



Effects of hypocaloric wholegrain rye vs refined wheat diets on weight loss, cardiometabolic risk factors and gut microbiota: A 12-week randomized

Downloaded from: <https://research.chalmers.se>, 2026-04-14 22:45 UTC

Citation for the original published paper (version of record):

Aberg, S., Nordin, E., Iversen, K. et al (2026). Effects of hypocaloric wholegrain rye vs refined wheat diets on weight loss, cardiometabolic risk factors and gut microbiota: A 12-week randomized controlled trial. *Clinical Nutrition*, 60. <http://dx.doi.org/10.1016/j.clnu.2026.106618>

N.B. When citing this work, cite the original published paper.



Randomized Control Trials

Effects of hypocaloric wholegrain rye vs refined wheat diets on weight loss, cardiometabolic risk factors and gut microbiota: A 12-week randomized controlled trial



Sebastian Åberg^{a,b,*}, Elise Nordin^{a,1}, Kia Nøhr Iversen^a, Panpan Qin^c,
Per M. Hellström^d, Karsten Kristiansen^c, Rikard Landberg^{a,e}

^a Department of Life Sciences, Division of Food and Nutrition Science, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

^b Institute of Neuroscience and Physiology, Department of Physiology, Sahlgrenska Academy, University of Gothenburg, 40530 Gothenburg, Sweden

^c 4 Laboratory of Integrative Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark

^d Department of Medical Sciences, Gastroenterology and Hepatology, Uppsala University, SE-751 85 Uppsala, Sweden

^e Wallenberg Laboratory, Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, SE 413 45 Gothenburg, Sweden

ARTICLE INFO

Article history:

Received 10 June 2025

Accepted 23 February 2026

Keywords:

Obesity

Whole grains

Rye

Weight-loss

Microbiota

Short chain fatty acids

SUMMARY

Background and aims: Wholegrain rye foods have shown promising effects on metabolic regulation and weight-loss, which may be mediated via gut microbiota and derived metabolites. This study aimed to investigate effects of hypocaloric diets with wholegrain rye versus commonly consumed refined wheat on body weight, fat mass, metabolic risk markers and gut microbiota. The study also explored determinants of diet-induced weight loss.

Methods: Participants with overweight or obesity were randomized (1:1) to 12-week hypocaloric diets, substituting habitual cereals with wholegrain rye or refined wheat foods. Body weight and composition were measured and fecal- and blood samples were collected at baseline, after 6 weeks and 12 weeks.

Results: Of 255 participants, 229 completed the study. Weight loss was 3.2 kg in the rye-group and 2.9 kg in the wheat-group, with no significant difference between groups ($p = 0.32$). Plasma acetate and butyrate were higher after 12 weeks in the rye-group versus wheat-group ($p = 0.003$) and microbial taxa, previously associated with negative health outcomes were reduced in the rye-group. Reductions in CRP by 17% ($p = 0.03$) were observed in the rye-group, while both CRP ($r = 0.17$, $p = 0.001$) and HOMA-IR ($r = 0.13$, $p = 0.02$) at baseline were associated with fat mass change in the wheat-group. Additionally, acetate at baseline was inversely associated with body weight change across groups ($r = -0.25$, $p < 0.001$). Baseline gut microbiota was not associated with weight loss after 12 weeks.

Conclusions: Wholegrain rye versus refined wheat foods as part of a hypocaloric diet did not result in larger weight loss. However, wholegrain rye reduced CRP and induced changes in gut microbiota and short-chain fatty acids which may have positive implications for cardiometabolic health. Notably, baseline HOMA-IR and CRP correlated with weight and fat mass reductions, suggesting that individuals with elevated inflammation and insulin resistance may benefit more from wholegrain rye foods. Gut microbiota at baseline was not associated with intervention-induced weight loss.

Trial Registration: ClinicalTrials.gov ID: NCT04203758. <https://classic.clinicaltrials.gov/ct2/show/NCT04203758?term=Rye&cond=Overweight+and+Obesity&cntry=SE&city=Gothenburg&draw=2&rank=3>.

© 2026 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Abbreviations: HOMA-IR, homeostatic model assessment for insulin resistance; SCFA, short chain fatty acids; LDL, low density lipoprotein; HDL, high density lipoprotein; BP, blood pressure; CRP, C-reactive protein; DXA, dual energy x-ray absorptiometry; PAL, physical activity level; 3DWF, 3-day weighed food record; VAS, visual analogue scales; AR, alkylresorcinols; NMR, nuclear magnetic resonance; GlycA, glycoprotein N-acetylation A; GlycB, glycoprotein N-acetylation B; SPC, supramolecular phospholipid composite peak; ITT, intention-to-treat; LOCF, last observation carried forward; tAUC, total area under the curve.

* Corresponding author. Department of Life Sciences, Division of Food and Nutrition Science, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden.

E-mail address: sebastian.berg@gu.se (S. Åberg).

¹ Shared first authorship.

1. Introduction

Globally, an estimated 2.5 billion adults were overweight in 2022, of whom 890 million were obese—conditions approximately accounting for 10 % of global deaths annually [1,2]. About 40 % of deaths due to high BMI are attributed to BMI <30, highlighting the severity of moderate overweight [3]. Lifestyle strategies to treat and prevent overweight and obesity are critically needed.

Diet is a major determinant of body weight changes and is important for the prevention, management, and treatment of overweight and obesity [4,5]. High whole grain intake has consistently been associated with lower BMI, body fat mass, and reduced incidence of obesity and related conditions such as type 2 diabetes, heart disease, and colorectal cancer [6,7]. These benefits are thought to be driven by improvements in glycemic control, blood lipid profiles, and reduced inflammation [8–10]. Effects on body weight and body fat mass have been inconclusive in intervention studies, partly because studies have not been primarily designed to study weight loss [11]. Moreover, studies have shown considerable heterogeneity, with intervention foods from different grains resulting in different effects on body weight and body composition [7]. Wholegrain rye has the highest content of dietary fiber among all cereals [12], and has shown beneficial effects on subjective appetite control when compared with refined grains [13]. Effects of wholegrain rye foods on appetite control and subsequent weight loss may be mediated by alterations in gut microbiota, particularly through increased production of short-chain fatty acids (SCFAs), which are known to act as signaling molecules in the gut–brain axis and may impact appetite regulation [14–16].

Some studies, but not all, have shown alterations of the gut microbiota induced by wholegrain interventions [17–21]. We recently found alterations of the gut microbiota paralleled by weight and body fat loss following a diet rich in wholegrain rye compared with refined wheat in a 12-week hypocaloric intervention, the RyeWeight1 study [22]. On average, participants in the rye-group lost 1.1 kg more bodyweight, but there was considerable inter-individual variation in weight loss, consistent with findings from other weight loss studies [23–25]. Factors such as age, sex, adherence to intervention diets, metabolic efficiency, thermogenic response to foods, and gut peptides have been suggested as determinants of individual differences in energy balance and weight loss [23]. Recent studies have shown that the gut microbiota predicted metabolic and/or weight loss response of high fiber diets, specifically related to the genus *Prevotella* [26–29]. Also, metabolic status, specifically fasting blood sugar and insulin have been associated with weight loss following high vs low glycemic load/whole grain rich diets [30]. However, this relationship has not yet been examined in large studies designed to evaluate weight loss in relation to wholegrain rye foods.

The primary aim of the current RyeWeight2 study was to replicate findings in the RyeWeight1 study, showing greater body weight and fat mass reduction following a 12-week hypocaloric diet with wholegrain rye foods versus refined wheat foods. The secondary aim was to evaluate the effects on metabolic risk markers, appetite, gut microbiota, and SCFAs, and explore their potential role as determinants of weight loss.

2. Methods

2.1. Study design, participants, and intervention diets

The RyeWeight2 study was conducted as a 12-week hypocaloric parallel randomized controlled trial in free-living

participants between January 2020 and June 2021 in Gothenburg, Sweden. The study design, protocol, and inclusion and exclusion criteria were identical to those of the previously published RyeWeight1 study [22]. However, the RyeWeight2 study was conducted in a different geographic population, with the purpose of replicating findings in the RyeWeight1 study to support a potential EFSA health claim (*article 13.5*) on rye fiber and weight-loss. Additionally, we investigated the effects of the interventions on gut microbiota composition and assessed whether baseline microbiota profiles or metabolic risk markers were associated with changes in weight and body composition [22].

Briefly, participants were recruited through advertisements in newspapers (online and in print), on social media through www.accindi.se, and posters in public areas in Gothenburg. Men and women aged 30–70 years, with a BMI of 27–35 kg/m², were eligible to participate in the study and invited to the research clinic for screening. At the clinic, medical history and lifestyle were examined and fasting blood samples were drawn. Blood samples collected during the screening process were delivered to the clinical chemistry laboratory at Sahlgrenska University hospital and analyzed for blood markers with the following inclusion criteria: hemoglobin ≥ 120 g/L, serum thyroid stimulating hormone ≤ 4.00 mIU/L, plasma low density lipoprotein (LDL) cholesterol < 5.3 mmol/L and plasma triglycerides ≤ 1.8 mmol/L. Exclusion criteria included chronic gastrointestinal conditions, thyroid disorder, medication for type II diabetes or body weight management. A full list of inclusion and exclusion criteria can be found in [Supplementary Text 1](#). After a 2-week run-in period following a hypocaloric refined wheat-based diet, participants were required to lose ≥ 0.5 kg of body weight (menstruating women not gaining weight). Those who met the criterion underwent baseline clinical examination and were then randomized 1:1 to either rye- or wheat-based diets for 12 weeks ([Fig. 1](#)). The randomization list was generated using R version 4.1.3, package *blockrand* version 1.3 and allocations were concealed in sealed envelopes. As enrollment was carried out continuously, mixed block randomization was employed. All research staff involved in measurements, including study nurses and dual energy x-ray absorptiometry (DXA) operators, were blinded. Blinding of participants was not feasible due to the visual differences between the intervention products.

During all study weeks, participants were advised to adhere to a hypocaloric diet with habitual cereals substituted with either wholegrain rye or refined wheat products. All cereals products were provided, and participants consumed a fixed amount of either wholegrain rye cereals (705 kcal) or refined wheat cereals (685 kcal) which corresponded to approximately 30–50 % of the participants' daily energy intake. The rye products provided approximately 30 g dietary fiber/day, whereas the wheat products provided 8 g fiber/day. Detailed nutritional data for the cereal products is provided in [Supplementary Table 1](#). Each participant met with a dietician to assess their habitual diet based on a 3-day weighed food record (3DWFR) and calculate their energy requirements based on NNR 2012 equations, assuming a physical activity level (PAL) of 1.4 [31]. The dietician suggested dietary changes to achieve a 500 kcal/day energy deficit based on the habitual dietary pattern and instructed participants to completely exclude cereals other than the provided intervention products. Participants were instructed to fill in a pre-coded compliance journal where they ticked off the fixed amount of intervention products daily and recorded any deviations from the intervention diet, adjustments in medication, or instances of illness. Participants were considered compliant if they consumed at least 80 % of

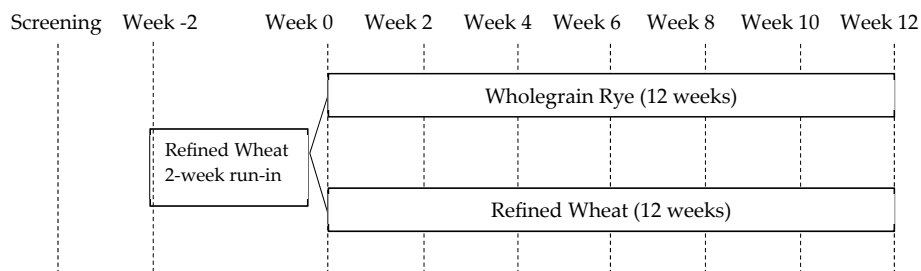


Fig. 1. Design of the RyeWeight2 study.

the prescribed intervention foods (by weight) over the 12-week intervention period.

The study was conducted by researchers from Chalmers University of Technology at a research clinic located at Department of Food and Nutrition and Sport Science, at University of Gothenburg in Gothenburg, Sweden. All participants gave written informed consent, after having received oral and written information about the study, prior to initiating the screening procedure. The study was approved by the Swedish Ethical Review Authority (application number: 2019-05307, approved 2020.01.10) and registered at www.clinicaltrials.gov (Identifier: NCT04203758, registration date: 2019.12.18). The study was conducted in accordance with the Declaration of Helsinki and reported in accordance with Consolidated Standards of Reporting Trials (CONSORT) [32].

2.2. Examination visits

Participants attended morning examination visits at weeks 0, 6, and 12, being fasted from the prior evening 10:00 PM, and they provided a fecal sample collected as close to the visit as possible. Anthropometric measurements and blood pressure were taken, followed by venous blood sample collection. Questionnaires were completed, and intervention products, compliance journals and materials provided. Full body DXA scans (Lunar iDXA, enCORE version 16, SP 1, GE Healthcare, Illinois, USA) were conducted on the same day, or at a nearby occasion. Additional visits to the research clinic occurred in weeks 2, 4, 8, and 10 for provision of intervention products, fecal collection kits, 3DWFR and compliance journals. To comply with COVID-19 safety measures, certain study visits that did not involve clinical examinations were conducted outdoors.

2.3. Anthropometric measurements

Body weight was measured to the nearest 0.1 kg with participants wearing light clothing (underwear and t-shirt) on a Tanita BC-545N scale (Tanita Corporation, Japan). Waist and hip circumference were measured to the nearest 0.5 cm. Participants were instructed to let arms hang down their sides and take a deep breath and waist was measured at the point of the navel and hip circumference was measured at the widest point of the hip. Measurements were done twice upon exhalation, and the average was calculated. Sagittal height was measured to the nearest 0.5 cm using a sagittal height measuring pin (BK-mätare, AJ Medical, Lidingö, Sweden) with participants lying on their back with legs bent. Participants were instructed to take a deep breath, and measurements were taken twice at the point of the navel upon exhalation. The average of the two measurements was calculated. Height measurements were conducted during screening, using a wall-mounted stadiometer (Hyssna Measuring Equipment AB, Hyssna, Sweden) and rounded to the nearest 0.5 cm.

2.4. Food records

Dietary intake was reported through 3DWFR at three occasions: baseline, 6-week and 12-week follow-up. Participants were provided with food scales and instructed to record everything they consumed during two weekdays and one day during the weekend using scales and household measures (e.g. deciliter and tablespoon) when weighing food was not possible. Daily energy and macronutrient intake were calculated using the software DietistNetPro (www.kostdata.se, Kost och Näringsdata AB, Bromma, Sweden) which contains a brand specific databased developed for use in Sweden and linked to the food composition database provided by the National Food Agency in Sweden. Approximations to standard portion sizes according to software were used when quantities of food items were missing. Additionally, foods items were categorized by food groups and the daily intake of each food group calculated based on the method used in Riksmaten, a national dietary intake survey conducted by the Swedish Food Agency [33].

2.5. Appetite assessment

Subjective appetite was assessed using 100 mm visual analogue scales (VAS) at three occasions: baseline, 6-week and 12-week follow-up. Participants were provided with all meals for these appetite assessment days and followed standardized meal plans strictly in a free-living setting. Meal plans were hypocaloric with a 500-kcal deficit based on estimated energy calculations and a set amount of rye or wheat foods according to allocation (Supplementary Table 1B). The full-day meal plan included a breakfast consisting of cereal puffs with milk, a lunch with tomato soup, crisp bread and cheese/jam, an afternoon snack consisting of crisp bread with cheese/jam and a goulash soup with soft bread and jam/cheese for dinner. Participants continuously answered questions about their perceived appetite every 30 min from 8:00 to 12:00 and every 60 min from 13:00 to 21:00. The following three questions were given in random order to evaluate appetite at each timepoint: "How hungry are you?", "How full are you?", "How big is your desire to eat?". Participants received an automated email through the online software Qualtrics (QualtricsXM, Seattle, Washington, USA). Participants followed a hyperlink to Qualtrics.com where the appetite questions were answered. All questions were also provided on paper, giving participants an analogue option and functioning as a back-up if the digital survey failed.

2.6. Questionnaires

Participants completed questionnaires on physical activity and gastrointestinal symptoms at baseline, week 6 and 12-week follow-up. Physical activity was assessed using the questionnaire

developed by Baecke et al., with separate scoring for work, sport, and leisure time activities [34]. Activity levels for sport activities not described by Baecke et al. were evaluated using the Compendium of Physical Activities by Ainsworth et al. [35]. Gastrointestinal symptoms were assessed using the GSRs-IBS questionnaire, and participants' answers were transformed into subscale symptom scores as per the method described by Wiklund et al. [36]. Eating behavior was assessed at screening, using the 21-item Three Factor Eating Questionnaire (TFEQ), which evaluates participants' behavior in three domains: cognitive restrained eating, uncontrolled eating, and emotional eating [37]. Additionally, participants completed a demographics questionnaire at screening, providing background information such as educational level, occupation, and housing.

2.7. Sample handling

Participants were provided with an EasySampler stool collection kit (GP Medical Devices ApS, Holstebro, Denmark), containing 2 fecal collection tubes (Sarstedt AG & Co., Nümbrecht, Germany) and were asked to fill 2–3 spoonful of feces in each tube. Participants were instructed to store the sample in a cooling bag with frozen cooling blocks for a maximum duration of 24 h before delivery to the clinic. Alternatively store the sample in a freezer ($-18\text{ }^{\circ}\text{C}$) for up to 72 h before transporting the sample to the clinic in a cooling bag with frozen cooling blocks. Fecal samples were stored in $-20\text{ }^{\circ}\text{C}$ for a maximum of 7 days at the clinic, then transferred to $-80\text{ }^{\circ}\text{C}$ freezers for long term storage. At clinical examinations week 0, 6, and 12, blood samples were collected in K2E EDTA-, serum-, sodium heparin- and sodium citrate tubes. Serum tubes were kept at room temperature before, during and after sampling, whereas other tubes were kept on ice during sample handling. EDTA, citrate and heparin tubes were centrifuged immediately after sampling at $4\text{ }^{\circ}\text{C}$ and 2500 g, for 10 min and separated into plasma, buffy coat (EDTA only) and erythrocytes (EDTA only) in 2 ml screw cap cryotubes (LVL technologies GmbH & Co. KG, Crailsheim, Germany), before being placed in $-20\text{ }^{\circ}\text{C}$ freezer. Serum tubes were kept at room temperature for 60 min prior to centrifugation and thereafter serum was transferred into 2 ml screw cap cryotubes and stored at $-20\text{ }^{\circ}\text{C}$. All samples were kept in $-20\text{ }^{\circ}\text{C}$ for a max duration of 7 days, where after they were transferred to $-80\text{ }^{\circ}\text{C}$ freezer for long term storage.

2.8. Sample analysis

All blood samples collected at week 0, week 6 and week 12 were analyzed at Lycksele clinical chemistry laboratory, Umeå University Hospital, for selected clinical risk markers. Glucose, CRP, insulin, total cholesterol, HDL cholesterol and triglycerides were measured in serum. LDL cholesterol was calculated using Friedewald's equation [38] and homeostatic model assessment insulin resistance (HOMA-IR) was calculated as $(\text{insulin (mIU/L)} \times \text{glucose (mmol/L)})/22.5$ [39]. All metabolic markers were analyzed with Cobas® Pro (Roche Diagnostics, Basel, Switzerland) according to laboratory accredited methods.

Plasma alkylresorcinols (AR), biomarkers of wholegrain rye and wheat intake [40,41], were analyzed as objective measure of compliance. AR concentrations were measured in EDTA plasma at Chalmers Mass Spectrometry Infrastructure using liquid chromatography-tandem mass spectrometry (LC-MS/MS), following a method developed at the platform [42]. The inter- and intra-batch coefficient of variance (CV) $< 15\%$. Total plasma AR concentration and a homologues C17:0-C25:0 were used as biomarkers of whole grain intake from rye and wheat sources, while

the AR C17:0/C21:0 homologue ratio was calculated as marker of the proportion whole grain from wheat and rye sources.

Inflammatory biomarkers glycoprotein N-acetylation (GlycA and GlycB) and supramolecular phospholipid composite peak (SPC) were analyzed with proton nuclear magnetic resonance (NMR) at Swedish Nuclear Magnetic Resonance Centre, Gothenburg. NMR data were acquired using a Bruker 600 MHz Avance III HD spectrometer, with GlycA, GlycB, and SPC data extracted through analysis algorithms (Bruker BioSpin) and custom in-house MATLAB scripts.

2.8.1. Fecal sample preparation for metagenome analysis

DNA was extracted from 451 fecal samples (35 mg each) using the NucleoSpin Soil kit (Macherey-Nagel, 740780.250 M) following the manufacturer's protocol. The DNA yield was measured with the Qubit 1X dsDNA Broad Range assay kit (Thermo Fisher Scientific, Q33266). For metagenomic shotgun sequencing, libraries were prepared using the MGIEasy Fast FS DNA Library Prep Set (MGI Tech Co., Ltd., 940-000030-00, Werheim, Germany) with a DNA input of 400 ng per sample. The library preparation process included DNA shearing for 12.5 min, followed by end repair, A-tailing, and a magnetic bead-based cleanup. Next, adapter ligation and cleanup were performed, followed by PCR amplification and magnetic bead-based size selection of the final library product. The quality of the libraries was assessed using the Qubit 1X dsDNA HS Assay Kit (Thermo Fisher Scientific, Q33231) and the Agilent High Sensitivity D1000 Assay Kit (Agilent Technologies, 5067-5584 and 5067-5585, St Clara, CA, USA). The pooled libraries were then circularized, barcoded, and used as templates for DNA nanoball preparation. The resulting nanoballs were analyzed on the DNBSEQ-T7 platform (MGI Tech Co., Ltd.) using the DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE150), according to the manufacturer's guidelines.

2.8.2. Processing of metagenome data

Raw metagenomic reads were subjected to quality control to remove low-quality sequences, adapters, and contaminants using Fastp [43] (version 0.23.2, default parameters). The high-quality reads were then filtered to remove human reads (GRCh38_noalt_as) using Bowtie2 [44] (version 2.5.3). The quality-filtered reads were aligned to the taxonomy database using the MetaPhlan (version 4.0.6, default parameters) [45] to obtain the relative abundance profiles.

2.9. Plasma SCFA analyses

A panel of nine SCFAs and gut fermentation products (formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, succinic acid, valeric acid, isovaleric acid, and capronic acid) were measured in heparin plasma at Chalmers University of Technology according to a method described by Fristedt et al. [46]. The within- and between batch CV% were < 15 and $< 20\%$ for determinations of most compounds in three QC samples representing low, medium and high concentrations.

2.10. Statistical analysis

The sample size estimation was calculated with an alpha of 0.025 and power of 0.80 to detect an effect of 1 kg difference in body weight and 1 kg difference in body fat mass after 12 weeks (two primary endpoints). In total, 106 participants in each treatment group were required to complete the intervention. Based on experience from previous studies we aimed to randomize 130 participants in each treatment group, allowing for a drop-out rate of 18% (Fig. 2). All data were analyzed according to complete case

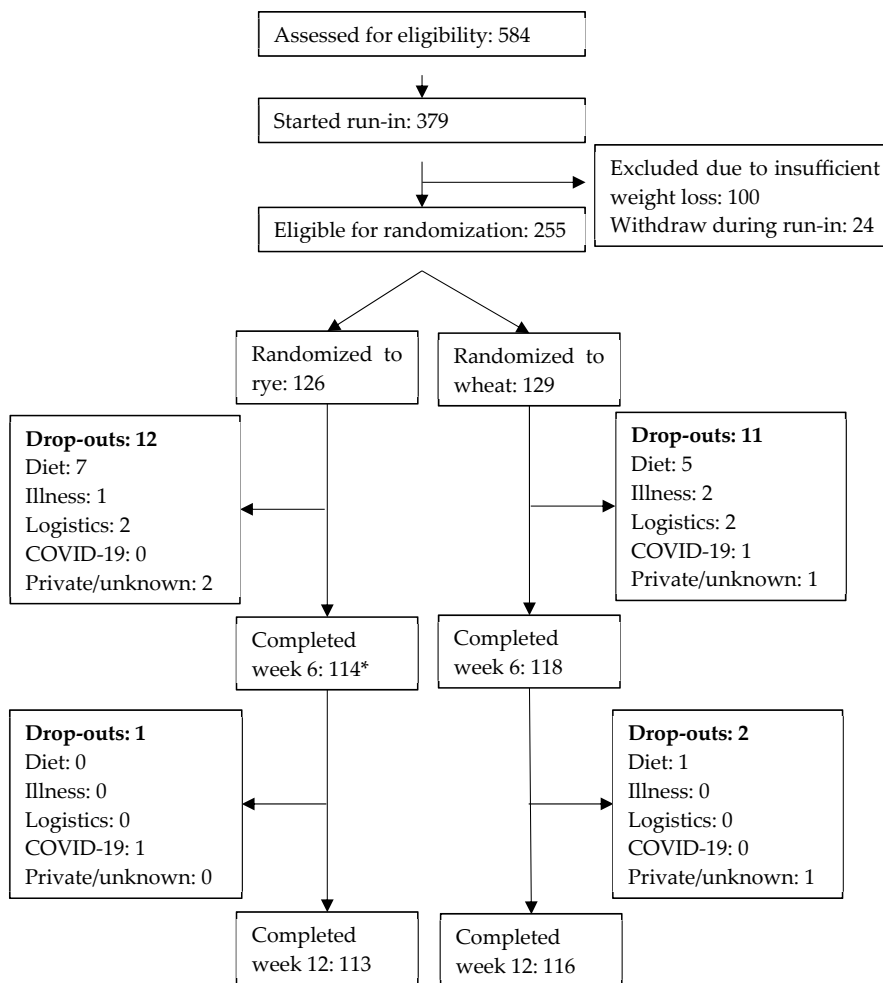


Fig. 2. Flowsheet of participants. * Including 3 participants who missed their week 6 examination but completed the study and attended week 12 (defined as complete cases).

analysis and additional intention to treat (ITT) analysis performed for primary outcomes body weight and fat mass change. Complete cases were defined as compliant to the study diet (reported consumption of >80 % of interventions foods during the 12 weeks intervention) and completion of the final examination at week 12. Intervention effects at 6 and 12 weeks were evaluated using linear mixed-effects models, with Week included as a covariate, a Diet × Week interaction term, and participant ID specified as a random effect. Additional covariates were included depending on the outcome variable, as described in the respective analyses. Results are presented as estimated marginal means ± standard error of the mean (SEM). All model residuals were assessed for normality and skewed data were log-transformed to meet model assumptions.

Dietary intake from 3DWFR, physical activity levels, and primary outcomes (body weight and fat mass) were analyzed using mixed models, adjusted for baseline values and including diet × week interaction terms. Body weight and fat mass change were considered in two ITT analysis where all randomized participants were included. In one model, missing data were imputed prior to analysis using last observation carried forward (LOCF), and the other model without imputation. For primary outcomes $p < 0.025$ was considered significant and all estimates were adjusted for multiple testing using Bonferroni correction. Anthropometric measures (e.g. waist and hip circumference, lean body mass) and metabolic markers were evaluated using baseline-

adjusted linear mixed models, with statistical significance assessed at $p < 0.05$ after Bonferroni correction. Models evaluating metabolic markers were additionally adjusted for body weight change. Data for metabolic markers were evaluated for normality using residual plots and histograms. Skewed data were log-transformed prior to analysis and estimates were back transformed before being reported. All other outcome measures were considered statistically significant at $p < 0.05$, unless otherwise is specified.

Exploratory analysis of baseline metabolic markers and diet-specific responses was evaluated using Spearman's correlations and confirmed by Fisher's r-to-z transformation. Furthermore, effect modification was tested using linear regression models including diet × baseline marker interaction terms, with metabolic status also assessed categorically based on clinical thresholds (e.g., HOMA-IR < or >2.0). Exploratory regression results were evaluated using Bonferroni correction ($p < 0.05$).

Total area under the curve (tAUC) was calculated for subjective appetite measures, using approximate integrals according to the trapezoidal rule [47]. Interventions effects were evaluated for postprandial periods (08:00–12:00, 12:00–19:00 and 19:00–21:00) following breakfast, lunch dinner and the whole day. Mean appetite scores were evaluated using a baseline-adjusted mixed model, whereas an unadjusted model was applied to assess the differences in appetite tAUC between interventions. Gastrointestinal symptoms were evaluated using Mann–Whitney U-test.

Intervention effects on SCFA concentrations and contrasts between intervention groups were evaluated in a baseline adjusted mixed model. Values exceeding ± 3 SD of the mean were excluded from analysis and values below limit of detection set to 0.

2.10.1. Microbiota

Principal coordinate analysis (PCoA) (Bray–Curtis dissimilarity matrix) was used to visualize clustering of microbiota composition. Although compositional approaches such as centered log-ratio (CLR) transformations and Aitchison distance are increasingly recommended for microbiome analyses, we applied alternative transformations and beta-diversity metrics commonly used in comparable studies, noting that our findings remain consistent with previous work using similar or alternative approaches. Richness and Shannon's diversity index was used for alpha diversity measures. For supervised analysis, microbiota composition was filtered with a 50 % prevalence cut-off at baseline or at least in one of the interventions, reducing the number of species from 2261 to 221. To handle zero values, a pseudo-count equal to 10^{-3} times the lowest value in the dataset was added. To explore the potential impact of the interventions on microbiota at species level, machine learning modeling was conducted using a random forest algorithm with unbiased variable selection. This was implemented through repeated double cross-validation using the R package MUVR [48]. The parameter settings for all MUVR models were as follows: $n_{\text{Outer}} = 8$, $n_{\text{Rep}} = 40$, and $\text{varRatio} = 0.85$. Intervention was used as a binary variable and species at 12 weeks as the predictor variable. The robustness of the models was assessed through permutation tests ($n = 100$) to ensure the results were not overfitted. The parameters for these permutation tests matched those used in the multilevel models. The variables with the greatest predictive power were identified through an automatic optimal variable selection process within the MUVR modeling.

Moreover, to compare the effect on the previously reported fecal microbiota in RyeWeight1 [49] with that of the present RyeWeight2 study, the effect on bacteria taxa that differed significantly between rye and wheat in the RyeWeight 1 study (*Agathobacter*, *Oscillospiraceae* UCG-003, *Haemophilus*, [*Ruminococcus*] *torques* group, [*Eubacterium*] *ventriosum* group, *Anaerotruncus*, *Anaerofilum*, and *Holdemania*) were evaluated in RyeWeight2, to investigate if the effects were replicated. For each genus, the species level was included to investigate which species were driving the effect on the genera level. All microbial genera and species selected from Random Forest modelling and included from the RyeWeight1-study were analyzed with linear modelling with microbial genera and species as dependent variable and intervention and baseline value of the microbial genera and species as independent variable. All variables were log-transformed, and the estimates were subsequently back-transformed to their original scale.

The MUVR algorithm was similarly used to investigate if the baseline microbiota (genus, species, strain level) was associated with weight loss response after intervention in the study. The target variable was the weight loss between week 12 and baseline, and the predictor variable was microbial taxa at baseline. Models were separately run for rye, wheat and a combination. The rationale behind combining the rye and wheat intervention was to exploratory test if the gut microbiota could predict weight loss in relation to cereal intake, independent of cereal type. The same modelling was performed but with log fold change of microbial features with features at 12 weeks in the nominator. In addition to modelling weight loss in kg, the same modelling was applied for change in weight loss in percent, fat mass (kg) and fat mass percent between baseline and 12 weeks of intervention.

In addition, univariate analysis with linear modelling was conducted with weight loss as dependent variable and species at baseline (modelled as value at baseline and as log fold change of species with 12-weeks in nominator and 0 weeks in denominator) as predictor variable. Body weight loss was modelled as weight loss (weight at week 12- weight at baseline) in kilo, percent, fat mass loss in kilo and fat mass loss in percent. Models for predicting weight loss were performed both separately and combined for the rye and wheat-based intervention. For the univariate analyses with weight loss as dependent variable and species at baseline, false discovery rate-adjusted p-values <0.05 were considered statistically significant.

Microbiota at the genus level was clustered into enterotypes (classified based on gut microbiota composition) by using the around medoid (PAM) clustering method [50], previously described for bacterial enterotype discovery [51]. Samples were clustered based on the between-sample Jensen-Shannon distance. Calinski-Harabasz index was used to estimate optimal number of clusters, and the silhouette validation technique was used to assess the robustness of the clusters. The dominant genus for each enterotype was identified as the genus with the highest relative abundance within that enterotype. The Dirichlet Multinomial Mixture (DMM) method [52] was used to verify the results from PAM clustering. For both clustering methods, the minimum average abundance of genera threshold was set to 0.001 %. Principal coordinate analysis of the JSD matrix was conducted using the ade4 package, and the most prevalent bacteria (top 10) in each enterotype were visualized with the ggplot2.

Microbial taxa were divided in strata (average abundance was set as cut off-off of low/high strata) of species known to be associated with butyrate [53] and acetate [54,55] production. Linear modelling was applied to investigate if the strata for each microbial taxa at baseline were differently associated with weight loss, including all data, irrespective of assigned intervention. For the linear modelling of the strata's, false discovery rate-adjusted p-values <0.05 were considered statistically significant. The selected taxa were correlated to butyrate respectively acetate levels at baseline in the study. Unless otherwise specified, microbiota analyses were reported using unadjusted p-values.

Statistical analyses were performed in programming software R version 4.1.3 (packages: lme4 (1.1–35.1), dplyr (1.1.4), tidyverse (2.0.0), emmeans (1.10.0), ggeffects (1.5.0), lmetest (09–40), missForest (1.4), caTools (1.18.2), pracma (2.4.4), corplot (0.92), MUVR (version 0.0.9), ggplot (version 3.3.5), vegan (2.5–7), aed4 (version 1.7–18).

3. Results

3.1. Participants

In total, 584 participants were screened, 379 fulfilled the inclusion criteria and started the run-in period. Among these, 255 achieved the weight loss target and were subsequently randomized into the 12-week parallel intervention phase (Fig. 2). During the 12-week parallel phase, 26 participants (10 %) dropped out from the intervention, mostly during the first half of the intervention period. The main reasons for drop-out were related to a disliking of the diet, gastrointestinal problems or difficulties consuming the diet (Fig. 2). Two participants chose isolation due to COVID-19 and reported family members belonging to risk groups ($n = 2$), persistent influenza-like symptoms ($n = 3$). One of these participants was confirmed to be positive for COVID-19, whereas the other two were unconfirmed. Three participants in the rye-group missed the clinical examination in week 6 due to symptoms requiring isolation but followed diet and continued the study

as planned and attended the clinical examination in week 12. These participants were therefore defined as complete cases for the primary analysis. Baseline characteristics of completed cases ($n = 229$) are shown in [Table 1](#).

3.2. Dietary intake

In complete case analysis both intervention groups reduced their energy intake with approx. 200–250 kcal/day, but the energy intake did not differ between the groups at any time-point ([Table 2](#)). An energy deficit of 250 kcal/day over a period of 12 weeks would in theory have resulted in approximately 3 kg of body weight loss. As expected, the dietary fiber intake differed between the groups at both week 6 and week 12. The difference in reported dietary fiber intake at week 12 (19 g/day vs 42 g/day) corresponds to the difference in fiber provided from intervention products. Intervention foods differed slightly in weight (198 g/day vs 231 g/day) which is reflected in differences in reported cereal consumptions from 3DWFR at week 6 and week 12 ([Supplementary Table 2](#)). The protein intake was slightly higher in the wheat-group, likely due to a marginally higher consumption of dairy products ([Table 2](#)). Both intervention groups reduced their intake of sweets, snacks, candy etc. ([Supplementary Table 2](#)), which is in line with strategies implemented by the study dietician to achieve daily calorie deficiency and weight loss.

3.3. Compliance

Self-reported compliance through journals indicated high adherence, with participants in both the rye and wheat-group consuming 96–98 % of the provided products ([Supplementary Table 3](#)). Hence, all participants who completed the intervention were deemed compliant. More detailed compliance data showed

that compliance was consistently high throughout the entire intervention period.

Objective measure of compliance, plasma concentrations and ratio of specific ARs, also indicated an overall good compliance ([Supplementary Table 4](#)). The groups had similar C17:0/C21:0 ratio and total AR at baseline, after 2-weeks of run-in period consuming refined wheat products. Total AR was 10 times higher and C17:0/C21:0 markedly higher in the rye-group at week 6, while unchanged in the wheat-group compared to baseline. At week 12, total AR was slightly lower (–13 %) in the rye-group and slightly higher in the wheat-group (23 %) compared to concentrations at week 6, yet remained seven times higher in the rye vs wheat-group. The C17:0/C21:0 ratio in the wheat-group was stable throughout the 12 weeks, and clearly higher in the rye-group at week 6 and week 12, showing a higher intake of whole grain, primarily from rye.

3.4. Physical activity and gastrointestinal symptoms

The diet groups did not differ in self-reported sports or work-related physical activity at baseline, 6-weeks or 12-week follow-up ([Supplementary Table 5](#)). The rye-group reported slightly higher leisure related physical activity at baseline, but this difference was not significant after adjusting for activity levels reported at screening ($p > 0.05$). There were no differences between groups at the 6- or 12-week follow-ups ($p > 0.05$). There were no differences in reported gastrointestinal symptoms between the rye- and wheat-group at screening or baseline ($p > 0.05$) ([Supplementary Table 6](#)). At week 6 and week 12, participants in the rye-group reported higher levels of symptoms related to bloating ($p < 0.001$), diarrhea ($p = 0.005$), and satiety ($p = 0.008$), compared to the wheat-group which experienced more constipation ($p = 0.008$). Higher reported bloating and diarrhea may be related to sensations of satiety. However, this was not observed in the current trial, and It should be noted that severity of

Table 1
Baseline characteristics of participants who completed the intervention.

	Rye $n = 113$	Wheat $n = 116$	All $n = 229$
Anthropometrics and demographics			
Females/males (n) (% males)	72/41 (36 %)	81/35 (30 %)	153/76 (33 %)
Age (years)	56.1 \pm 9.6 (58)	54 \pm 10.3 (55)	55 \pm 10.0 (56)
Body weight (kg)	86.9 \pm 10.4 (86.2)	87.1 \pm 11.7 (86.5)	87.0 \pm 11.1 (86.3)
BMI (kg/m ²)	29.7 \pm 2.1 (29.4)	29.5 \pm 2.4 (29.2)	29.6 \pm 2.3 (29.3)
Fat %	39.8 \pm 6.4 (41.7)	39 \pm 6.9 (39.7)	39.4 \pm 6.7 (40.5)
Body fat mass (kg)	34.3 \pm 5.6 (34)	33.8 \pm 7.1 (33.4)	34 \pm 6.4 (33.9)
Lean body mass (kg)	49.7 \pm 9.4 (48.3)	50.3 \pm 9.6 (47.8)	50 \pm 9.5 (48)
Three factor eating questionnaire			
Restrained eating	13.7 \pm 3 (14)	13.6 \pm 2.7 (13)	13.6 \pm 2.9 (13)
Emotional eating	13.7 \pm 4.4 (14)	13.2 \pm 4.2 (12)	13.4 \pm 4.3 (13)
Uncontrolled eating	19.7 \pm 4.8 (20)	19.8 \pm 4.9 (20)	19.8 \pm 4.8 (20)
Birth country (n)			
Sweden	97	94	191
European Union	4	7	11
Other	11	13	24
Education level (highest completed) (n)			
Primary/elementary school	3	2	5
2-year high-school program/vocational training	18	16	34
3–4 year high-school program	22	28	50
University	70	70	140
Income, brutto (gross) (n)			
0–149,000 SEK/year	6	15	21
150,000–299,000 SEK/year	20	20	40
300,000–449,000 SEK/year	44	31	75
450,000 SEK/year or more	43	49	92
Unknown	0	1	1

Data are presented as mean \pm SD and (median), unless otherwise specified.

Table 2
Average daily energy and macronutrient intake based on 3-day weighed food records.

		Baseline (screening)	Week 6	Week 12	p-value ^a		
					Baseline	Week 6	Week 12
Energy (kcal)	Rye	2130 ± 792 (2004)	1935 ± 577 (1839)	1919 ± 633 (1842)	-/	0.0657/0.2419	0.4533/0.7672
	Wheat	2158 ± 560 (2094)	1843 ± 406 (1823)	1884 ± 472 (1837)	0.4028		
Carbohydrate (g)	Rye	219 ± 95 (200)	208 ± 59 (197)	207 ± 67 (197)	-/	0.6564/0.4270	0.1381/0.0907
	Wheat	215 ± 68 (204)	205 ± 53 (200)	207 ± 51 (203)	0.4185		
Protein (g)	Rye	84 ± 28 (82)	89 ± 35 (82)	88 ± 31 (82)	-/	0.8522/0.8911	0.6256/0.6362
	Wheat	86 ± 26 (82)	90 ± 22 (87)	91 ± 22 (88)	0.9398		
Fat (g)	Rye	91 ± 40 (82)	71 ± 32 (64)	69 ± 32 (65)	-/	0.2829/0.5646	0.7971/0.8338
	Wheat	94 ± 29 (91)	67 ± 23 (64)	69 ± 26 (67)	0.1952		
Fiber (g)	Rye	22 ± 9 (21)	43 ± 11 (41)	42 ± 14 (40)	-/	<.0001/<.0001	<.0001/<.0001
	Wheat	22 ± 8 (22)	19 ± 5 (18)	19 ± 7 (17)	0.8930		
Alcohol (g)	Rye	8 ± 11 (3)	5 ± 8 (0)	6 ± 11 (0)	-/	0.1708/0.1134	0.2014/0.1282
	Wheat	10 ± 16 (5)	5 ± 9 (0)	5 ± 10 (0)	0.3387		
E% carbohydrate	Rye	41 ± 8 (41)	43 ± 6 (43)	44 ± 6 (44)	-/	0.0670/0.0938	0.1272/0.2283
	Wheat	40 ± 7 (40)	45 ± 6 (46)	45 ± 6 (45)	0.2608		
E% protein	Rye	16 ± 4 (16)	18 ± 3 (18)	18 ± 4 (18)	-/	0.0063/0.0093	0.0024/0.0043
	Wheat	16 ± 3 (16)	20 ± 3 (19)	20 ± 3 (19)	0.9847		
E% fat	Rye	38 ± 8 (38)	32 ± 7 (32)	32 ± 7 (32)	-/	0.9081/0.8159	0.6599/0.5049
	Wheat	39 ± 7 (39)	32 ± 7 (32)	32 ± 7 (33)	0.2549		
E% fiber	Rye	2 ± 1 (2)	5 ± 1 (5)	5 ± 1 (4)	-/	<.0001/<.0001	<.0001/<.0001
	Wheat	2 ± 1 (2)	2 ± 1 (2)	2 ± 1 (2)	0.3211		
E% alcohol	Rye	2 ± 3 (1)	2 ± 3 (0)	2 ± 4 (0)	-/	0.1658/0.2165	0.3480/0.2774
	Wheat	3 ± 5 (1)	2 ± 3 (0)	2 ± 3 (0)	0.5615		

Macronutrient intake among complete cases (n: rye = 113, wheat = 116) at baseline, week 6 and week 12.

^a Intervention groups compared in model with/without baseline adjustment.

symptoms reported in both groups were relatively mild and would not be considered clinically relevant.

3.5. Body weight, fat mass and anthropometrics

Both intervention groups experienced significant reductions in body weight and fat mass at the 12-week follow-up compared to baseline ($p < 0.001$) (Fig. 3). The rye-group lost on average 3.2 kg body weight and 2.9 kg fat mass while the wheat-group lost on average 2.9 kg body weight and 2.8 kg fat mass over the 12-week intervention period, but there were no significant differences between the groups either at week 6 or week 12 ($p > 0.05$) (Table 3). Results were similar for the intention-to-treat (ITT) analyses (Table 3). There were numeric reductions in most anthropometric measures ($p < 0.001$) within groups, which were expected as participants lost weight, but no differences between groups at either week 6 or week 12 were observed (Table 4).

3.6. Clinical markers

Only modest differences in clinical markers were observed between the intervention groups except for CRP where concentrations were 17 % lower in the rye-group compared to the wheat-group ($p = 0.025$) at week 12, after adjustment for baseline levels and weight loss induced by the intervention (Table 5B). NMR-derived inflammatory biomarkers GlycA, GlycB, and SPC all showed within-group reductions after 12 weeks, with no significant differences between the rye and wheat-group (Table 5A). Triglyceride levels increased in the rye-group, being 10 % higher ($p = 0.003$) compared to the wheat-group at week 6, a trend that persisted at week 12 but was not statistically significant. Additionally, HDL concentrations were lower in the rye-versus wheat-group after 6 weeks ($p = 0.024$). This difference was modest, likely not clinically relevant, and was attenuated by week 12 (Table 5B).

Within the wheat-group fasting insulin and HOMA-IR at baseline were associated with body weight ($r = 0.14$, $p = 0.01$,

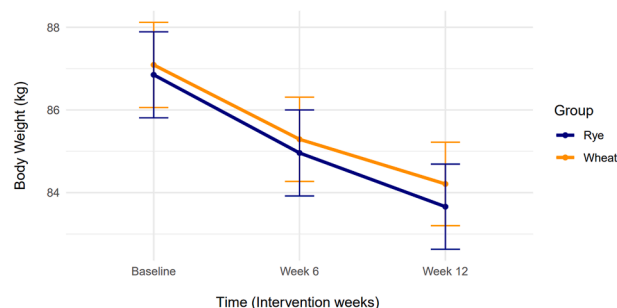


Fig. 3. Body weight changes in the rye- and wheat-group for the 12-week intervention. Data are presented as baseline-adjusted model estimates ± SEM; estimates were adjusted for multiple testing using the Bonferroni correction.

($r = 0.12$, $p = 0.03$) and fat mass ($r = 0.16$, $p = 0.003$), ($r = 0.13$, $p = 0.02$) respectively, at the 12-week follow-up. Furthermore, CRP ($r = 0.17$, $p = 0.001$) and GlycA ($r = 0.13$, $p = 0.04$) at baseline were associated with fat mass at week 12, while baseline HDL was inversely associated with body weight ($r = 0.11$, $p = 0.04$) at week 12 (Supplementary Table 7). Interestingly, none of these associations were observed within the rye-group, and Fisher r-to-z comparisons confirmed that the correlations for baseline insulin and HOMA-IR differed significantly between the wheat and rye groups (Supplementary Table 8).

3.7. Subjective appetite

No differences in hunger, fullness, or desire to eat, measured as VAS-score tAUC, were found between the groups in week 0, week 6 or week 12 (Supplementary Fig. 1). For certain timepoints, mean VAS-scores differed between groups at different occasions. The wheat-group reported lower hunger at 10:30 and lower desire to eat at 11:00 at the 6-week assessment, and lower desire to eat at 8:30 at the 12-week follow-up. The rye-group reported higher fullness at 21:00 compared to the wheat-group (Supplementary

Table 3
Body weight and fat mass change.

		Week 0 ^a	Week 6 ^a	Week 12 ^a	Δbetween groups week 6 ^b	Δbetween groups week 12 ^b	<i>p</i> week 6 ^b	<i>p</i> week 12 ^b
Complete cases,^c (n: rye = 113, wheat = 116)								
Body weight (kg)	Wheat	87.09 ± 1.03	85.29 ± 1.02	84.21 ± 1.01	0.09 (−0.29;0.47)	0.31 (−0.3;0.92)	0.637	0.319
	Rye	86.85 ± 1.04	84.96 ± 1.04	83.66 ± 1.03				
Fat mass (kg)	Wheat	33.81 ± 0.60	32.06 ± 0.60	30.98 ± 0.61	−0.004 (−0.308;0.3)	0.068 (−0.457;0.593)	0.978	0.799
	Rye	34.28 ± 0.60	32.54 ± 0.61	31.38 ± 0.62				
ITT,^d (n: rye = 126, wheat = 129)								
Body weight (kg)	Wheat	86.45 ± 0.99	84.67 ± 0.98	83.61 ± 0.97	0.13 (−0.25;0.51)	0.37 (−0.25;0.98)	0.495	0.240
	Rye	87.50 ± 1.00	85.57 ± 0.99	84.25 ± 0.99				
Fat mass (kg)	Wheat	33.94 ± 0.56	32.2 ± 0.57	31.13 ± 0.58	0.019 (−0.283;0.321)	0.104 (−0.418;0.626)	0.902	0.696
	Rye	34.55 ± 0.57	32.79 ± 0.58	31.62 ± 0.59				
ITT, LOCF,^e (n: rye = 126, wheat = 129)								
Body weight (kg)	Wheat	86.45 ± 0.99	84.82 ± 0.98	83.84 ± 0.98	0.05 (−0.32;0.42)	0.25 (−0.34;0.85)	0.796	0.400
	Rye	87.50 ± 1.00	85.80 ± 0.99	84.60 ± 0.99				
Fat mass (kg)	Wheat	33.94 ± 0.56	32.35 ± 0.58	31.39 ± 0.59	−0.035 (−0.338;0.268)	0.066 (−0.45;0.582)	0.822	0.801
	Rye	34.55 ± 0.57	33 ± 0.58	31.92 ± 0.59				

Abbreviations: ITT, intention-to-treat; LOCF, last observation carried forward. Data are presented as complete case analysis: estimated marginal means ± SEM, 'mean (95%confidence interval)'. Due to two primary endpoints, significance level is *p* > 0.025, significant *p*-values are marked in bold font.

^a Model estimated, not adjusted for baseline.

^b Derived from baseline adjusted model, model estimates and *p*-values were adjusted for multiple testing using Bonferroni correction. Rye-group is reference for confidence intervals.

^c Primary analysis (complete case).

^d ITT analysis, without imputation.

^e ITT analysis with imputation.

Table 4
Anthropometric measures.

		Week 0 ^a	Week 6 ^a	Week 12 ^a	Δbetween groups week 6 ^b	Δbetween groups week 12 ^b	<i>p</i> week 6 ^b	<i>p</i> week 12 ^b
BMI (kg/m ²)	Wheat	29.5 ± 0.21	28.9 ± 0.21	28.56 ± 0.22	0.03 (−0.1;0.16)	0.1 (−0.11;0.31)	0.656	0.344
	Rye	29.7 ± 0.21	29.01 ± 0.21	28.6 ± 0.22				
Waist (cm)	Wheat	105.0 ± 0.72	103.45 ± 0.72	102.5 ± 0.74	0.03 (−0.92;0.98)	0.24 (−0.78;1.26)	0.945	0.641
	Rye	106.9 ± 0.73	105.1 ± 0.73	103.9 ± 0.75				
Hip (cm)	Wheat	110.6 ± 0.58	109.4 ± 0.59	108.3 ± 0.56	0.03 (−0.62;0.68)	0.28 (−0.34;0.89)	0.929	0.376
	Rye	109.9 ± 0.59	108.8 ± 0.60	107.4 ± 0.57				
Sagittal height (cm)	Wheat	23.34 ± 0.21	22.6 ± 0.22	22.0 ± 0.22	0.00 (−0.34;0.35)	0.12 (−0.26;0.50)	0.978	0.526
	Rye	23.8 ± 0.21	22.9 ± 0.22	22.2 ± 0.22				
Waist/hip ratio (cm/cm)	Wheat	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.00 (−0.01;0.01)	0.00 (−0.01;0.01)	0.850	0.687
	Rye	0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.01				
Android fat (g)	Wheat	3284 ± 81	3058 ± 80	2903 ± 79	19 (−33;71)	5 (−73;83)	0.462	0.896
	Rye	3490 ± 82	3238 ± 81	3087 ± 80				
Gynoid fat (g)	Wheat	5516 ± 124	5204 ± 120	5044 ± 120	−21 (−79;36)	−18 (−106;70)	0.466	0.685
	Rye	5304 ± 125	5022 ± 122	4863 ± 121				
Lean mass (kg)	Wheat	50.32 ± 0.88	50.3 ± 0.89	50.27 ± 0.88	0.06 (−0.16;0.28)	0.24 (0;0.49)	0.589	0.150
	Rye	49.68 ± 0.89	49.59 ± 0.9	49.38 ± 0.9				
Body fat %	Wheat	39.01 ± 0.62	37.75 ± 0.65	36.94 ± 0.66	−0.03 (−0.28;0.21)	−0.09 (−0.49;0.3)	0.785	0.646
	Rye	39.81 ± 0.63	38.62 ± 0.65	37.86 ± 0.67				
VAT [†] mass (g)	Wheat	1372 ± 78	1251 ± 74	1175 ± 70	21 (−21;63)	11 (−43;66)	0.318	0.687
	Rye	1545 ± 79	1389 ± 75	1312 ± 71				
VAT [†] volume (cm ³)	Wheat	1455 ± 83	1326 ± 78	1246 ± 74	23 (−22;67)	12 (−46;70)	0.319	0.687
	Rye	1638 ± 84	1472 ± 79	1391 ± 75				

^a Model estimated, not adjusted for baseline.

^b Derived from baseline adjusted model, model estimates and *p*-values were adjusted for multiple testing using Bonferroni correction. Rye-group is reference for confidence intervals. [†]Visceral adipose tissue estimated from DXA. Data are presented as complete case analysis: estimated marginal means ± SEM, 'mean (95%confidence interval)'. Significance level is *p* > 0.05, significant *p*-values are marked in bold font. n: rye = 113, wheat = 116.

Fig. 2. Surprisingly, subjective appetite measured as tAUC, showed no association with changes in body weight or fat mass in the overall cohort, nor in the wheat- and rye-group separately (Supplementary Table 9).

3.8. Gut microbiota and SCFA

3.8.1. Gut microbiota

The sequencing generated a median of 38 130 884 [SD 13 718 639 (IQR 19 853 479)] sequences per sample. Two samples were discarded due to technical errors. Bacterial richness and Shannon's diversity index were marginally reduced in the wholegrain rye

versus refined wheat-based intervention mean [Confidence interval (CI)] [3.91 (3.83,3.99), 4.04 (3.96,4.12), *p* < 0.02], [292.75 (278.1,307.4), 319.15 (304.69,333.6), *p* < 0.02]. Unsupervised PCoA did not show any separation between the rye and wheat-group (Supplementary Fig. 3). Systematic differences in microbial species between the rye and wheat-group were found by Random Forest modelling (classification rate (CR) = 0.8, *p*_{permutation} < 0.0001). Moreover, some genera and species that differed between the rye and wheat-group in the previously reported RyeWeight1-study [49] also differed in RyeWeight2 (Fig. 4). Among rye and wheat discriminating species, *Bifidobacterium_adolescentis*, *Clostridium_sp_AF34_10BH*,

Table 5A
Clinical risk markers by diet.

		Week 0 ^a	Week 6 ^a	Week 12 ^a	Δbetween groups week 6 ^b	Δbetween groups week 12 ^b	p week 6 ^b	p week 12 ^b
Mean ± SEM		Mean difference (95 % CI)						
Systolic BP (mmHg)	Wheat	125.2 ± 1.1	124.0 ± 1.2	124.4 ± 1.2	-1.7 (-3.9;0.6)	0.8 (-1.5;3.1)	0.145	0.504
	Rye	124.0 ± 1.1	124.8 ± 1.2	122.7 ± 1.2				
Diastolic BP (mmHg)	Wheat	80.2 ± 0.7	78.7 ± 0.7	79.6 ± 0.7	-1.4 (-2.9;0.1)	0.3 (-1.2;1.9)	0.065	0.676
	Rye	79.8 ± 0.7	79.8 ± 0.7	79.0 ± 0.7				
Pulse (bpm)	Wheat	62.0 ± 0.8	60.3 ± 0.8	60.5 ± 0.8	0.1 (-1.2;1.4)	-0.3 (-1.9;1.3)	0.869	0.713
	Rye	62.6 ± 0.8	60.5 ± 0.8	61.2 ± 0.8				
Total cholesterol (mmol/l)	Wheat	4.83 ± 0.09	4.94 ± 0.08	4.85 ± 0.08	0.08 (-0.04;0.19)	-0.07 (-0.19;0.05)	0.181	0.274
	Rye	4.86 ± 0.09	4.89 ± 0.08	4.94 ± 0.08				
LDLcholesterol (mmol/l)	Wheat	2.96 ± 0.07	3.04 ± 0.07	2.94 ± 0.07	0.07 (-0.03;0.16)	-0.07 (-0.18;0.04)	0.150	0.192
	Rye	3.00 ± 0.07	3.01 ± 0.08	3.05 ± 0.07				
GlycA ^c	Wheat	9.84 ± 0.18	NA	9.36 ± 0.18	NA	0.31 (-0.05-0.66)	NA	0.087
	Rye	10.04 ± 0.18	NA	9.71 ± 0.18				
GlycB ^c	Wheat	3.23 ± 0.06	NA	3.07 ± 0.06	NA	0.1 (-0.02-0.22)	NA	0.090
	Rye	3.29 ± 0.06	NA	3.18 ± 0.06				
SPC ^c	Wheat	10.16 ± 0.26	NA	9.95 ± 0.26	NA	0.37 (-0.1-0.83)	NA	0.124
	Rye	10.46 ± 0.27	NA	10.41 ± 0.27				

Abbreviations: BP, blood pressure; GlycA, glycoprotein N-acetylation A; GlycB, glycoprotein N-acetylation B; SPC, supramolecular phospholipid composite peak.

^a Model estimates, not adjusted for baseline or weight loss.

^b Derived from model adjusted for baseline and bodyweight change (week 0-12), model estimates and p-values were adjusted for multiple testing using Bonferroni correction.

^c Arbitrary units.

Table 5B
Clinical risk markers by diet.

		Week 0 ^a	Week 6 ^a	Week 12 ^a	Δbetween groups week 6 ^b	Δbetween groups week 12 ^b	p week 6 ^b	p week 12 ^b
Geometric mean (95 % CI) ^c		Anti-log mean difference (95 % CI) ^c						
HDL cholesterol (mmol/l)	Wheat	1.34 (1.28;1.40)	1.35 (1.29;1.42)	1.37 (1.31;1.43)	1.03 (1.00;1.06)	1.02 (1.00;1.05)	0.024	0.079
	Rye	1.36 (1.30;1.43)	1.33 (1.27;1.40)	1.36 (1.30;1.42)				
Triglyceride (mmol/l)	Wheat	0.97 (0.91;1.04)	0.97 (0.91;1.04)	0.98 (0.92;1.05)	0.91 (0.86;0.97)	0.94 (0.88;1.00)	0.003	0.050
	Rye	0.98 (0.92;1.04)	1.07 (1.00;1.15)	1.05 (0.98;1.12)				
Glucose (mmol/l)	Wheat	5.18 (5.09;5.27)	5.14 (5.05;5.24)	5.16 (5.07;5.25)	1.00 (0.98;1.02)	1.00 (0.98;1.02)	0.965	0.937
	Rye	5.31 (5.21;5.40)	5.24 (5.15;5.34)	5.26 (5.17;5.36)				
Insulin (mIU/l)	Wheat	8.32 (7.59;9.12)	7.73 (7.09;8.43)	7.51 (6.86;8.22)	0.99 (0.91;1.06)	1.00 (0.92;1.09)	0.709	0.973
	Rye	9.04 (8.24;9.92)	8.31 (7.61;9.07)	7.93 (7.24;8.70)				
HOMA-IR	Wheat	1.92 (1.73;2.12)	1.77 (1.61;1.94)	1.72 (1.56;1.90)	0.98 (0.91;1.07)	1.00 (0.91;1.10)	0.701	0.987
	Rye	2.13 (1.92;2.36)	1.94 (1.76;2.13)	1.86 (1.68;2.05)				
HOMA2-IR	Wheat	1.70 (1.54;1.87)	1.56 (1.41;1.72)	1.52 (1.37;1.68)	0.99 (0.90;1.08)	0.97 (0.87;1.07)	0.803	0.512
	Rye	1.88 (1.69;2.08)	1.70 (1.56;1.87)	1.63 (1.48;1.80)				
CRP (mg/l)	Wheat	1.28 (1.1;1.49)	1.22 (1.05;1.41)	1.26 (1.08;1.46)	1.09 (0.94;1.26)	1.20 (1.02;1.41)	0.263	0.025
	Rye	1.31 (1.12;1.53)	1.13 (0.98;1.31)	1.06 (0.91;1.24)				
CRP2 (mg/l)	Wheat	1.21 (1.05;1.39)	1.18 (1.03;1.35)	1.20 (1.04;1.39)	1.15 (1.03;1.28)	1.21 (1.05;1.38)	0.010	0.006
	Rye	1.31 (1.13;1.51)	1.09 (0.95;1.25)	1.06 (0.92;1.22)				

Abbreviations: CRP, C-reactive protein; CRP2, C-reactive protein where values >10.00 mg/L has been removed; HOMA-IR, homeostatic model assessment insulin resistance.

^a Model estimates, not adjusted for baseline or weight loss.

^b Derived from model adjusted for baseline and bodyweight change (week 0-12), model estimates and p-values were adjusted for multiple testing using Bonferroni correction. Data are presented as complete case analysis: estimated marginal means ± SEM. Significance level is p > 0.05, marked in bold font.

^c Back transformed from natural logarithm scale.

Lacrimispora_amygdalina, were elevated in the wholegrain rye-group, while *Ruminococcus_torques*, *Romboutsia_timonensis*, *Clostridium_leptum*, *Streptococcus_salivarius*, *Ruthenibacterium_lactatiformans*, *Nitrosopumilus_SGB14899*, *Streptococcus_parasanguinis*, *Candidatus_Woodwardibium_SGB14954*, *Holdemania_filiformis*, *Clostridium_sp_AM49_4BH*, *Clostridiaceae_bacterium_Marseille_Q4149*, *Massiliimalia_massiliensis*, *Anaerofilum*, *Anaerofilum_spBX8*, *Anaerotruncus*, *Anaerotruncus_rubiinfantis* and *Holdemansia* were all reduced in the rye-group compared with the wheat-group (p < 0.05) (Fig. 5). *Agathobacter* and *Oscillospiraceae_UCG-003* were included in the Rye-Weight1 study, but these were not found in the metagenome data in RyeWeight2 and could therefore not be evaluated. The non-significant comparisons from Random Forest modelling are shown in Supplementary Fig. 4.

Gut microbial genus/species/strain at base line did not predict weight loss at 12 weeks (Q² < 0.2). The findings were irrespective if the data were analyzed for the two interventions separately or combined. The results were in concordance with univariate analysis where no model passed the p-value threshold. Regarding enterotyping, both PMM and DMM clustering identified two distinct enterotypes, driven by the genera *Prevotella* and *Bacteroides* (Supplementary Figs. 5-10). However, none of the enterotypes were significantly associated with weight loss in any of the models (p > 0.05).

Microbial communities typically associated with butyrate and acetate production, which have been reported to vary in individuals undergoing weight loss, did not show significant differences in our study (p > 0.05). Additionally, the selected microbial species did not exhibit a positive correlation with butyrate or acetate production (p > 0.05).

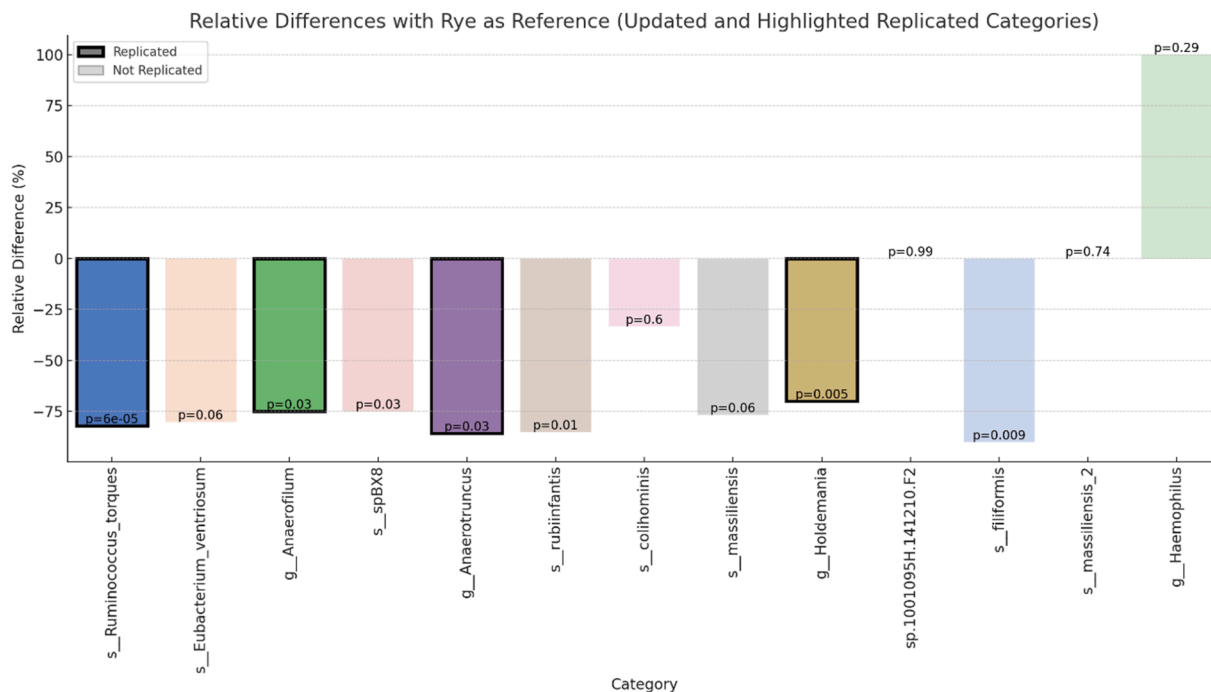


Fig. 4. Difference in relative abundance after 12 weeks (%). Bacteria that differed significantly ($p < 0.05$) between the wholegrain rye and refined wheat-group in the RyeWeight1 study were included, and intervention effects from the RyeWeight2 study presented. Species that showed significant differences at 12 weeks in both studies are highlighted.

3.8.2. SCFA

Plasma butyrate concentrations were 32% ($p = 0.005$) higher at week 6 and 38% ($p = 0.03$) at week 12 in the rye-group compared to the wheat-group (Table 6). Butyrate concentrations were unchanged in the wheat-group, subsequently relative increases in the rye-group were significant at 6 and 12 weeks compared to the wheat-group. Acetate decreased in the wheat-group, while concentrations were stable in rye-group resulting in relatively higher concentrations in the rye-versus wheat-group after 12 weeks of intervention -14% ($p = 0.03$) (Fig. 6).

Acetate concentrations at baseline were inversely correlated with weight ($r = -0.25$, $p < 0.001$) and fat mass ($r = -0.25$, $p < 0.01$) across intervention groups at 12 weeks (Supplementary Table 10). Although, participants with high versus low baseline acetate levels (50:50) lost 1.6 kg more body weight in the rye group ($p < 0.001$), compared to a difference of 0.6 kg in the wheat-group ($p = 0.01$) (Supplementary Table 13). In the wheat-group isovaleric acid showed an inverse correlation with weight ($r = -0.15$, $p = 0.003$) and with fat mass change ($r = -0.23$, $p < 0.001$), while baseline butyrate was positively correlated with body weight ($r = 0.18$, $p = 0.003$) at 12 weeks. Isobutyric acid at baseline was inversely associated with 12-week fasting insulin ($r = -0.31$, $p < 0.001$) and glucose ($r = -0.22$, $p < 0.001$) in the rye-group (Supplementary Table 10).

Increase in succinic acid over 12 weeks was inversely correlated with weight ($r = -0.22$, $p < 0.001$) and fat mass change ($r = -0.23$, $p < 0.001$) in the rye-group (Supplementary Table 11). Additionally, isovaleric acid showed an inverse correlation with body weight ($r = -0.13$, $p = 0.03$) and fat mass change ($r = -0.17$, $p = 0.004$) in the rye-group. Within the wheat-group, none of these associations were present, although increased propionate levels over 12 weeks was inversely correlated with weight change ($r = -0.15$, $p = 0.01$). Increased butyrate was inversely correlated with waist circumference ($r = -0.20$, $p < 0.001$) across diets, while propionic acid ($r = -0.20$, $p < 0.001$) and valeric acid ($r = -0.20$, $p < 0.001$) showed diet specific associations in the rye-group and

wheat-group respectively. More associations with baseline SCFAs and clinical risk markers are shown in Supplementary Tables 10 and 11.

4. Discussion

In the current 12-week RyeWeight2 trial, participants lost body weight and fat mass but with no differences between the intervention groups. This result is in contrast with the recent RyeWeight1 study, where weight loss in the rye-group was comparable to that in the present study, but the refined wheat-group experienced less weight loss [22]. An altered gut microbiota composition, changes in plasma SCFAs and reductions in CRP between rye and wheat-based interventions were found, in concordance with findings from the RyeWeight1 study [49]. Large inter-individual variability in body weight change was observed, with baseline fasting insulin, HOMA-IR, CRP and SCFA identified as potential determinants, showing inverse associations with weight and fat mass changes at 12 weeks, particularly in the wheat-group.

4.1. Body weight and fat mass

Few studies have examined the impact of wholegrain rye versus refined wheat cereals on body weight and to our knowledge, none except for the RyeWeight1 study have been specifically designed to evaluate weight loss as a primary outcome [56–58]. In a 6-week parallel trial, Suhr et al. evaluated effects on gastrointestinal symptoms and gut microbiota and observed a 1.3 kg greater weight loss in the wholegrain rye-group compared to the refined wheat-group [58]. In contrast to the present study, the RyeWeight1 study [22] found a greater reduction in both weight and fat mass in the rye-group compared to the wheat-group, consistent with the weight loss reported by Suhr et al. [58].

Although RyeWeight2 was designed to replicate the conditions of RyeWeight1, there were some differences that may explain the difference in results: Participants in RyeWeight2 were slightly

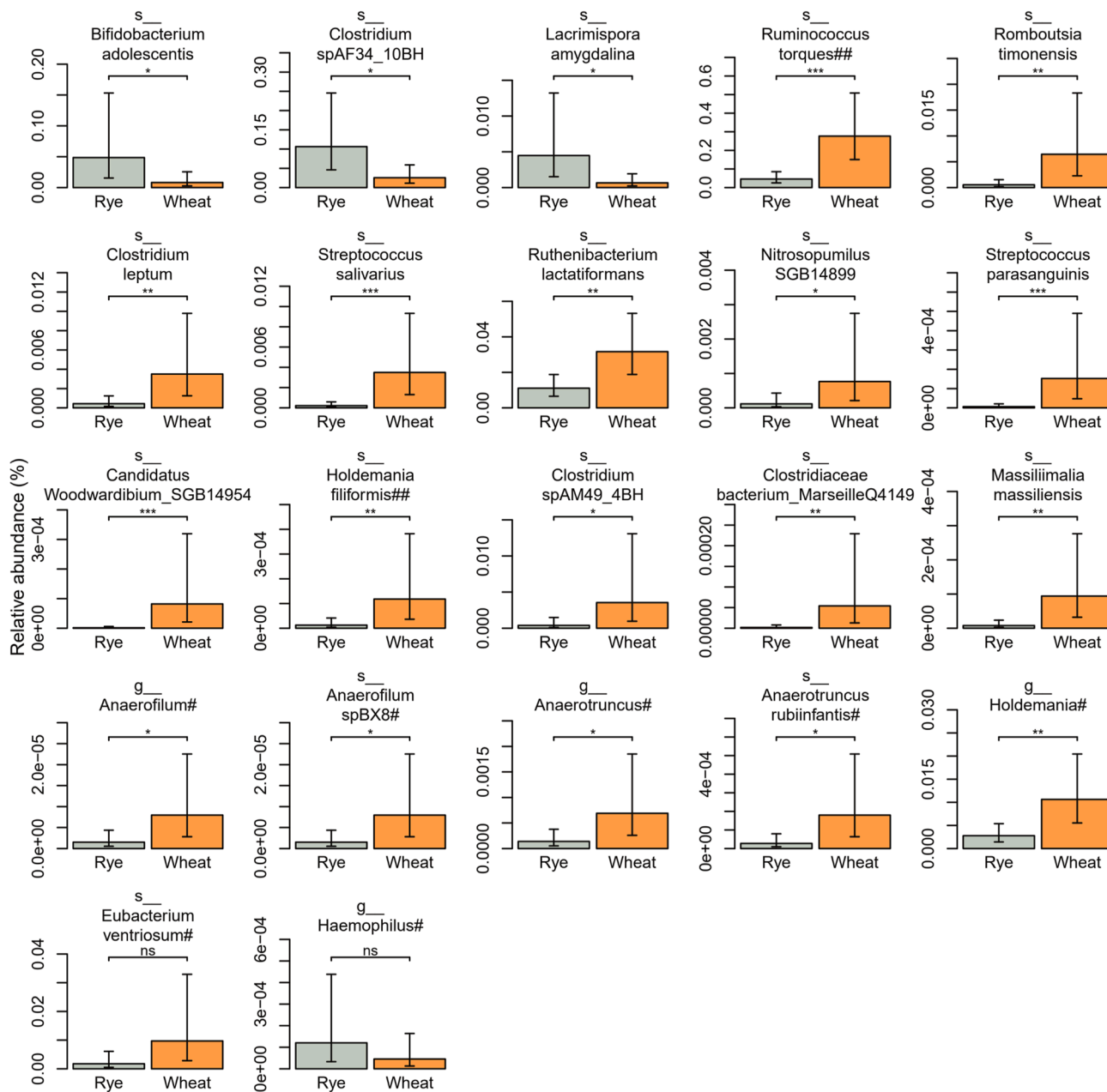


Fig. 5. Bacterial species selected from Random Forest modelling for the wholegrain rye and refined wheat-group and bacterial genera and species that different between the interventions in RyeWeight1 and evaluated in the present RyeWeight2 study are included. Data are presented with estimated marginal means and 95 % confidence interval. Only significant comparisons are presented ($p < 0.05$). p-values are presented with the star system: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. # Bacterial genera and species that differed between rye and wheat in the RyeWeight1 study and evaluated in the present RyeWeight2 study. ## Bacterial species that were both selected in the Random Forest modelling and differed between rye and wheat in the RyeWeight1 study. No */## indicates it was only selected in the Random Forest modelling.

younger (~2 years younger), had lower BMI (~1 kg/m²) and lower CRP (~15 %) (Supplementary Table 12). While participants in the rye-groups showed similar baseline characteristics across studies, wheat-group characteristics differed with HOMA-IR being 19 % lower in the RyeWeight2. This difference is notable in the light of the inverse correlation observed between baseline HOMA-IR and 12-week weight change. Participants in the wheat-group in the RyeWeight2 study with HOMA-IR < 2.0 lost 1 kg more than those with HOMA-IR > 2.0 (Supplementary Table 14). This weight loss difference corresponds to the observed difference between the

intervention groups in the two studies and could partly explain the discrepancy in results. Insulin resistance may impair lipolysis by prolonging postprandial insulin secretion [59,60], an effect potentially amplified in the wheat-group due to higher glucose and maltose release of wheat-based foods [61].

4.2. Clinical risk markers

Interestingly, the observed reduction in CRP in the rye-based diet versus the wheat-group is consistent with reductions found

Table 6
Plasma short chain fatty acids by diet.

		Week 0 ^a	Week 6 ^a	Week 12 ^a	Δbetween groups week 6 ^b	Δbetween groups week 12 ^b
Formic acid	Rye	66.9 (60.1;73.6)	69.9 (63.1;76.6)	69.7 (62.9;76.4)	0.4266	0.9255
	Wheat	66.2 (59.7;72.7)	67.6 (61.0;74.1)	70.1 (63.6;76.6)		
Acetic acid	Rye	60.6 (53.2;68.0)	65.7 (58.3;73.2)	62.2 (54.8;69.5)	0.2126	0.0349
	Wheat	66.5 (59.3;73.7)	64.0 (56.9;71.2)	57.1 (49.9;64.3)		
Propionic acid	Rye	0.60 (0.52;0.68)	0.61 (0.53;0.69)	0.62 (0.54;0.70)	0.6902	0.6035
	Wheat	0.53 (0.46;0.61)	0.58 (0.51;0.66)	0.59 (0.52;0.67)		
Butyric acid	Rye	0.37 (0.30;0.44)	0.49 (0.42;0.56)	0.51 (0.44;0.57)	0.0048	0.0027
	Wheat	0.41 (0.34;0.47)	0.40 (0.33;0.47)	0.42 (0.35;0.48)		
Isobutyric acid	Rye	0.15 (0.14;0.17)	0.16 (0.14;0.18)	0.15 (0.14;0.17)	0.5333	0.6082
	Wheat	0.15 (0.13;0.16)	0.15 (0.13;0.16)	0.15 (0.14;0.17)		
Succinic acid	Rye	2.22 (2.08;2.35)	2.22 (2.08;2.35)	2.27 (2.13;2.40)	0.2131	0.9875
	Wheat	2.30 (2.17;2.43)	2.36 (2.23;2.49)	2.30 (2.17;2.43)		
Valeric acid	Rye	0.05 (0.04;0.06)	0.04 (0.03;0.05)	0.05 (0.04;0.06)	0.5914	0.8679
	Wheat	0.04 (0.03;0.05)	0.05 (0.04;0.05)	0.05 (0.04;0.06)		
Isovaleric acid	Rye	0.51 (0.46;0.57)	0.53 (0.47;0.58)	0.57 (0.51;0.62)	0.8146	0.3538
	Wheat	0.48 (0.42;0.53)	0.51 (0.45;0.56)	0.52 (0.47;0.58)		
Capronic acid	Rye	0.14 (0.11;0.17)	0.16 (0.13;0.19)	0.18 (0.15;0.20)	0.3683	0.7404
	Wheat	0.17 (0.15;0.20)	0.18 (0.16;0.21)	0.18 (0.15;0.20)		

^a Geometric mean and 95 % confidence intervals, not baseline adjusted.

^b Difference between the groups at week 6/week 12 in a baseline adjusted model. Data are presented as complete case analysis. Significance level is $p < 0.05$, significant p -values are marked in bold font. n: rye = 113, wheat = 116.

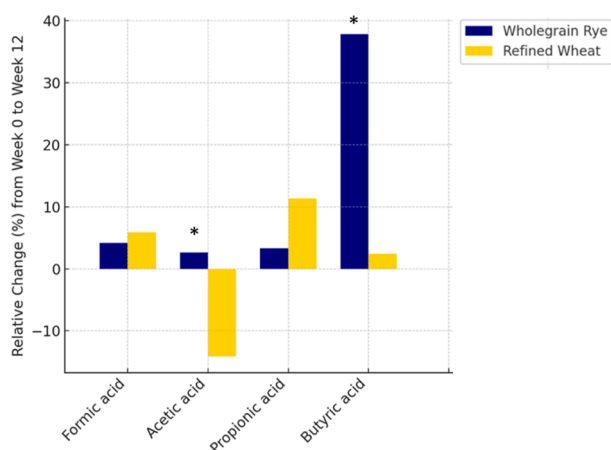


Fig. 6. Relative change (%) in selected short-chain fatty acid concentrations after 12 weeks of intervention in the rye- and wheat-group. *Indicate significant contrasts between intervention groups.

in the RyeWeight1 study, although the effect size was smaller in the current study [22]. These differences may be explained by larger and significant difference in weight loss between rye- and wheat-groups in RyeWeight1, since body weight loss is known as an important determinant of CRP [62]. Few studies have investigated the effects of whole grain rye foods on CRP and results are yet inconclusive [63]. In line with our results, Xue et al. found that CRP levels were lower in overweight participants consuming fermented wholegrain rye compared to those consuming refined wheat bread after 12 weeks [64]. While the absolute CRP concentrations observed in our study were low and within the clinically normal range, even modest reductions in low-grade systemic inflammation may have long-term health benefits [65,66]—particularly if such changes are sustained beyond the 12-week intervention period.

Moreover, in a cross-over study with 17 men with prostate cancer, CRP was lower after 6 weeks on high-fiber whole grain rye foods compared to refined wheat [67]. On the contrary, a 12-week wholegrain rye intervention Kallio et al., did not observe reductions in CRP, although inflammatory markers in adipose tissue

were considered and plasma concentrations of IL-1 β and IL-6 were reduced [68]. Both GlycA and GlycB have been suggested to better reflect inflammation than traditional biomarkers such as CRP and IL-6 [69], but no wholegrain interventions have yet measured these novel inflammatory markers. Observed reductions in GlycA, GlycB and SPC in the current study did not differ between the rye- and wheat-group. The increase in triglycerides at 6 weeks observed in the rye group may reflect a transient metabolic adaptation to increased fiber intake and SCFA production. Similar effects have been reported in high-fiber, high-carbohydrate interventions, potentially due to reduced clearance of VLDL triglycerides [70]. As this change was not sustained at 12 weeks and the magnitude was modest, it is unlikely to be clinically relevant.

When investigating the associations between CRP and GlycA and fat mass loss, an inverse association was found in the wheat-group but not in the rye-group. This indicates that sub-clinical inflammatory status influences participants' ability to reduce body fat mass following the hypocaloric wheat- but not the rye-based diet. This hypothesis is also supported by similar associations with CRP and fat mass loss found in the RyeWeight1 study [22].

4.3. Appetite

Overall, we did not observe any differences in subjective appetite between the wholegrain rye and refined wheat-group. These findings are in line with the results from the RyeWeight1 study. We recently showed good agreement between subjective appetite measures assessed by VAS in free-living participants and participants under controlled clinic-based conditions [71]. No correlations between appetite response and weight loss were found in this study or RyeWeight1, suggesting that self-reported satiety and hunger had little impact on weight loss under the conditions in the two studies. Reasons for that could be that the proportion of intervention foods in these study diets were insufficient to impact appetite sensations [13]. In contrast to most interventions assessing appetite responses to whole-grain rye, which have primarily included normal-weight individuals, our study focused on individuals with overweight or obesity—who are known to have altered appetite regulation [72,73]. This may also have contributed to no apparent relationship between appetite measures and weight loss.

4.4. Gut microbiota and SCFAs

We found several alterations in gut microbiota that have previously been associated with beneficial health outcomes. For example, we found an increase in *Bifidobacterium adolescentis* which is in line with other studies where subjects were provided with galacto-oligosaccharides and inulin [74,75]. In mice, *B. adolescentis* administration reduced fasting and postprandial blood sugar, improved glucose tolerance, and prevented pancreatic damage [76], though its effects in humans have been less explored. In contrast, we found that *Ruthenibacterium lactatiformans* decreased after rye versus wheat-based intervention, which is in line with a previously study where the genus was reduced by a mediterranean diet [77]. A similar effect was found for *Holdemania filiformis* which previously has shown to reduce by intake of berberine [78]. Interestingly, we found that [*Ruminococcus*] *torques* group, *Anaerotruncus*, *Anaerofilum*, and *Holdemania* were reduced in the rye-versus wheat-group and these genera have been associated to negative health outcomes as inflammation, obesity, and increased glucose levels [79–83]. The reduction of *Streptococcus salivarius* and *Streptococcus parasanguinis* in the rye-versus wheat-group is interesting, as both are normal members of the oral microbiome. However, notably, *Streptococcus parasanguinis* detected in the gut has in a large cross-sectional study been associated with subclinical coronary atherosclerosis [84]. The health impact of their reduction in the gut due to rye consumption needs to be further explored.

Observed decrease in [*Ruminococcus*] *torques* group, *Anaerotruncus*, *Anaerofilum*, *Holdemania* and trend of significant decrease in *Eubacterium ventriosum* and increase of *Haemophilus* in the rye-group were in concordance with alterations shown in the RyeWeight1-study where the 16SrRNA-method was used. This successful replication brings strong evidence of rye-related alterations of gut microbiota observed in both studies, despite methodological differences in gut microbiota assessment. Taken together, the effect of wholegrain rye versus refined wheat on microbiota composition appears favorable, since these alterations have been associated to beneficial health outcomes in previous studies.

Notably, our study could not replicate findings from a few previous studies where the genus *Prevotella* predicted weight loss in studies on a diet rich in whole grain and dietary fiber, mainly from rye. However, these were post hoc analyses of small studies from one research group [27,28,85]. We acknowledge that the enterotype concept remains debated, but we included it as an exploratory tool to provide context for overall microbiota patterns in relation to weight loss, since the effects on weight loss of a high fiber cereal food intervention have been attributed to enterotypes. Our study was large and provides robust, replicated results showing that the gut microbiota was not a determinant of weight loss, as suggested in several studies with high-fiber diets [27–29,86]. Instead, other metabolic factors at base line, such as HOMA-IR and weight loss may be of larger importance.

Many studies have shown that colonic microbiota and derived metabolites, particularly SCFAs, play a significant role in glucose metabolism, energy homeostasis and body weight control [15,79]. However, few intervention studies found higher plasma SCFA levels following whole grain intake, but the effects on SCFA levels, as well as the source of whole grains, varies across studies [21,87,88]. Absolut changes in plasma SCFAs observed in the current study were relatively small, but in line with increased acetate and butyrate in the rye-group vs wheat-group reported in the RyeWeight1 study [22]. Vetrani et al. showed increased plasma propionate following 12 weeks of mixed whole grain sources vs refined

cereals in participants with metabolic syndrome [89], while Damen et al. showed increased fecal butyrate and acetate in healthy volunteers (BMI ~ 21) consuming arabinoxylan fortified bread versus refined wheat bread for 12-weeks [90]. However, plasma SCFA levels may have stronger associations with BMI, GLP-1 and insulin sensitivity compared with fecal SCFAs [91]. In contrast to Damen et al., we show increased SCFAs in individuals with overweight and obesity measured in plasma.

In our study, plasma acetate at baseline was associated with weight and fat mass change across both diets over 12 weeks. However, the effect was more pronounced in the rye-group where participants with high vs low (50:50) acetate lost 1.6 kg more body weight (Supplementary Table 13). Acetate is the most abundant SCFA and has been shown to increase energy expenditure and fat oxidation via secretion of the gut hormones affecting appetite, which may mediate weight loss [92,93]. Beyond body weight regulation, circulating acetate and butyrate have also been associated with favorable metabolic profiles, including higher fasting GLP-1 levels and lower fasting glycerol, triglycerides, free fatty acids [91].

To our knowledge, this is the first wholegrain intervention to demonstrate that baseline plasma acetate levels was associated with whole grain intervention induced weight loss in overweight and obese participants. We also investigated whether bacterial species known to produce acetate and butyrate differed across -weight loss strata. The absence of such findings, and lack of correlation between these species and plasma SCFAs in our study may be attributed to the complex, yet poorly understood, mechanisms that have linked SCFA to weight loss in previous studies [94,95].

4.5. Strength and limitations

The RyeWeight2 study has several strengths. Participants were instructed to replace their habitual cereals with a fixed amount of intervention foods, thereby closely mimicking their habitual diet, which may be sustainable post-intervention. The RyeWeight2 study used the same set up as RyeWeight1, which offered unique replication. The RyeWeight1 and 2 studies are the largest intervention studies measuring body composition by DEXA following whole grain and refined wheat-based diets, providing valuable data on changes in body composition. Furthermore, adherence to intervention diets was evaluated by plasma alkylresorcinols, offering an objective measure of compliance.

The study also has some limitations. By design, no samples were taken before the initial 2-week run-in period on refined wheat which represented the baseline. Fecal samples were collected after the initial run-in period which may have affected gut microbiota composition and contaminated our analysis of specific genus, species or enterotypes associated with 12-week body weight changes. The run-in weight-loss requirement may limit generalizability, as about one-third of participants did not meet the threshold. Slightly higher protein intake observed in the wheat-group may affect satiety and subsequent energy intake as protein has shown such properties [96]. The increased gastrointestinal symptoms reported in the rye-group need to be considered when implementing a diet high in wholegrain rye foods. A key limitation is the use of different microbiome sequencing methods (16 S rRNA in RyeWeight1 and shotgun metagenomics in RyeWeight2), which differ in taxonomic resolution and sensitivity to protocol variations such as DNA extraction and library preparation, although all samples were stored under identical conditions at –80 °C. These methodological differences may introduce biases and limit direct comparability between studies. However, employing different methods alongside obtaining similar findings can also be

considered a strength, as it enhances the robustness and validity of the results. A further limitation is that exploratory correlation analyses—specifically those involving baseline clinical markers—and certain gut microbiota analyses were not adjusted for multiple testing. Consequently, these specific results should be interpreted with appropriate caution. Finally, while the imputation method used for missing data in the ITT analyses (LOCF) has known limitations, it was applied to maintain consistency with the RyeWeight1 study.

5. Conclusion

The hypothesis of greater reductions in body weight and fat mass with wholegrain rye versus refined wheat foods as part of a hypocaloric diet could not be confirmed in the present study. This contrasted with results from the RyeWeight1 study and may be due to younger participants with better insulin sensitivity in the wheat-group, achieving greater weight loss in the present study. However, reductions in CRP suggest that wholegrain rye cereals may have beneficial effects on subclinical inflammation compared to refined wheat, shown in both the RyeWeight1 and 2 study. Distinct shifts in gut microbiota composition and plasma SCFAs, with reductions in genera and species linked to negative health outcomes in the rye-group were in concordance with findings of the RyeWeight1 study. Exploratory analysis revealed that gut microbiota did not predict weight loss. However, individuals with higher baseline HOMA-IR, CRP, and plasma acetate showed modest reductions in weight and fat mass, especially in the wheat-based intervention. This suggests that those with elevated inflammation and advancing insulin resistance may benefit more from replacing refined wheat with wholegrain rye foods. Taken together, results from the present study, in line with a previous similar study, show that 12 weeks of rye intake causes positive metabolic effects and changes in microbiota which may have implications for cardiometabolic health.

Author contributions

The authors' responsibilities were as follows—; SÅ; conducted the study and collected the data; SÅ; and KNI: were responsible for study management, planning and execution; RL: was the guarantor of this work and, as such, had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; RL: conceived and designed the study, and critically revised the manuscript for intellectual content; KK: provided laboratory infrastructure for generation of the metagenome data; PQ: Initiating workflow and adaptations for processing of metagenome data; EN: performed the processing of the metagenome data; SÅ;, KNI and EN: was responsible for data curation and statistical analysis; SÅ; and EN: drafted the manuscript; SÅ;, RL, and EN: revised and edited the manuscript; and all authors: critically revised, read, and approved the final manuscript. No one eligible for authorship has been excluded from the list of authors. None of the authors reported a conflict of interest related to the study.

Data availability statement

Data and analytic code supporting this manuscript are available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

No generative AI or AI-assisted technologies were used in the creation of this manuscript. All writing, data analysis, and

interpretation were conducted by the authors without the assistance of AI tools.

Funding statement

This work was funded by Lantmännen ek. Förening and Barilla. Lantmännen and Barilla also provided rye- and wheat intervention products for the study.

Conflict of interest

R.L. is the initiator of the Nordic Rye Forum, which is a Nordic collaboration platform between academia, institutes and enterprises with an interest in rye and health. The Nordic Rye Forum obtains annual fees from industrial partners to fund the activities.

Acknowledgements

The authors thank all participants of this study. We thank study nurses from CTC in Gothenburg as well as Gothenburg University Center for Health and Performance for providing research facilities. We thank Per Hellström as being the responsible medical doctor. We also gratefully acknowledge colleagues at Department of Life Sciences, Division of Food and Nutrition Science at Chalmers University: Thérèse Hjort for her role as the primary study dietician; Karin Larsson for handling of biological samples; Rikard Fristedt for the analysis of short-chain fatty acids. We gratefully acknowledge collaborators at Laboratory of Integrative Biomedicine, Department of Biology, University of Copenhagen; Lelde Berzina for the laboratory preparations for metagenome analysis. The computations were enabled by resources provided by the Swedish National Infrastructure for Computing (SNIC), partially funded by the Swedish Research Council through grant agreement no. 2018-05973.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2026.106618>.

References

- [1] Institute for Health Metrics and Evaluation (IHME). GBD compare. Seattle, WA: IHME, University of Washington. [cited 2024 Jul 17]. The global distribution of health impacts from obesity. Available from: <https://ourworldindata.org/obesity>.
- [2] Brauer M, Roth GA, Aravkin AY, Zheng P, Abate KH, Abate YH, et al. Global burden and strength of evidence for 88 risk factors in 204 countries and 811 subnational locations, 1990–2021: a systematic analysis for the global burden of disease study 2021. *Lancet* 2024 May 18;403(10440):2162–203 [cited 2025 Aug 4]; Available from: <https://www.sciencedirect.com/science/article/pii/S0140673624009334>.
- [3] Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *New England J Med* 2017 Jul 6;377(1):13–27. Available from: <https://www.nejm.org/doi/full/10.1056/NEJMoa1614362>.
- [4] WHO Regional Office for Europe. WHO European regional obesity report 2022. 2022 [cited 2024 Sep 11]. Available from: <http://apps.who.int/bookorders>.
- [5] European Food Safety Authority (EFSA). *Dietary reference values for nutrients summary report. EFSA supporting publications, vol. 14. Wiley; 2017 Dec.*
- [6] Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* 2019;393(10170):434–45. [https://doi.org/10.1016/S0140-6736\(18\)31809-9](https://doi.org/10.1016/S0140-6736(18)31809-9).
- [7] Pol K, Christensen R, Bartels EM, Raben A, Tetens I, Kristensen M. Whole grain and body weight changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled studies. *Am J Clin Nutr* 2013;98(4):872–84.
- [8] Milesi G, Rangan A, Grafenauer S. Whole grain consumption and inflammatory markers: a systematic literature review of randomized control trials. *Nutrients* 2022 Jan 1;14(2) [cited 2024 Aug 6]; Available from: <https://pmc/articles/PMC8778110/>.

- [9] Holl ander PLB, Ross AB, Kristensen M. Whole-grain and blood lipid changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled studies. *Am J Clin Nutr* 2015 Sep 1;102(3):556–72.
- [10] Ying T, Zheng J, Kan J, Li W, Xue K, Du J, et al. Effects of whole grains on glycemic control: a systematic review and dose-response meta-analysis of prospective cohort studies and randomized controlled trials. *Nutr J* 2024 Dec 1;23(1):1–18. cited 2024 Aug 6]; Available from: <https://nutritionj.biomedcentral.com/articles/10.1186/s12937-024-00952-2>.
- [11] Maki KC, Palacios OM, Koecher K, Sawicki CM, Livingston KA, Bell M, et al. The relationship between whole grain intake and body weight: results of meta-analyses of observational studies and randomized controlled trials. *Nutrients* 2019 Jun 1;11(6) [cited 2022 Jan 14]; Available from: <https://pmc/articles/PMC6627338/>.
- [12] Andersson R, Fransson G, Tietjen M, Åman P. Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *J Agric Food Chem* 2009 Mar 11;57(5):2004. –8. [cited 2022 Nov 24]; Available from: <https://pubs.acs.org/doi/full/10.1021/jf801280f>.
- [13] Sanders LM, Zhu Y, Wilcox ML, Koecher K, Maki KC. Effects of whole grain intake, compared with refined grain, on appetite and energy intake: a systematic review and meta-analysis. *Adv Nutr* 2021 Jul 30;12(4):1177. –95. [cited 2022 Mar 14]; Available from: <https://academic.oup.com/advances/article/12/4/1177/6126746>.
- [14] Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science* 2018 Mar 9;359(6380):1151. –6. [cited 2024 Aug 6]; Available from: <https://www.science.org/doi/10.1126/science.aao5774>.
- [15] Byrne CS, Chambers ES, Morrison DJ, Frost G. The role of short chain fatty acids in appetite regulation and energy homeostasis. *Int J Obes (Lond)* 2015 Sep 10;39(9):1331 [cited 2024 Aug 6]; Available from: <https://pmc/articles/PMC4564526/>.
- [16] Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proc Nutr Soc* 2015 Aug 21;74(3):328–36 [cited 2024 Aug 6]; Available from: <https://www.cambridge.org/core/journals/proceedings-of-the-nutrition-society/article/control-of-appetite-and-energy-intake-by-scfa-what-are-the-potential-underlying-mechanisms/A1EFBE12AD6F9838EBE3D7314D1EE1B4>.
- [17] Munch Roager H, Vogt JK, Kristensen M, Hansen LBS, Ibr gger S, Maerkedahl RB, et al. Original article: whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. *Gut* 2019 Jan 1;68(1):83 [cited 2024 Jul 17]; Available from: <https://pmc/articles/PMC6839833/>.
- [18] Lappi J, Saloj rvi J, Kolehmainen M, Mykk nen H, Poutanen K, de Vos WM, et al. Intake of whole-grain and fiber-rich rye bread versus refined wheat bread does not differentiate intestinal microbiota composition in Finnish adults with metabolic syndrome. *J Nutr* 2013 May 1;143(5):648–55.
- [19] Ampatzoglou A, Atwal KK, Maidens CM, Williams CL, Ross AB, Thielecke F, et al. Increased whole grain consumption does not affect blood biochemistry, body composition, or gut microbiology in healthy, low-habitual whole grain consumers. *J Nutr* 2015 Feb 1;145(2):215–21.
- [20] Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2011;5(2):220–30. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3105703&tool=pmcentrez&rendertype=abstract>.
- [21] Costabile A, Klinder A, Fava F, Napolitano A, Fogliano V, Leonard C, et al. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *Br J Nutr* 2008 Jan;99(1):110–20 [cited 2024 Jul 18]; Available from: <https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/wholegrain-wheat-breakfast-cereal-has-a-prebiotic-effect-on-the-human-gut-microbiota-a-doubleblind-placebocontrolled-crossover-study/D497A73F0AFE6E2B0E6C15E87400B2CA>.
- [22] Iversen KN, Carlsson F, Andersson A, Micha lsson K, Langton M, Ris rus U, et al. A hypocaloric diet rich in high fiber rye foods causes greater reduction in body weight and body fat than a diet rich in refined wheat: a parallel randomized controlled trial in adults with overweight and obesity (the RyeWeight study). *Clin Nutr ESPEN* 2021 Oct 1;45:155–69.
- [23] Dent R, McPherson R, Harper ME. Factors affecting weight loss variability in obesity. *Metabolism* 2020 Dec 1;113:154388.
- [24] Dent R, McPherson R, Harper ME. Variability in weight loss in highly compliant women on a controlled dietary regimen. *Obes Res Clin Pract* 1999;7(Suppl.1):98.
- [25] Gerrits MF, Ghosh S, Kavaslar N, Hill B, Tour A, Seifert EL, et al. Distinct skeletal muscle fiber characteristics and gene expression in diet-sensitive versus diet-resistant obesity. *J Lipid Res* 2010 Aug;51(8):2394 [cited 2024 Sep 6]; Available from: <https://pmc/articles/PMC2903798/>.
- [26] Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized nutrition by prediction of glycemic responses. *Cell* 2015 Nov 19;163(5):1079–94.
- [27] Hjorth MF, Roager HM, Larsen TM, Poulsen SK, Licht TR, Bahl MI, et al. Pre-treatment microbial prevotella-to-bacteroides ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. *Int J Obes (Lond)* 2018 Mar 1;42(3):580 [cited 2024 Jul 18]; Available from: <https://pmc/articles/PMC5880576/>.
- [28] Hjorth MF, Bl edel T, Bendtsen LQ, Lorenzen JK, Holm JB, Kiilerich P, et al. Prevotella-to-Bacteroides ratio predicts body weight and fat loss success on 24-week diets varying in macronutrient composition and dietary fiber: results from a post-hoc analysis. *Int J Obes (Lond)* 2019 Jan 1;43(1):149 [cited 2024 Jul 18]; Available from: <https://pmc/articles/PMC6331389/>.
- [29] Christensen L, Vuholm S, Roager HM, Nielsen DS, Krych L, Kristensen M, et al. Prevotella abundance predicts weight loss success in healthy, overweight adults consuming a whole-grain diet Ad libitum: a post hoc analysis of a 6-Wk randomized controlled trial. *J Nutr* 2019 Dec 1;149(12):2174–81.
- [30] Hjorth MF, Ritz C, Blaaq EE, Saris WHM, Langin D, Poulsen SK, et al. Pre-treatment fasting plasma glucose and insulin modify dietary weight loss success: results from 3 randomized clinical trials. *Am J Clin Nutr* 2017 Aug 1;106(2):499–505.
- [31] Nordic Council of Ministers. Nordic nutrition recommendations 2012. Integrating nutrition and physical activity. Nordic Nutrition Recommendations; 2012. p. 1–627. Available from: <https://www.norden.org/en/publications/publikationer/2014-002%5Cnhttp://urn.kb.se/resolve?urn=urn:nbn:se:norden:org:diva-2561>.
- [32] Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010 Mar 24;340(7748):698–702 [cited 2024 Jun 18]; Available from: <https://www.bmj.com/content/340/bmj.c332>.
- [33] Elisabeth Amcoff av, Edberg A, Enghart Barbieri H, Karin Lindroos A, N ls n C, Pearson and Eva Warensj  Lemming M. Livsmedels- och n ringsintag bland vuxna i Sverige, metodrapport, Riksmaten vuxna 2010–11. 2014.
- [34] Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies'2. 1982 [cited 2022 Sep 26]; Available from: <https://academic.oup.com/ajcn/article/36/5/936/4693663>.
- [35] Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, et al. 2011 compendium of physical activities: a second update of codes and MET values. *Med Sci Sports Exerc* 2011 Aug;43(8):1575–81 [cited 2024 Jul 16]; Available from: https://journals.lww.com/acsm-msse/fulltext/2011/08000/2011_compendium_of_physical_activities__a_second.25.aspx.
- [36] Wiklund IK, Fullerton S, Hawkey CJ, Jones RH, Longstreth GF, Mayer EA, et al. An irritable bowel syndrome-specific symptom questionnaire: development and validation. *Scand J Gastroenterol* 2003 Sep 1;38(9):947–54 [cited 2024 Jul 16]; Available from: <https://www.tandfonline.com/doi/abs/10.1080/00365520310004209>.
- [37] Three-factor eating Questionnaire–Revised 21-Item version. <https://psycnet.apa.org/doi/landing?doi=10.1037%2Ft80113-000>.
- [38] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density Lipoprotein Cholesterol in Plasma, Without use of the Preparative Ultracentrifuge. *Clin Chem* 1972;18(6):499 [cited 2024 Apr 25]; Available from: <https://academic.oup.com/clinchem/article/18/6/499/5676160>.
- [39] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diab Care* 2004 Jun 1;27(6):1487–95. <https://doi.org/10.2337/diacare.27.6.1487>.
- [40] Landberg R, Hanhineva K, Tuohy K, Garcia-Aloy M, Biskup I, Llorach R, et al. Biomarkers of cereal food intake. *Gene Nutr* 2019 Oct 12;14(1):1–16 [cited 2024 Jul 30]; Available from: <https://genesandnutrition.biomedcentral.com/articles/10.1186/s12263-019-0651-9>.
- [41] Landberg R, Marklund M, Kamal-Eldin A, Åman P. An update on alkylresorcinols – occurrence, bioavailability, bioactivity and utility as biomarkers. *J Funct Foods* 2014 Mar 1;7(1):77–89.
- [42] Ross AB, Svelander C, Savolainen OI, Lind MV, Kirwan JP, Breton I, et al. A high-throughput method for liquid chromatography–tandem mass spectrometry determination of plasma alkylresorcinols, biomarkers of whole grain wheat and rye intake. *Anal Biochem* 2016 Apr 15;499:1–7.
- [43] Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 2018 Sep 1;34(17). <https://doi.org/10.1093/bioinformatics/bty560>. i884–90. [cited 2024 Aug 15].
- [44] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012 Apr;9(4):357–9 [cited 2024 Aug 15]; Available from: <https://pubmed.ncbi.nlm.nih.gov/22388286/>.
- [45] Beghini F, McIver LJ, Blanco-Mig uez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with biobakery 3. *eLife* 2021 May 1;10.
- [46] Fristedt R, Ruppert V, Trower T, Cooney J, Landberg R. Quantitation of circulating short-chain fatty acids in small volume blood samples from animals and humans. *Talanta* 2024 May 15;272:125743.
- [47] Gabri lsson J, Weiner D. Pharmacokinetic and pharmacodynamic data analysis: concepts and applications. In: Pharmacokinetic and pharmacodynamic data analysis: concepts and applications. 3rd ed. Stockholm, Sweden: Swedish pharmaceutical press; 2000. p. 141–53.
- [48] Shi L, Westerhuis JA, Ros n J, Landberg R, Brunius C. Variable selection and validation in multivariate modelling. *Bioinformatics* 2019 Mar 15;35(6):972–80.
- [49] Iversen KN, Dicksved J, Zoki C, Fristedt R, Pelve EA, Langton M, et al. The effects of high fiber rye, compared to refined wheat, on gut microbiota composition, plasma short chain fatty acids, and implications for weight loss and metabolic risk factors (the RyeWeight study). *Nutrients* 2022 Apr 1;14(8).
- [50] Kaufman Leonard, Peter Rousseeuw J. Finding groups in data: an introduction to cluster analysis. New Jersey: John Wiley & Sons, Inc.; 1990.

- [51] Arumugam M, Raes J, Pelletier E, Paslier D Le, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. *Nature* 2011 May 12;473(7346):174–80.
- [52] Holmes I, Harris K, Quince C. Dirichlet multinomial mixtures: generative models for microbial metagenomics. *PLoS One* 2012 Feb 3;7(2).
- [53] Singh V, Lee GD, Son HW, Koh H, Kim ES, Unno T, et al. Butyrate producers, “the sentinel of gut”: their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics. *Front Microbiol* [Internet] 2023 Jan 12 [cited 2024 Sep 3];13. Available from: <https://pubmed.ncbi.nlm.nih.gov/36713166/>.
- [54] Hosmer J, McEwan AG, Kappler U. Bacterial acetate metabolism and its influence on human epithelia. *Emerg Top Life Sci* 2024;8:1–13. Portland Press Ltd.
- [55] Rey FE, Faith JJ, Bain J, Muehlbauer MJ, Stevens RD, Newgard CB, et al. Dissecting the in vivo metabolic potential of two human gut acetogens. *J Biol Chem* 2010 Jul 16;285(29):22082–90.
- [56] Lappi J, Mykkänen H, Knudsen KEB, Kirjavainen P, Katina K, Pihlajamäki J, et al. Postprandial glucose metabolism and SCFA after consuming wholegrain rye bread and wheat bread enriched with bioprocessed rye bran in individuals with mild gastrointestinal symptoms. *Nutr J* 2014 Nov 4;13(1):1–9 [cited 2024 Sep 2]; Available from: <https://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-13-104>.
- [57] Leinonen KS, Poutanen KS, Mykkänen HM. Rye bread decreases serum total and LDL cholesterol in men with moderately elevated serum cholesterol. *J Nutr* 2000 Feb 1;130(2):164–70.
- [58] Suhr J, Vuholm S, Iversen KN, Landberg R, Kristensen M. Wholegrain rye, but not wholegrain wheat, lowers body weight and fat mass compared with refined wheat: a 6-week randomized study. *Eur J Clin Nutr* 2017;71(8):959–67. 71:8 [Internet]. 2017 Mar 22 [cited 2024 Aug 26]; Available from: <https://www.nature.com/articles/ejcn201712>.
- [59] Röhling M, Martin K, Ellinger S, Schreiber M, Martin S, Kempf K. Weight reduction by the low-insulin-method—A randomized controlled trial. *www.mdpi.com/journal/nutrients*.
- [60] Meyer-Gerspach AC, Cajacob L, Riva D, Herzog R, Drewe J, Beglinger C, et al. Mechanisms regulating insulin response to intragastric glucose in lean and non-diabetic obese subjects: a randomized, double-blind, parallel-group trial. *PLoS One* 2016 Mar 1;11(3) [cited 2024 Sep 29]; Available from: <https://doi.org/10.1371/journal.pone.0147796>.
- [61] Lu J, Hansson H, Johansson DP, Landberg R, Langton M. Microstructure and viscosity of in vitro-digested rye and wheat food products. *Food Hydrocoll* 2024 Apr;109990. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0268005X24002649>.
- [62] Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on C-Reactive protein: a systematic review. *Arch Intern Med* 2007 Jan 8;167(1):31–9 [cited 2024 Oct 2]; Available from: <https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/411497>.
- [63] Milesi G, Rangan A, Grafenauer S. Whole grain consumption and inflammatory markers: a systematic literature review of randomized control trials. *Nutrients* 2022 Jan 1;14(2):374 [cited 2024 May 14]; Available from: <https://www.mdpi.com/2072-6643/14/2/374/htm>.
- [64] Xue K, Liu Y, Iversen KN, Mazidi M, Qu Z, Dong C, et al. Impact of a fermented high-fiber rye diet on *Helicobacter pylori* and cardio-metabolic risk factors: a randomized controlled trial among *Helicobacter pylori*-positive Chinese adults. *Front Nutr* 2021 Jan 15;7:608623 [cited 2024 Sep 13]; Available from: <https://doi.org/10.3389/fnut.2021.608623>.
- [65] Tong KI, Hopstock LA, Cook S. Association of C-reactive protein with future development of diabetes: a population-based 7-year cohort study among Norwegian adults aged 30 and older in the Tromsø study 2007–2016. *BMJ Open* 2023 Sep 1;13(9):e070284 [cited 2025 Aug 7]; Available from: <https://bmjopen.bmj.com/content/13/9/e070284>.
- [66] Kim-Mitsuyama S, Soejima H, Yasuda O, Node K, Jinnouchi H, Yamamoto E, et al. Reduction in hsCRP levels is associated with decreased incidence of cardiovascular events in Japanese hypertensive women but not in men. *Sci Rep* 2020 Dec 1;10(1):1–10 [cited 2025 Aug 7]; Available from: <https://doi.org/10.1038/s41598-020-73905-4>.
- [67] Landberg R, Andersson SO, Zhang JX, Johansson JE, Stenman UH, Adlercreutz H, et al. Rye whole grain and bran intake compared with refined wheat decreases urinary C-peptide, plasma insulin, and prostate specific antigen in men with prostate cancer. *J Nutr* 2010 Dec 1;140(12):2180–6.
- [68] Kallio P, Kolehmainen M, Laaksonen DE, Pulkkinen L, Atalay M, Mykkänen H, et al. Inflammation markers are modulated by responses to diets differing in postprandial insulin responses in individuals with the metabolic syndrome. *Am J Clin Nutr* 2008 May 1;87(5):1497–503.
- [69] Fung E, Chan EYS, Ng KH, Yu KM, Li H, Wang Y. Towards clinical application of GlycA and GlycB for early detection of inflammation associated with (pre)diabetes and cardiovascular disease: recent evidence and updates. *J Inflamm (UK)* 2023 Dec 1;20(1):1–10 [cited 2024 May 8]; Available from: <https://doi.org/10.1186/s12950-023-00358-7>.
- [70] Parks EJ, Krauss RM, Christiansen MP, Neese RA, Hellerstein MK. Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J Clin Invest* 1999;104(8):1087 [cited 2025 Aug 6]; Available from: <https://doi.org/10.1172/JCI7292>.
- [71] Åberg S, Palmnäs-Bédard M, Karlsson T, Hjorth T, Iversen KN, Landberg R. Evaluation of subjective appetite assessment under free-living vs. controlled conditions: a randomized crossover trial comparing whole-grain rye and refined wheat diets (VASA-Home). *Nutrients* 2023 Jun 1;15(11):2456 [cited 2023 Jun 30]; Available from: <https://www.mdpi.com/2072-6643/15/11/2456/htm>.
- [72] Dalton M, Finlayson G, Esdaile E, King N. Appetite, satiety, and food reward in obese individuals: a behavioral phenotype approach. *Curr Nutr Rep* 2013;2(4):207–15.
- [73] Blundell JE, Finlayson G. Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption? *Physiol Behav* 2004 Aug 1;82(1):21–5.
- [74] Looijesteijn E, Schoemaker MH, van den Belt M, Hester ER, Kortman GAM, Viskaal-van Dongen M, et al. A double-blind intervention trial in healthy women demonstrates the beneficial impact on bifidobacterium with low dosages of prebiotic galacto-oligosaccharides. *Front Nutr* 2024 Aug 19;11:1440319 [cited 2024 Sep 3]; Available from: <https://doi.org/10.3389/fnut.2024.1440319>.
- [75] Birkeland E, Gharagozlian S, Birkeland KI, Valeur J, Måge I, Rud I, et al. Prebiotic effect of inulin-type fructans on faecal microbiota and short-chain fatty acids in type 2 diabetes: a randomised controlled trial. *Eur J Nutr* 2020 Oct 1;59(7):3325–38 [cited 2024 Sep 3]; Available from: <https://doi.org/10.1007/s00394-020-02407-3>.
- [76] Wang G, Si Q, Yang S, Jiao T, Zhu H, Tian P, et al. Lactic acid bacteria reduce diabetes symptoms in mice by alleviating gut microbiota dysbiosis and inflammation in different manners. *Food Funct* 2020 Jul 22;11(7):5898–914 [cited 2024 Sep 17]; Available from: <https://pubs.rsc.org/en/content/articlehtml/2020/fo/c9fo02761k>.
- [77] Meslier V, Laiola M, Roager HM, De Filippis F, Roume H, Quinquis B, et al. Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* 2020 Jul 1;69(7):1258. –68. Available from: <https://doi.org/10.1136/gut-2019-037587>.
- [78] Zhang Y, Gu Y, Ren H, Wang S, Zhong H, Zhao X, et al. Gut microbiome-related effects of berberine and probiotics on type 2 diabetes (the PRE-MOTE study). *Nat Commun* 2020;11(1):1–12. 11:1 [Internet]. 2020 Oct 6 [cited 2024 Sep 3]; Available from: <https://doi.org/10.1038/s41467-020-18414-8>.
- [79] Wiredu Ocansey DK, Hang S, Yuan X, Qian H, Zhou M, Valerie Olovo C, et al. The diagnostic and prognostic potential of gut bacteria in inflammatory bowel disease. *Gut Microbes* 2023;15. Taylor and Francis Ltd.
- [80] Schaus SR, Vasconcelos Pereira G, Luis AS, Madlambayan E, Terrapon N, Ostrowski MP, et al. Ruminococcus torques is a keystone degrader of intestinal mucin glycoprotein, releasing oligosaccharides used by Bacteroides thetaiotaomicron. *mBio* 2024 Jul 8.
- [81] Dai H, Hou T, Wang Q, Hou Y, Zhu Z, Zhu Y, et al. Roles of gut microbiota in atrial fibrillation: insights from Mendelian randomization analysis and genetic data from over 430,000 cohort study participants. *Cardiovasc Diabetol* 2023 Dec 1;22(1) [cited 2024 Sep 1]; Available from: <https://doi.org/10.1186/s12933-023-01639-4>.
- [82] Bailén M, Bressa C, Martínez-López S, González-Soltero R, Montalvo Lominchar MG, San Juan C, et al. Microbiota features associated with a high-fat/low-fiber diet in healthy adults. *Front Nutr* 2020 Dec 18;7.
- [83] Latorre-Pérez A, Hernández M, Iglesias JR, Morán J, Pascual J, Porcar M, et al. The Spanish gut microbiome reveals links between microorganisms and Mediterranean diet. *Sci Rep* 2021 Dec 1;11(1).
- [84] Sayols-Baixeras S, Dekkers KF, Baldanzi G, Jönsson D, Hammar U, Lin YT, et al. Streptococcus species abundance in the gut is linked to subclinical coronary atherosclerosis in 8973 participants from the SCAPIS cohort. *Circulation* 2023 Aug 8;148(6):459–72 [cited 2024 Oct 3]; Available from: <https://doi.org/10.1161/CIRCULATIONAHA.123.063914>.
- [85] Christensen L, Vuholm S, Roager HM, Nielsen DS, Krych L, Kristensen M, et al. Prevotella abundance predicts weight loss success in healthy, overweight adults consuming a whole-grain diet Ad libitum: a post hoc analysis of a 6-Wk randomized controlled trial. *J Nutr* 2019 Dec 1;149(12) [cited 2023 Apr 25];2174–81. Available from: <https://doi.org/10.1093/ajph/113/12/2174>.
- [86] Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. *Int J Obes (Lond)* 2017 Jul 1;41(7):1099 [cited 2024 Oct 9]; Available from: <https://doi.org/10.1038/s41325-017-0185-5>.
- [87] So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, et al. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *Am J Clin Nutr* 2018 Jun 1;107(6):965–83.
- [88] Oliver A, Chase AB, Weihe C, Orchanian SB, Riedel SF, Hendrickson CL, et al. High-fiber, whole-food dietary intervention alters the human gut microbiome but not fecal short-chain fatty acids. *mSystems* 2021 Apr 27;6(2) [cited 2024 Aug 7]; Available from: <https://doi.org/10.1128/mSystems.00115-21>.
- [89] Vetrani C, Costabile G, Luongo D, Naviglio D, Rivellese AA, Riccardi G, et al. Effects of whole-grain cereal foods on plasma short chain fatty acid concentrations in individuals with the metabolic syndrome. *Nutrition* 2016 Feb 1;32(2):217–21.
- [90] Damen B, Cloetens L, Broekaert WF, François I, Lescoart O, Trogh I, et al. Consumption of breads containing in situ-produced arabinoxylan oligosaccharides alters gastrointestinal effects in healthy Volunteers. *J Nutr* 2012 Mar 1;142(3):470–7.

- [91] Müller M, Hernández MAG, Goossens GH, Reijnders D, Holst JJ, Jocken JWE, et al. Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. *Sci Rep* 2019;9(1): 1–9. 9:1 [Internet]. 2019 Aug 29 [cited 2024 Aug 7];Available from: <https://www.nature.com/articles/s41598-019-48775-0>.
- [92] Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients* 2019 Aug 1;11(8) [cited 2024 Aug 7];Available from: [pmc/articles/PMC6723943/](https://pubmed.ncbi.nlm.nih.gov/31111111/).
- [93] Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* 2014;5(1):1–11. 5:1 [Internet]. 2014 Apr 29 [cited 2024 Aug 7];Available from: <https://www.nature.com/articles/ncomms4611>.
- [94] Ilyés T, Silaghi CN, Crăciun AM. Diet-related changes of short-chain fatty acids in blood and feces in obesity and metabolic syndrome. *Biology (Basel)* 2022 Nov 1;11(11) [cited 2024 Oct 1];Available from: [pmc/articles/PMC9687917/](https://pubmed.ncbi.nlm.nih.gov/3687917/).
- [95] Wen X, Zhang B, Wu B, Xiao H, Li Z, Li R, et al. Signaling pathways in obesity: mechanisms and therapeutic interventions. *Signal Transduct Targeted Ther* 2022;7(1):1–31. 7:1 [Internet]. 2022 Aug 28 [cited 2024 Oct 1];Available from: <https://www.nature.com/articles/s41392-022-01149-x>.
- [96] Hill AJ, Blundell JE. Macronutrients and satiety: the effects of a high-protein or high-carbohydrate meal on subjective motivation to eat and food preferences. *Nutr Behav* 1986;3(2):133–44.