LIGNAN-RICH WHOLE GRAIN CONSUMPTION - EFFECTS ON GUT MICROBIOTA AND CARDIOMETABOLIC RISK FACTORS

INTRODUCTION

Cereals have been an important part of the human diet since the advent of agriculture about 10 000 years ago and during the majority of that time they have been consumed as whole grain. It is only within the last hundred years that most people have consumed refined grains [1]. Whole grain (WG) refers to *'intact, ground, cracked or flaked caryopsis, whose principal anatomical components- the starchy endosperm, germ and bran are present in the same relative proportions as they exist in the intact caryopsis'* (definition by AACC, 1999 adopted by U.S. Food and Drug Administration (FDA) in (2006)). Epidemiological studies in different populations have consistently shown inverse associations between whole grain intake and incident of type 2 diabetes, cardio vascular disease, colorectal cancer and some of their risk factors. WG is a rich source of dietary fiber, minerals, vitamins and bioactive compounds, but content and features varies considerably between different cereals particularly with regards to dietary fiber content, composition and bioactive compounds such as plant lignans [2].

From a Nordic perspective, WG is an interesting dietary factor in relation to type 2 diabetes and cardio vascular disease, as intake is among the highest in the world and because cereal grains is a large source of energy intake in several Nordic populations. Most epidemiological studies have been conducted in US- populations where WG intake is low and wheat is the main source. WG rye is believed to be nutritionally superior to whole grain wheat due to high content of dietary fiber, which includes soluble fermentable fructans, arabinoxylans and beta-glucans, as well as other bioactive compounds such as lignans [3]. Among cereal foods, fibre-rich rye products have consistently shown beneficial effects on glucose metabolism and self-reported satiety, suppression of hunger and/or energy intake in a subsequent meal [4, 5, 6, 7, 8]. Such effects have not been demonstrated with similar consistency for WG wheat. It is also clear that different fiber sources show differences in gut microbial fermentation patterns in vitro, but it is not clarified to what extent it will affect the microbial composition and/or function in the gut during a short-term intervention [9]. Recent studies have shown that the microbiota seems to respond rapidly to changes in dietary intakes of macronutrients and that richness of the microbiota is affected by diet and related to metabolic risk factors. [10, 11, 12]

Lignans are bioactive plant compounds found in high concentrations in seeds and in lower amounts in grains, fruits and vegetables. In the Nordic countries, whole-grain cereals and vegetables are the main sources of plant lignans, and particularly whole-grain rye has high contents [13, 14]. In the large intestine, plant lignans are converted to enterodiol and enterolactone by the colonic microbiota, and thereafter absorbed through the colonic barrier [15]. Enterolactone has attracted most interest as it accounts for more than 95% of the circulating levels of enterolignans. The biological activity of enterolignans is possibly related to the estrogen-like structure, and especially enterolactone has been found to bind weakly to estrogen receptors, have estrogenic effects in cultured cells and to modulate the response to endogenous estrogens [16]. Animal models have shown that enterolignans may play an important role in prevention of cancer at the early stages leading to lower cancer incidence as well as in the progression of already established tumors [17, 18].

The beneficial effects of WG and lignans are to a large extent dependent on intestinal microbes. The microbiota has a central role for its host's health and provides its host with several important functions such as protection against enteric pathogens, contribution to a proper immune function and to the host metabolism by production of vitamins and bioactive

compounds, modifying dietary components and by extracting energy from the diet. Researchers have tried to understand the link between the microbiota and health for almost a century, but this research area is still in its infancy. In depth analysis of the intestinal microbiome has not been possible until recently due to limitations in the technology to study microbial ecosystems in a comprehensive way. The development of next generation sequencing technologies, has substantially improved the analytical capacity and it is now possible to get a comprehensive view on both the microbial composition and the functions they encode. Metagenomic analyses of the gut microbiome have provided valuable insights on the genetic diversity of the genes of involved in degradation of carbohydrates [19] and as potential biomarkers for disease states, such as type 2 diabetes [20] and cardio vascular disease [21].

Recent studies suggest that the composition of baseline gut microbiota is associated with beneficial postprandial glucose response [22, 23] and 6 months weight-loss [24] after intake of cereal fibre-rich diet [25], in particular related to the *Prevotella* and *Bacteroides* genera. Furthermore, it has been suggested that individuals can be grouped into two to three "enterotypes", i.e. strata based on characteristic microbiota composition. The two most frequently reported enterotypes are dominated by either *Prevotella* or *Bacteroides*. Enterotypes have been associated with long-term dietary patterns, where *Prevotella* associated with plant-based foods with complex carbohydrates high in dietary fibre, and *Bacteroides* associated with Western diet, high in simple carbohydrates, fat and animal protein [26]. It remains unknown, however, whether baseline profile of gut microbiota could modify effects on metabolic risk factors such as blood lipid profiles after a high-fibre WG rye versus WG wheat diet.

Despite a large number of epidemiological studies in relation to health outcomes, surprisingly little is known about what gut micro-organisms and enzyme systems that are responsible for the enterolactone production or how the microbiota may be affected by different food intakes [27]. Therefore, the overall aim with this project is to study the impact of whole grain foods and lignan intake on the microbiota composition and function. Moreover, a second aim is to study how composition and activity of microbiota is correlated to potential effects on cardiometabolic risk factors after intervention.

MATERIAL AND METHODS

This project was a complementary extension to the large project "ELIN- The effects of enterolignans in chronic disease" funded by The Danish Strategic Research Fund 2013-2017 (http://www.cancer.dk/elin/). The material derived from a human intervention study that evaluated effects of WG wheat (with a low lignan content) and WG rye (high lignan content) on cardiometabolic risk factors among men with the metabolic syndrome. The intervention study was conducted during 2015 in Uppsala and was funded by the ELIN project.

Ethics statement

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 clinicaltrials.gov (NCT02987595). All study procedures were conducted in accordance with the Helsinki Declaration.

Participants

In total, 49 men aged between 49 and 74 years were recruited 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 body mass index (BMI), waist

circumference, blood pressure measurements (performed seated after 5 min rest), routine clinical tests (blood lipids, liver enzymes, haemoglobin, glucose, insulin, CRP) and questionnaires (concerning lifestyle and medical history). Eligible participants, who had at least two of the following risk factors for metabolic syndrome, 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. In total, 40 participants completed the study.

Study design

The study was a randomized, controlled, single-blinded cross-over trial where a whole grain rye diet (WGR) was evaluated against a whole grain wheat diet (WGW) during 8-week periods separated by an 8-week wash-out period. After the washout period, the two groups continued the dietary intervention with the whole-grain products opposite from what they started out with. During the last 4 weeks of the whole-grain rye diet, lignan capsules were added to the diet. Participants were randomly assigned to begin either with WGW or WGR and were instructed to replace their habitual consumption of cereal products with the provided intervention products but otherwise maintain their usual diet throughout the study. Fecal samples was collected to study the effects of WG wheat, WG rye and lignan content on composition and structure of the gut microbiota. Whole grain rye products corresponding to 30% of daily energy intake was compared with corresponding WG wheat products, during 4wk. After 4 wk, pure lignans was added to the WG rye treatment for another 4 wk to evaluate isolated effects of lignans on the outcomes.

Study procedures

All study visits were scheduled in the morning between 7.00am and 10.00am and followed the same procedure. Each participant was examined at baseline, after four weeks and eight weeks of the first intervention period, after eight weeks of washout, and after four weeks and eight weeks of the second intervention period (in total six times during 24 weeks). At each occasion, fasting blood was collected from each participant for routine clinical tests (blood lipids, liver enzymes, haemoglobin, glucose, insulin, CRP). Furthermore, each participant collected one faecal sample prior to all study visits. Faecal collection kits and instructions were provided and the samples were kept in a study freezer bag at -18 ºC in the home freezer until it was brought to the clinic at the following study visit. The fecal samples were then stored at – 80 ºC.

Dietary products

The test diets were designed to contribute 30% of the total energy intake during the intervention. Each intervention diet provided about 30g of dietary fibre per day. Products included breakfast cereals, rolled cereals for porridge etc., crisp bread and pasta. Diets were designed to ensure that whole-grain intake (g/d) and dietary fibre content remained similar for the two intervention. Treatments differed in terms of dietary fibre quality and lignan content. To obtain similar dietary fibre contents in the two diets, additional wheat bran was added to one of the whole-grain wheat products. The whole-grain products included in the diets were both commercially available products from the supermarket and specially developed products for the purpose of the ELIN-study. Lantmännen developed 100% whole-grain wheat and rye puffs (with 17% added wheat bran to the wheat product) and Wasabröd/Barilla Sweden developed a 100% whole-grain wheat crisp bread product. Conventional, on-the-market products included rolled rye and wheat contributed by Lantmännen, a rye crisp bread by Wasa/Barilla and Il Fornaio contributed with whole-grain wheat and rye pasta. The dose of lignan capsules contained 280 mg of pure secoisolaricirecinol (SDG) lignans (purity 20 %) isolated from flaxseed hulls, corresponding to doses previously used in human intervention studies (25-26). The lignan extract used in the

study was provided by Source Naturals, Threshold Enterprises LTD, California, USA. The product is commercially available as a dietary supplement.

Analysis of the fecal microbiota

DNA was isolated from the collected fecal samples using QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany) according to the protocol from the manufacturer, but with the exception that a bead beating step was added to the protocol 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 x 45 seconds using a Precellys instrument (Bertin Technologies, Montigny-le-Bretonneux, France). The purified DNA was stored at -20°C until analysis. 16S rRNA gene amplicons were generated from the V3-V4 region of the 16S gene using the primers 341f and 805r [28]. The PCR products were purified with AMPure XP magnetic beads and the purified products were subsequently used in a second PCR run in order to attach sample specific barcodes and illumina adaptors to each amplicon. The amplicons were again purified with the AMPure XP magnetic beads, quantified using a Qubit fluorometer and pooled into equimolar amounts and subsequently sent for Illumina Miseq sequencing at the National Genomics Infrastructure hosted by Science for Life Laboratory in Stockholm.

The sequence data were processed according to Muller et al. [29] In brief, the paired end sequence reads were quality trimmed using the Cutadapt tool in Python version 2.7 (Python Software Foundation, http://www.python.org). Paired end reads were joined according to the SeqPrep method (https://github.com/jstjohn/SeqPrep) with a minimum overlap of 150 bp using QIIME version 1.7.0/1.8.0 [30]. The joined reads were then used to assign sequences to operational taxonomic units (OTUs) using the open reference OTU picking strategy at a threshold of 97 %, using U-CLUST against Greengenes core set (gg_13_8) [31]. The representative sequences were aligned against the Greengenes core set using PyNAST [32]. Chimeric sequences were removed by ChimeraSlayer [33] and taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier with a minimum confidence threshold of 80 %. The final OTU table was further filtered to include only OTUs present in at least three samples and each sample was subsampled to contain equal numbers of sequences (5300 sequences/sample).

Study participants were subdivided *a priori* [34] depending on their baseline microbiota composition into a high *Prevotella* group (ratio of *Prevotella* genus ≥ 0.20; n=10), a high *Bacteroides* group (ratio of *Bacteroides* genus ≥ 0.35; n=12), and a low *Prevotella* and *Bacteroides* group (n=18). The latter, low group was characterized by high proportion of firmicutes, in particular from the *Ruminococcaceae* and *Lachnospiraceae* families (40-83%) and was therefore referred to as the firmicutes group.

Enterolignan analyses

Enterolactone concentrations in plasma samples were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [35]. Clinical blood measurements were analyzed at the clinical laboratory at Akademiska Hospital in Uppsala, Sweden. Lignans in the WG products and enterolignan in plasma samples were analyzed using an LC-MS method at Aarhus University, at the Department of Animal Science [35].

Statistical analysis

Statistical evaluation of the effect of treatment on glucose, insulin, lipids, BMI, waist and blood pressure were performed by mixed effects modelling (PROC MIXED) on completed cases in the SAS® statistical software release 9.4 (SAS Institute, Cary, NC). Diet, period and diet x 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 two intervention arm baseline values) was included as a covariate. All concentration variables were log-transformed before analysis to normalise 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 analysed by random forest analyses using the MUVR package [36] in the R environment for statistical computing v 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/). The MUVR algorithm performs random forest modelling 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 [36, 37]. Consequently, since microbiota variables (species and genera) were selected from multivariate modelling, up- and downregulation of relative abundances is reported without univariate p-values. Random forest analysis was performed in multilevel mode [36, 38] 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 two interventions at 8 weeks as independent variables, using counts from both OTU and genus level combined. Within treatment effects were similarly investigated using log fold change between 8 weeks and baseline. In addition, to investigate the effect of lignan capsules, log fold change between 4 and 8 weeks in the WG rye arm was used. Microbiota data were filtered prior to analysis, removing those OTUs that were not present in at least 50% of samples from at least one of the investigated groups (with treatment x time point considered as grouping factors). Zero counts were substituted with 1e-5 before log-transformation. Significance of multivariate models was assessed by permutation tests (n=100) [37, 39]. Evaluation of the effect of baseline microbiota composition on clinical outcomes (total, HDL and LDL cholesterol, triacylglycerides, glucose, insulin, BMI as well as systolic and diastolic blood pressure) was performed by linear modelling in R. Models included enterotype (prevotella, bacteroides or firmicutes) as fixed factor. Within treatment effects were investigated separately for the WG rye and WG wheat treatments, using differences in clinical outcome variables between 8 weeks and baseline as dependent variable. Between treatment effects were similarly investigated using differences in clinical outcome variables between the two interventions at 8 weeks.

RESULTS

From a total of 107 interested men, 49 were eligible, enrolled and randomized to the study of which 40 completed the intervention. Five participants dropped out before the second study visit (<4 weeks) due to lack of motivation, time or vigour on the WG diet, and due to the high prescribed amount of WG products. Two participants dropped out before the third study visit (<8 weeks) due to stomach-ache from high WG intake or from the introduction of lignan capsules. One participant dropped out during the washout period because it was too much to consume all the products. Finally, one participant dropped out before the fifth study visit as he experienced stomach-ache after starting the rye product period.

The median age at recruitment was 68 (range 49-74) and the median BMI was 30.5 kg/m^2 (range: 25.7-40.8). The reported habitual WG product intake was high (almost 120 g/d, 0-650). However, the median total alkylresorcinols 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) [40]. Based on the ratio of the alkylresorcinols C17:0/C21:0, the primary source of WG among the participants was wheat. Thirty-three participants met the international definition of the metabolic syndrome. The metabolic profile of the remaining seven participants was characterised primarily by a combination of high waist circumference, increased fasting glucose and elevated blood pressure.

Compliance based on product diaries and alkylresorcinols

Self-reported compliance, assessed by product checklists filled by the participants, was high for both rye (median: 96.7%, range: 72-111) and wheat (median: 97.6%, min-max: 40-104) diets. Compliance was also assessed by alkylresorcinols. An increase in total alkylresorcinol concentration was seen in both groups after 4 and 8 weeks treatment. The C17:0/C21:0 ratio increased in the rye treatment arm ($p<0.0001$) and decreased in the wheat treatment arm ($p<.0001$) confirming high intakes of WG rye and WG wheat, respectively.

Enterolignans in plasma

As expected, plasma enterolactone and enterodiol concentrations increased following the lignan supplementation in the rye intervention at 4 weeks to the 8 week visit and were significantly higher compared with WG wheat at wk 8 ($p<0.0001$). No differences were observed between baseline and 4 weeks in the rye intervention or between any two time points in the wheat intervention.

Effects on cardiometabolic risk factors

No differences were observed between intervention diets for BMI, waist circumference, systolic or diastolic blood pressure. After 4 weeks of WG rye intervention, both total- and LDL cholesterol were lower compared to WG wheat intervention. This difference was no longer apparent after 8 weeks of intervention. No difference between treatments was observed for either HDL cholesterol or triglycerides.

Effect of treatment on gut microbiota composition

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

WG rye compared to WG wheat resulted in lower relative abundance of the *Clostridium* as well as an unannotated *Clostridiales* genus. In addition, several OTUs of the *Roseburia* and *Coprococcus* genera as well as unannotated *Lachnospiraceae* were either increased or decreased. Within the WG rye intervention treatment, 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*, *Oscillospira* genera and unannotated *Lachnospiraceae* and *Ruminococcaceae*.

Association of baseline gut microbiota composition with treatment 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 difference in any measured outcome variables across metabotypes at base-line ($p>0.05$). In individuals with a high proportion of *Prevotella*, total cholesterol decreased after WG rye compared with base-line ($p=0.006$). Conversely, in those with high *Bacteroides*, total

cholesterol decreased after WG wheat compared with base-line (p=0.034). These results were also reflected in the difference in total cholesterol between treatments (p=0.003). Similar results, albeit less pronounced, were observed also for LDL cholesterol and triacylglycerides. Moreover, triglycerides were also increased after the WG rye intervention in the group characterized by high levels of firmicutes. In addition, diastolic blood pressure showed a differential effect between the WG wheat and WG rye arms for the bacteroides vs firmicutes enterotypes (p=0.018).

DISCUSSION

Consistent evidence from observational studies support that consumption of WG foods are beneficial for prevention of type 2 diabetes [41, 42, 43]. However, there is a lack of studies to evaluate effects of specific types of WGs [44]. In this randomized, controlled crossover intervention with WG rye and additional lignan supplementation vs. WG wheat diets, we found a lowering effect of WG rye on total and LDL cholesterol at 4 weeks compared with baseline as well as compared with WG wheat. However, this effect was attenuated after 8 weeks. No effects of treatments were found on anthropometry or blood pressure. Interestingly, effects on blood lipid outcomes in the WG rye intervention was for the first time associated with microbial enterotype at baseline. Cholesterol-lowering effects of WG rye compared to refined wheat, have been observed in some intervention studies. Both total and LDL-cholesterol was decreased in men, but not in women, in a randomized crossover trial comparing WG rye to white wheat breads in a dose-dependent manner favoring high intakes of rye [45]. Cholesterol absorption was lower and synthesis higher (negative balance) for rye diet compared with wheat in another parallel intervention on subjects with MetS [46]. The lipid-lowering properties of rye may be caused by arabinoxylans through e.g. effects on bile acid reabsorption that may have been lowered due to viscous arabinoxylans from the WG rye but high lignan contents in rye products may also elicit beneficial effects as suggested in some intervention studies with flaxseed lignan supplements [47]. 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 weeks on the rye diet where the decrease after the first 4 weeks yielded less potential for additional lowering [48].

Treatment effects on gut microbiota composition

It was previous reported that an intervention with 12-weeks of WG rye vs. refined wheat bread had no effect on intestinal microbiota composition [49]. Moreover, in a one-week crossover intervention, WG bread did not affect glycemic response or gut microbiota composition compared to refined bread [22], although glycemic response was associated with baseline microbiota compositions. Contrary to those previous studies, there was a reproducible effect of the WG rye intervention on the microbiota composition in 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 is generally considered beneficial for host health [50]. Bifidobacteria are commonly a target group for prebiotic therapies and it has been show that prebiotic oligosaccharides, such as inulin, stimulate growth of bifidobacteria [51]. Moreover, rye kernels and rye kernel bread were shown to contribute to increased relative abundances of *Bifidobacterium* in vitro [52]. This may be linked to conversion of arabinoxylan to arabinoxylan oligosaccharides, which can be utilized and stimulate growth of bifidobacteria [53]. Moreover, the in vitro study by Ibrugger and coworkers (2014), also showed that rye was associated with reduced levels of the Clostridium coccoides group, from which many of the genera within the Lachnospiraceae family are represented [52]. Several species within the Lachnospiraceae family have frequently been associated with obesity and/or metabolic syndrome [54, 55, 56,

57]. Although mechanisms are unclear, it has been hypothesised that the Lachnospiraceae family might serve as a metabolic regulator that could contribute to higher energy harvesting [54]. Conversely, the decrease in several Lachnospiraceae species could imply decreased energy harvest in overweight and/or MetS following WG rye consumption. These systematic effects on microbiota composition were likely related to consumption of WG rye and not from the lignan capsules, as evidenced by the lack of systematic differences between 4 and 8 weeks. WG wheat, on the other hand, did not show strong systematic effects on the microbiota composition at 8 weeks intervention. This could potentially indicate that WG wheat consumption is more similar to baseline diet than WG rye or that WG wheat does not induce as large systematic effects on microbiota composition, potentially reflecting a lower content of biologically active phytochemicals compared to WG rye. However, the microbiota composition at 4 weeks differed from both baseline and 8 weeks (data not shown). This could indicate that WG wheat indeed induced effects on microbiota composition which, however, are transient. Since objective measurement of alkylresorcinols confirmed compliance, we hypothesize that the observed effect transience is likely due to small effect size or biological adaptation. Regardless, the modest classification rate between treatments was likely related to diluting the clear treatment effect of WG rye intervention with the less pronounced systematic effects of WG wheat intervention on the microbiota composition.

Baseline enterotype associated with treatment outcomes

To investigate potential associations between enterotypes and treatment outcomes, we employed a pragmatic enterotyping scheme in which individuals were stratified based on *Prevotella* and *Bacteroides*, so that individuals in these strata had low abundance of the other respective genus. We then observed that the remaining individuals in general had a high proportion of firmicutes, in line with previous results [34, 58]. Interestingly, the *Prevotella* and *Bacteroides* enterotypes were differentially associated with lipid profile for the two interventions: An improvement in lipid profile (lower triacylglycerides and total and LDL cholesterol) was observed for the enterotype rich in *Prevotella* after WG rye, but not after WG wheat. In contrast, lower triacylglycerides (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 *Prevotella*-to-*Bacteroides* ratio was associated with improved improved glucose metabolism [59] and increased weight-loss [60, 61] on a high-fibre vs low-fibre diet. It is therefore implied that WGs rich in dietary fibre in general should benefit predominantly the *Prevotella* but not the *Bacteroides* enterotype. However, a recent study failed to demonstrate differential response of a WG barley diet on postprandial glucose responses when comparing treatment effects across groups with high vs low *Prevotella/Bacteroides* ratios [62]. Moreover, 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 fibre and/or bioactive compounds. Hamaker and Truncil [63], introduced the concept of "discrete fiber structures" where bacteria develop preferences for specific fibre features such as form of starch, length of fructan etc. According to this concept, specific fiber structures align differently to the polysaccharide utilizing enzymes in bacteria which can lead to differential utilization of the fiber [64].

Conclusions

WG rye compared with WG wheat lowered LDL cholesterol after 4 wk in men with metabolic syndrome. Supplementing the WG rye with lignans appeared to have no impact on metabolic factors. WG rye resulted in higher abundance of the *Bifidobacterium* genus and lower abundance of Lachnospiroceae OTUs. Moreover, we showed for the first time differential

effect of WG wheat and rye intake on blood lipids and blood pressure across three different enterotypes. The blood lipid profile was improved by WG rye intervention for the *Prevotella* enterotype, and by WG wheat intervention for the *Bacteroides* enterotype. These findings need to be validated in a randomized controlled trial setting where similar treatments are provided across enterotype strata.

SCIENTIFIC PUBLICATIONS FROM THE PROJECT

The study has been included as a manuscript included in the Doctoral thesis of Anne-Kirstine Eriksen, 2019. The role of whole grains and lignans in lifestyle diseases-emphasis on prostate cancer and type 2 diabetes and their risk factors, Acta Universitatis Agriculturae Sueciae 2019:25.

This manuscript is currently under review in the American Journal of Clinical Nutrition. Anne K Eriksen*, Carl Brunius, Mohsen Mazidi, Per Hellström, Ulf Risérus, Kia N Iversen, Rikard Fristedt, Li Sun, Yi Huang, Natalja P Nørskov, Knud-Erik Bach-Knudsen, Cecilie Kyrø, Kirsten Frederiksen, Anja Olsen, Anne Tjønneland, Johan Dicksved, Rikard Landberg (2019). Effects of whole-grain rye and lignan supplementation on cardio-metabolic risk factors in men with metabolic syndrome are associated with baseline gut microbiota enterotype: a randomized cross-over trial. Submitted manuscript.

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