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The Journal of Nutrition
Nutrition and Disease

Whole-Grain Intake, Reflected by Dietary Records and Biomarkers, Is Inversely Associated with Circulating Insulin and Other Cardiometabolic Markers in 8- to 11-Year-Old Children[1,2]

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Abstract

Background: Whole-grain consumption seems to be cardioprotective in adults, but evidence in children is limited.

Objective: We investigated whether intakes of total whole grain and dietary fiber as well as specific whole grains were associated with fat mass and cardiometabolic risk profile in children.

Methods: We collected cross-sectional data on parental education, puberty, diet by 7-d records, and physical activity by accelerometry and measured anthropometry, fat mass index by dual-energy X-ray absorptiometry, and blood pressure in 713 Danish children aged 8–11 y. Fasting blood samples were obtained and analyzed for alkylresorcinols, biomarkers of whole-grain wheat and rye intake, HDL and LDL cholesterol, triacylglycerols, insulin, and glucose. Linear mixed models included puberty, parental education, physical activity, and intakes of energy, fruit and vegetables, saturated fat, and n–3 (ω-3) polyunsaturated fatty acids.

Results: Median (IQR) whole-grain and dietary fiber intakes were 52 g/d (35–72 g/d) and 17 g/d (14–22 g/d), respectively. Fourteen percent of children were overweight or obese and most had low-risk cardiometabolic profiles. Dietary whole-grain and fiber intakes were not associated with fat mass index but were inversely associated with serum insulin [both $P < 0.01$; e.g., with 0.68 pmol/L (95% CI: 0.26, 1.10 pmol/L) lower insulin · g whole grain^{-1} · MJ^{-1}]. Whole-grain oat intake was inversely associated with fat mass index, systolic blood pressure, and LDL cholesterol (all $P < 0.05$) as well as insulin ($P = 0.003$), which also tended to be inversely associated with whole-grain rye intake ($P = 0.11$). Adjustment for fat mass index did not change the associations. The C17-to-C21 alkylresorcinol ratio, reflecting whole-grain rye to wheat intake, was inversely associated with insulin ($P < 0.001$).

Conclusions: Higher whole-grain intake was associated with lower serum insulin independently of fat mass in 8- to 11-y-old Danish children. Whole-grain oat intake was linked to an overall protective cardiometabolic profile, and whole-grain rye intake was marginally associated with lower serum insulin. This supports whole grains as healthy dietary components in childhood. This trial was registered at clinicaltrials.gov as NCT01577277. *J Nutr* 2017;147:816–24.

Keywords: alkylresorcinols, fiber, cardiovascular, metabolic syndrome, obesity, children

Introduction

In parallel with the obesity epidemic, increasing numbers of children in the Western world now show elevated cardiovascular risk markers and insulin resistance (1), which increases the risk of metabolic syndrome and type 2 diabetes in adulthood (2) and may be prevented with healthy dietary habits and physical activity in childhood. Higher whole-grain intake has been associated with a lower risk of myocardial infarction (3), lower all-cause mortality (4), a lower risk of type 2 diabetes and insulin resistance (5), and protection against weight gain (6) in adults. Most randomized controlled trials investigating cardiometabolic

[1] Supported by Nordea-fonden, Denmark (grant 02-2010-0389).
[2] Author disclosures: CT Damsgaard, A Biltoft-Jensen, I Tetens, KF Michaelsen, MV Lind, A Astrup, and R Landberg, no conflicts of interest.
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Manuscript received November 21, 2016. Initial review completed December 16, 2016. Revision accepted February 10, 2017.
First published online March 29, 2017; doi:10.3945/jn.116.244624.

effects of whole grains in adults have been small and often inconsistent, but overall suggest small effects on body fatness (7) and beneficial effects on LDL cholesterol (8) and potentially also on blood pressure (9) and insulin (10). Moreover, specific effects may be attributed to specific types of whole grains, particularly oats (11) and rye (12), which are rich in soluble (viscous) fiber and a mixture of soluble and insoluble fibers, respectively (13).

To our knowledge, no randomized controlled trials have investigated the effect of whole-grain intake on cardiometabolic risk markers in healthy children. There is some evidence from observational studies to indicate beneficial associations in children and adolescents, especially with regard to insulin sensitivity (14–17). However, most previous observational studies in children assessed whole-grain and fiber intake by using an FFQ or a single 24-h dietary recall and only a few have been able to adjust for objectively measured physical activity and other healthy dietary characteristics, which may be important confounders. Furthermore, none have used objective biomarkers of intake, such as plasma alkylresorcinols, which were previously evaluated as biomarkers of whole-grain rye and wheat intake in adults (18, 19) and in Danish children (20). Due to the consumption of traditional Danish whole-grain rye bread and rolled oats, Danish children have high intakes of whole grain, which provides a unique opportunity for investigating associations with health outcomes. We explored whether intakes of whole grain and dietary fiber, as well as specific whole-grain types, were associated with fat mass index and cardiometabolic risk profile, including blood pressure, fasting plasma lipids, and insulin, in a largely representative sample of 8- to 11-y-old Danish children.

Methods

Study design and participants. This cross-sectional study included baseline data from the Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet (OPUS)[8] School Meal Study, which was a randomized controlled trial originally designed to investigate the effects of Nordic school meals on cardiometabolic health and cognitive performance in 8- to 11-y-old Danish children (21). The study was conducted according to the guidelines in the Declaration of Helsinki, approved by the Danish National Committee on Biomedical Research Ethics (H-1-2010-124), and registered at clinicaltrials.gov as NCT01577277. All children from the third and fourth grades at 9 schools in the eastern part of Denmark were invited to participate, and baseline measurements were conducted during August to November 2011. As previously described (21), schools were mainly invited by a study investigator with a strong network within Danish municipal schools. Inclusion criteria for the schools were as follows: *1*) location in the eastern part of Denmark, *2*) ≥4 classes in total at the third or fourth grade, *3*) kitchen facilities available for the school meal intervention, and *4*) high motivation for participation. Moreover, our aim was that ≥50% of the schools would belong to municipalities with low income and educational level, which was the case for 3 of the 9 included schools (21). Children were excluded if they had severe food-related allergies, food intolerances, or malabsorption; severe mental handicaps; or participated in other research projects that involved blood sampling or radiation. Among the 1021 children invited, the parents of 834 of the children (82%) gave informed written consent for participation (21). The present study is based on baseline data on the 713 children for whom we obtained data on anthropometry, body composition, dietary intake, whole-blood EPA + DHA, physical activity, puberty, and parental education. A total of 708 children also had available data on blood pressure and blood lipids, 674 children had data on serum insulin and glucose, and plasma alkylresorcinols were analyzed in 564 children. Missing data were mainly due to incomplete dietary recordings or unsuccessful blood sampling in some children.

Parental education, puberty, diet, and physical activity. Each family underwent a 2-h interview with regard to socioeconomic status and demographic characteristics, during which instructions on diet and physical activity recording were given. Parental educational level was defined by the highest level obtained in the household and categorized as described by Statistics Denmark (22). Pubertal status was self-evaluated by the child according to Tanner stages (21). Because very few children were in stages III–V, the variable was recoded to indicate whether the child had entered puberty (stages II–V) or not (stage I).

With support from their parents, the children recorded their daily intakes of food and beverages every night for 7 consecutive days by using an Internet-based dietary assessment software developed for the study (23). We previously validated this tool in 8- to 11-y-old Danish children for energy intake with the use of accelerometer-derived total energy expenditure as the reference method ($r = 0.31$, $P < 0.001$; $n = 81$) (24), for intakes of whole-grain wheat and rye by using plasma alkylresorcinols ($r = 0.40$, $P < 0.001$; $n = 593$) (20), for fish intake against whole-blood EPA + DHA ($r = 0.38$, $P < 0.0001$; $n = 658$) (25), and for fruit and vegetable intakes against plasma carotenoids ($r = 0.58$, $P < 0.01$; $n = 73$) (26). Intakes of energy, fruit and vegetables, macronutrients, and total dietary fiber (including cereal and noncereal sources and using the AOAC 985.29 method) were calculated by using the software system GIES (version 1.000 d-2010-02-26) developed by the National Food Institute, Technical University of Denmark, and with the use of data from the National Danish Food Composition database. Whole grain was defined as the whole kernel of grain or cereal (including germ, endosperm, and bran) in which the whole kernel could be ground, broken, or left intact, but the components, for the respective cereals, should be included in the same proportion as in the intact whole kernel. Grain types were defined as wheat, spelt, rye, oats, barley, corn, rice, millet, and sorghum. Whole-grain contents in the foods eaten by the children were estimated from market data, as previously described (20). On the basis of reported energy intake and estimated basal metabolic rate (BMR), both in megajoules per day (27, 28), 58 children (8.1%) were classified as underreporters (energy intake: BMR ≤1.05) and 12 children (1.7%) as overreporters of energy intake (energy intake: BMR ≥2.29).

During the same 7 d, the children wore a tri-axis accelerometer (GT3X or GT3X+; ActiGraph) in an elastic belt secured tightly at the right hip. The children were instructed to remove the accelerometer only during water activities. Data were reintegrated to 1-min epochs by using ActiLife (version 6.0.0; ActiGraph), as previously described (29). The 62 children (7.4% of the original study population of 834 children) who wore the accelerometer for <10 h on <3 weekdays or <1 weekend day were excluded. Moderate-vigorous intensity physical activity was defined as number of minutes spent with an activity of ≥2296 counts/min (30). Median (range) days of valid recording were 5 (3–6) weekdays and 2 (1–2) weekend days, with a mean ± SD monitor wear time (excluding sleep time) of 900 ± 34 min/d.

Clinical measurements and blood sampling. Clinical measurements and blood sampling were performed by standard procedures in the morning, as described previously (31). Only 3.0% of the children were not fasted. Up to 40 mL venous blood was drawn from the antecubital vein, and plasma and serum were separated from the samples and stored at −80°C for later analysis. Height was measured to the nearest 0.1 cm by using a portable stadiometer (CMS Weighing Equipment), and the mean of 3 measurements was calculated. Body weight was measured to the nearest 0.1 kg on a digital scale (Tanita 800S; Tanita). Children wore light clothing and were asked to empty their bladder before measurement. Sex- and age-adjusted BMI *z* scores were calculated by using WHO AnthroPlus software (32), and the prevalence of underweight, overweight, and obesity was calculated as described by Cole et al. (33, 34). Children's whole-body composition was measured by DXA scan (Lunar Prodigy; GE Healthcare) with the use of Encore software version

[8] Abbreviations used: BMR, basal metabolic rate; CVD, cardiovascular disease; OPUS, Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet.

13.5 (GE Healthcare), and fat mass index was calculated as total fat mass per height squared (kg/m^2). Blood pressure was measured in the supine position and after a 10-min rest by an automated device (UA-787 Plus; A&D Medical) with the use of the appropriate cuff size. A second device (ProBP 3400 SureBP; Welch Allyn, Inc.) was used for children with arm circumferences <18 cm. Measurements were performed 3 times, and the mean of the last 2 measurements was used.

Blood analyses. Plasma HDL cholesterol and TGs were measured on a Vitros 5.1 FS (Ortho-Clinical Diagnostics); LDL cholesterol was calculated by Friedewald's equation (35). Serum insulin was measured by immunoassay on an ADVIA Centaur XP (Siemens Healthcare), and concentrations were converted from milli-international units per liter to picomoles per liter by multiplying with 6.945. All samples from the same child were analyzed in the same batch and the inter- and intra-assay CVs were as follows: 2.0% and 1.2% (HDL cholesterol), 1.5% and 0.8% (TGs), and 2.5% and 3.1% (insulin). Plasma glucose was assessed immediately after blood sampling on a Hemocue Glucose 201 (Hemocue Denmark), and the interassay CV was 4.0%. Whole-blood FA composition was measured by high-throughput GC as previously described (31). The intra- and interassay CVs were 1.3% and 4.5% for EPA and 2.4% and 6.4% for DHA, respectively. The amount of EPA + DHA is given in percentage of weight of total whole-blood FAs.

Plasma alkylresorcinols were measured by gas chromatography–mass spectrometry on a Trace GC Ultra coupled to a DSQII mass spectrometer (Thermo Scientific) by using the principle described by Landberg et al. (36) and modified slightly as previously described (20). All samples from the same child were analyzed in the same batch and the intra- and interassay CVs for total alkylresorcinols were 6.0% and 15.6%, respectively. We used total alkylresorcinols (reflecting whole-grain wheat and rye intakes) as well as the C17-to-C21 alkylresorcinol homolog ratio (reflecting the relative intakes of whole-grain rye and wheat) (19) in the present study.

Statistical analysis. Descriptive data are presented as means ± SDs or as medians (IQRs) separately for girls and boys and were compared by using an unpaired *t* test or Mann-Whitney *U* test (for nonnormally distributed variables). Included and nonincluded children were compared by using an unpaired *t* test and chi-square test, and under-, normal, and overreporters of energy intake were compared using 1-factor ANOVA with Tukey's post hoc test.

Potential associations between the exposure variables (whole-grain intake and dietary fiber intake expressed per energy intake and plasma alkylresorcinols) and the outcome variables (BMI *z* score, fat mass index, waist circumference, systolic and diastolic blood pressure, LDL and HDL cholesterol, TGs, insulin, and glucose) were analyzed by use of linear mixed models. These models included school and class as random effects; sex, puberty (yes or no), and parental education as fixed effects; and age, moderate–vigorous physical activity (minutes per day), energy intake (megajoules per day), and intakes of fruit and vegetables (grams per 10 MJ), saturated fat intake (percentage of energy), and whole-blood EPA + DHA (percentage of weight), which is a biomarker of fish and n–3 long-chain PUFA intake, as covariates. Energy intake was included because it may affect the relation between dietary intake exposure and the outcomes (37). Physical activity, fruit and vegetables, and the fish biomarker were included as potential confounders that reflect a generally healthy lifestyle and that have been inversely associated with cardiovascular disease (CVD) in adults (38, 39) and with the cardiometabolic risk markers in this population (31). Likewise, saturated fat intake has been positively associated with CVD risk (40). All of the analyses except for those of BMI *z* scores and fat mass index were also adjusted for height, and blood pressure models were additionally adjusted for the blood pressure device used. Models in which alkylresorcinols were exposure variables were also adjusted for plasma TGs, because plasma alkylresorcinols are transported in TG-rich lipoproteins (41) and are strongly correlated with plasma TGs in this population (42). To investigate whether potential associations with the cardiometabolic markers were mediated through or independently of fat mass, the models were further adjusted for fat mass index in secondary analyses. Finally, to check whether the results were biased by dietary misreporting, the analyses in which whole-grain or dietary fiber intake were exposures were repeated after the exclusion of under- and overreporters of energy intake. β-Values are expressed per g/MJ increase in whole-grain or fiber intake or per 10-nmol/L increase in alkylresorcinols.

For each outcome, we further explored potential associations with specific types of whole grains by substituting total whole-grain intake with intakes of whole-grain wheat, oat, and rye simultaneously in the models and in those models in which associations were found by also substituting total alkylresorcinols with the C17-to-C21 ratio in subsequent models. Model checking was based on visual inspection of residual and normal probability plots. Fat mass index, waist circumference, plasma TGs, and insulin were log-transformed before analysis to obtain normality; and estimates were back-transformed to their original scale (43). Data were analyzed by using SPSS version 22 (IBM Corporation) and R (The R Foundation for Statistical Computing, version 3.1.3), and significance was established at $P < 0.05$.

Results

Baseline characteristics. As shown in **Table 1**, most children were of normal weight, had ≥1 parent with a higher education, and low cardiometabolic risk profiles. Boys were slightly older than girls, had higher BMI *z* scores but lower fat mass index, were more physically active, and had higher energy intakes. Approximately half of the girls and only approximately one-fourth of the boys had entered puberty. In line with these differences, girls had higher diastolic blood pressure, lower HDL cholesterol, and higher plasma TGs and insulin than boys (Table 1). Whole-grain and dietary fiber intakes were high [median (IQR): 52 (35–72) and 17 (14–22) g/d, respectively], with 44% and 40% of the children fulfilling the current Danish recommendation of 75 g whole grains/10 MJ (44) and 20–30 g dietary fiber/10 MJ (interpreted as 25 g/10 MJ) (45). Fruit and vegetable intake was higher in girls than in boys [median (IQR): 374 g/10 MJ (280–484 g/10 MJ) compared with 320 g/10 MJ (230–437 g/10 MJ); $P < 0.001$], whereas saturated fat intake and whole-blood EPA + DHA were 13% ± 2% of energy and 3.6% ± 1.0% of weight, respectively, with no sex differences ($P > 0.75$).

The 713 children included in the present study comprised 86% of the original OPUS School Meal Study population and did not differ from the nonincluded children with regard to age, sex, or BMI *z* scores, but had parents with slightly higher education ($P = 0.02$). Underreporters of energy intake had higher BMI *z* scores than did normal and overreporters (both $P < 0.001$).

Associations between intakes of whole grains or dietary fiber and the cardiometabolic risk markers. Intakes of whole grain and dietary fiber were not associated with fat mass index (**Table 2**) or waist circumference (data not shown) but were inversely associated with serum insulin, and these associations remained after adjustment for fat mass index (Table 2). The exclusion of energy under- and overreporters slightly increased the *P* value of the association between whole-grain intake and insulin (β: −0.55 pmol/L · g^{-1} · MJ^{-1}; 95% CI: −0.99; −0.12 pmol/L · g^{-1} · MJ^{-1}; $P = 0.01$, $n = 610$) and rendered the association between dietary fiber and insulin nonsignificant (β: −2.66 pmol/L; 95% CI: −6.06; 0.74 pmol/L per g/10 MJ; $P = 0.12$), but also markedly reduced the sample size ($n = 610$). As expected, plasma total alkylresorcinol concentration was consistently positively associated with plasma TGs and tended to be positively associated with HDL cholesterol, after adjustment for TGs (Table 2). None of the other cardiometabolic markers were associated with the exposure variables.

TABLE 1 Characteristics of the 713 included children[1]

	Girls (n = 345)	Boys (n = 368)
Parental education, %		
≤Lower secondary education	5.5	4.4
Upper secondary education	3.5	2.2
Vocational education	33.0	31.5
Short higher education	10.2	9.5
Bachelor's degree or equivalent	28.7	29.9
Master's degree or higher	19.1	22.6
Age, y	9.9 ± 0.6	10.0 ± 0.6**
Fat mass index, kg/m^2	4.1 (2.9–5.7)[2]	3.1 (2.2–4.8)***
Waist circumference, cm	62.5 (58.9–68.2)	62.4 (59.3–68.2)
BMI-for-age z score	0.06 ± 1.02	0.22 ± 1.11*
Weight status,[3] %		
Underweight	11.6	8.2
Normal weight	74.7	78.3
Overweight	12.5	11.1
Obese	1.2	2.5
Entered puberty, %	47	23***
Time spent in MVPA,[4] min/d	38 ± 16	57 ± 24***
Dietary intake		
Energy, MJ/d	7.0 ± 1.4	8.2 ± 1.7***
Protein, % of energy	15 ± 2	16 ± 2
Carbohydrate, % of energy	53 ± 5	53 ± 5
Fat, % of energy	32 ± 4	32 ± 4
Whole grain,[5] g/10 MJ	66 (47–93)	72 (50–96)
Rye	39 (26–51)	39 (24–54)
Wheat	12 (7–20)	13 (8–21)
Oats	6 (1–24)	10 (1–31)
Dietary fiber, g/10 MJ	24 ± 6	24 ± 6
Plasma alkylresorcinols,[6] nmol/L	42 (25–66)	49 (26–72)
C17:C21	0.3 (0.2–0.4)	0.3 (0.2–0.4)
Systolic blood pressure,[7] mm Hg	107 ± 9	108 ± 8
Diastolic blood pressure,[7] mm Hg	69 ± 7	67 ± 6**
LDL cholesterol,[7] mmol/L	2.36 ± 0.56	2.31 ± 0.56
HDL cholesterol,[7] mmol/L	1.39 ± 0.29	1.48 ± 0.32***
TGs,[7] mmol/L	0.66 (0.54–0.87)	0.58 (0.48–0.71)***
Insulin,[8] pmol/L	45 (35–63)	39 (30–54)***

[1] Values are means ± SDs unless otherwise indicated. *,**,***Different from girls: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[2] Median; IQR in parentheses (all such values).
[3] Based on age- and sex-specific cutoffs defined by Cole et al. (33, 34).
[4] MVPA, moderate–vigorous physical activity defined as ≥2296 counts/min.
[5] The median (IQR) whole-grain intake in the total population was 52 (35–72) g/d (mean ± SD: 56 ± 30 g/d).
[6] n = 277 girls and n = 287 boys (total n = 564).
[7] n = 344 girls and n = 364 boys (total n = 708).
[8] n = 325 girls and n = 349 boys (total n = 674).

Associations with specific whole-grain types. To explore potential associations between specific types of whole grains and the cardiometabolic markers, whole-grain rye, wheat, and oats in grams per megajoule were included simultaneously as independent variables in the mixed models instead of total whole-grain intake. These analyses showed that the intake of whole-grain oats was inversely associated with fat mass index, systolic blood pressure, LDL cholesterol, and serum insulin (**Table 3**). Whole-grain rye intake also tended to be inversely associated with circulating insulin (P = 0.11). Further adjustment for fat mass index (Table 3) did not change this. Exclusion of energy over- and underreporters also did not change these results (data not shown).

To further verify the associations with specific whole-grain types, the C17-to-C21 alkylresorcinol ratio (reflecting the proportion between intakes of whole-grain rye and wheat) was included in the models of fat mass index, systolic blood pressure, insulin, and LDL cholesterol instead of total alkylresorcinols. The C17-to-C21 ratio was inversely associated with serum insulin (β: −1.55; 95% CI: −2.41; −0.70 pmol/L per 0.1 increase in the ratio; $P < 0.001$) (**Figure 1**). This result did not change when the ratio was further adjusted for total alkylresorcinols to account not only for the proportion between the homologs but also for the concentration of alkylresorcinols reflecting total whole-grain wheat and rye intake (β: −1.55; 95% CI: −2.41; −0.70 pmol/L per 0.1 increase in the ratio; $P < 0.001$). The C17-to-C21 ratio was not associated with the other outcomes ($P > 0.20$).

Discussion

This cross-sectional study in a well-characterized population of Danish schoolchildren showed that energy-adjusted intakes of whole grains and dietary fiber were inversely associated with serum insulin. Among the whole-grain types, oat intake was associated with lower serum insulin, fat mass index, systolic blood pressure, and LDL cholesterol and whole-grain rye intake tended to be inversely associated with serum insulin, which was supported by an inverse association between the C17-to-C21 alkylresorcinol ratio and insulin. Apart from this, alkylresorcinols were not associated with the cardiometabolic markers, which seems to support our results because alkylresorcinols do not reflect whole-grain oat intake. The associations were adjusted for a number of potential confounders and were independent of children's fat mass.

Two large American cross-sectional studies in adolescents showed inverse associations between whole-grain intake and insulin or insulin resistance (14, 17), which is in line with our results. However, these previous studies measured whole-grain intake by 24-dietary recall (14) or FFQ (17), which likely gives a lower precision than do 7-d dietary records (46). A representative cross-sectional study in British children and adolescents did not measure insulin or glucose, and found no association between whole-grain intake and cholesterol, but showed an inverse association with systolic blood pressure (47). However, these analyses were not adjusted for potential confounders other than sex and age. To our knowledge, no randomized controlled trials have investigated the effects of whole grain or whole-grain oats compared with refined grains on blood pressure in children, but some trials in adults have shown blood pressure–reducing effects of whole grains (9, 11, 48, 49). Three of these studies provided mainly whole-grain oats (11, 48, 49) and 2 of these also found tendencies or effects on glucose and insulin (48, 49). Moreover, a recent meta-analysis confirmed that whole-grain oats lower LDL cholesterol in adults (8), which is in line with our findings. The reported associations between whole grain and whole-grain oat intakes and the cardiometabolic markers were not found when substituting whole-grain intake with plasma total alkylresorcinols. However, because the associations were mainly driven by oats, no associations with plasma alkylresorcinols would be expected, because alkylresorcinols only capture whole-grain wheat and rye intakes. In line with this, total alkylresorcinols have shown moderate associations (r values of ~0.30–0.50) with total whole-grain intake in previous studies in adults (18, 19) as well as in a previous article from the OPUS School Meal Study (r = 0.32) (20). Whole-grain rye tended to be inversely associated with serum insulin in the present study, and this was supported by the inverse association

TABLE 2 Associations between measures of whole-grain and dietary fiber intake and markers of body fatness and cardiometabolic risk in children aged 8–11 y[1]

	Multivariable adjusted[2]		Multivariable adjusted + fat mass index[3]	
	β (95% CI)	*P*	β (95% CI)	*P*
BMI *z* score				
Whole-grain intake, g/MJ	−0.01 (−0.03, 0.02)	0.60	—	—
Dietary fiber intake, g/MJ	0.07 (−0.12, 0.24)	0.45	—	—
Plasma alkylresorcinols, 10 nmol/L	−0.00 (−0.02, 0.02)	0.70	—	—
Fat mass index, kg/m^2				
Whole-grain intake, g/MJ	−0.02 (−0.06, 0.01)	0.23	—	—
Dietary fiber intake, g/MJ	0.06 (−0.23, 0.35)	0.69	—	—
Plasma alkylresorcinols, 10 nmol/L	−0.00 (−0.03, 0.03)	0.81	—	—
Systolic blood pressure, mm Hg				
Whole-grain intake, g/MJ	−0.12 (−0.29, 0.05)	0.17	−0.11 (−0.28, 0.06)	0.22
Dietary fiber intake, g/MJ	−1.00 (−2.10, 0.58)	0.26	−0.75 (−2.08, 0.59)	0.27
Plasma alkylresorcinols, 10 nmol/L	−0.04 (−0.17, 0.10)	0.62	−0.046 (−0.182, 0.091)	0.65
Diastolic blood pressure, mm Hg				
Whole-grain intake, g/MJ	−0.08 (−0.22, 0.06)	0.28	−0.07 (−0.21, 0.07)	0.35
Dietary fiber intake, g/MJ	−0.19 (−1.28, 0.90)	0.73	−0.18 (−1.26, 0.90)	0.75
Plasma alkylresorcinols, 10 nmol/L	0.02 (−0.09, 0.13)	0.68	0.03 (−0.08, 0.14)	0.62
LDL cholesterol, mmol/L				
Whole-grain intake, g/MJ	−0.01 (−0.02, 0.01)	0.40	−0.00 (−0.02, 0.01)	0.55
Dietary fiber intake, g/MJ	−0.03 (−0.12, 0.07)	0.54	−0.03 (−0.12, 0.07)	0.57
Plasma alkylresorcinols, 10 nmol/L	0.01 (−0.01, 0.01)	0.34	0.01 (−0.00, 0.01)	0.28
HDL cholesterol, mmol/L				
Whole-grain intake, g/MJ	0.00 (−0.01, 0.01)	0.99	−0.00 (−0.01, 0.01)	0.86
Dietary fiber intake, g/MJ	−0.02 (−0.07, 0.03)	0.50	−0.02 (−0.07, 0.03)	0.47
Plasma alkylresorcinols, 10 nmol/L	0.00 (−0.00, 0.01)	0.14	0.00 (−0.00, 0.01)	0.16
TGs, mmol/L				
Whole-grain intake, g/MJ	−0.00 (−0.00, 0.00)	0.73	−0.00 (−0.01, 0.00)	0.87
Dietary fiber intake, g/MJ	0.01 (−0.03, 0.05)	0.55	0.01 (−0.03, 0.05)	0.54
Plasma alkylresorcinols, 10 nmol/L	0.01 (0.01, 0.01)	<0.0001	0.01 (0.01, 0.01)	<0.0001
Insulin, pmol/L				
Whole-grain intake, g/MJ	−0.68 (−1.10, −0.26)	0.002	−0.54 (−0.94, −0.14)	0.008
Dietary fiber intake, g/MJ	−4.36 (−7.66, −1.07)	0.009	−3.87 (−6.97, −0.77)	0.01
Plasma alkylresorcinols, 10 nmol/L	0.05 (−0.26, 0.35)	0.76	0.10 (−0.19, 0.39)	0.49

[1] β-Values are expressed as outcome values per gram per megajoule increase in whole-grain or fiber intake or per 10-nmol/L increase in alkylresorcinols; n = 708–713 in models with whole-grain or dietary fiber intake, n = 564 in models with alkylresorcinols.
[2] Adjusted for school and class (as random effects) and age; sex; height; puberty; parental education; time spent in moderate–vigorous physical activity; whole-blood EPA + DHA; and intakes of energy, fruit and vegetables, and saturated fat intake (as fixed effects). However, to avoid collinearity, fat mass index and BMI *z* scores were not adjusted for height. All blood pressure models further included adjustment for the blood pressure device used, and models with alkylresorcinols as an exposure were adjusted for plasma TGs (except when TGs was the outcome).
[3] Additionally adjusted for fat mass index.

between the C17-to-C21 ratio (reflecting the proportion between whole-grain rye and wheat) and insulin. Inverse associations between C17:C21 and insulin or type 2 diabetes have also been shown in several studies in adults [e.g. (50, 51)]. Randomized controlled trials investigating the effects of whole-grain rye on glucose homeostasis have shown inconsistent results, with some finding no differences in blood insulin or glucose (12, 52) and some finding reductions (53). To our knowledge, no randomized trials have investigated the effects of whole-grain intake on serum insulin in children, so this needs further investigation in the future.

Although no associations were seen between total whole-grain intake and children's anthropometric measurements, whole-grain oat intake was associated with a lower fat mass index. This is somewhat in line with the findings of a recent meta-analysis of randomized controlled trials in adults, which showed no effect of whole grains on body weight but a small effect on body fat percentage (7). Only 2 of the included trials that performed measurements of body fat administered oats, so it is speculative whether oats have specific effects on body fat mass. In contrast with our findings, a recent observational study based on NHANES data showed an inverse association between total whole-grain intake and BMI in 6- to 18-y-olds (54). However, although the authors adjusted their regression models for energy intake and physical activity, potential confounding from other healthy dietary components than whole grains was not taken into account, so randomized controlled trials in children are needed.

The potential mechanisms behind the effects of whole grains on insulin and insulin resistance are likely explained mainly by the high (soluble) fiber content and by the food structure of whole-grain products, which may provide a more intact structure and larger particle sizes than milled cereals (55, 56). These

TABLE 3 Associations between specific whole-grain type intakes and fat mass index, systolic blood pressure, and circulating LDL cholesterol and insulin concentrations in 8- to 11-y-old children[1]

	Multivariable adjusted[2]		Multivariable adjusted + fat mass index[3]	
	β (95% CI)	*P*	β (95% CI)	*P*
Fat mass index, kg/m^2				
Whole-grain rye, g/MJ	0.02 (−0.04, 0.07)	0.51	—	—
Whole-grain wheat, g/MJ	−0.03 (−0.13, 0.07)	0.57	—	—
Whole-grain oats, g/MJ	−0.06 (−0.11, −0.00)	0.03	—	—
Systolic blood pressure, mm Hg				
Whole-grain rye, g/MJ	0.02 (−0.24, 0.28)	0.88	0.02 (−0.24, 0.28)	0.88
Whole-grain wheat, g/MJ	0.11 (−0.37, 0.60)	0.65	0.13 (−0.36, 0.62)	0.60
Whole-grain oats, g/MJ	−0.31 (−0.56, −0.07)	0.01	−0.29 (−0.54, −0.05)	0.02
LDL cholesterol, mmol/L				
Whole-grain rye, g/MJ	0.01 (−0.01, 0.03)	0.16	0.01 (−0.01, 0.03)	0.15
Whole-grain wheat, g/MJ	−0.02 (−0.05, 0.02)	0.30	−0.02 (−0.05, 0.02)	0.35
Whole-grain oats, g/MJ	−0.02 (−0.04, −0.00)	0.03	−0.02 (−0.04, −0.00)	0.049
Insulin, pmol/L				
Whole-grain rye, g/MJ	−0.53 (−1.17, 0.12)	0.11	−0.49 (−1.09, 0.12)	0.12
Whole-grain wheat, g/MJ	−0.59 (−1.77, 0.59)	0.32	−0.49 (−1.60, 0.62)	0.39
Whole-grain oats, g/MJ	−0.90 (−1.50, −0.30)	0.003	−0.67 (−1.23, −0.10)	0.02

[1] β-Values are expressed as outcome values per gram per megajoule increase in whole-grain intake; $n = 674$ in models of insulin and $n = 708$ in models of blood pressure and LDL cholesterol.
[2] Models were mutually adjusted for whole-grain rye, wheat, and oats as well as adjusted for school and class (as random effects) and age; sex; height; puberty; parental education; time spent in moderate–vigorous physical activity; whole-blood EPA + DHA; and intakes of energy, fruit and vegetables, and saturated fat intake (as fixed effects). However, to avoid collinearity, fat mass index was not adjusted for height. The blood pressure model was further adjusted for the blood pressure device used.
[3] Additionally adjusted for fat mass index.

substances and physicochemical characteristics affect viscosity and may delay gastric emptying and inhibit the rate of absorption of macronutrients. This may give an overall lower glycemic and insulinemic response to ingestion and may even increase satiety. Like Danish children in general, the children in the present study mainly consumed whole-grain oats in the form of rolled oats with milk for breakfast, whereas whole-grain rye was mainly consumed as traditional Danish whole-grain sourdough rye bread as open sandwiches eaten at lunch. Rolled oats are rich in soluble fibers such as β-glucans, whereas the Danish rye bread contains a high proportion of whole-rye kernels and has a coarse structure, which might explain the associations between whole-grain consumption and serum insulin. The potential mechanisms behind the effects of whole-grain oats on blood pressure are speculative but have been proposed to be mediated via insulin sensitivity (11). In contrast, the β-glucans in whole-grain oats are likely to reduce LDL cholesterol by lowering the reabsorption of bile acids in the intestine, leading to increased hepatic conversion of cholesterol into bile acids and therefore increased hepatic uptake of LDL cholesterol (57).

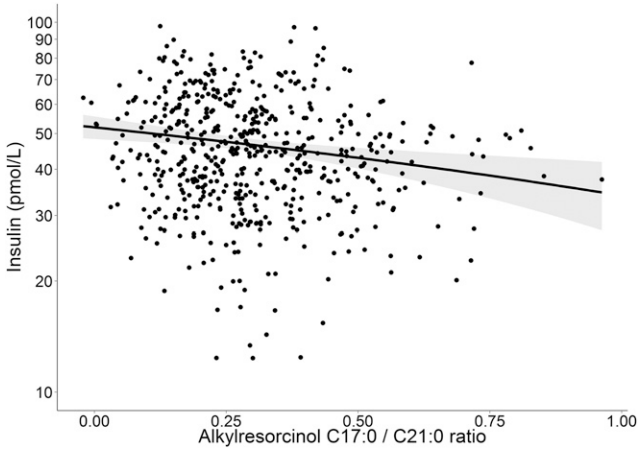

FIGURE 1 The C17-to-C21 alkylresorcinol ratio was inversely associated with circulating insulin concentration in 8- to 11-y-old children; $n = 564$. Regression lines and 95% CIs are shown: $\beta = -1.55$ (−2.41, −0.70) per 0.1 increase in the ratio ($P < 0.001$). Because the insulin models were log-linear, the *y* axis was logarithmized to best depict the linear relation with C17:C21. The plot was adjusted for school; class; age; sex; height; puberty; time spent in moderate–vigorous physical activity; parental education; whole-blood EPA + DHA; and intakes of energy, fruit and vegetables, and saturated fat.

The implications of our findings for children's long-term health are speculative, but in adults, blood pressure, LDL cholesterol, and insulin resistance are associated with CVD mortality (58–60). Atherosclerosis is a gradual, life-long process, blood pressure and LDL cholesterol show tracking from childhood and adolescence to adulthood (61), and children who are diagnosed with the metabolic syndrome are more likely to have metabolic syndrome as adults (2). On the basis of this indirect evidence, low concentrations of the cardiometabolic markers in childhood could be important for long-term cardiovascular health. The estimated slopes of the observed associations were small. However, with an IQR in whole-grain intakes of almost 50 g/10 MJ, this would correspond to, e.g., a 3- to 4-pmol lower fasting serum insulin/L in high compared with low consumers. If sustained over time, such differences may be important from a public health perspective. For children of this age, this dietary difference between high and low consumers would correspond to ~1 small serving (1 dL) of rolled oats or ~2.5 slices of

whole-grain oatmeal bread/d. Remarkably, the associations between whole-grain and fiber intake and the cardiometabolic markers were independent of body fatness in the present study. This may indicate that whole grains, particularly oats, could benefit cardiometabolic health in general child populations, regardless of weight status, and that potential beneficial effects may be induced without weight loss. However, this needs further investigation.

The present study is based on a unique study population with detailed measurements of dietary intakes and whole-grain types by 7-d records, fat mass by DXA scans, and assessment of a range of cardiometabolic risk markers under standardized conditions and by fasting blood samples. The participating children were largely representative of Danish children (62) and their intakes of whole grains and dietary fiber were similar to those reported among Danish children in the most recent national dietary survey (63, 64). Whole-grain intakes were high (i.e., mean and median of 56 and 52 g/d, respectively) compared with children in other Western countries such as the United States and the United Kingdom, where intakes have been estimated to be ~12–13 g/d (47, 54), and the results indicate that cardiometabolic benefit can be achieved at these high intakes. As for other cross-sectional studies, causality cannot be inferred from the presented data. However, the results are strengthened by the careful adjustment for parental education, objectively measured physical activity, and intakes of energy, fruit and vegetables, saturated fat, and a biomarker of fish and n–3 long-chain PUFA intake, which minimizes the risk of residual confounding from an overall healthy lifestyle and increases the likelihood that associations reflect actual aspects of whole grains per se. Apart from the limitation that the alkylresorcinol biomarker does not reflect whole-grain oat intake, plasma alkylresorcinols have a half-life of ~5 h (65), and thereby reflect relatively acute intakes but have been shown to reflect long-term intake in populations with regular and frequent whole-grain intakes (66). Another issue is the association between alkylresorcinols and TGs, inherent in the fact that alkylresorcinols are transported in TG-rich lipoproteins (41). However, this was overcome by adjustment for TGs in the statistical models.

In conclusion, this study showed that higher whole-grain intake was associated with lower serum insulin independently of fat mass in a large sample of 8- to 11-y-old Danish children. Among the whole-grain types, oat intake was associated with lower serum insulin, fat mass index, systolic blood pressure, and LDL cholesterol and whole-grain rye intake tended to be inversely associated with serum insulin, which was supported by an inverse association between the C17-to-C21 alkylresorcinol ratio and insulin. These cross-sectional findings should be investigated further in randomized controlled trials that administer whole grains to children.

Acknowledgments

CTD designed and conducted the research, performed the statistical data analysis, wrote the first draft of the manuscript, and had primary responsibility for the final content; AB-J designed and conducted the research and processed the dietary data; IT designed the research and supervised the dietary data collection; RL analyzed the alkylresorcinols and provided valuable interpretation; MVL helped analyze the data and provided valuable interpretation; AA designed the research; and KFM designed the research and supervised the data collection. All authors critically reviewed and approved the final version of the manuscript.

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