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Published in:
Journal of Nutrition

Link to article, DOI:
10.3945/jn.116.244624

Publication date:
2017

Document Version
Peer reviewed version

Citation (APA):
Damsgaard, C. T., Biltoft-Jensen, A. P., Tetens, I., Michaelsen, K. F., Lind, M. V., Astrup, A., & Landberg, R. (2017). 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. Journal of Nutrition, 147(5), 816-824. DOI: 10.3945/jn.116.244624

Whole grain intake, reflected by dietary records and biomarkers, is inversely associated with insulin and other cardiometabolic markers in 8-11 year-old children[1-3]

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[1]Abbreviations: OPUS; Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet.

[2]Supported by Nordea-fonden, Denmark (grant no. 02-2010-0389).

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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 8-11-year-olds. Fasting blood samples were analyzed for alkylresorcinols, biomarkers of whole grain wheat and rye intake, HDL and LDL cholesterol, triacylglycerol, insulin, and glucose. Linear mixed models included puberty, parental education, physical activity, and intake of energy, fruit and vegetables, saturated fat, and n-3 PUFA.

Results: Median (IQR) whole grain and dietary fiber intakes were 52 (35-72) g/d and 17 (14-22) g/d, respectively. Fourteen% of the children were overweight/obese and most had low-risk cardiometabolic profiles. Dietary whole grain and fiber intake were not associated with fat mass index but were inversely associated with plasma insulin (both $P<0.01$); e.g. with 0.68 (95% CI 0.26; 1.10) pmol/L lower insulin per g/MJ whole grain. 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:C21 alkylresorcinol ratio, reflecting whole grain rye:wheat intake, was inversely associated with insulin ($P<0.001$).

Conclusions: Higher whole grain intake was associated with lower plasma insulin independently of fat mass in Danish 8-11-year-olds. Whole grain oat intake was linked to an overall protective

cardiometabolic profile, and whole-grain rye seemed associated with lower insulin. This supports whole grains as healthy dietary components in childhood.

Keywords: Alkylresorcinols; fiber; cardiovascular; metabolic syndrome; obesity.

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). This increases the risk of metabolic syndrome and type II 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 lower risk of myocardial infarction (3), lower all-cause mortality (4), type II diabetes and insulin resistance (5) and protection against weight gain (6) in adults. Most randomized controlled trials investigating cardiometabolic effects of whole grains in adults have been small and somehow inconsistent but overall suggest small effects on body fatness (7), 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) 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 have assessed whole grain and fiber intake by food frequency questionnaire or a single 24-h recall and only 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 have previously been 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 gives unique opportunities for investigating associations with health outcomes.

We explored whether intake 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 Danish 8-11-year-olds.

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) 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 8-11 year-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 (no. H-1-2010-124), and registered at www.clinicaltrials.gov as NCT01577277. All children from third and fourth grade at nine schools in the Eastern part of Denmark were invited to participate, and baseline measurements were conducted during August to December 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: 1) location in the eastern part of Denmark; 2) at least four classes in total at 3rd or 4th 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 should belong to municipalities with low income and education level, which was the case for three of the nine 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 children (82%) gave informed written consent for participation (21). The present study is based on baseline data from the 713 children for whom we obtained data on anthropometry, body composition, dietary intake, whole-blood EPA + DHA, physical activity, puberty, and parental education. Hereof, 708 children also had available data on blood pressure and blood lipids, 674 children had data on plasma insulin and glucose, and plasma alkylresorcinols were analysed 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 about socioeconomic status and demographics, 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). As very few children were in stages 3–5, the variable was recoded to entered puberty (stages II - V) or not (stage I).

With support from their parents, the children recorded their daily intake of food and beverages every night for 7 consecutive days using a web-based dietary assessment software developed for the study (23). We have previously validated this tool in 8-11-year-old Danish children for energy intake using accelerometer-derived total energy expenditure as reference method (r=0.31, P<0.001, n=81) (24), for intake of whole-grain wheat and rye 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 intake 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 non-cereal sources and using the AOAC 985.29 method) were calculated using the software system GIES (Version 1.000 d-2010-02-26) developed by the National Food Institute, Technical University of Denmark, and using 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) where the whole kernel could be ground, broken, or 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). Based on reported energy intake and estimated basal metabolic rate (BMR), both in MJ/d (27,28) 58 children (8.1%) were classified as under-reporters (energy intake : BMR ≤ 1.05) and 12 children (1.7%) as over-reporters of energy intake (energy intake : BMR ≥ 2.29).

During the same 7 days the children wore a tri-axis accelerometer (GT3X or GT3X+, ActiGraph, Pensacola, FL) in an elastic belt tightly at the right hip. The children were instructed only to remove the accelerometer during water activities. Data was reintegrated to 1-min epochs using ActiLife (version 6.0.0, ActiGraph, Pensacola, FL), 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 activity of ≥2296 counts/min (30). Median (range) days of valid recording was 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 was separated from the samples and stored at -80ºC for later analysis. Height was measured to the nearest 0.1 cm using a portable stadiometer (CMS Weighing Equipment), and the mean of three 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 prior to measurement. Sex- and age-adjusted BMI z-scores were calculated 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 Medical) using Encore software version 13.5 and fat mass index was calculated as total fat mass/height^2 in 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) using the appropriate cuff size. A second device (ProBP 3400 Sure BP; Welch Allyn Inc.) was used for children with arm circumferences <18 cm. Measurements were performed three times, and the mean of the last two measurements was used.

Blood analyses

Plasma HDL cholesterol and triacylglycerol 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 pmol/L to mIU/L by dividing by 6.945. All samples from the same child were analyzed in the same batch and the inter- and intra-assay CV were: 2.0% and 1.2% (HDL cholesterol); 1.5% and 0.8% (triacylglycerol), and 2.5% and 3.1% (insulin). Plasma glucose was assessed immediately after blood sampling on a Hemocue Glucose 201 (Hemocue Denmark) and the inter-assay CV was 4.0%. Whole blood fatty acid composition was measured by high-throughput gas chromatography as previously described (31). The intra- and inter-assay CV 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 weight% of total whole blood fatty acids.

Plasma alkylresorcinols were measured by gas chromatography-mass spectrometry (GC-MS) on a Trace GC Ultra coupled to a DSQII mass spectrometer (Thermo Scientific), 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 inter-assay CV for total alkylresorcinols were 6.0% and 15.6%, respectively. We used total alkylresorcinols (reflecting whole grain wheat and rye intake) as well as the C17:C21 alkylresorcinol homologue ratio (reflecting the relative intakes of whole grain rye and wheat) (19) in the present study.

Statistical analysis

Descriptive data are presented as mean ± SD or median (IQR) separately for girls and boys and were compared using unpaired t test or Mann-Whitney U test (for non-normally distributed variables). Included and excluded children were compared using unpaired t test and chi-square test, and under-, normal-, and over-reporters of energy intake were compared using 1-way 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, triacylglycerol, insulin, and glucose) were analyzed by use of linear mixed models. These models included school and class as random effects, sex, puberty (yes/no) and parental education as fixed effects, and age, moderate-vigorous physical activity (min/d), energy intake (MJ/d), intake of fruit and vegetables (g/10 MJ), saturated fat intake (energy%), and whole-blood EPA + DHA (weight%), which is a biomarker of fish and n-3 LCPUFA intake, as covariates. Energy intake was included as it may impact the relationship between dietary intake exposure and the outcomes (37), and physical activity, fruit and vegetables and the fish biomarker were included as potential confounders that reflect a generally healthy lifestyle and have been inversely associated with CVD in adults (38,39) and for the biomarker also with the cardiometabolic risk markers in this population (31). Likewise saturated fat intake has been positively associated with CVD risk (40). All 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 where alkylresorcinols were exposure variables were also adjusted for plasma triacylglycerol, since plasma alkylresorcinols are transported in TG-rich lipoproteins (41), and are strongly correlated with plasma triacylglycerol 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 where whole grain or dietary fiber intake were exposures were repeated after exclusion of under- and over-reporters 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 where associations were found by also substituting total alkylresorcinols with the C17:C21 ratio in subsequent models.

Model checking was based on visual inspection of residual and normal probability plots. Fat mass index, waist circumference, plasma triacylglycerol, and insulin were log-transformed before analysis to obtain normality and estimates were back-transformed to their original scale (43). Data were analyzed with SPSS version 22 (IBM Corporation) and R (The R Foundation for Statistical Computing, version 3.1.3) and statistical significance was established at $P<0.05$.

Results

Baseline characteristics

As shown in **Table 1** most children were normal weight, had at least one 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. About half of the girls and only about 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 triacylglycerol and insulin than boys (Table 1). Whole grain and dietary fiber intakes were high (median [IQR]: 52 [35-72] g/day and 17 [14-22] g/day, respectively) with 44% and 40% of the children fulfilling the current Danish recommendation of 75 g/10 MJ whole grains (44) and 20-30 g/10 MJ dietary fiber (interpreted as 25 g/10 MJ) (45). Fruit and vegetable intake was higher in girls compared to boys (median [IQR]: 374 [280-484] g/10 MJ vs. 320 [230-437] g/10 MJ, $P<0.001$) whereas saturated fat intake and whole-blood EPA + DHA were 13 ± 2 energy% and 3.6 ± 1.0 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 102 non-included children with regard to age, sex or BMI z-scores, but had parents with slightly higher education ($P=0.02$). Under-reporters of energy intake had higher BMI z-scores than normal- and over-reporters (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 plasma insulin and these associations remained after adjustment for fat mass index (Table 2). Exclusion of energy under- and over-reporters slightly increased the P value of the association between whole grain intake and insulin (β: -0.55 pmol/L; 95% CI: -0.99; -0.12 pmol/L per g/MJ) (P=0.01, n=610) and rendered the association between dietary fiber and insulin non-significant (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 triacylglycerol, and tended to be positively associated with HDL cholesterol, after adjustment for triacylglycerol (Table 2). None of the other cardiometabolic markers were associated with the exposure variables.

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 oat in g/MJ where included simultaneously as independent variables in the mixed models, instead of total whole grain intake. These analyses showed that whole grain oat was inversely associated with fat mass index, systolic blood pressure, LDL cholesterol, and plasma insulin (**Table 3**). Whole grain rye intake also showed a slight tendency towards an inverse association with plasma insulin, which did not reach statistical significance ($P=0.11$). Further adjustment for fat mass index (Table 3) or exclusion of energy over- and under-reporters (data not shown) did not change these results (data not shown).

To further verify the associations with specific whole grain types the C17: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:C21 ratio was inversely associated with plasma 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 homologues 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:C21 ratio was not associated with the other outcomes ($P>0.20$).

Discussion

This cross-sectional study among a well-characterized population of Danish school children showed that energy-adjusted intake of whole grains and dietary fiber were inversely associated with plasma insulin. Among the whole grain types oat intake was associated with lower plasma insulin, fat mass index, systolic blood pressure, and LDL cholesterol and whole grain rye intake tended to be inversely associated with plasma insulin, which was supported by an inverse association between the C17:C21 alkylresorcinol ratio and insulin. Apart from this alkylresorcinols were not associated with the cardiometabolic markers and this seems to support our results as 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 among adolescents showed inverse associations between whole grain intake and insulin/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 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 cholesterols, 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 oat vs. refined grain 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 two of these also found tendencies or effects on glucose and insulin (48,49). Moreover, a recent meta-analysis confirmed that whole grain oat lowers 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, since the associations were mainly driven by oat, no associations with plasma alkylresorcinols would be expected, as alkylresorcinols only capture whole grain wheat and rye intake. In line with this, total alkylresorcinols have shown moderate association (*r*-values around 0.30-0.50) with total whole grain intake in previous studies in adults (18,19) as well as in a previous paper from the OPUS School Meal Study (*r*=0.32) (20). Whole grain rye tended to be inversely associated with plasma insulin in the present study, and this was supported by the inverse association between the C17:C21 ratio (reflecting the proportion between whole grain rye and wheat) and insulin. Inverse associations between C17:C21 and insulin or type 2 diabetes has 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 plasma insulin in children, so this needs further investigation in the future.

Although no associations were seen between total whole grain intake and children's anthropometry whole grain oat intake was associated with 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 two of the included trials that performed measurements of body fat administered oat, so it is speculative whether oat has 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 6-18 year-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 compared with milled cereals (55,56). These substances and physico-chemical 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 plasma insulin. The potential mechanisms behind the effects of whole-grain oat on blood pressure are speculative, but have been proposed to be mediated via insulin sensitivity (11). In contrast, the β-glucans in whole grain oat are likely to reduce LDL cholesterol by lowering the reabsorption of bile acids in the intestines, leading to increased hepatic conversion of cholesterol into bile acids and therefore increased hepatic uptake of LDL cholesterol (57).

The implications of our findings for the 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). Based on this indirect evidence, low levels 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 it would correspond to e.g. a 3-4 pmol/L lower fasting plasma insulin in high compared to 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 about 1 small serving (1 dl) of rolled oats or about 2.5 slices of whole grain oatmeal bread per day. 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 oat, 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 intake 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 intake of wholegrains 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 (ie. mean and median of 56 g/d and 52 g/d, respectively) compared to children in other Western countries such as the US and the UK, where intakes have been estimated to around 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, intake 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 are reflecting 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 around 5 h (65) and thereby reflect relatively acute intakes, but have been shown to reflect long-term intake in populations with a regular and frequent whole grain intake (66). Another issue is the association between alkylresorcinols and triacylglycerol, inherent to the fact that alkylresorcinols are transported in TG-rich lipoproteins (41). However, this was overcome by adjustment for triacylglycerol in the statistical models.

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

Authorship

C.T.D. designed and conducted the research, performed the statistical data analysis, wrote the first draft of the paper, and had primary responsibility for the final content; A. B.-J. designed and conducted the research and processed the dietary data; I.T. designed the research and supervised the dietary data collection; R.L. analyzed the alkylresorcinols and provided valuable interpretation; M.V.L. helped analyze the data and provided valuable interpretation; A.A. designed the research;

and K.F.M. designed the research and supervised the data collection. All authors critically reviewed and approved the final version of the manuscript.

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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	19.1	22.6
Age, years	9.9 ± 0.6	10.0 ± 0.6**
Fat mass index,[2] kg/m^2	4.1 (2.9-5.7)	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 with MVPA,[4] min/d	38 ± 16	57 ± 24***
Dietary intake		
Energy, MJ/d	7.0 ± 1.4	8.2 ± 1.7***
Protein, energy %	15 ± 2	16 ± 2
Carbohydrate, energy %	53 ± 5	53 ± 5
Fat, 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)
Oat	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] mmHg	107 ± 9	108 ± 8
Diastolic blood pressure,[7] mmHg	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***
Triacylglycerol,[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] All values are means ± SDs unless stated otherwise. Asterisks indicate significant difference from girls, *P<0.05, **P<0.01, ***P<0.001.

[2]Values are medians (IQRs).

[3]Based on age- and sex-specific cut-offs defined by Cole et al. (33,34).

[4]MVPA, moderate-vigorous physical activity defined as ≥2296 counts/min.

[5]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).

Table 2. Associations between measures of whole grain and dietary fiber intake and markers of body fatness and cardiometabolic risk in the children[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 (mmHg)				
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 (mmHg)				
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
Triacylglycerol (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 g/MJ 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 with moderate-vigorous physical activity, energy intake, intake of fruit and vegetables,

saturated fat intake, and whole-blood EPA + DHA (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 exposure were adjusted for plasma triacylglycerol (except for when triacylglycerol was the outcome).

[3]Additionally adjusted for fat mass index.

Table 3. Associations between specific whole grain types and fat mass index, systolic blood pressure, LDL cholesterol, and serum insulin[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 oat (g/MJ)	-0.06 (-0.11; -0.00)	0.03	-	-
Systolic blood pressure (mmHg)				
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 oat (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 oat (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 oat (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 g/MJ 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 oat as well as adjusted for school and class (as random effects) and age, sex, height, puberty, parental education, time spent with moderate-vigorous physical activity, energy intake, intake of fruit and vegetables, saturated fat intake, and whole-blood EPA + DHA (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.

Figure legend

Figure 1. The C17:C21 alkylresorcinol ratio was inversely associated with plasma insulin in the children. Regression lines and 95% CI are shown β = -1.55 (-2.41; -0.70) per 0.1 increase in the ratio, P<0.001, n = 564. As the insulin models were log-linear the y-axis was logaritmized to best depict the linear relationship with the C17:C21 ratio. The plot was adjusted for school, class, age, sex, height, puberty, time spent with moderate-vigorous physical activity, parental education, intake of energy, fruit and vegetables, and saturated fat as well as whole-blood EPA + DHA.