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Original Research Article

Effects of Wholegrain Compared to Refined Grain Intake on Cardiometabolic Risk Markers, Gut Microbiota, and Gastrointestinal Symptoms in Children: A Randomized Crossover Trial

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A B S T R A C T

Background: Wholegrain intake is associated with lower risk of cardiometabolic diseases in adults, potentially via changes in the gut microbiota. Although cardiometabolic prevention should start early, we lack evidence on the effects in children.

Objectives: This study investigated the effects of wholegrain oats and rye intake on serum low-density lipoprotein (LDL) cholesterol and plasma insulin (coprimary outcomes), other cardiometabolic markers, body composition, gut microbiota composition and metabolites, and gastrointestinal symptoms in children with high body mass index (BMI).

Methods: In a randomized crossover trial, 55 healthy Danish 8- to 13-y-olds received wholegrain oats and rye ("WG") or refined grain ("RG") products ad libitum for 8 wk in random order. At 0, 8, and 16 wk, we measured anthropometry, body composition by dual-energy absorptiometry, and blood pressure. Fasting blood and fecal samples were collected for analysis of blood lipids, glucose homeostasis markers, gut microbiota, and short-chain fatty acids. Gut symptoms and stool characteristics were determined by questionnaires. Diet was assessed by 4-d dietary records and compliance by plasma alkylresorcinols (ARs).

Results: Fifty-two children (95%) with a BMI z-score of 1.5 ± 0.6 (mean $\pm$ standard deviation) completed the study. They consumed 108 ± 38 and 3 ± 2 g/d wholegrain in the WG and RG period, which was verified by a profound difference in ARs ($P < 0.001$). Compared with RG, WG reduced LDL cholesterol by 0.14 (95% confidence interval: −0.24, −0.04) mmol/L ($P = 0.009$) and reduced total:high-density lipoprotein cholesterol ($P < 0.001$) and triacylglycerol ($P = 0.048$) without altering body composition or other cardiometabolic markers. WG also modulated the abundance of specific bacterial taxa, increased plasma acetate, propionate, and butyrate and fecal butyrate and reduced fatigue with no other effects on gut symptoms.

Conclusion: High intake of wholegrain oats and rye reduced LDL cholesterol and triacylglycerol, modulated bacterial taxa, and increased beneficial metabolites in children. This supports recommendations of exchanging refined grain with wholegrain oats and rye among children.

This trial was registered at clinicaltrials.gov as NCT04430465.

Keywords: cholesterol, dietary fibers, overweight, insulin, adolescent

Introduction

High consumption of wholegrain has been associated with lower risk of cardiovascular disease (CVD) and type 2 diabetes [1,2]. The evidence from randomized trials is more inconsistent [3–5], but some trials have shown beneficial effects of wholegrain intake on body weight [6], blood lipids [7], and glucose homeostasis [8,9] in adults. The pathophysiology of cardiometabolic disease begins in childhood [10], and a recent study showed that a cardiometabolic risk profile including high blood pressure, dyslipidemia, and adiposity in childhood was positively associated with CVD events in midlife [11]. This highlights the need to address overweight and cardiometabolic risk markers in childhood to prevent later development of cardiometabolic disease.

Abbreviations: AR, alkylresorcinol; B, baseline; CVD, cardiovascular disease; CRP, C-reactive protein; FMI, fat mass index; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NEXS, Department of Nutrition Exercise and Sports; RG, refined grain (control treatment in KORN); SCFA, short-chain fatty acid; TG, triacylglycerol; WG, wholegrain oats and wholegrain rye (intervention in KORN).

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Observational studies in children and adolescents have demonstrated inverse associations between wholegrain intake and BMI [12–14], triacylglycerol (TG) [15], and insulin [14,16,17]. Furthermore, intake of wholegrain oats has been associated with lower fat mass, blood pressure, LDL cholesterol, and insulin, while wholegrain rye tended to be associated with lower insulin in children [16]. To our knowledge, only 2 wholegrain interventions have been conducted among children or adolescents. A randomized crossover study in schoolgirls with overweight found that advice to promote wholegrain increased HDL cholesterol [18] and reduced fasting glucose and TG [18], markers of inflammation [19], and waist circumference [20]. Also, a quasi-experimental school-based study found that advice to increase wholegrain consumption reduced measures of adiposity [21].

The effect of wholegrain from oats and rye on cardiometabolic risk markers in adults [9,22] may be attributed to their content of specific dietary fibers, including β-glucans, arabinoxylans, lignins, fructans, and cellulose, which have been shown to have beneficial effects on gut health and functionality [23]. The gel-forming property of β-glucans in oats and some arabinoxylans in rye may delay gastric emptying, increase the excretion of bile acids, and reduce nutrient absorption, which may in turn improve the lipid profile and glycemic response and promote weight control [24,25]. Additionally, cellulose and lignin from wholegrain rye may increase the stool water-content, leading to a decreased transit time [26], which is important to minimize gastrointestinal symptoms [23] and plays a role in prevention of colon cancer [27]. Furthermore, β-glucans, fructans, and to some degree arabinoxylans are fermentable, and intake of these has been shown to alter the gut microbiota composition and increase the production of specific microbial metabolites [28]. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate comprise products from saccharolytic fermentation that have been linked to improved lipid and glucose metabolism through multiple pathways in the gut and peripheral tissues [29].

In this randomized crossover trial, we investigated the effect of intake of wholegrain oats and wholegrain rye compared with refined grain on cardiometabolic risk markers, BMI, and body composition, with LDL cholesterol and insulin as coprimary outcomes, in 8- to 13-y-old children with high BMI. To address possible underlying mechanisms and the overall impact on children's gastrointestinal wellbeing, we also investigated changes in gut microbiota composition, SCFA in feces and blood, gut symptoms, and stool characteristics.

Methods

Study design

The study was a randomized 2 × 8-wk crossover trial with no washout period. It was conducted at Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen, Denmark from September 2020 to May 2021. Participating children were instructed to substitute their habitual intake of grain products with wholegrain oats and wholegrain rye (hereafter referred to as "WG") and refined grain (hereafter referred to as "RG") study products during 2 8-wk periods in random order. Participants were asked not to alter their habitual physical activity or background diet throughout the study. Measurements were conducted at examination visits at the department at 0, 8, and 16 wk.

Participants

Healthy 8- to 13-y-old children living in the Capital Region of Denmark were identified via the Danish Civil Registration System and recruited to the study via digital invitation to the parental authority holders from August through December 2020. Eligible children were self-reported overweight as defined by a BMI +1 SD above the median according to sex and age-standardized Danish Growth Curves [30]. Furthermore, the children had to be Danish-speaking, eat cereals and bread daily, and at least one of their parents had to be able to read and speak Danish. Children were excluded if they had allergies or intolerances to the study products, used dietary fiber or probiotic supplements, followed energy-restricted or special diets, had serious illness, used medication that could interfere with the study including antibiotics within a month prior to the first examination visit, participated in other studies involving dietary supplements, drugs, or blood sampling, or lived in a household with another participating child. The parents and child received written and oral information about the study, and written consent was obtained from all custody holders of the children prior to enrollment. The study was approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark (no. H-19010737) on 25 June, 2019 and registered with clinicaltrials.gov (NCT04430465) on 12 June, 2020.

Randomization and masking

A staff member who was not involved in the study created a random list of treatment sequences (WG/RG or RG/WG) using block randomization (size 6) and linked it to a list of 55 consecutive numbers. Another impartial staff member created 55 sealed envelopes containing the sequences and marked them with the randomization numbers. The order in which the children completed their baseline visit determined their randomization number and thus their sequence allocation. It was not feasible to blind the study participants or staff during the intervention, but participants and investigators involved in outcome assessment were blinded until the baseline measurements were completed and during laboratory analysis.

Study products

We provided study products typically consumed for breakfast, lunch, and dinner and as snacks in Denmark. These included ready-to-eat breakfast cereals, soft breads, crisp breads, pasta, whole kernels, and rice. All products in the WG period were based on oats and rye. We defined wholegrain and wholegrain products according to the aligned Danish and global consensus definition with a minimum requirement of around half of total product dry matter being wholegrain [31,32]. The products in the RG period were chosen based on their low content of wholegrain and primarily contained refined wheat, rice, and corn. Each RG product was matched with a corresponding WG product based on sugar and fat content. Children were instructed to eat the provided products ad libitum, with a product intake goal of 150 to 200 g/d corresponding to 75 g wholegrain and 14 to 19 g dietary fiber in the WG period. Throughout the intervention periods, the children were asked to record their daily intake of the study products using grams or household measures (slices of bread or deciliters) and any deviations from the instructions in study product diaries. For this, the youngest children were assisted by their parents.

The wholegrain content in the study products was based on up-to-date nutritional information from the food producers. Macronutrient and dietary fiber content were analyzed in milled freeze-dried breads and dry-products. Protein and fat contents were analyzed by Nordic-Baltic Committee on Food Analysis methods, and carbohydrate and total energy content were calculated subsequently (Eurofins Food & Feed Testing Sweden). Total dietary fiber content was analyzed at the Swedish University of Agricultural Sciences, Uppsala, Sweden and determined as the sum of klason lignin, neutral sugar, and uronic acid

residues using the Uppsala method [33] and subsequently combined with fructans [34]. The content of β-glucans was analyzed enzymatically as previously described [35] in the same laboratory. The content of arabinoxylans was calculated as the sum of arabinose and xylose residues as previously described [36]. Klason lignin complex comprised the noncarbohydrate fraction of dietary fiber and included acid insoluble residues minus acid insoluble ash (33). A list of WG and RG products with their nutritional contributions is available in Supplemental Table 1.

Outcomes

The a priori defined coprimary outcomes were plasma insulin and serum LDL cholesterol. Secondary outcomes included BMI z-scores, fat mass index (FMI), waist circumference, systolic and diastolic blood pressure, serum HDL cholesterol, total cholesterol, TG, and C-reactive protein (CRP) as well as gut symptoms and stool characteristics. Other outcomes included 16S rRNA gene amplicon derived gut microbiota composition (α- and β-diversity, relative abundance of gut bacteria at genus level) and plasma and fecal SCFA and serum interleukin-6 (IL-6).

Measurements

Questionnaires

Questionnaires were answered online prior to each examination visit except for parental education and the child's pubertal stage, which were administered at baseline only. Parents were instructed to fill questions about household education and assist the youngest children with the rest of the questionnaires. The longest education obtained in the household was classified into 4 levels: vocational, short academic, bachelor's degree, and master's degree or higher. Pubertal stage was evaluated using Tanner stage illustrations of pubic hair for boys and breast development for girls. Physical activity level and screen time were assessed using a modified version of the validated Nordic Physical Activity Questionnaire as daily time spent on moderate to vigorous physical activity and sedentary time spent in front of a screen [37] and were reported as weekly averages.

We used a previous questionnaire [38] adjusted to children to evaluate gastrointestinal symptoms. The questionnaire assesses stool frequency and severity of 7 gut symptoms; abdominal pain, bloating, flatulence, constipation, diarrhea, fatigue and nausea, using Likert scales. Stool frequency was evaluated on an 8-point scale corresponding to "rarer than 1 time per week," "1-2 times per week," "3-4 times per week," "5-6 times per week," "1 time per day," "2 times per day," "3 times per day," and "more often than 3 times per day" and gut symptoms on a 5-point scale corresponding to "never", "rarely", "sometimes", "often," and "very often". Stool consistency was assessed using the Bristol stool chart for children [39]. Information on potential illness and use of antibiotics in the preceding treatment period was collected by interview at each examination visit.

Dietary intake

Dietary intake was recorded by the parents with help from the children during the last week prior to each examination visit, using a 4-d weighted dietary record on 3 consecutive weekdays and 1 weekend day. Children and parents were instructed to weigh and record all consumed energy-containing foods and drinks in the web-based software Madlog Classic (MADLOG), which calculates energy and nutrient intakes based on the Danish national food composition database (FOODCOMP 7.01, 2009, National Food Institute, Technical University of Denmark). Household measures were used only when weighing was not possible. The dietary records were checked for completeness and corrected after dialog with the children and parents at the examination visits. Intake of study products was included in the 4-d dietary records, but the reported intake of study products was based on the daily recordings from study product diaries.

Anthropometry, blood pressure, and body composition

Children arrived in the fasting state on the examination days. They were weighed to the nearest 0.1 kg using a Tanita BWB-800 digital scale wearing light clothes or underwear. Height was measured to the nearest 0.1 cm with the head in the Frankfurt plane using a Seca 216 stadiometer. Waist circumference was measured to the nearest 0.1 cm at the umbilical level on exhalation using a nonelastic measuring tape. Systolic and diastolic blood pressure were measured in the supine position after 10 min of rest using an automated device (Connex ProBP 3400 digital, Welch Allyn) with an appropriate cuff size. Height, waist circumference and blood pressure were measured in triplicate, and the mean was used. Age- and sex-adjusted z-scores for BMI were calculated based on WHO's growth standards, using the WHO 2007 R macro package WHO2007_R.zip [40]. The BMI z-scores were separated into BMI categories based on conventional intervals: normal weight (−1 SD to +0.99 SD), overweight (+1 SD to +1.99 SD) and obese (≥2 SD) [41]. Body composition was measured by whole body dual-energy X-ray absorptiometry scan using a GE Lunar Prodigy (GE Healthcare) scanner with GE Healthcare software version 17, SP1, following a standardized breakfast meal and after the children had emptied the bladder. FMI was calculated as kg of total body fat divided by the squared height in m.

Biological sampling and analyses

Fasting blood samples were collected at each examination visit. Fecal samples were collected at home by the children, if necessary aided by a parent, with EasySampler (GP Medical Devices A/S) and stored at −18°C for a maximum of 3 d in their home freezers prior to the visit. The parents brought the samples to the department in cooler bags, where they were stored at −80°C until laboratory analyses.

Fluoride-citrate whole blood for analysis of glucose, lithium-heparin plasma for analysis of SCFA, and EDTA plasma for analyses of insulin and ARs were centrifuged at 2300 × *g* at 4°C for 10 min, within 30 min after blood sampling. Serum samples for analyses of lipids, CRP, and IL-6 were left to coagulate at room temperature for 30 min prior to centrifugation. All samples were stored in 2 mL cryotubes at −80°C until analysis, except for glucose, which was analyzed immediately after sampling.

The sequence in sample preparation and analysis was randomized, but all biological samples from each participant were included in the same analysis batch. Plasma glucose and serum LDL cholesterol, HDL cholesterol, total cholesterol, TG, and CRP were analyzed at NEXS on a Pentra 400 (HORIBA ABX). All CRP values ≥10 mg/L were taken as an indication of infection [42] and were excluded ($n = 1$), and measurements below the detection limit of 0.1 mg/L were set to 0.05 mg/L ($n = 16$). IL-6 was analyzed at NEXS with a Human IL-6 Quantikine high sensitivity kit (R&D Systems/Bio-Techne). Plasma insulin was analyzed with chemiluminescent immunoassay on a LIAISON XL (DiaSorin) at the Department of Clinical Biochemistry, Rigshospitalet, Copenhagen. Concentrations of SCFA and total AR in plasma were analyzed at Department of Life Sciences, Chalmers University of Technology, Gothenburg, Sweden using liquid chromatography with tandem mass

spectrometry (LC-MS/MS) on a QTRAP 6500+ (AB SCIEX) as previously described [43,44]. Total AR (C17:0, C19:0, C21:0, C23:0, and C25:0 AR homologues) reflects WG intake from rye and wheat, and it was included as a biomarker of compliance with the intervention. For the analysis of SCFA in fecal samples, sample preparation was performed according to Cheng et al. [45], and the prepared extracts were analyzed by LC-MS/MS as described for plasma samples [44]. Gut microbiota composition was determined by 16S rRNA gene amplicon sequencing (Illumina NextSeq). Details on DNA extraction, library preparation, and sequencing have been described previously [46].

Adverse events

Parents were asked to report any health-related events during the intervention to the study staff. At each examination visit, the child and a parent were interviewed about adverse events or use of medication since the last visit. Adverse events and use of medication during the intervention were recorded and evaluated for severity and possible relation to the intervention by the responsible study physician.

COVID-19 lockdown and homeschooling

The study was conducted during the COVID-19 pandemic and the consequential lockdown of schools and universities in Denmark. The present study was exempted for lockdown but was conducted under a strict modified protocol that assured minimal physical contact between investigators and participants and various safety measures during the examination visits, and rescheduling of visits if participants or their families felt ill. Lockdown of schools and after-school sport activities for children ≥11 y became effective in the middle of our intervention on December 9, 2020 and for the rest of the schoolchildren on December 21, 2020, and it ceased on February 8, 2021. We used information about homeschooling as a proxy for change in typical lifestyle routines, e.g., meal patterns, food intake, and levels of physical activity. At each visit, the families were interviewed about the number of homeschooling days during the preceding period, which was reported as the percentage of homeschooling days out of all school days.

Sample size

Sample size was calculated based on data from the 81 children (8–12 y) with overweight who participated in our previous crossover trial OPUS among [16]. The children were measured at baseline and followed up after 3 and 6 mo, and the average correlations between repeated measurements of LDL cholesterol and insulin were $r = 0.79$ and $r = 0.54$, respectively. Assuming a linear mixed model, $\alpha = 0.05$, $\beta = 0.80$, and meaningful treatment differences of 0.15 mmol/L for LDL cholesterol and 5 pmol/L for insulin (corresponding to 6% and 7%, respectively), a sample size of $n = 40$ was required. To allow for 15% to 20% dropout and unsuccessful blood sampling, we recruited a total of 55 children.

Statistical analysis

Descriptive values are presented as mean ± SD or median (25th and 75th percentiles) for normally distributed and skewed variables, respectively. Variables at baseline (B) and during the WG and RG periods were compared using linear mixed model with treatment (B/WG/RG) as fixed effect and subject as random effect, and the WG period was compared to the RG period using paired *t* test. Correlations between the reported wholegrain intake and plasma AR were conducted with Spearman's ρ statistics. Due to the national COVID-19 lockdown, homeschooling was introduced by the government after the baseline visit. Homeschooling was unequally distributed between allocation sequences as it constituted 7.1% and 32.1% of weekdays in the WG and RG periods, respectively, in the WG/RG group compared to 41.1% and 12.3% in the RG/WG group ($P < 0.001$, chi-square test). Also, homeschooling was positively associated with FMI ($\beta = 2.64$, $P = 0.010$, linear mixed model including treatment [WG/RG] and FMI as fixed effects and subject as random effect). Therefore, homeschooling was included as a covariate in the statistical models.

To investigate the effects of WG compared with RG, outcomes were analyzed in linear mixed models for continuous outcomes and cumulative link mixed models for ordinal outcomes (gut symptoms and stool characteristics). Treatment effects were analyzed as mean differences (95% confidence interval) between WG and RG. For continuous outcomes, we modeled repeated measurements as response variables, and treatment (WG/RG), homeschooling (% of all school days), and baseline values as fixed effects, while subject was modeled as random effect to adjust for within-subject correlation. A proxy carryover effect was tested for all continuous outcomes using an extended model that additionally included a treatment × sequence interaction term, and results are reported by allocation sequence in the supplementary material. Additionally, as a sensitivity analysis, we conducted similar analyses without adjustment for homeschooling, which can be found in the supplementary material. For ordinal outcomes, a similar model without homeschooling was applied, and the diarrhea symptom was releveled into the 3 first levels due to few observations in the last 2 levels. For gut microbiota-related outcomes, we applied linear mixed models with all repeated measurements as response variables, treatment (B/WG/RG) and homeschooling as fixed effects and subject as random effect. Models were verified based on residual and normal probability plots, and insulin, TG, CRP, and IL-6 were log-transformed prior to the analysis, and estimates were back-transformed, as previously described [47]. For selected cardiometabolic markers, ad hoc analyses were carried out to explore correlations with intakes of wholegrain, total dietary fibers, and subtypes of dietary fibers using Pearson's ρ statistics.

Construction of the operational taxonomic unit table followed the previously described procedure [46]. α-diversity (Chao1 and Shannon) indices were generated using the package phyloseq version 1.42.0. Abundance data was normalized by sequencing depths. Differences in α-diversity and relative abundance of different genus across baseline and intervention groups were compared by linear mixed models, consistent with the modeling used for cardiometabolic outcomes. Bray–Curtis was used to calculate the distance matrix, and principal coordinate analysis was chosen as the ordination method, combined with adonis (n permutations = 999).

All statistical analyses followed the intention to treat principle (available case analyses) and were performed in R version 4.2.1, using particularly the lmerTest package version 3.1-3 [48], the ordinal package version 2022.11-16 [49], and the vegan package version 2.6-2. Statistical significance was established at $P < 0.05$.

Results

Children's characteristics

The children had a mean age of 11 y at baseline, and 76% came from households with a bachelor's degree or higher (Supplemental Table 2). A total of 22%, 60%, and 18% were classified as having normal weight, overweight, and obesity, respectively. There was an even number of girls and boys, and 75% and 44% of these, respectively, had entered puberty (Supplemental Table 2). None of the examined baseline characteristics appeared different between allocation sequences (Table 1). Physical

TABLE 1
Baseline characteristics by allocation sequence

	WG/RG ($n = 28$)	RG/WG ($n = 27$)
Female sex (n, %)	16, 57	12, 44
Age (y)	11.0 ± 1.8	11.0 ± 1.9
Height (cm)	151.7 ± 11.5	150.6 ± 9.2
Moderate to vigorous physical activity[1] (min/d)	50 (37.5–123.2)	73 (39–134)
Screen time[1] (min/d)	51 (13–130)	60 (27–133)
Body weight (kg)	50.1 ± 11.5	49.3 ± 11.8
BMI (z-score)	1.5 ± 0.6	1.5 ± 0.6
Fat mass index (kg/m^2)	8.1 ± 2.2	8.2 ± 1.9
Waist circumference (cm)	78.0 ± 8.8	77.2 ± 9.2
Diastolic blood pressure (mmHg)	65 ± 4	66 ± 4
Systolic blood pressure (mmHg)	103 ± 5	104 ± 6
LDL cholesterol (mmol/L)	2.2 ± 0.5	2.4 ± 0.7
HDL cholesterol (mmol/L)	1.4 ± 0.2	1.3 ± 0.3
Total cholesterol (mmol/L)	3.9 ± 0.6	4.1 ± 0.8
Total: HDL cholesterol	2.9 ± 0.6	3.2 ± 0.8
Triacylglycerol[1] (mmol/L)	0.53 (0.48–0.91)	0.65 (0.50–0.85)
Insulin[1] (pmol/L)	60 (48–88)	71 (49–86)
Glucose (mmol/L)	5.1 ± 0.4	5.1 ± 0.3
C-reactive protein[1] (mg/L)	0.31 (0.23–0.72)	0.44 (0.20–0.82)
IL-6[1] (pmol/L)	0.88 (0.59–1.16)	0.90 (0.56–1.52)

Abbreviations: RG, refined grain; WG, wholegrain.
Data are shown as mean ± standard deviation unless otherwise indicated. $n = 27$ for all blood measurements in the WG/RG allocation group.
[1] Median (25th–75th percentiles).

activity and screen time did not change throughout the study ($P \geq 0.149$). In total, 52 (95%) of the children completed the study; 3 children allocated to the WG/RG sequence withdrew on study day 14, 14, and 38 (Figure 1). No important harms or unintended effects were observed during the study.

Dietary intake and compliance

Overall, children's dietary compliance was high based on self-reported wholegrain intake and plasma AR (Table 2), and the reported wholegrain intake was positively associated with plasma AR ($r = 0.48$, $P < 0.001$). During the WG period, most children (94%) consumed ≥75g/10 MJ of wholegrain, as recommended in the Danish food-based dietary guidelines, with equal amounts of wholegrain oats and wholegrain rye ($P = 0.133$), and all children consumed <11 g/MJ wholegrain during the RG period. Intakes of wholegrain oats, wholegrain rye, and total wholegrain were independent of allocation sequence ($P \geq 0.763$). The intake of study products did not differ between periods, but during the WG period, the children consumed more than double the amount of total dietary fibers, including β-glucans, fructans, klason lignin, and arabinoxylans from study products compared with the RG period (Table 2). The WG products provided less energy, available carbohydrate, and protein and more fat than the RG products. Overall energy, fat, and protein intake did not differ between periods; however, overall carbohydrate intake was slightly higher in the RG period compared with the WG period ($P = 0.044$) (Table 2).

Cardiometabolic risk markers

WG reduced plasma LDL cholesterol, total cholesterol, total:HDL cholesterol, and TG (all $P \leq 0.048$) (Table 3). WG also increased butyrate in both feces and plasma ($P \leq 0.001$) and acetate and propionate in plasma (Table 3). We found no differences between WG and RG on plasma insulin or glucose (Table 3). Furthermore, there were no effects on BMI or any other measures of adiposity (all $P \geq 0.487$), blood pressure, or serum IL-6, but a tendency of reduced serum CRP (Table 3).

The change in LDL cholesterol was correlated with the change in intake of wholegrain ($r = -0.27$, $P = 0.056$) and total dietary fibers ($r = -0.29$, $P = 0.043$) as well as β-glucans (Pearson's $r = -0.24$, $P = 0.088$), fructans ($r = -0.27$, $P = 0.055$), klason lignin ($r = -0.28$, $P = 0.048$), and arabinoxylans ($r = -0.29$, $P = 0.038$), although not all reached statistical significance. The changes in plasma and fecal butyrate during the intervention were correlated with the change in intake of wholegrain ($r > 0.36$, $P < 0.012$) and total dietary fibers ($r > 40$, $P < 0.005$) as well as with β-glucans, fructans, klason lignin, and arabinoxylans (all $r > 0.31$, $P < 0.05$). Furthermore, the changes in plasma and fecal butyrate were both correlated with the change in LDL cholesterol ($r > -0.38$, $P < 0.008$).

There was no treatment-allocation sequence interaction for the outcomes, except for FMI (Table 3). Stratified results by allocation sequence can be found in Supplemental Table 3. Treatment effects in the sensitivity analysis (where homeschooling was excluded as a covariate) remained consistent with the primary adjusted results; however, treatment-allocation sequence interactions were also observed for BMI z-score and waist circumference apart from FMI in the sensitivity analyses (Supplemental Table 4).

Gut microbiota and symptoms and stool frequency

Bifidobacterium, *Blautia*, *Faecalibacterium*, and *Clostridium* predominated the children's gut microbiota with relative abundances of 13%, 11%, 12%, and 6%, respectively (Supplemental Figure 1D). WG increased the relative abundance of *Faecalibacterium* ($P = 0.031$) and *Dialister* ($P = 0.032$) and reduced *Collinsella* ($P = 0.001$) and *Ruminococcus* ($P \leq 0.035$) compared with RG (Figure 2). There were no differences between the treatments in Chao1 or Shannon α-diversity ($P \geq 0.202$) (Supplemental Figure 1A, B) or β-diversity ($P = 0.192$) (Supplemental Figure 1C). Lastly, WG reduced the odds of self-reported fatigue ($P = 0.017$) and tended to increase stool frequency compared with RG (Supplemental Figure 2).

Discussion

In this randomized 8-wk crossover trial, high intake of WG reduced LDL cholesterol, total:HDL cholesterol and TG in children with high BMI without altering glucose homeostasis, blood pressure, BMI, or body composition. This indicates that wholegrain from oats and rye has beneficial effects on children's lipid profiles independent of changes in body weight and fat mass. We also found treatment differences in the relative abundances of specific microbial genus, accompanied by increased SCFA concentrations in both feces and plasma. The changes in plasma and fecal butyrate were correlated with the changes in intakes of wholegrain and dietary fibers as well as LDL cholesterol. Finally, the children experienced less fatigue and tended to have more frequent stools when consuming WG compared with RG.

In contrast to the present study, a 6-wk randomized crossover study among Iranian schoolgirls did not find an effect on LDL cholesterol but found increased HDL cholesterol and reduced TG [18], glucose [18], inflammatory markers [19], and waist circumference [20] in response to wholegrain intake. Furthermore, promotion of wholegrain reduced BMI, fat percentage, and waist circumference in a Malaysian school-based quasi-experimental study [21]. However, the 2 previous interventions mainly gave advice on wholegrain intake, which may not

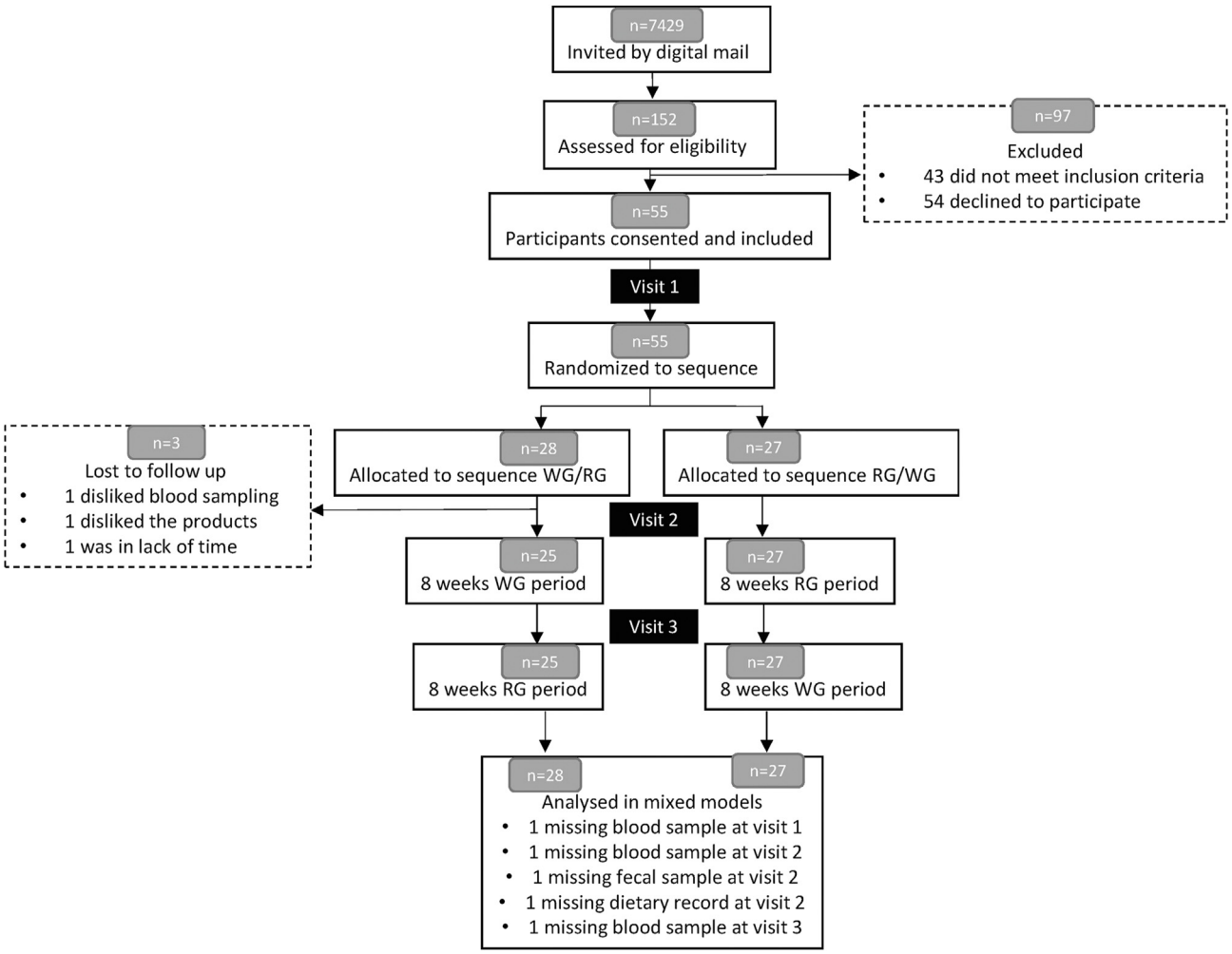

FIGURE 1. Participant flow chart. Abbreviations: RG, refined grain; WG, wholegrain.

be as effective, resulting in a wholegrain intake of only 26.5 g/d in the crossover study [18], while the quasi-experimental study did not report on wholegrain intake [21]. Furthermore, these previous studies in children applied mixed wholegrains, which may also explain differences in their findings compared to the present study.

Wholegrain oats hold a distinct physiochemical property attributed to their content of viscous β-glucans [50] that has been suggested to improve blood lipids by reducing the absorption of lipid and carbohydrate and inhibiting bile acid reabsorption, thus upregulating de novo synthesis of bile at the cost of circulating LDL cholesterol [51]. The children in the present study consumed on average 2.8 g β-glucans per day during the WG period, and LDL cholesterol was reduced by 0.13 mmol/L. This reflects the reduction in LDL cholesterol of 0.25 mmol/L after at least 3 g/d of β-glucans that has been demonstrated in a meta-analysis of randomized trials in adults [52] and aligns with the universally adopted efficacious daily dose of 3 g β-glucans in health claims [53]. In the present study, we were not able to separate the effects of wholegrain oats and wholegrain rye due to high correlation between the 2 types, but both may have contributed. The mechanisms underlying the effect of wholegrain rye may mimic those of oats, since soluble arabinoxylans in rye share some physiochemical properties with β-glucans [50], and wholegrain rye has previously been shown to reduce LDL cholesterol in adults [54]. In addition, due to the viscous and structural properties of their fibers, wholegrain from oats and rye possess high water-holding capacity [50], which may increase fecal bulk and explain the observed tendency of increased stool frequency in present study. Accordingly, previous studies in healthy adults found that intake of wholegrain rye compared to refined grain improved several markers of gut health, including increased stool frequency [38,55,56].

High dietary fiber diets have often been associated with increased bacterial diversity in observational studies [57]; however, we did not find any changes in α- or β-diversity in the present study. This is in accordance with 2 previous Danish trials [38,58], a Swedish trial [44], and a meta-analysis of dietary fiber interventions [59]. Although we could not quantify the children's baseline intake of wholegrain due to limitations in the national Danish food composition database, it is worth noting that Danish children have a habitual mean wholegrain intake of about 58 g/d [60], which is higher than children in most other countries [61]. Thus, increase in microbial diversity may have been minimal in our study population relative to changes observed with low intake of wholegrain at baseline.

In the present study, WG increased the relative abundance of *Faecalibacterium* and *Dialister* while it reduced *Ruminococcus* and *Collinsella*, and this was accompanied by higher butyrate, acetate, and propionate in plasma as well as butyrate in feces. Although randomized studies generally show inconsistencies in the affected taxa [62], some trials, especially using wholegrain rye, have shown similar effects, including increased abundance of several strains of *Faecalibacterium* [58] and decreased abundance of *Ruminococcus*

TABLE 2
Biomarker of WG intake and dietary intake from 4-d food record and study products

	B	WG	RG	P[2]
Plasma biomarker of WG intake				
Total alkylresorcinols[1] (nmol/L)	47.3 (35.3–89.7)^{A}	90.2 (44.3–151.9)^{B}	26.6 (15.9–37.9)^{C}	<0.001
Dietary intake from 4-d food record				
Energy intake (MJ/d)	7.8 ± 1.8^{A}	7.7 ± 2.2^{A}	7.0 ± 1.6^{B}	≥0.005
Carbohydrate (E %)	53 ± 5^{A}	51 ± 6^{B}	53 ± 6^{A}	≥0.036
Fat (E %)	32 ± 5	33 ± 7	31 ± 5	≥0.076
Protein (E %)	15 ± 2^{A}	17 ± 3^{B}	16 ± 3^{B}	≥0.005
Dietary intake from study products				
Total product (g/d)	—	175 ± 54	184 ± 74	0.373
Total wholegrain (g/d)	—	108 ± 38	3 ± 2	<0.001
WG rye	—	58 ± 21	0 ± 0	—
WG oat	—	50 ± 33	0 ± 0	—
Energy (MJ/d)	—	2.09 ± 0.68	2.46 ± 1.03	0.033
Carbohydrate (g/d)	—	82.1 ± 26.6	113.1 ± 48.8	<0001
Fat (g/d)	—	8.0 ± 3.2	4.8 ± 1.9	<0001
Protein (g/d)	—	16.0 ± 5.0	18.6 ± 7.9	0.048
Total dietary fiber (g/d)	—	16 ± 5.0	6.0 ± 2.1	<0001
β-glucans	—	2.8 ± 1.3	0.2 ± 0.1	<0001
Fructans	—	1.9 ± 0.7	0.7 ± 0.3	<0001
Klason lignin		1.0 ± 0.3	0.4 ± 0.2	<0.001
Arabinoxylans		6.0 ± 1.9	2.3 ± 0.8	<0.001

Abbreviations: B, baseline; E %, energy percentage; RG, refined grain; WG, wholegrain.
Data shown as mean ± standard deviation unless otherwise indicated. $n = 55$ at baseline except from total alkylresorcinols ($n = 53$); $n = 52$ in WG except from total alkylresorcinols ($n = 51$); $n = 52$ in RG except from total alkylresorcinols ($n = 51$) and dietary intake from 4-d food record ($n = 51$).
[1] Median (25th–75th percentiles).
[2] P value for comparison of biomarkers of WG intake and intake from 4-d food record was calculated by linear mixed model with treatment (B/WG/RG) as fixed effect and subject as random effect and intake from study products by paired t test. Different superscripts (A, B, C) indicate statistical differences within rows ($P < 0.05$).

TABLE 3
Body composition, cardiometabolic risk markers and SCFA following WG and RG (empirical mean ± SD) and estimated mean differences (95 % CI)

	WG	RG	WG – RG[3]	P	
				Treatment[3]	Treatment × sequence[4]
BMI (z-score)	1.5 ± 0.6	1.5 ± 0.6	−0.01 (−0.04, 0.03)	0.676	0.102
Fat mass index (kg/m^2)	8.3 ± 2.0	8.3 ± 2.0	−0.04 (−0.16, 0.07)	0.487	0.045
Waist circumference (cm)	79.3 ± 8.3	79.1 ± 8.8	0.13 (−0.39, 0.64)	0.636	0.231
Diastolic blood pressure (mmHg)	65 ± 4	65 ± 4	−0.36 (−1.23, 0.51)	0.422	0.539
Systolic blood pressure (mmHg)	103 ± 5	104 ± 6	−0.85 (−2.04, 0.35)	0.170	0.301
LDL cholesterol (mmol/L)	2.3 ± 0.6	2.4 ± 0.6	−0.14 (−0.24, −0.04)	0.009	0.077
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.4 ± 0.3	0.03 (−0.02, 0.07)	0.206	0.398
Total cholesterol (mmol/L)	4.0 ± 0.7	4.2 ± 0.7	−0.15 (−0.28, −0.02)	0.033	0.475
Total:HDL cholesterol	3.0 ±0.61	3.2 ± 0.7	−0.20 (−0.31, −0.09)	<0.001	0.088
Triacylglycerol[1] (mmol/L)	0.70 (0.53–0.81)[1]	0.81 (0.56–0.92)[1]	−0.10 (−0.20, 0.00)[2]	0.048	0.250
Insulin[1] (pmol/L)	75.9 (51.0–88.9)[1]	69.9 (52.3–105.3)[1]	−1.04 (−10.37, 8.30)[2]	0.828	0.094
Glucose (mmol/L)	5.0 ± 0.3	5.1 ± 0.4	−0.03 (−0.17, 0.11)	0.672	0.372
C-reactive protein[1] (mg/L)	0.28 (0.14–0.71)[1]	0.44 (0.16–1.12)[1]	−0.13 (−0.25, 0.00)[2]	0.064	0.792
IL-6[1] (pmol/L)	0.88 (0.57–1.36)[1]	0.91 (0.55–1.18)[1]	−0.01 (−0.17, 0.15)[2]	0.917	0.571
Plasma acetate (μmol/L)	103 ± 78	76 ± 61	29.73 (9.71, 49.74)	0.005	0.895
Plasma propionate (μmol/L)	1.1 ± 0.6	1.0 ± 0.5	0.15 (0.02, 0.29)	0.036	0.721
Plasma butyrate (μmol/L)	1.0 ± 0.7	0.7 ± 0.4	0.33 (0.14, 0.52)	<0.001	0.963
Fecal acetate (μmol/g)	172 ± 94	166 ± 91	5.16 (−21.90, 32.22)	0.710	0.507
Fecal propionate (μmol/g)	52 ± 27	54 ± 31	−2.84 (−10.31, 4.63)	0.460	0.397
Fecal butyrate (μmol/g)	75 ± 45	57 ± 29	18.11 (7.68, 28.54)	0.001	0.075

Abbreviations: CI, confidence interval; RG, refined grain; SCFA, short-chain fatty acid; SD, standard deviation; WG, wholegrain.
[1] Empirical median (25th–75th percentiles).
[2] Estimates and 95% CI for absolute differences between medians after back-transformation to their original scale.
[3] Mixed model with treatment, homeschooling, and baseline value as fixed effects and subject as random effect. $n = 55$ for all analyses, except for IL-6 ($n = 53$).
[4] Extended mixed model that, in addition to [3], included a treatment × sequence interaction term.

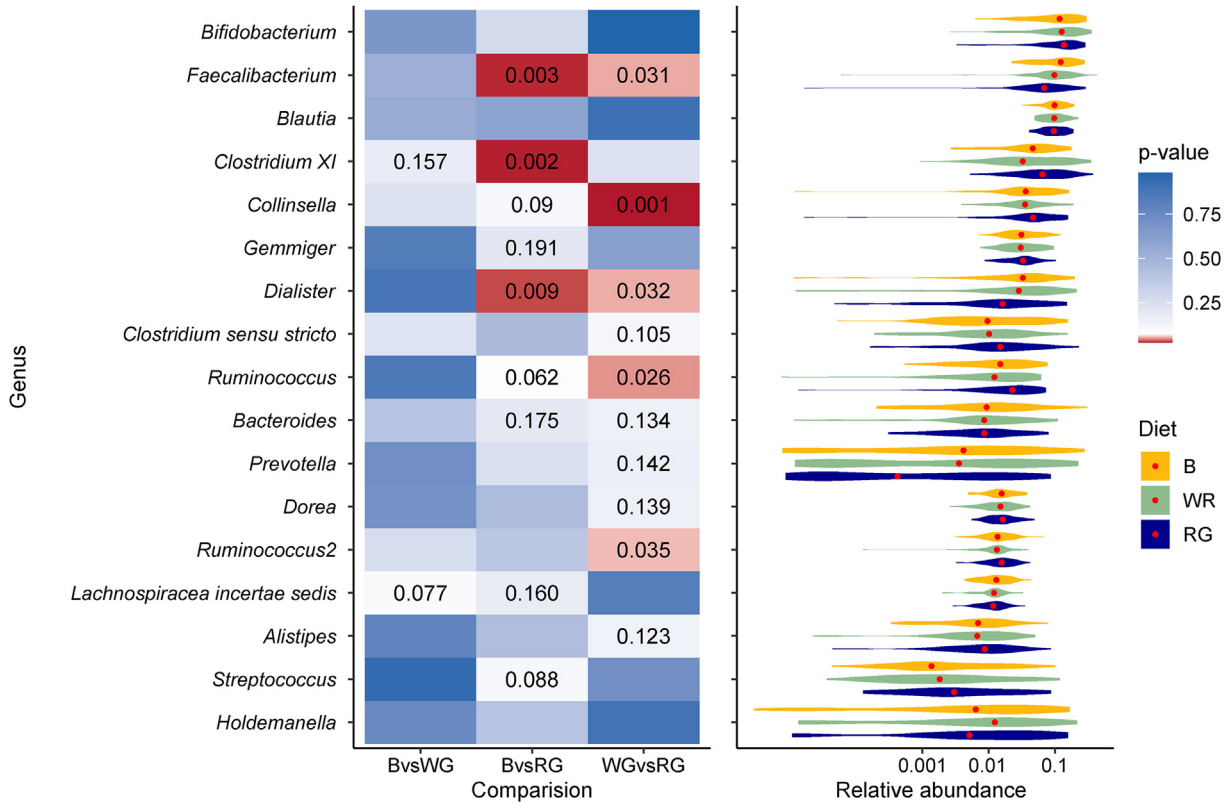

FIGURE 2. Genus-specific relative abundance comparisons between baseline (B), wholegrain (WG), and refined grain (RG). *P* values from mixed model with treatment (B/WG/RG) and homeschooling as fixed effects and subject as random effect. $N = 55$ for all analysis. All *P* values < 0.2 are stated.

[44,54], while high dietary fiber diets have been linked to reduced *Collinsella* abundance [63,64]. Increased butyrate has previously been observed in response to wholegrain rye intake in adults but mainly in feces [38,44,55,56].

The observed simultaneous changes in the microbiota composition, SCFA, and lipid profile in response to WG, and the intercorrelation between cereal dietary fibers, butyrate, and LDL cholesterol indicates a causal link of action. Fructans, some arabinoxylans, and β-glucans have high fermentation capacity in the gut [50], and these may, directly or via cross-feeding mechanisms, have fueled *Faecalibacterium,* which is one of the major butyrate-producing bacteria in the human gut [65]. Butyrate has been proposed to affect lipid metabolism via G-protein coupled receptors 41 and 43, which are expressed in liver, skeletal muscle, and adipose tissue [66]. Although most trials indicate changes in fecal SCFA with wholegrain, a rarely seen increase in plasma butyrate [44], as in our study, supports an effect on hepatic lipid metabolism.

Strengths of the present study include the low dropout rate and high dietary compliance, which was monitored by use of both diaries and ARs, which has previously been demonstrated to efficiently address incompliance with wholegrain intervention [67]. The selection of commercially available grain products, provided ad libitum, mimicked the effect of the universally adopted recommendation of high wholegrain intake [68]. The children in present study reached an intake corresponding to the highest quartile of wholegrain intake among Danish children [60] so that almost all of the children fulfilled the Danish food-based dietary guideline of consuming 75 g/10 MJ. Refined grain was chosen as comparator treatment as it reflects the very low wholegrain intake in many countries outside Scandinavia [61]. Noticeably, the treatment differences in serum TG could be due to increased intake of available carbohydrate, especially from the RG products, which can increase plasma TG [69]. This highlights the benefit of substituting refined grain with wholegrain, rather than just adding wholegrain to an existing diet high in refined grain. We consider the risk of carryover effects as negligible based on our sensitivity analyses. This is supported by the fact that cardiometabolic risk markers have shown to change in response to diet over just a few weeks among children [18,21], and changes in gut microbiota composition have been suggested to occur already within a few days of dietary intervention [28]. We conducted parts of the present study during the national lockdown of the COVID-19 pandemic, but due to a dedicated protocol, we were able to successfully complete the study and control the statistical analyses for potential changes in normal routines caused by the lockdown. Lastly, due to multiple testing and stratification in secondary analyses, there is a risk of type 1 errors. However, the consistency between outcomes supports the validity of the presented findings.

In conclusion, high intake of wholegrain from oats and rye compared with refined grain for 8 wk reduced serum LDL cholesterol as well as total:HDL cholesterol and TG without affecting plasma insulin among 8- to 13-y-old children with high BMI. These changes were accompanied by increases in SCFA in both feces and plasma, whereas glucose, blood pressure, BMI, body composition, and inflammatory markers were unaltered. Furthermore, we found genus-specific changes in the microbiota with WG compared with RG, without compromising microbial diversity. This study contributes to a growing body of research demonstrating the cardiometabolic benefits of replacing refined grain with wholegrain, even in children, and regardless of effects on body fat. Further randomized trials among children, including long-term studies and trials

focusing on specific wholegrain types, are necessary to gain a deeper understanding of the underlying mechanisms.

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Author contributions

The authors' responsibilities were as follows—MTBM: helped plan and conducted the study, analyzed data, wrote the paper, and had primary responsibility for final content; CTD, RL: designed the study; RL: supervised the analyses of SCFAs, bile acids, and ARs in plasma samples and coordinated dietary fiber analysis; DN, OMRA, YZ: carried out gut microbiota characterization and bioinformatics; CTD, LL: provided sparring in writing the manuscript and the interpretation of results; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript are not available for sharing because data are not anonymized due to Danish legislation and therefore considered as sensitive personal data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.10.025.

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