
# Effects of whole-grain wheat, rye, and lignan supplementation on cardiometabolic risk factors in men with metabolic syndrome: A

# Effects of whole-grain wheat, rye, and lignan supplementation on cardiometabolic risk factors in men with metabolic syndrome: a randomized crossover trial

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## ABSTRACT

**Background:** A whole-grain (WG)–rich diet has shown to have potential for both prevention and treatment of the metabolic syndrome (MetS), which is a cluster of risk factors that increase the risk of type 2 diabetes and cardiovascular disease. Different WGs may have different health effects. WG rye, in particular, may improve glucose homeostasis and blood lipids, possibly mediated through fermentable dietary fiber and lignans. Recent studies have also suggested a crucial role of the gut microbiota in response to WG.

**Objectives:** The aim was to investigate WG rye, alone and with lignan supplements [secoisolariciresinol diglucoside (SDG)], and WG wheat diets on glucose tolerance [oral-glucose-tolerance test (OGTT)], other cardiometabolic outcomes, enterolignans, and microbiota composition. Moreover, we exploratively evaluated the role of gut microbiota enterotypes in response to intervention diets.

**Methods:** Forty men with MetS risk profile were randomly assigned to WG diets in an 8-wk crossover study. The rye diet was supplemented with 280 mg SDG at weeks 4–8. Effects of treatment were evaluated by mixed-effects modeling, and effects on microbiota composition and the role of gut microbiota as a predictor of response to treatment were analyzed by random forest plots.

**Results:** The WG rye diet (± SDG supplements) did not affect the OGTT compared with WG wheat. Total and LDL cholesterol were lowered (−0.06 and −0.09 mmol/L, respectively; $P < 0.05$) after WG rye compared with WG wheat after 4 wk but not after 8 wk. WG rye resulted in higher abundance of *Bifidobacterium* [fold-change (FC) = 2.58, $P < 0.001$] compared with baseline and lower abundance of *Clostridium* genus compared with WG wheat (FC = 0.54, $P = 0.02$). The explorative analyses suggest that baseline enterotype is associated with total and LDL-cholesterol response to diet.

**Conclusions:** WG rye, alone or with SDG supplementation, compared with WG wheat did not affect glucose metabolism but caused transient LDL-cholesterol reduction. The effect of WG diets appeared to differ according to enterotype. This trial was registered at www.clinicaltrials.gov as NCT02987595. *Am J Clin Nutr* 2020;111:864–876.

**Keywords:** whole grain, rye, wheat, lignan, glucose-tolerance test, cardiometabolic, lipid profile, enterotype

## Introduction

Metabolic syndrome (MetS) is characterized by obesity, insulin resistance, hypertension, dysglycemia, and dyslipidemia and an increased risk of type 2 diabetes (T2D) (1), cardiovascular disease (CVD), and mortality (2). Diet is a key risk factor for

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Supplemental Figures 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Data described in the manuscript can be made available upon request.

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Abbreviations used: BEE, basal energy expenditure; cr, classification rate; CVD, cardiovascular disease; MetS, metabolic syndrome; MUVR, Multivariate Methods with Unbiased Variable Selection in R; OGTT, oral-glucose-tolerance test; OTU, operational taxonomic unit; P, percentile; SDG, secoisolariciresinol diglucoside; T2D, type 2 diabetes; WG, whole-grain.

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MetS (3). Cereals represent the major energy and dietary fiber source in the Western diet. While cereals are often consumed as refined grain, the preferred form from a health perspective is whole grains (WGs).

WGs are generally rich in vitamins, minerals, fatty acids, dietary fiber, and phytochemicals such as lignans, but there is large variation within and between different grains (4). Observational studies have consistently shown inverse associations for a high intake of WGs and the development of T2D, CVD, and mortality (5). Underlying mechanisms are not fully elucidated, but WGs may mediate benefits through improving glucose/insulin homeostasis (6), blood pressure, lipid profiles, and body-weight regulation (5) related to the content, composition, and physiochemical properties of dietary fiber and associated bioactive compounds. Dietary fiber causing high digesta viscosity leads to lower postprandial glucose and insulin response by delayed gastric emptying and improved lipid profile (7). Moreover, dietary fiber provides substrate for many gut bacteria for the production of SCFAs and other metabolites that may link gut microbiota with improved health outcomes (8). WG rye is especially rich in dietary fiber and has been shown to improve glycemic and insulin responses and lipid profile in both acute and long-term randomized trials (9), whereas no blood lipid–lowering effects have been observed for WG wheat. Comparisons between WG rye and WG wheat on health outcomes have rarely been made (10, 11), despite apparent differences in content of total and fermentable dietary fiber and bioactive compounds such as lignans.

Diversity and composition of gut microbiota have been associated with diseases such as MetS, T2D, and CVD (12) and may be modulated by diet (13). It has been suggested that individuals can be grouped according to enterotypes (i.e., strata based on characteristic microbiota composition): the 2 most frequently reported enterotypes are dominated by *Prevotella* and *Bacteroides,* respectively (14, 15). A recent study found that gut microbiota composition modified postprandial glucose response following a barley-kernel fiber-rich bread intervention (16). However, it remains unknown whether baseline profile of gut microbiota could modify effects on metabolic risk factors such as blood lipid profiles after a WG rye compared with a WG wheat diet.

We conducted a randomized crossover trial to investigate the impact of WG rye compared with WG wheat diets on glucose tolerance as the primary endpoint and related cardiometabolic risks and gut microbiota as secondary outcomes in men with signs of MetS. Furthermore, we evaluated whether high amounts of plant lignans in the form of secoisolariciresinol diglucoside (SDG) added to the WG rye diet after 4 wk had metabolic benefits on primary and secondary outcomes. Finally, we performed an exploratory investigation of whether the gut microbiota at baseline was associated with individual metabolic response to the different intervention arms.

## Methods

### Study population

Study participants ($n = 49$ men aged 49–74 y) were recruited between June 2015 and March 2016 from Uppsala county, Sweden, through advertisements in local newspapers, the Internet, and through direct invitation of subjects with matching inclusion criteria from the Uppsala part of the EpiHealth cohort (17). The study was conducted at the Clinical Nutrition Laboratory at Uppsala University, located at the Uppsala University Hospital. The study participants were screened based on a physical examination that included anthropometry (weight, height, and waist circumference), blood pressure (performed seated after a 5-min rest), routine clinical tests (blood lipids, liver enzymes, hemoglobin, glucose, insulin, C-reactive protein), and lifestyle and medical history questionnaires. Approval of participation was granted by the medical doctor responsible for the screening. Eligible participants, who had ≥2 of the following MetS risk factors, were included in the study: waist circumference ≥102 cm, serum triglycerides ≥1.7 mmol/L, HDL cholesterol <1.03 mmol/L, blood pressure ≥130/85 mmHg, or fasting plasma glucose ≥5.6 mmol/L (18). Participants were excluded based on the following: use of lipid lowering medication, smoking and/or snuffing, high physical activity level (>7 h/wk), excessive alcohol consumption (>21 units/wk), use of antibiotics within the previous 6 mo, chronic diseases including type 1 and 2 diabetes, CVD (except for high blood pressure), and cancers diagnosed within the preceding 5 y. One participant was included despite use of lipid-lowering medication by mistake. A sensitivity analysis without this subject was performed, and since results did not change, this subject was included in the analyses. All participants gave written informed consent at the screening visit after obtaining oral and written information about the study. The study was approved by the Regional Ethical Committee in Uppsala (Dnr 2015/098) and registered at clinical trials.gov (NCT02987595). All study procedures were conducted in accordance with the Helsinki Declaration.

### Recruitment and screening

All volunteers who responded to the study advertisements were contacted and received information about the study design and procedures through a telephone screening. For those who were potentially eligible, a screening appointment was scheduled, including a health screen, fasting (12-h) blood sampling, and questionnaire information regarding medical history and bowel function as well as physical activity and dietary habits to assess eligibility. In total, 107 men were screened, of whom 25 were excluded based on the screening results, and 33 lost interest in the study after they received detailed information of the study design; 49 men were thus enrolled (**Figure 1**). Nine participants dropped out of the study due to lack of time or motivation, the prescribed high amount of products, or stomachache.

### Study design

The study was a randomized crossover trial (**Figure 2**) where a WG rye diet was evaluated against a WG wheat diet during 8-wk periods separated by an 8-wk washout period. During the last 4 wk of the WG rye diet, SDG lignan capsules were added to the diet. Study participants were randomly assigned to intervention sequence (wheat-rye or rye-wheat) by mixed-block randomization (size 2 or 4) continuously during recruitment by the same investigator. All staff involved in analyses of outcome variables were blinded to the intervention allocation. Participants

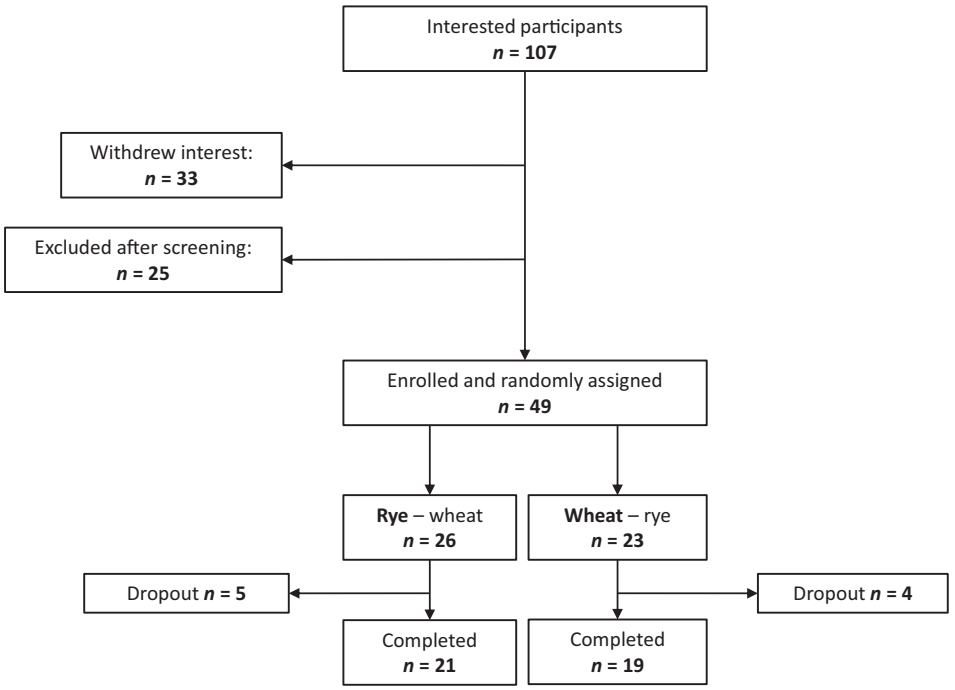


**FIGURE 1** Flowchart of study subjects.

were not blinded since the appearance of the test products revealed the intervention diet sequence. Test products were given to replace the habitually consumed cereal products, but otherwise, participants were instructed to maintain their usual diet.

The primary outcome of the study was the difference in 2-h oral-glucose-tolerance test (OGTT) between the 2 interventions. Secondary outcomes included differences in total cholesterol, HDL and LDL cholesterol, triglycerides, systolic and diastolic blood pressure, BMI, and enterolignans between the interventions after 4 wk (rye compared with wheat) and 8 wk (rye + SDG supplementation compared with wheat). Furthermore, using an exploratory approach, we investigated differences in gut microbiota composition and fecal SCFAs between diets. Participants stratified into 3 enterotypes based on gut microbiota composition at baseline and effects of intervention were investigated across enterotype subgroups.

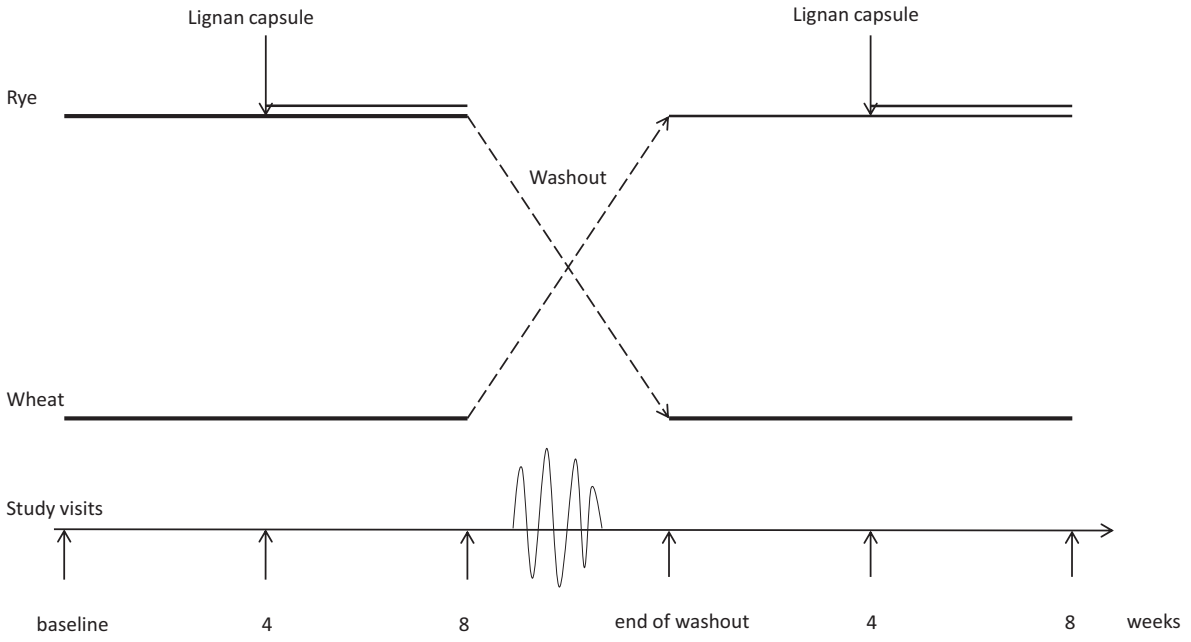


**FIGURE 2** Crossover study design.

Sample size was calculated based on effects estimated by means of a paired 2-sided *t* test based on the assumption of no crossover effect or interaction between participants, treatments, and periods. In order to detect an 8% difference with assumed SEs similar to previous observed effects on 2-h blood glucose after OGTT (19) with a 2-tailed $\alpha$ error of 5% and a statistical power of 90%, a sample size of $n = 40$ was needed. To account for an expected 20% dropout rate, the aim was to recruit 50 participants.

### Study procedures

At each study visit, participants were instructed to be fasting from 20:00 on the previous night and to avoid alcohol and strenuous exercise for the past 24 h. All visits were scheduled in the morning beginning with weight and waist circumference measurements and a fasting blood sample. Then, a 75-g OGTT was carried out, after which blood samples were collected by trained nurses at 30, 60, and 120 min. Each participant was examined 6 times: at baseline and 4, 8, 16 (after washout), 20, and 24 wk after baseline (Figure 2). On each sampling occasion, 38 mL fasting blood was collected in 4 EDTA-coated tubes (7 mL each), 1 PAXgene tube (7 mL), and 1 heparin tube (3 mL). Blood plasma was separated from erythrocytes and buffy coat by centrifugation at 4°C for 10 min at 2500 × *g* and placed into 2-mL screw-cap microtube aliquots (Sarstedt). Twenty-four-hour urine samples were collected in 3-L containers on the day prior to each study visit. Participants were instructed to add 10 mL nitric acid in the container at first urination to prevent bacterial growth. At each study visit, the total amount of urine was noted and 5 samples of 2 mL were frozen for analysis. Furthermore, each participant collected a fecal sample prior to every study visit. Fecal collection kits and instructions were provided, and the samples were kept in a freezer bag at −18°C in the home freezer until they were brought to the clinic. All samples were stored at −20°C for ∼1 wk and then transferred to −80°C storage until analysis.

### Intervention products

The test diets were designed to contribute 30% of daily energy intake. This was determined based on age, weight, and physical activity level and the equation for calculating basal energy expenditure (BEE) from the Nordic Nutrition recommendation (20). For a 50-y-old participant of 90 kg with low physical activity level (PAL) (1.4) the corresponding energy intake was calculated as BEE of $0.0485 \times 90\ \text{kg} + 3.67 \times 1.4 = 11.25$ MJ/d. Intervention products for this participant therefore contributed 11.25 MJ/d × 239 kcal/MJ × 0.3 = 807 kcal/d. Treatment diets were isocaloric and contained 100% WG. To obtain similar dietary fiber contents in the 2 diets (∼30 g/d), additional wheat bran was added to one of the WG wheat products (WG puffs; see below). Thus, diets differed only in dietary fiber quality and lignan content (**Table 1**).

WG products included breakfast cereals, rolled cereals (e.g., porridge), crisp bread, and pasta and included both commercially available products from the supermarket and products specially developed for this study. Lantmännen developed 100% WG wheat (with 17% added wheat bran) and rye puffs, and Wasabröd/Barilla Sweden developed a wheat crisp bread product. Conventional products included rolled rye and wheat contributed by Lantmännen, a rye crisp bread by Wasabröd/Barilla, and wheat and rye pasta from Il Fornaio.

In previous interventions with flaxseed lignans, doses of ∼300 mg/d were applied for evaluating effects on blood glucose

**TABLE 1** Nutritional composition of rye and wheat intervention food products and SDG lignan capsules for a randomized crossover trial[1]

| | Whole-grain rye products | | | | Whole-grain wheat products | | | |
|---|---|---|---|---|---|---|---|---|
| Content/100 g product | Pasta | Extruded puffs | Crisp bread | Rolled rye | Pasta | Extruded puffs | Crisp bread | SDG capsules, μg/capsule |
| Total energy, kJ | 1605 | 1558 | 1577 | 1557 | 1636 | 1545 | 1733 | NA |
| Total energy, kcal | 382 | 371 | 377 | 371 | 390 | 368 | 412 | NA |
| Carbohydrate, g | 74.7 | 70.0 | 71.2 | 69.5 | 70.0 | 60.4 | 64.7 | 0.5 |
| Fat, g | 2.2 | 2.2 | 1.5 | 2.2 | 3.4 | 3.6 | 8.5 | NA |
| Protein, g | 8.6 | 9.0 | 9.0 | 8.6 | 13.5 | 13.5 | 13.7 | NA |
| Total dietary fiber,[2] g | 13.4 | 16.8 | 16.5 | 18.3 | 11.4 | 19.4 | 10.9 | NA |
| Soluble fiber,[3] g | 5.7 | 6.9 | 6.7 | 7.4 | 2.6 | 3.1 | 1.9 | NA |
| Insoluble fiber, g | 7.8 | 10.0 | 10.3 | 10.9 | 8.8 | 16.3 | 9.0 | NA |
| Fructans, g | 2.2 | 3.9 | 2.5 | 3.0 | 1.2 | 1.6 | 0.5 | NA |
| Arabinoxylans, g | 2.4 | 2.6 | 3.0 | 3.0 | 2.1 | 3.6 | 2.0 | NA |
| Total lignans, μg | 4231 | 2250 | 2755 | 3542 | 791 | 2238 | 1654 | 75,100 |
| Total free lignans, μg | 660 | 173 | 254 | 618 | 132 | 237 | 171 | 70,500 |
| Secoisolariciresinol, μg | 39 | 21 | 30 | 32 | 43 | 111 | 49 | 73,000 |
| Isolariciresinol, μg | 88 | 47 | 62 | 55 | 18 | 95 | 25 | 1350 |
| Matairesinol, μg | 40 | 17 | 27 | 36 | 1 | 6 | 2 | 40 |
| Pinoresinol, μg | 72 | 34 | 71 | 49 | 15 | 34 | 14 | 40 |
| Syringaresinol, μg | 3505 | 1865 | 2164 | 2982 | 533 | 1335 | 1320 | 40 |
| Mediresinol, μg | 248 | 150 | 255 | 212 | 127 | 417 | 182 | 2 |
| Lariciresinol, μg | 240 | 116 | 146 | 177 | 54 | 240 | 62 | 500 |

[1] Values are means of 2 measurements. NA, not applicable; SDG, secoisolariciresinol diglucoside.
[2] The sum of total dietary fiber determined by the Uppsala method with inclusion of fructans (21).
[3] The sum of soluble dietary fiber determined by the Uppsala method with inclusion of fructans (21).

(22, 23). For the current study, a dose of 280 mg/d (purity 20%) of SDG lignans isolated from flaxseed hulls (product name: Lignan Extract) was provided by Source Naturals, Threshold Enterprises Ltd. We determined the exact amount in the product, which corresponded to the prescribed 70 mg SDG/capsule, but trace amounts of other lignans (lar, iso, etc.) were measured when analyzing the capsules by LC-MS/MS (Table 1). The product was commercially available as a dietary supplement.

### Dietary assessment, questionnaires, and assessment of compliance

At the baseline visit, participants completed a Swedish food-frequency questionnaire (24). Furthermore, participants were instructed to complete weighed 3-d dietary records prior to each study visit. Diet compliance was evaluated from personal checklists (for each 4-wk period), stating the prescribed amount of each type of test product. Furthermore, compliance was evaluated by plasma concentrations of alkylresorcinols, which are compounds found in WG rye and wheat and therefore used as biomarkers of WG intake. The alkylresorcinol homologs C17:0 and C21:0 are associated with the type of WG intake. The sum of the homologs reflects total WG intake, whereas the ratio reflects proportion of WG rye to WG wheat (25). Alkylresorcinols in plasma were analyzed at Chalmers Mass Spectrometry Infrastructure using a well-established LC-MS/MS method (26). Compliance to SDG capsules was evaluated by plasma secoisolariciresinol (method described below).

### Clinical measurements

Clinical blood measurements (glucose; insulin; LDL, HDL, and total cholesterol; and triglycerides) were analyzed at the clinical laboratory at Uppsala University Hospital, Uppsala, Sweden. CVs for all assays were $<10\%$.

### Lignans and enterolignans

Enterolignan concentrations in plasma samples (27) and lignans in WG products and capsules (28) were measured by LC-MS/MS on a microLC 200 series from Eksigent/AB Sciex and QTrap 5500 mass spectrometer from AB Sciex at Aarhus University, Department of Animal Science.

### Gut microbiota

DNA was isolated from the collected fecal samples using QIAamp DNA Stool Mini kit (Qiagen) according to the protocol with an additional bead-beating step in order to increase efficiency in lysis of bacterial cell walls. The bead beating was carried out with 0.1-mm Silica/Zirconia beads for $2 \times 45$ s using a Precellys homogenizer (Bertin Technologies). The purified DNA was stored at $-20^{\circ}$C until analysis. 16S ribosomal RNA gene amplicon libraries were generated from the V3-V4 region of the 16S gene using the primers 341f and 805r (29), and the libraries were sent for Illumina Miseq sequencing at the National Genomics Infrastructure hosted by Science for Life Laboratory in Stockholm.

The bioinformatics data processing was performed as described previously by Müller et al. (30) and was performed using Quantitative Insights into Microbial Ecology (QIIME) (31). The final operational taxonomic units (OTUs) table was further filtered to include only OTUs present in $\geq 3$ samples, and each sample was subsampled to contain equal numbers of sequences (5300 sequences/sample). For an exploratory investigation into potential associations between enterotypes and treatment outcomes, we employed a pragmatic enterotyping scheme in which study participants were subdivided (14) depending on their baseline microbiota composition into a high-*Prevotella* group (ratio of *Prevotella* genus $\geq 0.20$; $n = 10$), a high-*Bacteroides* group (ratio of *Bacteroides* genus $\geq 0.35$; $n = 12$), and a low–*Prevotella* and *Bacteroides* group ($n = 18$) (see **Figure 3**). The latter low group was characterized by a high proportion of *Firmicutes* in line with previous results (14, 32) and is therefore referred to as the *Firmicutes* group. In contrast to others (33) we did not observe a bimodal distribution and therefore could not use cutoff values based on such conditions (14, 33). Instead, we selected cutoff values from an iterative process where different cutoff values were investigated against effects on clinical outcomes (LDL cholesterol) and where the outcome of the settings was monitored by log proportion of *Prevotella* plotted against log proportion of *Bacteroides*. The selected cutoff values resulted in clear separation into 3 enterotypes (**Supplemental Figure 1**). Our approach is highly explorative and needs to be verified in larger study settings.

### SCFAs in fecal samples

SCFAs (acetate, propionate, and butyrate) were analyzed in fecal samples by GC-MS according to a method described elsewhere (34). Briefly, 20 mg fecal sample was homogenized in 4 mL water along with metal beads for 5 min at 2000 $\times$ *g* and 4ºC in a vortex mixer [VWR, Darmstadt, Germany (DVX-2500)]. A total volume of 400 $\mu$L of the supernatant was transferred to an Eppendorf tube and mixed with an internal standard solution mixed with metaphosphoric acid. The extract was washed with propylformate, and the solution was centrifuged at 4°C at 16,000 $\times$ *g* for 15 min. A total volume of 150 $\mu$L of the upper phase was pipetted to a GC vial and analyzed in random order along with blanks and reference standards for the standard curve. The sample was injected on a Shimadzu GC-MSTQ8030. SCFAs were separated on a 30-m $\times$ 0.25-μm Zebron ZB-FFAP (Phenomenex, Torrance, USA) column using helium as carrier gas. The temperature program started at 40°C and was ramped in a gradient up to 250°C. Acetate, propionate, and butyrate were quantified in single-ion monitoring mode. The analysis was conducted at Chalmers.

### Statistical analyses

Statistical evaluation of the effect of treatment on glucose, insulin, lipids (total, HDL and LDL cholesterol, triglycerides), BMI, waist circumference, blood pressure (systolic and diastolic), and enterolignans was performed by mixed-effects

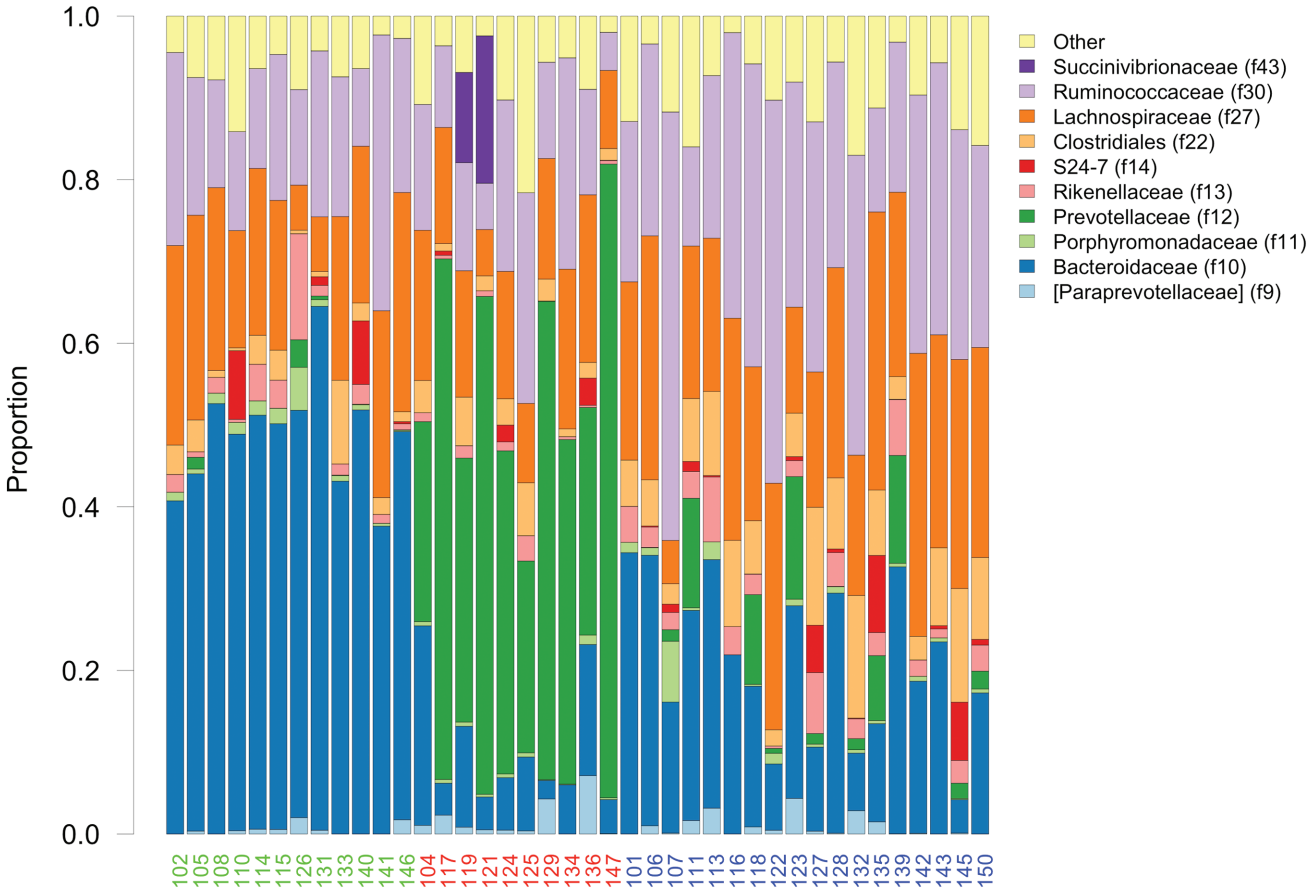


**FIGURE 3** Baseline gut microbiota composition at the family level (f) across 3 enterotypes: high *Prevotella* (red numbers), high *Bacteroides* (green numbers), and low *Prevotella* and *Bacteroides* (blue numbers) in the ELIN crossover intervention study ($n = 40$).

modeling (PROC MIXED) on completed cases in the SAS statistical software release 9.4 (SAS Institute). Diet, period, and diet × period were included as fixed factors and subject as a random factor in the models. The baseline value for each dependent variable (calculated as the average of the 2 intervention arm baseline values) was included as a covariate. All concentration variables were log-transformed before analysis to normalize residual distributions, and estimates were then back-transformed to the original scale. Homoscedasticity and normality were assessed by residual and quantile-quantile plots for all models.

The effect of treatment on microbiota composition was analyzed by random forest analyses using the Multivariate Methods with Unbiased Variable Selection in R (MUVR) package (35) in the R environment for statistical computing version 3.5.1 (R Foundation for Statistical Computing; https://www.R-project.org/). The MUVR algorithm performs random forest modeling with unbiased selection of the most informative features through recursive feature elimination within a repeated double cross-validation procedure, which also reduces the likelihood of false-positive findings (35). Random forest analysis was performed in multilevel mode (35, 36) (i.e., considering the sample dependency from repeated measurements). Treatment effects were investigated using log fold-change in relative abundance of microbial taxa between the 2 interventions at 8 wk as independent variables, using counts from both OTU and genus-level combined. Within-treatment effects were similarly investigated using log fold-change between 8 wk and baseline. In addition, to investigate the effect of lignan capsules, log fold-change between 4 and 8 wk in the WG rye arm was used. Microbiota data were filtered prior to analysis, removing those OTUs that were not present in ≥50% of samples from ≥1 of the investigated groups (with treatment × time point considered as grouping factors). This reduced the number of OTUs from 4854 (85% zero counts) to 428 (38% zero counts). Zero counts were substituted with $1 \times 10^{-5}$ before log-transformation. The significance of multivariate models was assessed by permutation tests ($n = 100$). Up- and downregulation of microbiota variables (OTUs and genera) selected from multivariate modeling was further assessed by paired *t* tests (**Figure 4**).

The exploratory evaluation of the effect of baseline microbiota composition on the above-listed clinical outcomes was performed by linear modeling in R. Models included enterotype (*Prevotella*, *Bacteroides*, or *Firmicutes*) as a fixed factor. Within-treatment effects were investigated separately for the WG rye and WG wheat treatments using differences in clinical outcome variables between either 4 or 8 wk and baseline as the dependent variable. Between-treatment effects were similarly investigated using differences in clinical outcome variables between the 2 interventions at either 4 or 8 wk.

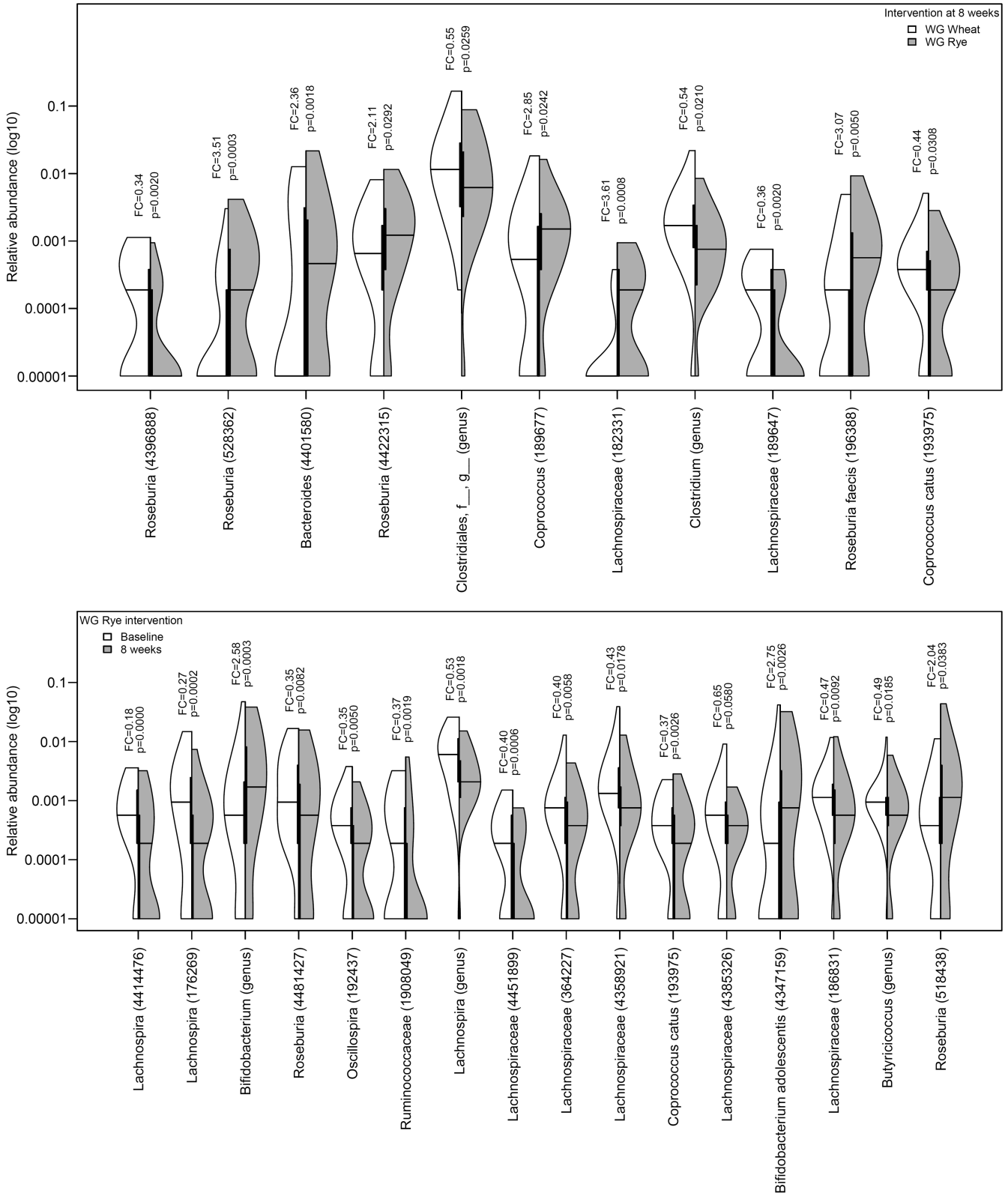


**FIGURE 4** Relative abundance for species (OTUs; numbers in parenthesis) and genera between treatment (top; vertical center lines separate WG wheat in white on the left and WG rye in gray on the right) and within WG rye treatment (bottom; vertical center lines separate baseline in white on the left and 8 wk in gray on the right) with corresponding mean FC and *P* values obtained from paired *t* test. Horizontal lines correspond to median values and thicker black center lines correspond to IQR (box) ranges. FC, fold-change; OTU, operational taxonomic unit; WG, whole-grain.

## Results

Baseline characteristics of the participants are shown in **Table 2**. The median age at recruitment was 68 y [percentile (P) 5–P95: 49–74] and the median BMI (in kg/m$^2$) was 30.5 (P5–P95: 26.8–38.5). The reported median habitual WG product intake was high (almost 120 g/d; P5–P95: 0–650). However, the median total alkylresorcinol concentration only corresponded to a low WG intake (46.8 nmol/L; 17.6–146.9, corresponding to about the average intake of 35 g/d in the Swedish population) (25). Based on the ratio of the alkylresorcinols C17:0/C21:0, the primary source of WG among the participants was wheat (Table 2). Thirty-three participants met the international definition of MetS. The metabolic profile of the remaining 7 participants was characterized primarily by a combination of high waist circumference and increased fasting glucose and elevated blood pressure (**Table 3**). The intakes of macronutrients, fiber types, and lignans from the intervention diets are described in **Table 4**.

### Compliance based on product diaries and alkylresorcinols

Self-reported compliance, assessed by product checklists, was high for both rye (median: 96.7%; range: 72–111%) and wheat (median: 97.6%; minimum–maximum: 40–104%) diets. Compliance was also assessed by alkylresorcinols that increased in both groups after 4 and 8 wk of treatment. The C17:0 to C21:0 ratio increased in the rye treatment arm ($P < 0.0001$) and decreased in the wheat treatment arm ($P < 0.0001$), confirming high intakes of WG rye and WG wheat, respectively. Secoisolariciresinol concentration increased after SDG capsules were introduced at week 4 in the rye compared with the wheat diet ($P < 0.0001$), indicating compliance to the SDG capsules (**Table 5**).

### Enterolignans in plasma and urine

Plasma and urine enterolactone and enterodiol concentrations increased following lignan supplementation to the rye diet between 4- and 8-wk visits and were significantly higher compared with the wheat diet at 8 wk ($P < 0.0001$; Table 5). No differences were observed between baseline and 4 wk in the rye intervention or between any 2 time points in the wheat intervention.

### Effects on glucose and insulin after OGTT

Plasma glucose and insulin concentration profiles in response to OGTT did not differ between or within diets (Table 5). We also evaluated effects on HOMA-IR and the Matsuda index, but no significant differences were found between the diets (data not shown).

### Effects on cardiometabolic risk factors

No differences were observed between intervention diets for BMI, waist circumference, triglycerides, and systolic or diastolic blood pressure (Table 5). After 4 wk of the rye diet, LDL- and total-cholesterol concentrations were lower compared with the wheat diet. This difference was no longer apparent after 8 wk of the intervention. No difference between diets was observed for either HDL cholesterol or triglycerides.

### Effect of intervention on gut microbiota composition

Microbiota composition differed between the rye and wheat diets at 8 wk [classification rate (cr): 71%; $P = 0.033$] as well as within the rye diet from baseline to 8 wk of the intervention (cr: 85%; $P = 0.0016$), but not within the wheat diet (cr: 68%; $P = 0.074$) (Figure 4). Moreover, no difference in microbiota composition was observed following lignan capsule supplementation (i.e., from 4 to 8 wk of intervention in the rye arm; cr: 30%; $P = 0.952$).

Rye compared with wheat resulted in lower relative abundance of the *Clostridium* as well as an unannotated *Clostridiales* genus (Figure 4). In addition, several OTUs of the *Roseburia* and *Coprococcus* genera as well as unannotated *Lachnospiraceae* were either increased or decreased. Within the rye diet, an increased relative abundance was observed for the *Bifidobacterium* genus, whereas downregulation was observed for the *Lachnospira* and *Butyricicoccus* genera as well as for species of the *Coprococcus* and *Oscillospira* genera and unannotated *Lachnospiraceae* and *Ruminococcaceae*. Again, both up- and downregulation occurred between species of the *Roseburia* genus (Figure 4).

### Exploratory analysis: association of baseline gut microbiota enterotype with outcomes

Of the investigated outcome variables, treatment differences in total and LDL cholesterol, triglycerides, as well as diastolic blood pressure were associated with enterotype. We found no significant differences in any measured outcome variables across metabotypes at baseline. In individuals with a high proportion of *Prevotella*, total cholesterol decreased after the rye diet compared with baseline ($P = 0.006$). Conversely, in those with high *Bacteroides*, total cholesterol decreased after the wheat diet compared with baseline ($P = 0.034$). These results were also reflected in the difference in total cholesterol between treatments ($P = 0.003$; **Supplemental Figure 2**). Similar results, albeit less pronounced, were observed also for LDL cholesterol and triglycerides. Moreover, triglycerides were also increased after the rye diet in the group characterized by high *Firmicutes*. In addition, diastolic blood pressure showed a differential effect between the wheat and rye arms for the *Bacteroides* compared with *Firmicutes* enterotypes ($P = 0.018$; Supplemental Figure 2).

### SCFAs in fecal samples

Among the SCFAs, only butyrate differed significantly between overall treatment, with higher concentrations after the consumption of WG rye/lignans compared with WG wheat ($P < 0.03$). When testing the combination of SCFAs and treatment contrasts by enterotype, propionate tended to be higher after the rye treatment in the *Prevotella* enterotype after 4 and 8 wk compared with the *Bacteroides* enterotype (**Supplemental Figures 3** and **4**). However, the sample size is small, and results need to be interpreted with caution.

**TABLE 2** Baseline characteristics of participants in the ELIN crossover intervention study[1]

| | Mean ± SD | Median (P5–P95) |
|---|---|---|
| Age, y | 65 ± 8 | 68 (49–74) |
| Waist circumference,[2] cm | 113.4 ± 8.1 | 111.5 (103.5–132) |
| BMI,[2] kg/m$^2$ | 30.9 ± 3.3 | 30.5 (26.8–38.5) |
| Systolic blood pressure,[3] mmHg | 141.1 ± 12.5 | 140 (120–160) |
| Diastolic blood pressure,[3] mmHg | 83.6 ± 9.0 | 80 (70–102.5) |
| Total cholesterol, mmol/L | 5.47 ± 0.7 | 5.45 (4.35–6.8) |
| HDL cholesterol, mmol/L | 1.23 ± 0.3 | 1.20 (0.90–1.9) |
| LDL cholesterol, mmol/L | 3.22 ± 0.7 | 3.10 (2.25–4.4) |
| Triglycerides, mmol/L | 1.59 ± 0.8 | 1.41 (0.87–2.8) |
| Fasting glucose, mmol/L | 5.7 ± 0.5 | 5.65 (4.90–6.75) |
| Fasting insulin, mmol/L | 8.8 ± 5.4 | 7.65 (3.1–19.0) |
| Glucose AUC, mmol/L | 949.7 ± 160.6 | 951 (734–1241) |
| Insulin AUC, mmol/L | 5967 ± 4668 | 4898 (2184–17,651) |
| Plasma enterolactone, nmol/L | 27.3 ± 26.3 | 17.0 (7.2–73.4) |
| Urine enterolactone, nmol/L | 6413.3 ± 6678.9 | 4357.4 (513.5–25,246.4) |
| Plasma enterodiol, nmol/L | 1.63 ± 1.29 | 1.21 (0.22–4.55) |
| Urine enterodiol, nmol/L | 505.4 ± 1026.1 | 214.6 (31.2–1453.9) |
| Total alkylresorcinols, nmol/L | 62.90 ± 46.7 | 46.76 (17.6–146.9) |
| C17:C21 alkylresorcinol ratio | 0.37 ± 0.2 | 0.33 (0.11–0.8) |
| Secoisolariciresinol, mmol/L | 2.5 ± 1.6 | 2.2 (1.2–4.9) |
| Habitual cereal intake, g/d | 139.2 ± 117.8 | 133.5 (0–650) |
| Habitual intake of WG products, g/d | 126.6 ± 117.0 | 119.6 (0–650) |

[1] $n = 40$. AUC was determined following 75 g of oral-glucose-tolerance test after 120 min. P, percentile; WG, whole-grain.
[2] $n = 1$ missing.
[3] $n = 2$ missing.

## Discussion

In this randomized crossover trial with WG rye and additional SDG supplementation compared with WG wheat diets, we found no effect on response to OGTT, indicting no major effects on insulin sensitivity. For secondary outcomes, the only observed effect was a lowering of total and LDL cholesterol at 4 wk after the rye compared with the wheat diet (and with baseline), but this effect was attenuated after 8 wk. Microbiota composition differed between rye and wheat diets and between baseline and 8 wk within the rye intervention. No difference in composition was observed within the wheat diet or following lignan capsules. Interestingly, we found in exploratory analyses that effects on blood lipid outcomes following WG interventions were associated with enterotype at baseline.

**TABLE 3** Overview of metabolic syndrome components and number of completing participants meeting these

| | *n* |
|---|---|
| Components of the metabolic syndrome | |
| Waist circumference >102 cm | 39 |
| Systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg | 35 |
| HDL cholesterol ≤1.03 mmol/L | 11 |
| Triglycerides ≥1.70 mmol/L | 7 |
| Fasting glucose ≥5.6 mmol/L | 38 |
| Number of participants meeting the international definition of the metabolic syndrome | 33 |

High compliance to test diets was observed based on the ratio of plasma alkylresorcinol homologs C17:0 and C21:0 (high ratio = high rye intake, low ratio = high wheat intake) for the WG diet and on secoisolariciresinol for SDG capsules.

The lipid-lowering properties of rye may be caused by arabinoxylan through, for example, effects on bile acid reabsorption that may have been lowered due to viscous arabinoxylan from the WG rye. Cholesterol-lowering effects of WG rye compared with refined wheat have been observed in a few intervention studies (37, 38). The evidence for a lipid-lowering effect seems more pronounced among individuals with high initial cholesterol concentrations, which may partly explain the attenuation of effect on LDL cholesterol after 8 wk in the current study (39).

Consistent evidence from observational studies supports that the consumption of WG foods is beneficial for prevention of T2D (40). However, studies that evaluate the effects of specific types of WGs are lacking (41). For the current study, we hypothesized that WG rye would have a greater impact on the investigated cardiometabolic health outcomes compared with WG wheat. The higher fermentability and subsequent formation of SCFAs as well as the lower postprandial insulin responses (42) after consuming rye compared with wheat found in other studies support this hypothesis, but overall findings on investigated parameters under fasting conditions in the current study give limited support for that, except for effects on blood lipids (see below). Soluble fiber in rye may induce beneficial effects from delaying gastric emptying, which affects glucose absorption rate (43). Lignans, which are present in relatively high amounts in especially rye (44), have been linked with improved glycemic control (glycated hemoglobin) among persons with T2D (22)

**TABLE 4** Macronutrient and lignan intake as reported in product diaries for intervention periods: baseline–4 wk and 4–8 wk: a randomized crossover trial[1]

| | Intervention: baseline–4 wk | | Intervention: 4–8 wk | |
|---|---|---|---|---|
| | WG rye | WG wheat | WG rye + SDG capsules | WG wheat |
| Total energy, kcal | 814 | 826 | 820 | 830 |
| Carbohydrate, g | 155 | 137 | 156 | 138 |
| Fat, g | 4 | 12 | 4 | 12 |
| Protein, g | 19 | 28 | 19 | 28 |
| Fiber, g | 35.1 | 27.5 | 35.4 | 27.9 |
| Total lignans, μg | 6961 | 3214 | 2,794,528 | 3338 |
| Total free lignans, μg | 927 | 367 | 262,613 | 383 |
| Secoisolariciresinol, μg | 68 | 131 | 2,709,621 | 131 |
| Isolariciresinol, μg | 139 | 84 | 5151 | 87 |
| Matairesinol, μg | 65 | 5 | 215 | 7 |
| Pinoresinol, μg | 123 | 40 | 274 | 43 |
| Syringaresinol, μg | 5716 | 2269 | 5937 | 2375 |
| Medieresinol, μg | 474 | 467 | 482 | 473 |
| Lariciresinol, μg | 375 | 217 | 2233 | 223 |

[1] Values are means of self-reported intervention product intake per day for each intervention period: 0–4 wk and 4–8 wk. SDG, secoisolariciresinol diglucoside; WG, whole-grain.

and lowering of fasting plasma glucose among persons with high cholesterol (23). Only limited and inconsistent results from observational studies exist supporting the association between enterolignans in urine (45, 46) or lignan intake (47) and risk of T2D. However, higher lignan intake was associated with reduced insulin secretion among men from the Health Professionals Follow-Up Study (48), but this was not supported by the current study despite the highly compliant intake of SDG capsules, verified by lignan concentrations in plasma. To sum up, there is consistent evidence of a beneficial role of WGs for T2D

**TABLE 5** Effects of a crossover WG and lignan intervention on cardiometabolic outcomes comparing rye (± SDG lignans) and wheat at 4 and 8 wk of intervention[1]

| | 4 wk: WG rye vs. wheat[2] | 8 wk: WG rye + SDG vs. wheat[2] | $P$[3] | | |
|---|---|---|---|---|---|
| | | | Treatment | Time | Treatment × time |
| Primary outcomes, AUC | | | | | |
| Glucose, mmol/L | −0.02 ± 0.02 | 0.02 ± 0.02 | 0.86 | 0.85 | 0.12 |
| Insulin, mmol/L | −0.02 ± 0.07 | 0.07 ± 0.07 | 0.70 | 0.56 | 0.16 |
| Secondary outcomes | | | | | |
| Plasma ENL, nmol/L | −0.01 ± 0.19 | 2.09 ± 0.19*** | <0.0001 | <0.0001 | <0.0001 |
| Urine ENL, nmol/L | 0.13 ± 0.19 | 2.18 ± 0.19*** | <0.0001 | <0.0001 | <0.0001 |
| Plasma END, nmol/L | 0.53 ± 0.29 | 5.08 ± 0.29*** | <0.0001 | <0.0001 | <0.0001 |
| Urine END nmol/L | 0.29 ± 0.23 | 4.90 ± 0.23*** | <0.0001 | <0.0001 | <0.0001 |
| Total cholesterol, mmol/L | −0.06 ± 0.02** | −0.02 ± 0.02 | 0.04 | 0.85 | 0.09 |
| HDL cholesterol, mmol/L | −0.05 ± 0.02 | −0.02 ± 0.02 | 0.06 | 0.94 | 0.28 |
| LDL cholesterol, mmol/L | −0.09 ± 0.03** | −0.01 ± 0.03 | 0.03 | 0.54 | 0.03 |
| Triglycerides, mmol/L | 0.07 ± 0.04 | 0.06 ± 0.04 | 0.06 | 0.99 | 0.87 |
| BMI,[4] kg/m$^2$ | 0.08 ± 0.13 | 0.07 ± 0.13 | 0.54 | 0.46 | 0.91 |
| Waist circumference,[4] cm | 0.26 ± 0.60 | 0.74 ± 0.60 | 0.33 | 0.01 | 0.45 |
| Systolic blood pressure,[5] mmHg | 1.94 ± 2.16 | 1.68 ± 2.16 | 0.32 | 0.91 | 0.91 |
| Diastolic blood pressure,[5] mmHg | 3.30 ± 1.76 | 1.09 ± 1.76 | 0.16 | 0.13 | 0.23 |
| Compliance markers | | | | | |
| ARs, nmol/L | 0.06 ± 0.12 | 0.14 ± 0.12 | 0.32 | 0.72 | 0.48 |
| AR 17:0:21:0 ratio | 0.52 ± 0.05*** | 0.52 ± 0.05*** | <0.0001 | 0.10 | 0.98 |
| Secoisolariciresinol, nmol/L | 0.35 ± 0.23 | 4.75 ± 0.23*** | <0.0001 | <0.0001 | <0.0001 |

[1] $n = 40$. AR, alkylresorcinol; END, enterodiol; ENL, enterolactone; SDG, secoisolariciresinol diglucoside; WG, whole-grain.

[2] Least-square mean estimates ± SEs at the specific time point with $P$ values calculated as contrasts (using the SAS SLICE statement) at the significance levels $^{**}P < 0.01$, $^{***}P < 0.001$, for comparisons where statistically significant treatment or treatment × period effects were found.

[3] Mixed-effects modeling with diet, period, and diet × period included as fixed factors and subject as a random factor. Baseline values for each dependent variable (calculated as the average of the 2 intervention arm baseline values) included as a covariate.

[4] $n = 1$ missing.

[5] $n = 2$ missing.

prevention. On the contrary, the proposed effects from rye and lignans are not consistently reflected in the few human studies, including the current study. The apparent lack of effect of the rye compared with wheat intervention on the primary endpoint could be due to the already high reported intake of WG wheat and rye at baseline among the subjects. Furthermore, it has been suggested that initial gut microbiota composition affects response to dietary interventions (49). The lack of observed effect on metabolic outcomes in the current study could therefore be partly explained by the relatively small sample size, which would increase the risk of unequal distribution of responders and nonresponders. Furthermore, the expected difference of 8% in 2-h OGTT might have been too ambitious, and the sample size thus too small to detect smaller true effects. Studies where intervention foods high in WG are tested in populations with low habitual consumption are warranted.

### Treatment effects on gut microbiota composition

A previous 12-wk intervention comparing WG rye with refined wheat bread reported no effect on intestinal microbiota composition (50). Moreover, in a 1-wk crossover intervention, WG bread did not affect glycemic response or gut microbiota composition compared with refined bread (51), although glycemic response was associated with baseline microbiota compositions. Contrary to those previous studies, we found an effect of the WG rye intervention on the microbiota composition in the exploratory analysis of the present study, with an increase in *Bifidobacterium* and a decrease in several OTUs within the *Lachnospiraceae* family. The *Bifidobacterium* genus consists of saccharolytic, acetate-producing bacteria normally found as a part of the intestinal microbiota and generally considered beneficial for host health (52). Bifidobacteria are commonly a target group for prebiotic therapies, and it has been shown that prebiotic oligosaccharides, such as inulin, stimulate growth of bifidobacteria (53). Rye kernels and rye kernel bread were previously shown to contribute to increased relative abundances of *Bifidobacterium* in vitro (54). This may be linked to conversion of arabinoxylan to arabinoxylan oligosaccharides, which can be utilized and stimulate growth of bifidobacteria (55). Since higher abundance of bifidobacteria did not result in any clear metabolic effects in the current study, it could be speculated whether bifidobacteria in fact colonize the gut or simply reside on the WG residues. Our results are in line with those from a study by Kjølbæk and colleagues (56), where increased abundance of bifidobacteria was observed but not followed by effects on metabolic outcomes. The effects on microbiota composition were likely related to the consumption of WG rye and not from the lignan capsules, as evidenced by the lack of differences between 4 and 8 wk. WG wheat, on the other hand, did not show effects on the microbiota composition at 8 wk of the intervention. This indicates that WG wheat consumption may be more similar to baseline diet than WG rye, or that WG wheat does not induce as large effects on microbiota composition. However, the microbiota composition at 4 wk differed from both baseline and 8 wk (**Supplemental Figure 5**). The vast majority of microbiota species that were either up- or downregulated from baseline to 4 wk were regulated in the opposite direction from 4 wk to 8 wk, indicating that the WG wheat diet–induced effects on microbiota composition are transient.

### Baseline enterotype associated with treatment outcomes

Interestingly, the *Prevotella* and *Bacteroides* enterotypes were differentially associated with lipid profile for the 2 interventions. An improvement in lipid profile (lower triglycerides and total and LDL cholesterol) was observed for the enterotype rich in *Prevotella* after WG rye but not after WG wheat. In contrast, lower triglycerides (and borderline total and LDL cholesterol) was observed for the *Bacteroides* enterotype after WG wheat but not after WG rye. Previous studies have associated *Prevotella* to *Bacteroides* ratio with differential outcomes to dietary interventions, where a high ratio was associated with improved glucose metabolism (57), higher total cholesterol (58), and increased weight loss (59–61) with a high-fiber compared with a low-fiber diet. It is therefore implied that WGs rich in dietary fiber in general should benefit predominantly the *Prevotella* but not the *Bacteroides* enterotype. Our results indicate that the enterotypes respond differently not only to the amount but also to the type of WG, possibly reflecting differential responses to different profiles of dietary fiber and/or bioactive compounds. This is supported by data from an in vitro study where it was found that fecal samples from individuals with the *Prevotella* and *Bacteroides* enterotypes showed different capabilities in fermenting 3 different types of dietary fiber (62). WG rye contains fructans and more soluble arabinoxylans (63) than wheat (64) and is therefore more fermentable by the gut microbiota. It appears that the cholesterol-lowering effect after 4 wk of WG rye in the *Prevotella* enterotype in the current study is concurrent with higher fecal propionate concentrations and a nonsignificant tendency for acetate and butyrate (Supplemental Figures 3 and 4). Propionate has been shown to reduce lipogenesis and cholesterol synthesis in the liver (65) and may, in this way, have contributed to the cholesterol-lowering effect observed in the present study. Our finding of higher propionate for the *Prevotella* enterotype is in agreement with the results reported by Chen et al. (62), but further studies of larger sample sizes are needed to verify findings across enterotypes.

## Conclusions

WG rye compared with WG wheat did not affect glucose metabolism in men with MetS, but transient effects on total and LDL cholesterol were observed with a reduction following diet with WG rye compared with WG wheat after 4 wk of an intervention, which was not present after 8 wk. Supplementing the WG rye with SDG, which resulted in the highest enterodiol and enterolactone concentrations measured in fasting samples after an intervention, appeared to have no impact on any of the investigated metabolic factors. WG rye resulted in a higher abundance of the *Bifidobacterium* genus and a lower abundance of the *Lachnospira* and *Butyricicoccus* genera. Moreover, exploratory analysis of our study suggests for the first time an associated differential effect of WG wheat and rye intake on blood lipids and blood pressure across 3 different enterotypes. The blood lipid profile was improved by WG rye intervention for the *Prevotella* and by WG wheat for the *Bacteroides* enterotype.

These findings need to be validated in a randomized trial setting where similar treatments are provided across enterotype strata.

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