.27, 0.74) | 0.46 (0.25, 0.86) | 0.68 (0.54, 0.86) |
| Model 3 | 1 | 0.76 (0.47, 1.24) | 0.47 (0.28, 0.80) | 0.49 (0.26, 0.95) | 0.69 (0.54, 0.89) |
| All women | | | | | |
| Cases/control subjects, *n* | 114/88 | 102/87 | 81/87 | 53/88 | 350/350 |
| Model 1 | 1 | 0.84 (0.56, 1.28) | 0.62 (0.39, 0.97) | 0.37 (0.22, 0.62) | 0.72 (0.60, 0.86) |
| Model 2 | 1 | 1.23 (0.74, 2.03) | 0.92 (0.53, 1.59) | 0.65 (0.36, 1.19) | 0.87 (0.70, 1.08) |
| Model 3 | 1 | 1.25 (0.74, 2.10) | 0.91 (0.52, 1.61) | 0.66 (0.36, 1.22) | 0.87 (0.70, 1.09) |
| Swedish women | | | | | |
| Cases/control subjects, *n* | 62/42 | 49/45 | 42/43 | 30/53 | 183/183 |
| Model 1 | 1 | 0.70 (0.39, 1.24) | 0.58 (0.31, 1.08) | 0.30 (0.15, 0.60) | 0.69 (0.54, 0.87) |
| Model 2 | 1 | 1.59 (0.72, 3.49) | 1.14 (0.51, 2.55) | 0.65 (0.28, 1.55) | 0.89 (0.67, 1.19) |
| Model 3 | 1 | 1.86 (0.80, 4.33) | 1.46 (0.60, 3.54) | 0.80 (0.32, 2.03) | 0.95 (0.70, 1.27) |
| Danish women | | | | | |
| Cases/control subjects, *n* | 52/46 | 53/42 | 39/44 | 23/35 | 167/167 |
| Model 1 | 1 | 1.04 (0.57, 1.91) | 0.69 (0.36, 1.34) | 0.49 (0.23, 1.04) | 0.78 (0.58, 1.04) |
| Model 2 | 1 | 1.10 (0.56, 2.17) | 0.83 (0.39, 1.77) | 0.71 (0.30, 1.66) | 0.87 (0.62, 1.22) |
| Model 3 | 1 | 1.03 (0.50, 2.10) | 0.70 (0.31, 1.59) | 0.66 (0.27, 1.58) | 0.83 (0.59, 1.17) |

[1]Calculated with conditional logistic regression. Values are ORs; 95% CIs in parentheses unless otherwise noted. Model 1 (AR total + C17:0/C21:0) was a crude model conditioned on the following matching factors: year of birth, sex, age, fasting status (time since last meal), and country; model 2 (AR total + C17:0/C21:0) was adjusted for BMI (in kg/m$^2$); and model 3 (AR total + C17:0/C21:0) was further adjusted for smoking status, education, physical activity, alcohol intake, coffee intake, and energy intake. AR, alkylresorcinol; Q, quartile; T2D, type 2 diabetes.

case-control study was nested within the prospective cohort. A wide exposure range is preferred in epidemiologic studies. This population is therefore probably well suited for studies of the association between whole-grain foods in relation to T2D because of a wide range of whole-grain rye and wheat intakes, but we cannot rule out the possibility that the intakes may have been beyond the beneficial range (6). In this study, whole-grain wheat and rye intakes were assessed with a specific dietary biomarker,

whereas other studies have typically assessed total whole-grain intake with questionnaires that include oats, which are known to affect blood lipids positively and thereby lower diabetes risk (45). In addition, a large number of participants and comprehensive information on confounders were available from validated FFQs.

Our study also has several potential limitations. First, the observational study design can only identify associations, which may not necessarily imply causality. The half-life of plasma alkylresorcinols are relatively short (46), leading to random fluctuations in plasma alkylresorcinols with a resulting attenuation bias of observed ORs as well as a loss of statistical power to find any existing associations. However, the sample size is relatively large, and the reproducibility of the Swedish part of the study has been shown to be adequate (44). Plasma alkylresorcinol concentrations are to some extent affected by other factors than whole-grain intake, such as sex, age, and BMI (44, 47–51). We carefully adjusted or matched on these factors, but residual confounding cannot be ruled out. Moreover, plasma alkylresorcinol concentrations are affected by between-subject differences in absorption and/or metabolism, which may contribute to errors in the biomarker reflection of true whole-grain wheat and rye intake (44, 52). Moreover, plasma alkylresorcinols are biomarkers of whole-grain wheat and rye but not for other cereals, such as oats, which were also consumed in these cohorts (38). Misclassifying an outcome is also likely. It is known that many people have undiagnosed diabetes (33); thus, some of the controls that were supposed to be diabetes-free at matching might in fact have been undiagnosed patients with diabetes.

To check the results found when plasma total alkylresorcinol concentration was used as the exposure, further analyses were conducted with whole-grain intake assessed from FFQs. These results supported the findings obtained with alkylresorcinols. Different FFQs were used for 440 participants from NSHDS than for 1422 from DCH and NSHDS. However, all FFQs have been validated and contain similar questions on whole-grain intake.

Moderate correlation between plasma alkylresorcinol homologs and whole-grain intake of wheat and rye or rye separately and no correlation with whole-grain wheat probably resulted from the misclassification of whole-grain wheat intake estimated by FFQs as a result of the large variation of whole-grain content among whole-grain wheat products.

Results may not be generalizable beyond middle-aged Caucasian, since the DCH and NSHDS cohorts comprised relative homogenous populations of mostly middle-aged Caucasian participants (53, 54).

In summary, we found no support for a beneficial role of whole-grain wheat and rye intake as measured with plasma total alkylresorcinol as a biomarker or from FFQs and T2D in a Scandinavian population with a high intake of these cereals. However, the C17:0-to-C21:0 ratio was associated with lower T2D risk. This suggests that whole-grain intake with a higher proportion of rye relative to wheat may be favorable for T2D prevention. This finding needs to be verified in future studies.

The authors' responsibilities were as follows—IB: conducted biomarker analyses, performed statistical analyses, interpreted the data, and drafted and revised the manuscript; CK: performed statistical analyses, interpreted the data, and drafted and revised the manuscript; MM: interpreted the data and drafted and revised the manuscript; AO, RMvD, AT, KO, BL, and IJ: interpreted the data and provided critical intellectual input; RL: conceived and designed the study, analyzed the biomarkers, interpreted the data, and drafted and revised the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

## REFERENCES

1. International Diabetes Federation. IDF diabetes atlas. 6th ed. Brussels (Belgium): International Diabetes Federation; 2013.
2. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care 2011;34:1249–57.
3. Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet 2014;383:1999–2007.
4. Fung TT, Hu FB, Pereira MA, Liu S, Stampfer MJ, Colditz GA, Willett WC. Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. Am J Clin Nutr 2002;76:535–40.
5. Montonen J, Knekt P, Järvinen R, Aromaa A, Reunanen A. Whole-grain and fiber intake and the incidence of type 2 diabetes. Am J Clin Nutr 2003;77:622–9.
6. Aune D, Norat T, Romundstad P, Vatten LJ. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. Eur J Epidemiol 2013;28:845–58.
7. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. J Nutr 2012;142:1304–13.
8. Okarter N, Liu RH. Health benefits of whole grain phytochemicals. Crit Rev Food Sci Nutr 2010;50:193–208.
9. Andersson A, Tengblad S, Karlström B, Kamal-Eldin A, Landberg R, Basu S, Åman P, Vessby B. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. J Nutr 2007;137:1401–7.
10. Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, Mutch W, Wahle K, Horgan G, Thies F. Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: a randomized controlled trial. Am J Clin Nutr 2010;92:733–40.
11. Brownlee IA, Moore C, Chatfield M, Richardson DP, Ashby P, Kuznesof SA, Jebb SA, Seal CJ. Markers of cardiovascular risk are not changed by increased whole-grain intake: the WHOLEheart study, a randomised, controlled dietary intervention. Br J Nutr 2010;104:125–34.
12. Rakha A, Åman P, Andersson R. Characterisation of dietary fibre components in rye products. Food Chem 2010;119:859–67.
13. Andersson AAM, Dimberg L, Åman P, Landberg R. Recent findings on certain bioactive components in whole grain wheat and rye. J Cereal Sci 2014;59:294–311.
14. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Åkesson B, Landin-Olsson B, Rosqvist F, Iggman D, Schwab U, Herzig KH, et al. Plasma alkylresorcinols C17:0/C21:0 ratio, a biomarker of relative whole-grain rye intake, is associated to insulin sensitivity: a randomized study. Eur J Clin Nutr 2014;68:453–8.
15. Juntunen KS, Laaksonen DE, Poutanen KS, Niskanen LK. High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. Am J Clin Nutr 2003;77:385–91.
16. Kallio P, Kolehmainen M, Laaksonen DE, Kekalainen J, Salopuro T, Sivenius K, Pulkkinen L, Mykkanen HM, Niskanen L, Uusitupa M, et al. Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome : the FUNGENUT Study. Am J Clin Nutr 2007;85:1417–27.
17. Kallio P, Kolehmainen M, Laaksonen DE, Pulkkinen L, Atalay M, Mykkanen H, Uusitupa M, Poutanen K, Niskanen L. Inflammation markers are modulated by responses to diets differing in postprandial insulin responses in individuals with the metabolic syndrome. Am J Clin Nutr 2008;87:1497–503.
18. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Åkesson B, Rosqvist F, Schwab U, Herzig KH, Hukkanen J, Savolainen MJ, et al. Whole grain rye intake, reflected by a biomarker, is associated with favorable blood lipid outcomes in subjects with the metabolic syndrome—a randomized study. PLoS One 2014;9:e110827.
19. Landberg R, Andersson S, Zhang J, Johansson J, Adlercreutz H, Kamal-Eldin A. Rye whole grain and bran intake compared with refined wheat decreases urinary C-peptide, plasma insulin, and prostate specific antigen in men with prostate cancer. J Nutr 2010;140:2180–6.

20. Kristal AR, Peters U, Potter JD. Is it time to abandon the food frequency questionnaire? Cancer Epidemiol Biomarkers Prev 2005;14:2826–8.
21. Illner AK, Freisling H, Boeing H, Huybrechts I, Crispim SP, Slimani N. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. Int J Epidemiol 2012;41:1187–203.
22. Landberg R, Kamal-Eldin A, Andersson A, Vessby B. 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.
23. Linko AM, Juntunen KS, Mykkanen HM, Adlercreutz H. Whole-grain rye bread consumption by women correlates with plasma alkylresorcinols and increases their concentration compared with low-fiber wheat bread. J Nutr 2005;135:580–3.
24. Ross AB, Kamal-Eldin A, Åman P. Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. Nutr Rev 2004;62:81–95.
25. Landberg R, Åman P, Kamal-Eldin A. A rapid gas chromatography-mass spectrometry method for quantification of alkylresorcinols in human plasma. Anal Biochem 2009;385:7–12.
26. Chen Y, Ross AB, Åman P, Kamal-Eldin A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. J Agric Food Chem 2004;52:8242–6.
27. Hallmans G, Ågren Å, Johansson G, Johansson A, Stegmayr B, Jansson JH, Lindahl B, Rolandsson O, Söderberg S, Nilsson M, et al. Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort - evaluation of risk factors and their interactions. Scand J Public Health Suppl 2003;61:18–24.
28. Tjønneland A, Olsen A, Boll K, Stripp C, Christensen J, Engholm G, Overvad K. Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: a population-based prospective cohort study of 57,053 men and women in Denmark. Scand J Public Health 2007;35:432–41.
29. Tjønneland A, Overvad K, Haraldsdóttir J, Bang S, Ewertz M, Jensen OM. Validation of a semiquantitative food frequency questionnaire developed in Denmark. Int J Epidemiol 1991;20:906–12.
30. Van Guelpen B, Hultdin J, Johansson I, Stegmayr B, Hallmans G, Nilsson TK, Weinehall L, Witthoft C, Palmqvist R, Winkvist A. Folate, vitamin B12, and risk of ischemic and hemorrhagic stroke: a prospective, nested case-referent study of plasma concentrations and dietary intake. Stroke 2005;36:1426–31.
31. Johansson I, Hallmans G, Wikman A, Biessy C, Riboli E, Kaaks R. Validation and calibration of food-frequency questionnaire measurements in the Northern Sweden Health and Disease cohort. Public Health Nutr 2002;5:487–96.
32. Johansson I, Van Guelpen B, Hultdin J, Johansson M, Hallmans G, Stattin P. Validity of food frequency questionnaire estimated intakes of folate and other B vitamins in a region without folic acid fortification. Eur J Clin Nutr 2010;64:905–13.
33. Carstensen B, Kristensen JK, Marcussen MM, Borch-Johnsen K. The National Diabetes Register. Scand J Public Health 2011;39:58–61.
34. Wierzbicka R, Wu H, Franek M, Kamal-Eldin A, Landberg R. Determination of alkylresorcinols and their metabolites in biological samples by gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2015;1000:120–9.
35. American Association of Cereal Chemists. Definition of whole grain [Internet]. [cited 2010 Apr 21]. Available from: http://www.aaccnet.org/definitions/wholegrain.asp.
36. Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, Kroke A, Trichopoulos D, Trichopoulou A, Lauria C, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002;5:1125–45.
37. Kyrø C, Skeie G, Dragsted LO, Christensen J, Overvad K, Hallmans G, Johansson I, Lund E, Slimani N, Johnsen NF, et al. Intake of whole grains in Scandinavia is associated with healthy lifestyle, socio-economic and dietary factors. Public Health Nutr 2011;14:1787–95.
38. Kyrø C, Skeie G, Dragsted LO, Christensen J, Overvad K, Hallmans G, Johansson I, Lund E, Slimani N, Johnsen NF, et al. Intake of whole grain in Scandinavia: intake, sources and compliance with new national recommendations. Scand J Public Health 2012;40:76–84.
39. Liu S, Manson JE, Stampfer MJ, Hu FB, Giovannucci E, Colditz GA, Hennekens CH, Willett WC. A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. Am J Public Health 2000;90:1409–15.
40. Linko-Parvinen AM, Landberg R, Tikkanen MJ, Adlercreutz H, Penalvo JL. Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. J Nutr 2007;137:1137–42.
41. Cox RP, Garcia-Palmieri MR. Cholesterol, triglycerides, and associated lipoproteins. In: Walker H, Hall W, Hurst J, editors. Clinical methods: the history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths; 1990. p. 153–60.
42. Knudsen MD, Kyrø C, Olsen A, Dragsted LO, Skeie G, Lund E, Åman P, Nilsson LM, Bueno-de-Mesquita HB, Tjønneland 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.
43. McKeown NM, Hruby A, Landberg R, Herrington DM, Lichtenstein AH. Plasma alkylresorcinol, biomarkers of whole-grain intake, are not associated with progression of coronary artery atherosclerosis in postmenopausal women with coronary artery disease. Public Health Nutr 2016;19:326–31.
44. Landberg R, Åman P, Hallmans G, Johansson I. 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 2013;67:259–63.
45. Wolever TMS. Do whole grain cereals really reduce LDL-cholesterol by 0,72 mmol/L? Reply to Wolever. J Nutr 2013;143:1521–2.
46. Marklund M, Stromberg EA, Lærke HN, Knudsen KEB, Kamal-Eldin A. Simultaneous pharmacokinetic modeling of alkylresorcinols and their main metabolites indicates dual absorption mechanisms and enterohepatic elimination in humans. J Nutr 2014;144:1674–80.
47. Montonen J, Landberg R, Kamal-Eldin A, Åman P, Knueppel S, Boeing H, Pischon T. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. Eur J Clin Nutr 2010;64:698–703.
48. 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.
49. Ross AB, Bourgeois A, Ndung H, 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.
50. Ma J, Ross AB, Shea MK, Bruce SJ, Jacques PF, Saltzman E, Lichtenstein AH, Booth SL, McKeown NM. Plasma alkylresorcinols, biomarkers of whole-grain intake, are related to lower BMI in older adults. J Nutr 2012;142:1859–64.
51. McKeown NM, Yoshida M, Shea MK, Jacques PF, Lichtenstein AH, Rogers G, Booth SL, Saltzman E. Whole-grain intake and cereal fiber are associated with lower abdominal adiposity in older adults. J Nutr 2009;139:1950–5.
52. Landberg R, Marklund M, Kamal-Eldin A, Åman P. An update on alkylresorcinols—occurrence, bioavailability, bioactivity and utility as biomarkers. J Funct Foods 2014;7:77–89.
53. Hellmann SS, Njor SH, Lynge E, von Euler-Chelpin M, Olsen A, Tjønneland A, Vejborg I, Jovanovic Andersen Z. Body mass index and participation in organized mammographic screening: a prospective cohort study. BMC Cancer 2015;15:294.
54. Lukanova A, Toniolo P, Lundin E, Micheli A, Akhmedkhanov A, Muti P, Zeleniuch-Jacquotte A, Biessy C, Lenner P, Krogh V, et al. Body mass index in relation to ovarian cancer: a multi-centre nested case-control study. Int J Cancer 2002;99:603–8.