Randomized Controlled Trial

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)

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SUMMARY

Background and aim: A high intake of whole grain foods is inversely associated with body mass index (BMI) and body fat in observational studies, but mixed results have been found in interventional studies. Among whole grains, rye is the richest source of dietary fiber and meals containing high-fiber rye foods have shown increased satiety up to 8 h, compared to meals containing refined wheat products. The aim of the study was to determine the effect of consuming high fiber rye products, compared to refined wheat products, on body weight and body fat loss in the context of an energy restricted diet.

Methods: After a 2-week run-in period, 242 males and females with overweight or obesity (BMI 27 -35 kg/m^2), aged 30–70 years, were randomized (1:1) to consume high fiber rye products or refined wheat products for 12 weeks, while adhering to a hypocaloric diet. At week 0, week 6 and week 12 body weight and body composition (dual energy x-ray absorptiometry) was measured and fasting blood samples were collected. Subjective appetite was evaluated for 14 h at week 0, 6 and 12.

Results: After 12 weeks the participants in the rye group had lost 1.08 kg body weight and 0.54% body fat more than the wheat group (95% confidence interval (CI): 0.36; 1.80, p < 0.01 and 0.05; 1.03, p = 0.03, respectively). C-reactive protein was 28% lower in the rye vs wheat group after 12 weeks of intervention (CI: 7; 53, p < 0.01). There were no consistent group differences on subjective appetite or on other cardiometabolic risk markers.

Conclusion: Consumption of high fiber rye products as part of a hypocaloric diet for 12 weeks caused a greater weight loss and body fat loss, as well as reduction in C-reactive protein, compared to refined wheat. The difference in weight loss could not be linked to differences in appetite response.

Clinical trial registration: www.clinicaltrials.gov, Identifier: NCT03097237.

1. Introduction

One of the most important health challenges of today is to reduce the prevalence of non-communicable diseases which together cause over 60% of total the mortality globally [1]. Obesity is a key risk factor for the development of non-communicable diseases and lifestyle changes have driven obesity prevalence to epidemic proportions [2,3]. Different types of lifestyle modifications that target this issue needs to be investigated [4]. Diet is one of the major determinants for body weight changes and a key tool in the prevention, management and treatment of overweight and obesity [5].

A high whole grain intake is recommended by authorities in many countries, including Sweden, where whole grain foods is recommended whenever cereals are consumed [6]. A high whole grain intake has been shown to have positive effects on several health outcomes [7−9] including inverse association with body mass index (BMI) and body fat in observational studies, but results from intervention studies are inconsistent [10]. These discrepancies might be explained by the fact that most of the studies reporting the effect of wholegrain on body weight are not designed for evaluation of the effect of whole grains on body weight loss. Furthermore, whole grains from different cereals differ in many aspects, such as fiber content and composition, and it is reasonable to hypothesize that the health effects, including impact on body weight and body composition, may vary accordingly [6,11].

Among cereals, rye which is commonly consumed in eastern and Northern Europe, has the highest dietary fiber content and has therefore been suggested to be superior to other whole grains in terms of improving health related outcomes [11]. High fiber whole grain rye food have been shown to increase satiety for up to 8 h after consumption, compared to refined wheat products [12−16]. One hypothesis, which has been supported by a recent meta-analysis, is that increased acute satiety could lead to reduced energy intake and in turn lead to weight loss [17]. Furthermore, rye has been linked to a range of other beneficial health effects such as reduced postprandial insulin [18], reductions in cholesterol [19,20] and reductions in low-grade inflammation [20,21].

The aim of the present study, the RyeWeight study, was therefore to investigate whether a diet rich in high fiber whole grain rye vs refined wheat, as part of a hypocaloric diet, leads to larger weight loss and larger reductions in body fat during a 12-week intervention.

2. Subjects and methods

2.1. Study design and ethics statement

The RyeWeight study was designed as a parallel randomized controlled study in free-living participants, aiming to compare the effect of a high fiber whole grain rye diet, compared to a refined wheat diet, on weight loss and body fat reduction. After a 2-week run-in period, where all participants consumed wheat products, participants were randomized (1:1) to consume either rye products or wheat products for 12 weeks (Fig. 1). Rye and wheat products were provided to the participants free of charge. During all 14 weeks all participants were instructed to adhere to a hypocaloric diet aiming at a 500 kcal energy deficit. The first participant was enrolled in the study in September 2016 and the last participant finalized the intervention in December 2018. The study was conducted at a research clinic in Uppsala Science Park and at the clinical nutrition research lab at Uppsala University Hospital.

All participants gave written consent, after having received oral and written information about the study, prior to initiating the screening procedure. The study was approved by the Ethical Review Board in Uppsala (Dnr: 2016/254) and registered at www.clinicaltrials.gov (Identifier: NCT03097237). The study was conducted in accordance with the Declaration of Helsinki.

2.2. Study population

Participants were recruited through advertisements in newspapers and on the internet, as well as through postings and distribution of flyers in public places such as shopping malls and public libraries.

Men and women aged 30−70 years, with a BMI of 27−35 kg/m^2, were eligible to participate in the study. Furthermore, participants were required to have 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 at the time of screening. Exclusion criteria included blood pressure ≥160/105 mmHg, use of nicotine products, strenuous physical activity more than 10 h/week, history of gastrointestinal disease or major gastrointestinal surgery (e.g. inflammatory bowel disease, malabsorption, bowel resection, gastric bypass surgery), type-1 diabetes, pharmaceutically treated type-2 diabetes, thyroid disorder, use of medication or supplements with the aim of body weight management within the past 6 months, adherence to a weight loss diet within the past 6 months, pregnancy or lactation, heart attack or stroke within past 12 months, and inability to consume any of the foods included in the study (e.g. due to allergy).

BMI and blood pressure were measured after an overnight fast at the first screening visit. Blood samples collected at the first screening visit were sent to the Clinical Chemistry Laboratory at Uppsala University Hospital within 4 h after collection and analyzed within the same day. Participants filled in questionnaires and underwent an interview with the study coordinator to determine eligibility according the inclusion and exclusion criteria.

After the first screening visit, participants were invited to another screening visit ≤5 days later. Before the second screening visit participants were required to complete a 3-day weighed food record (3DWFR). The purpose of doing a 3DWFR at this stage was to a) record the participant's habitual dietary intake, b) briefly screen for any dietary habits that could be incompatible with the protocol (e.g. vegan diets, low-calorie diets) and c) test the participant's willingness to adhere to instructions.

At the second screening visit, results from analysis of blood samples were assessed and exclusion criteria were re-checked to make sure that the participant was eligible for participation. The participant was provided with intervention foods and a fecal collection kit, all future visits were scheduled, and the participant was instructed to start the run-in period. During the run-in period participants were required to lose at least 0.5 kg in order to be enrolled and randomized into the 12-week parallel intervention phase. For women menstruating during the run-in period, the requirement for enrollment was no weight gain during the run-in period. Participants were not informed about the weight loss requirement during the run-in period.

2.3. Intervention products

The intervention products consisted of breakfast cereals, crisp bread and soft bread, in both the rye group and in the wheat group (Table 1). Breakfast cereals consisted of extruded rye and wheat puffs, as well as rolled rye flakes and semolina wheat, packed in 30 g portions. Participants were instructed to consume two packages per day but could choose freely if they wanted puffs or flakes/semolina. Participants in the rye group had four different rye crisp breads to choose from and were instructed to consume 4−6 slices per day (53−60 g/day), depending on the variety they
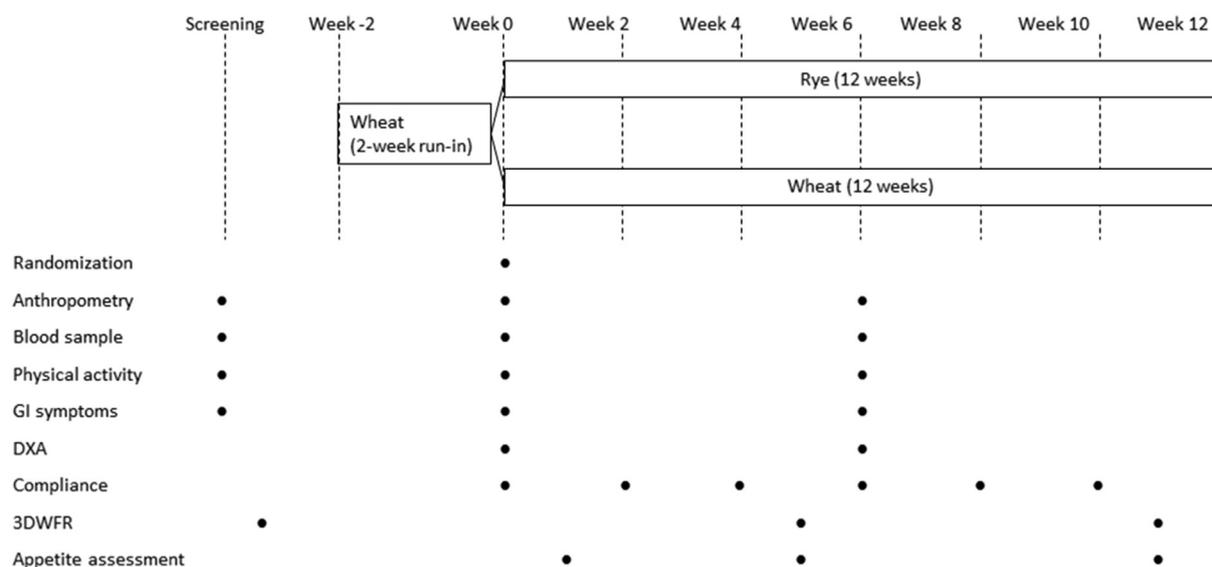
Fig. 1. Study design. Abbreviations: DXA, dual energy x-ray absorptiometry; 3DWFR, 3-day weighed food record.

choose (due to difference in slice weight between the varieties). The wheat group had only one type of crisp bread and were instructed to consume 5 slices per day (66 g/day). Crisp bread was packed in portion packs to help participants consume correct amounts and make it easier to bring products when eating outside the house. Soft bread was provided frozen in the form of wheat buns or slices rye bread. The participants in the wheat group were instructed to eat one wheat bun (70 g) per day, while the participants in the rye group were instructed to consume 2—2.5 slices of rye bread per day to reach a total of 16 slices per week (on average 119 g per day). All products were packed in neutral white or transparent packaging material, but due to the visual differences between rye and wheat, participants could not be blinded to their allocation. The daily amount of interventions products amounted to approx. 650 kcal in both groups, which corresponded to approximately 30—50% of the participants daily energy intake. The fiber content and composition differed substantially between the groups, with the rye products providing approx. 30 g fiber/day and the wheat products providing 8 g fiber/day (Table 1, supplemental material 1).

Participants were instructed not to consume any other cereals than the ones they received from the study, except for very small amounts of "hidden" cereal (e.g. thickening in sauces). Every day during the study, the participants filled in a pre-coded compliance journal where they ticked off the products they consumed. Furthermore, participants were instructed to note deviations from the intervention diet, changes in habitual medication, or any cases of illness in the journal as well.

2.4. Weight loss intervention

The aim for participants was to reach an energy deficit of 500 kcal/day, while maintaining a macronutrient distribution as recommended in Nordic Nutrition Recommendations (NNR) 2012 (45—60 E% carbohydrate, 10—20 E% protein, 25—40 E% fat) [9]. All participants had a personal meeting with a dietician around the time they were scheduled to start the run-in period. The dieticians reviewed the 3DWFR and interviewed the participant to get an understanding of their habitual diet and calculated their energy requirement following the equations, according to Henry et al. [22], as presented in NNR 2012, assuming a physical activity level (PAL)

of 1.4 [9]. Based on the habitual dietary pattern the dietician provided suggestions for dietary change based on the Step-wise Weight-determined Accumulative Change Plan (SWAP) model, developed by Bertz et al. [23], in order to reach the desired energy deficit. The original SWAP model did not address fast-food intake, but as most fast-food contain cereals which participants were instructed to avoid, it was considered important for dieticians to address this and a step concerning fast-food was added to the model.

The following modified SWAP model was used in the Rye-Weight-study:

1. Minimize the intake of sweets, cakes and soft drinks (limit to a small amount (<100 g) once per week).
2. Minimize the intake of fast-food
3. Choose "keyhole" labelled products to minimize the intake of sugar and fat
4. Increase the intake of vegetables (half the plate for lunch and dinner).
5. Decrease portion sizes (while still maintaining a meal composition following the NNR recommendation).

Dieticians would implement the steps of the SWAP model into the participants habitual diet in prioritized order. Hence, if a participant had a high intake of foods mentioned in step one, this would be the main focus, whereas if the intake of foods mentioned in step one was not identified as being too high, then the focus was moved to step two and so forth.

Dieticians contacted each participant by email or telephone after their visit to the clinic in week 2 for a follow-up consultation. Follow-up consultations were done through email or phone depending on the individual participants preference. Lastly, the participants were informed that they could request additional follow-up consultations with the dieticians at any time during the study, if they had any problems or questions relating to the diet.

2.5. Randomization and blinding

After the 2-week run-in period the participants attended an examination visit (week 0). First participants were weighed to evaluate whether they had lost sufficient weight during the run–in

Table 1

Composition of intervention products, per 100 g edible product. Participants were instructed to consume 60 g breakfast cereals (puffs or flakes/semolina), 4–6 slice of crisp bread (53–66 g) and one serving of soft bread (Wheat: 70 g, rye: 119 g) per day, why the intake as per protocol varied depending on the participants choice of crisp bread and breakfast cereals.

		Product weight (g)	Energy (kcal)	CHO (g)	Protein (g)	Fat (g)	Dietary fiber (g)[a]			Arabinoxylans (g)			Fructans (g)	Klason lignin (g)	Glucose (g)	Total AR (mg)	C17:0/C21:0 ratio
							Total	Extr.	Unextr.	Total	Extr.	Unextr.					
Per 100 g edible product																	
Rye products																	
Extruded rye puffs		100	345.6	64.0	9.0	2.2	16.78	6.86	9.92	7.16	2.29	4.87	3.85	1.28	3.59	24.9	1.15
Rolled rye flakes		100	345.8	63.7	8.6	2.2	18.33	7.41	10.92	8.34	3.29	5.05	3.02	1.34	4.69	35.8	0.98
Rye crisp bread 'Rågi'		100	339.8	65.9	8.8	1.4	14.14	5.60	8.54	6.59	2.47	4.10	2.30	0.65	3.74	39.5	0.93
Rye crisp bread 'Husman'		100	335.6	63.1	9.0	1.5	16.95	6.65	10.30	8.16	2.98	5.18	2.45	1.24	4.18	39.7	1.00
Rye crisp bread 'Sport'		100	337.5	63.6	8.4	1.6	17.50	6.11	11.39	8.07	2.44	5.64	2.61	1.09	4.80	43.3	0.97
Rye crisp bread 'Delikatess'		100	336.1	61.0	10.0	1.5	19.27	7.39	11.88	8.43	2.54	5.89	3.79	1.19	4.96	38.3	0.97
Soft rye bread		100	225.6	34.4	7.5	4.1	10.62	3.75	6.87	4.54	1.50	3.04	1.52	1.04	2.92	32.9	0.86
Wheat products																	
Extruded wheat puffs		100	367.7	73.4	11.5	1.9	5.42	2.51	2.91	2.36	1.24	1.12	1.05	0.78	0.75	0.8	nd[c]
Wheat semolina		100	353.5	75.0	10.0	0.8	2.95	1.33	1.63	1.17	0.56	0.61	0.62	0.17	0.54	nd[b]	nd[b]"
Wheat crispbread		100	392.4	65.6	12.4	7.7	5.56	1.52	4.04	2.56	0.86	1.69	0.39	0.50	1.50	4.1	0.05
Soft wheat bread		100	252.2	43.3	13.7	1.9	3.53	1.03	2.50	1.24	0.76	0.49	0.15	0.71	1.05	1.6	nd[c]
Average daily amount of intervention products prescribed per protocol																	
Rye	Mean	235	664	114.6	19.2	7.0	32.7	12.3	20.4	14.4	4.9	9.5	5.4	2.6	8.4	80.01	0.93
	(minimum)	(232)	(656)	(112.5)	(18.8)	(6.9)	(30.2)	(11.5)	(18.7)	(13.2)	(4.5)	(8.7)	(4.8)	(2.3)	(7.6)	(75.00)	(0.91)
	(maximum)	(239)	(679)	(117.5)	(19.8)	(7.2)	(34.2)	(12.9)	(21.6)	(15.2)	(5.4)	(10.0)	(6.2)	(2.7)	(9.2)	(86.62)	(0.95)
Wheat	Mean	196	652	118.1	24.2	7.2	8.7	2.9	5.8	3.6	1.6	2.0	0.9	1.1	2.1	4.09	0.03
	(minimum)	(196)	(648)	(117.6)	(23.8)	(6.9)	(7.9)	(2.5)	(5.4)	(3.3)	(1.4)	(1.8)	(0.7)	(0.9)	(2.0)	(3.86)	(0.03)
	(maximum)	(196)	(656)	(118.6)	(24.7)	(7.6)	(9.4)	(3.2)	(6.2)	(4.0)	(1.8)	(2.1)	(1.0)	(1.3)	(2.2)	(4.33)	(0.04)

[a] Including arabinoxylans, fructans, klason lignin and glucose.

[b] no alkylresorcinol detected in the product.

[c] No C17:0 detected in the product. Abbreviations: CHO, carbohydrate; extr, extractable; unextr, unextractable; AR, alkylresorcinols.

period and were eligible for enrollment. If a participant was found to be eligible, the examination continued as planned (see below) and the participant was randomized. If the participant had not lost sufficient weight, the examination was stopped, and the participant was not randomized and was withdrawn from the study. The randomization list was generated using the online tool www. randomization.com, by random mixed block randomization with block sizes 6, 8 and 10. Randomization was done by the study coordinator.

Participants were not blinded due to the visual differences between rye and wheat products. Nurses and technicians conducting the examinations of the participants were however blinded to participants allocation. Dieticians met with participants before randomization and were therefore not aware of the allocation at the first consultation, however it is likely that some participants revealed their allocation during the follow-up consultations.

2.6. Study visits

At week 0, week 6 and week 12 participants attended an examination visit, which was conducted in the morning between the hours of 7:00—11:30. Participants arrived at the clinic in fasted state and brought with them a fecal sample. Participants were instructed to avoid food and beverages from 20:00 the night before, except for up to 500 ml plain tap water. Furthermore, participants were instructed to avoid alcohol and intense physical activity for 24 h prior to their visit to the clinic. Upon arrival to the clinic bodyweight, hip and waist circumference, and sagittal diameter was measured. 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, Tokyo, Japan). Waist and hip circumference were measured to the nearest 0.5 cm with a measuring tape (Measuring tape 201, SECA Medical Measuring Systems and Scales, Hamburg, Germany). Waist was measured at the point of the navel and hip was measured at the widest point of the hip. Participants were instructed to let arms hang down their sides and take a deep breath. Measurement was done twice upon exhalation, and an average was calculated. Sagittal diameter was measured to the nearest 0.5 cm using a sagittal diameter measuring pin (BK-mätare, AJ Medical, Lidingö, Sweden) with participant lying on their back with legs bend. Participants were instructed to take a deep breath and measurement was done twice at the point of the navel upon exhalation. Average of the two measurements was calculated. Height was measured only at the first screening visit using a wall mounted roll-up measuring tape (Measuring tape 206, SECA Medical Measuring Systems and Scales, Hamburg, Germany), with participants wearing no shoes.

Thereafter participants were instructed to rest for 10 min in a supine position before blood pressure was measured. Blood pressure was measured twice using an automatic blood pressure monitor (OMRON M6 AC, OMRON HEALTHCARE Co. Ltd., Kyoto, Japan), and if measurements differed more than 5 mmHg a third measurement was done. The average of all two/three measurements was calculated. Immediately hereafter blood samples were collected with participant remaining in the supine position. Finally, participants filled in questionnaires (see below) and were provided with intervention products and materials needed prior to next visit (e.g. compliance journal and appetite assessment forms).

Participants underwent a total body dual energy x-ray absorptiometry (DXA) scan (Lunar Prodigy, GE Medical Systems, Chicago, Illinois, USA) for estimation of body composition. The DXA scan was conducted either on the same day as the examination, or on another day in the same week. For majority of the participants (88% of conducted scans), DXA scans were conducted immediately after the examinations without breaking the fast. Participants who had DXA scans conducted on another day were instructed to fast (avoid all food or beverages) for minimum 3 h prior to the DXA scan. The precision errors on triple DXA scans in 15 participants, including repositioning, were 0.8—1.5% depending on type of measurement. The long-term coefficient of variation was less than 1% for a spine phantom. The validity of fat mass derived by Lunar Prodigy has been evaluated against the 4-compartment model, the tool that is currently considered the gold standard method of body composition appraisal, resulting in 1.7—2.0% higher fat mass estimates with the narrow fan-beam DXA equipment [24].

Besides examination visits in week 0, 6 and 12, the participants visited the clinic in weeks 2, 4, 8 and 10 to collect intervention products and other materials needed for future visits (e.g. fecal collection kits and 3DWFR).

2.7. Appetite assessment

Participants conducted a full day appetite assessment at home between visits in week 0—2, week 4—6 and week 10—12. During this day, the participants followed a standardized meal plan, including intervention products according to allocation. During the day the participants answered questions about their appetite every 30 min from 8:00—12:00 and every 60 min from 13:00—22:00.

At each time point participants answered three questions in random order: "How hungry are you?", "How full are you?", "How strong is your desire to eat?". Questions were answered on a 100-point visual analogue scale with following words anchored at each end: "Not hungry at all/I have never felt more hungry", "Not full at all/Extremely full" and "Not strong at all/Extremely strong". Questions were sent to participants by email using the online survey tool Qualtrics (Qualtrics[xm], Seattle, Washington, USA), and the participants could answer questions on their mobile device, computer or similar. All participants were provided with questions on papers as well, in case they could not use email, or experienced technical problems during the day. When answering questions on paper, participants were asked to note the time and date on the questionnaires, place the questionnaires in sealed envelopes (one envelope per timepoint) and bring them to the clinic at next visit. During the day of the appetite assessment the participants had a checklist that contained all scheduled questions and meals. Participants were asked to note any deviations in the checklist and hand it in at their next visit to the clinic. During the day of the appetite assessment the participants followed a meal plan that was adjusted to match the energy need for each participant with an energy deficit of 500 kcal. The meal plans included intervention products according to group allocation and all foods needed for the days was provided to the participants. Immediately after answering the first questions at 8:00 the participants consumed a breakfast meal consisting of puffs and milk. For lunch, immediately after 12:00 questions, participants consumed a ready-to-eat goulash soup with crisp bread and butter/cheese. At 15:00 participants had a snack meal consisting of crisp bread, yoghurt and jam/butter. Immediately after answering questions at 18:00 participant consumed a dinner of tomato soup, soft bread and cheese/butter. At 20:00 participant had a snack meal of crisp bread, butter/jam and sesame crackers. Participants were allowed 300 ml of water, tea or coffee with each meal. At first appetite assessment day participants chose and recorded their choice of beverage and were then asked to have the same beverages on later appetite assessment days. If participants felt very thirsty during the day, they could consume maximum 100 ml extra water at least 30 min before next question was scheduled to be answered.

2.8. Dietary assessment

Participants were instructed to conduct a 3DWFR between the screening visits, between week 4–6 and between week 10–12. Participants were instructed to record everything they ate or drank during two weekdays and one weekend day. They were instructed to use scales as much as possible, and to use household measures (e.g. deciliter or slices) to estimate amounts when it was not possible to weigh the food. 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. In case of missing information about the amount of specific food items in the 3DWFR standard portions in the software was used. Additionally, foods were grouped into food groups and the daily intake of each food group was calculated. The grouping of foods was based on the method used in a national dietary intake surveys conducted by the Swedish Food Agency, Riksmaten [25].

2.9. Compliance evaluation

Compliance was evaluated using pre-coded compliance journals, as previously described, and the daily intake of intervention products was calculated. As pre-defined in the study protocol, a participant was considered to be compliant if he/she consumed minimum 80% of the daily amount of products (weight/weight) on average over the 12-week intervention period. Plasma alkylresorcinols (AR), biomarkers of whole grain rye and wheat intake [26], were analyzed as a supporting measure of compliance.

2.10. Questionnaires

At screening, week 0, week 6 and week 12 participants filled in questionnaires on physical activity and gastrointestinal symptoms. Physical activity was assessed using the questionnaire developed by Baecke et al., and an activity score for work, sport and leisure time activities was calculated as per the method described by the developer of the questionnaire [27]. Activity level of sports reported by the participants that were not mentioned in the original publication of the questionnaire were evaluated using the compendium of physical activity by Ainsworth et al. [28]. Gastrointestinal symptoms was assessed using the GSRS-IBS questionnaire and participants answers were transformed into subscale symptom scores using the method described by Wiklund et al. [29].

Eating behavior was assessed at the first screening visit using the 21-item Three Factor Eating Questionnaire (TFEQ), which is evaluating participant's behavior with regards to three different domains; cognitive restrained eating, uncontrolled eating and emotional eating [30]. Furthermore, the participants filled in a demographic questionnaire developed for study to record background information, such as educational level, occupation and housing.

2.11. Biological samples

At week 0, week 6 and week 12 blood samples were collected in 6 ml K2E EDTA tubes, 4 ml sodium heparin tubes and 5 ml serum glass tubes (BD Vacutainer, Beckton Dickinson, Franklin Lakes, New Jersey, USA). As a standard procedure in our laboratory we also collected samples in 4.5 ml sodium citrate tubes and 2.5 ml PAXgene tubes, which were processed and stored for potential later use. Serum tubes were kept at room temperature before, during and after sampling, whereas EDTA, citrate and heparin tubes were kept on ice from before sampling until centrifugation.

EDTA, heparin and citrate tubes were centrifugated immediately after sampling at 4 °C and 2500 g, for 10 min, whereafter plasma was transferred into screw cap micro tubes (Sarstedt AG & Co., Nümbrecht, Germany), before being placed in −20 °C freezer. Erythrocytes and buffy coat from the EDTA tubes were likewise transferred to micro tubes and stored for potential later use as a standard procedure in our laboratory. Serum tubes were kept at room temperature for 60 min, prior to centrifugation and thereafter the serum was transferred into screw cap microtubes and stored at −20 °C. PAXgene tubes were kept at room temperature for 2 h and transferred to −20 °C for a minimum of 24 h before being transferred to −80 °C. Spot fecal samples were collected as a standard procedure in our laboratory and stored for later use. All samples were kept in −20 °C freezer for max duration of 7 days, and then transferred to −80 °C freezer for long term storage in a biobank.

Blood samples were analyzed at the Department of Clinical Chemistry at Uppsala University Hospital for selected metabolic biomarkers. Insulin was measured in serum using a noncompetitive immunoassay. Remaining markers were analyzed in sodium heparin plasma using immunoturbidimetric assay (C-reactive protein (CRP)), hexokinase method (glucose) and enzymatic colorimetric assays (lipids). All measurement was done on Cobas® Pro (Roche Diagnostics, Basel, Switzerland) according to standardized procedures at the laboratory (coefficient of variance<10%). Some samples had CRP concentration below the detection limit (0.2 mg/L) and were therefore assigned a value of half the detection limit (2.6% of the samples). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as (insulin (mIU/L) x glucose (mmol/L))/22.5 [31].

AR were measured in EDTA plasma at Chalmers Mass Spectrometry Infrastructure using liquid chromatography tandem mass spectrometry according to a method developed at the platform [32]. Total plasma AR concentration was calculated as a sum of homologues C17:0-C25:0 and was used as a biomarker 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. Since the C17:0 homologue is primarily present in rye, a higher ratio C17:0/C21:0 indicate that a larger proportion of the consumed whole grain cereals are from rye, while a low ratio indicate that the intake is primarily from wheat [33]. The inter- and intra assay coefficient of variance was <15% for all batches analyzed.

2.12. Statistical analyses

The number of subjected needed for the study was estimated based on two primary endpoints (body weight and body fat percentage), with power of 80% and alpha of 2.5%. The number of subjects needed to detect an effect of 1 kg difference in body weight (standard deviation: 2.4 kg [34]) and 1% difference in body fat percentage (standard deviation 1.5% [35]) after 12 weeks, was estimated to be 106 participants in each treatment group. Based on experience from previous studies, we aimed to randomize 260 participants (130/treatment group) to allow for 18% drop-out rate. Due to having two primary endpoints, Bonferroni adjustment was applied to the primary outcomes, and therefore the significance level was set to p < 0.025 for the primary outcomes. For remaining outcomes significance level was p < 0.050.

Differences between treatments were evaluated using the PROC MIXED procedure in SAS statistical software version 9.4 (SAS institute, Cary, USA). Week and week*diet were included as fixed factors, and a REPEATED statement specifying an unstructured

correlation structure with week as the repeated factor was included. Models were comparing the groups at week 6/week 12 and were adjusted for baseline (week 0). Furthermore, models evaluating metabolic markers were also adjusted for weight change. Normality was evaluated using residual plots and histograms. Data was log transformed prior to analysis if deviating from a normal distribution, and estimates were back transformed to the original scale after analysis.

Primary outcomes of the study were complete cases analysis of the difference in body weight and body fat percentage, between diets after 12 weeks of intervention as defined in the study protocol. Secondary outcomes were differences of additional anthropometric and clinical outcomes measures (e.g. body fat mass, lean body mass, waist-hip ratio, blood pressure) between diets after 6 and 12 weeks of intervention, body weight and body fat percentage after 6 weeks of intervention and differences in appetite responses. In the complete cases analysis, a completed case was defined as a participant who had completed the 12-week intervention and participated in the final examination at week 12.

As a secondary analysis, two intention to treat (ITT) analyses were conducted on the primary and secondary outcomes. In these analyses, all randomized participants were included irrespective of whether they completed the 12-week intervention or dropped out during the intervention. ITT analysis was conducted using last observation carried forward (LOCF), where missing values were imputed by the last observed value, as well as by linear mixed modelling using the PROC MIXED procedure without imputation of missing data, as defined in the statistical analysis plan.

Area under the curve (AUC) for appetite response was calculated using the trapezoid method. AUC was calculated for the entire day (8:00–22:00), morning (8:00–12:00), afternoon (12:00–18:00) and evening (18:00–22:00). AUC appetite data was analyzed using the same method as anthropometric outcomes (above), though without baseline adjustment. Repeated appetite response was analyzed separately for each week, in the same way as AUC appetite, but using time of day rather than week as the repeated factor.

Correlations were calculated using the PROC CORR procedure, specifying Pearson's correlation for normally distributed data and Spearman's rank correlation when data was not normally distributed.

3. Results

In total 590 participants were screened for the study of which 317 fulfilled the criteria and initiated the run-in period (Fig. 2). During the run-in period, 36 subjects withdrew from the study, mainly due to not being willing to follow intervention diet or difficulties fitting the logistics of the study into their daily life. Furthermore, 39 participants were excluded from the study due to not achieving sufficient weight loss during the run-in period (lost <0.5 kg). In total, 242 participants completed the run-in period with sufficient weight loss and were enrolled and randomized into the 12-week parallel intervention. During the 12-week intervention 14% of the participants dropped out. Main reason for drop-out was related to the diet. Participants became tired of the products or experienced stomach problems (mainly bloating and flatulence) due to the change in diet. Furthermore, some participants dropped out due to difficulties fitting study visits into working hours and/or family life. Four participants dropped out due to various illnesses, none of which are related to the study or the intervention. In total, 207 participants completed the 12-week intervention (Fig. 2).

At baseline, the groups did not differ in terms of anthropometrics and demographic factors and sex distribution was similar

(40% males). Furthermore, no baseline difference in terms of the factors evaluated in the TFEQ was observed between the groups (Table 2).

Physical activity level remained stable throughout the study and the were no differences between the groups at any time point (Supplemental Table 1).

For gastrointestinal symptoms, the groups did not differ in terms of abdominal pain or diarrhea (Supplemental Fig. 1). However, during the 12-week intervention, participants in the rye group felt more bloated and less constipated compared the participants in the wheat group. Nevertheless, it should be noted that both groups had relatively low symptom scores throughout the study (1–2.5 of 5), indicating that participants had relatively few symptoms both before and during the study.

3.1. Compliance

Compliance, assessed by compliance journals, showed a high degree of compliance to the intervention with participants reporting consumption of about 94–95% of the products on average over the 12-week intervention, as well as during the run-in period (Table 3). The participants in the rye group consumed an average of 30.7 g of dietary fiber per day from the intervention products, whereas the wheat group consumed 8.5 g of dietary fiber per day (Table 3).

Similarly, concentration of AR in plasma also indicated an overall good compliance (Table 4). The groups had similar and low concentration of total AR and C17:0/C21:0 ratio at baseline, which is to be expected as they had consumed refined wheat products during the 2-week run in period. Both total AR concentration and C17:0/C21:0 was markedly higher in the rye group at week 6 and week 12, compared to the wheat group, showing a higher intake of whole grain, primarily sourced from rye. It should be noted that the total AR concentration seemed to decrease slightly in the rye group between week 6 and week 12, which could indicate that the compliance was somewhat better during the first part of the intervention. The AR concentration in the wheat group remained stable throughout the entire intervention period indicating good compliance to a refined wheat diet.

3.2. Dietary intake

Energy intake did not differ between the groups before or during the intervention, and the daily energy intake was reduced by 100–200 kcal during the intervention in both groups (Table 5). The dietary fiber intake in the wheat group remained relatively stable throughout the intervention, whereas the dietary fiber intake increased in the rye group and was significantly higher than the intake in the wheat group at week 6 and 12. The difference between groups in dietary fiber intake estimated from the 3DWFR corresponds approximately to the difference in dietary fiber intake found in the compliance journals (approx. 20 g/day) (Tables 3 and 5). The protein intake seemed to be slightly higher in the wheat group, compared to the rye group, in week 12, but otherwise the macronutrient intake did not differ between the groups (Table 5).

Table 5 show intake of different food groups calculated from the 3DWFR. The total cereal food intake was higher in the rye group, compared to the wheat group throughout the intervention, but this can be explained by the fact that weight of the prescribed rye products was higher than the prescribed amount of wheat products (235 vs. 196 g/day, Table 1) in order to reach the same amount of
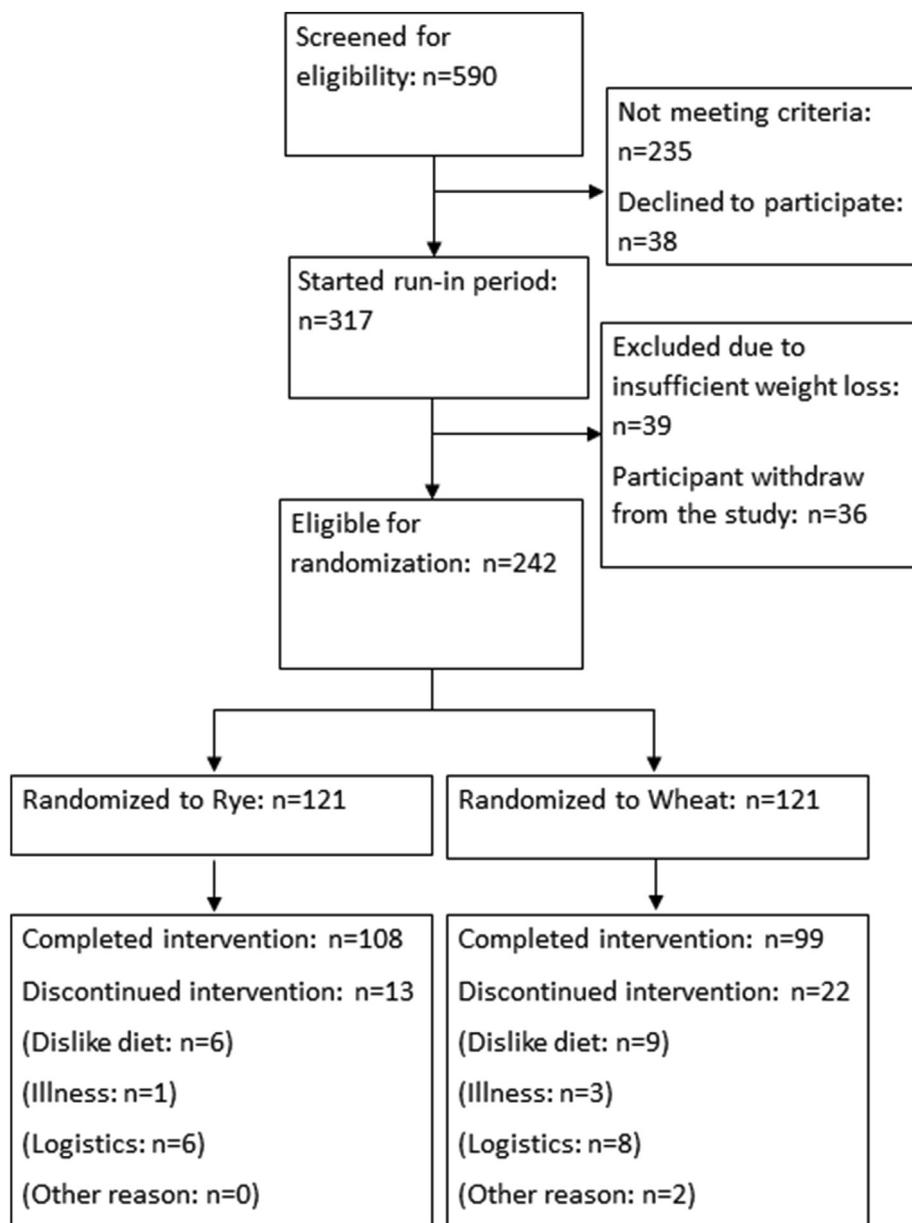
Fig. 2. Flow diagram of participants.

energy from intervention products. Dairy consumption was higher in the wheat group compared with the rye group (60—70 g/day difference). This may be due to fact that the participants in the wheat group preferred puffs, commonly eaten with milk or yoghurt, over semolina porridge, whereas the participants in the rye group had a more even distribution between puffs and rye flakes. In the rye group, puffs accounted for 64% of the breakfast cereal intake, while puffs accounted for 74% of the breakfast cereal intake in the wheat group. The higher dairy intake could potentially explain the higher protein intake in the wheat group.

Both the rye and wheat group reduced their intake of sweets and snacks by approx. 50% (Table 5). This is in line with the first step of the SWAP model used by dieticians, and it is therefore plausible that reduction of food from this category has been the main focus across all participants.

3.3. Body weight and body fat − primary outcomes

Evaluation of the primary outcomes, completed cases analysis of body weight and body fat percentage at week 12, showed that the body weight in the rye group was significantly lower than the bodyweight in the wheat group ($p = 0.0036$) (Table 6). On average, the rye group lost 2.9 kg (hereof 2.7 kg fat) during the 12-week intervention, while the wheat group lost 1.8 kg (hereof 1.8 kg fat) (Tables 6 and 7). The rye group had a lower body fat percentage at week 12, compared to the wheat group, but this difference was borderline significant after adjustment for multiple endpoints ($p = 0.0307$, significance level: $p < 0.025$). Both approaches for ITT analyses confirmed the difference between the groups in body weight and body fat mass, and furthermore, the difference in body fat percentage was significant in both ITT analyses approaches even

Table 2
Baseline characteristics of enrolled participants.

	Rye (n = 121)	Wheat (n = 121)
Anthropometrics and demographics		
Sex (f/m) (%females)	71/50 (59%)	76/45 (63%)
Age (years)	56.5 ± 9.7	56.9 ± 9.5
Body weight (kg)	88.2 ± 12.5	88.8 ± 12.5
BMI (kg/m^2)	29.7 ± 2.4	30.3 ± 2.4
Body fat (%)	39.1 ± 6.6	40.8 ± 7.2
Education level (highest completed)		
Primary school	7 (6%)	11 (9%)
2 year high-school program/vocational training	20 (17%)	18 (15%)
3—4 year high-school program	15 (12%)	23 (19%)
University	78 (65%)	67 (55%)
Other/unknown	1 (1%)	2 (2%)
Current occupation		
Employed	83 (69%)	69 (57%)
Self-employed	12 (10%)	12 (10%)
Temporarily unemployed	0 (0%)	1 (1%)
Parental leave	0 (0%)	1 (1%)
Studying	1 (1%)	1 (1%)
Retired	23 (19%)	29 (24%)
Sick leave/early retirement	0 (0%)	3 (2%)
Other/unknown	2 (2%)	5 (4%)
Housing		
Apartment, rented	14 (12%)	17 (14%)
Apartment, owned	35 (29%)	31 (31%)
House, rented	1 (1%)	3 (2%)
House, owned	68 (56%)	66 (55%)
Other/unknown	3 (2%)	4 (4%)
Share household with other adults (≥18 y)	100 (83%)	96 (79%)
Share household with children (<18 y)	38 (31%)	32 (26%)
Living alone	17 (14%)	23 (19%)
Country of birth		
Sweden	102 (84%)	110 (91%)
Other country in the European Union	7 (6%)	4 (3%)
Outside the European Union	11 (9%)	6 (5%)
Unknown	1 (1%)	1 (1%)
Three factor eating questionnaire (TFEQ)		
Restrained eating	13.5 ± 2.9	13.8 ± 2.9
Emotional eating	13.6 ± 4.2	14.2 ± 4.5
Uncontrolled eating	20.4 ± 4.6	20.3 ± 5.0

Data is n (%) or mean ± SD.

Table 3
Daily average intake of intervention products over 2 weeks (run-in period) and 12 weeks (rye group and wheat group) according to compliance journals.

	Weight (g)	Energy (kcal)	Carbo-hydrate (g)	Protein (g)	Fat (g)	Dietary fiber (g)	% of products eaten (weight/weight)[a]
Run-in period (n = 207)	186.4 ± 14.4 (191)	619.5 ± 50.4 (637.6)	111.9 ± 9.3 (115)	23.2 ± 1.7 (23.7)	6.9 ± 0.6 (7.2)	8.4 ± 0.7 (8.5)	95.1 ± 7.4 (97.4)
Wheat group (n = 99)	185.4 ± 11.8 (188.9)	618.3 ± 39.1 (627.8)	111.6 ± 7.1 (113.4)	23.1 ± 1.5 (23.5)	7 ± 0.6 (7.1)	8.5 ± 0.6 (8.6)	94.6 ± 6.0 (96.4)
Rye group (n = 108)	221.4 ± 20.6 (228.4)	626.8 ± 54.3 (644)	108.4 ± 9.2 (111.2)	18.1 ± 1.6 (18.7)	6.6 ± 0.7 (6.8)	30.7 ± 2.6 (31.5)	94.2 ± 8.8 (97.2)

Data is mean ± SD(median).
[a] Consumed amount of the prescribed products, 100% indicate perfect compliance.

Table 4
Total plasma alkylresorcinol concentration and the ARC17:0/C21:0 homologue ratio. N: rye = 108, wheat = 99. Data is geometric mean (95% confidence interval).

	Week 0	Week 6	Week 12
Total alkylresorcinol (nmol/L)			
Wheat	79 (70; 88)	71 (64; 79)	73 (65; 83)
Rye	72 (64; 80)	531 (478; 589)	457 (405; 515)
C17:0/C21:0 homologue ratio			
Wheat	0.06 (0.05; 0.07)	0.06 (0.05; 0.06)	0.07 (0.06; 0.08)
Rye	0.06 (0.05; 0.06)	0.40 (0.36; 0.45)	0.41 (0.37; 0.46)

Data is geometric mean (95% confidence interval).

after adjustment for multiple endpoints (Supplemental Table 2). Body weight differed between the groups already after 6 weeks of intervention (0.6 kg, p = 0.0071), though to a lesser extent than after 12 weeks (1.1 kg, p = 0.0036).

3.4. Secondary anthropometric outcomes and clinical markers

BMI, fat mass, waist and hip circumference were lower in the rye group than in the wheat group after 12 weeks of intervention, which is to be expected due the higher weight loss (Table 7). Lean mass did not differ between the groups and did not decrease in either of the groups, showing that the weight reduction was primarily due to a reduction in fat mass. Waist-to-hip ratio was not affected by the intervention, which is likely because waist and hip circumference was reduced to a similar extent during the intervention, meaning that the ratio remained unchanged. Blood pressure did not differ between the groups and remained stable throughout the intervention (Table 8). However, it should be noted that the average blood pressure in both groups were within normal range (approx. 125/80 mmHg) at week 0, leaving little room for improvement.

Table 5
Average daily dietary intake according to 3-day weighed food records.

		Baseline n = 107/99[a]	Week 6 n = 108/99[a]	Week 12 n = 107/97[a]	*p-value* (week 0)	*p-value* (week 6)	*p-value* (week 12)
Energy (kcal/day)	Rye	2173 ± 5312 (2122)	1953 ± 435 (1884)	2001 ± 496 (1976)	0.402	0.969	0.687
	Wheat	2118 ± 550 (2020)	1958 ± 469 (1871)	2026 ± 468 (1909)			
Protein (g/day)	Rye	87 ± 23 (82)	89 ± 22 (84)	87 ± 23 (87)	0.984	0.366	0.012
	Wheat	87 ± 24 (84)	92 ± 21 (91)	95 ± 25 (91)			
Fat (g/day)	Rye	93 ± 28 (91)	75 ± 26 (70)	80 ± 28 (78)	0.959	0.711	0.807
	Wheat	94 ± 30 (88)	77 ± 31 (73)	81 ± 29 (76)			
Carbohydrate (g/day)	Rye	219 ± 65 (211)	201 ± 38 (200)	202 ± 49 (197)	0.593	0.449	0.145
	Wheat	208 ± 64 (207)	205 ± 50 (200)	207 ± 44 (201)			
Dietary fiber (g/day)	Rye	22 ± 8 (21)	38 ± 9 (38)	37 ± 9 (38)	0.055	<0.001	<0.001
	Wheat	21 ± 6 (20)	20 ± 7 (19)	19 ± 5 (18)			
E% protein	Rye	17 ± 4 (16)	18 ± 3 (18)	18 ± 3 (17)	0.520	0.139	0.001
	Wheat	17 ± 3 (16)	19 ± 3 (18)	19 ± 3 (18)			
E% fat	Rye	38 ± 6 (39)	34 ± 6 (34)	35 ± 6 (36)	0.150	0.443	0.934
	Wheat	40 ± 7 (40)	35 ± 8 (35)	35 ± 7 (35)			
E% carbohydrate	Rye	40.1 ± 6.1 (41)	40.1 ± 6.1 (41)	40.1 ± 6.1 (41)	0.206	0.680	0.678
	Wheat	39.4 ± 7.1 (40)	39.4 ± 7.1 (40)	39.4 ± 7.1 (40)			
E% dietary fiber	Rye	2.1 ± 0.6 (2)	4.0 ± 1.0 (4)	3.8 ± 1.0 (4)	0.204	<0.001	<0.001
	Wheat	2.0 ± 0.5 (2)	2.1 ± 0.6 (2)	1.9 ± 0.6 (2)			
E% alcohol	Rye	3 ± 4 (2)	3 ± 3.0 (0)	2 ± 4 (0)	0.696	0.166	0.282
	Wheat	3 ± 3 (1)	2.2 ± 3 (0)	3 ± 4 (1)			
Fruit and vegetables (g/day)	Rye	364 ± 175 (346)	382 ± 179 (365)	351 ± 164 (335)	0.065	0.229	0.060
	Wheat	336 ± 174 (313)	364 ± 194 (342)	317 ± 151 (310)			
Potatoes (g/day)	Rye	71 ± 70 (53)	77 ± 69 (65)	80 ± 70 (70)	0.427	0.337	0.493
	Wheat	63 ± 58 (52)	65 ± 58 (50)	87 ± 73 (68)			
Cereals (g/day)	Rye	234 ± 120 (223)	232 ± 88 (218)	227 ± 74 (222)	0.069	<0.001	<0.001
	Wheat	209 ± 98 (193)	184 ± 46 (187)	181 ± 44 (191)			
Meat (g/day)	Rye	134 ± 77 (121)	127 ± 75 (112)	127 ± 76 (114)	0.677	0.212	0.561
	Wheat	129 ± 64 (127)	110 ± 79 (100)	129 ± 91 (108)			
Seafood and caviar (g/day)	Rye	50 ± 56 (33)	46 ± 41 (40)	49 ± 51 (40)	0.273	0.091	0.223
	Wheat	46 ± 41 (40)	38 ± 37 (37)	42 ± 41 (33)			
Eggs (g/day)	Rye	30 ± 30 (23)	26 ± 32 (17)	28 ± 30 (17)	0.250	0.059	0.140
	Wheat	36 ± 40 (20)	36 ± 41 (22)	36 ± 39 (22)			
Dairy (excluding cheese) (g/day)	Rye	271 ± 149 (258)	348 ± 152 (333)	357 ± 174 (333)	0.335	0.006	0.025
	Wheat	295 ± 196 (243)	416 ± 200 (407)	417 ± 211 (383)			
Cheese (g/day)	Rye	42 ± 28 (40)	39 ± 32 (32)	37 ± 24 (34)	0.606	0.809	0.737
	Wheat	44 ± 35 (34)	40 ± 37 (33)	38 ± 33 (30)			
Oil, butter and margarine (g/day)	Rye	16 ± 12 (13)	15 ± 12 (12)	15 ± 12 (13)	0.048	0.322	0.308
	Wheat	20 ± 17 (18)	17 ± 19 (12)	18 ± 16 (15)			
Sweets, snacks, candy, cakes, desserts, sweeteners (g/day)	Rye	109 ± 82 (96)	42 ± 47 (30)	51 ± 50 (38)	0.167	0.962	0.626
	Wheat	93 ± 76 (80)	4 ± 57 (26)	49 ± 62 (33)			

Data is mean ± SD(median).
[a] n = rye/wheat.

Table 6
Primary outcomes (complete case analysis).

		Week 0[a]	Week 6[a]	Week 12[a]	Δbetween groups wk 6[b]	Δbetween groups wk 12[b]	p wk 6[b]	p wk 12[b]
Body weight (kg)	Wheat	89.14 ± 1.26	87.83 ± 1.24	87.35 ± 1.27	0.60 (0.17; 1.04)	1.08 (0.36; 1.80)	**0.0071**	**0.0036**
	Rye	88.83 ± 1.21	86.93 ± 1.19	85.97 ± 1.21				
Body fat (%)	Wheat	40.69 ± 0.69	39.83 ± 0.73	39.37 ± 0.75	0.22 (−0.1; 0.53)	0.54 (0.05; 1.03)	0.1831	0.0307
	Rye	38.88 ± 0.66	37.75 ± 0.7	36.92 ± 0.72				

Data is 'mean ± SEM', 'mean (95%confidence interval)'. Due to two primary endpoints, significance level is p < 0.025, significant p-values are marked in bold font. n: rye = 108, wheat = 99.
[a] LSMEANS, not adjusted for baseline.
[b] Derived from baseline adjusted model, rye group is reference for confidence intervals.

Table 8 show results of clinical biomarkers measured as secondary outcomes. LDL cholesterol differed between groups after 6 weeks of intervention (p = 0.013), while this difference was attenuated at week 12 (p = 0.095). Interestingly, the difference was caused by an increased LDL cholesterol concentration in the refined wheat group, rather than a decrease in the rye group.

CRP was lower in the rye group, compared to the wheat group, after both 6 (p = 0.019) and 12 (p = 0.001) weeks of intervention. The difference was driven by a reduction in CRP concentration in the rye group, and after 12 weeks of intervention the concentration in the rye group was 39% lower than the concentration in the wheat group. Removing observations higher than 10 mg/L (2.6% of the samples), which could indicate acute inflammation rather than low grade inflammation, reduced the difference between the groups but the CRP concentration in the rye group was still lower than the wheat group after both 6 (21%, p = 0.033) and 12 (28%, p = 0.008) weeks of intervention.

Both CRP and LDL cholesterol are known to be positively affected by weight reduction, but we found no correlations between weight loss and LDL (Week 6: r = 0.05, p = 0.45. Week 12:

Table 7
Secondary outcomes (complete case analysis).

		Week 0[a]	Week 6[a]	Week 12[a]	Δbetween groups wk 6[b]	Δbetween groups wk 12[b]	p wk 6[b]	p wk 12[b]
BMI (kg/m^2)	Wheat	30.35 ± 0.25	29.89 ± 0.25	29.72 ± 0.26	0.19 (0.05; 0.33)	0.33 (0.09; 0.57)	**0.0098**	**0.0067**
	Rye	29.83 ± 0.24	29.29 ± 0.24	28.88 ± 0.25				
Waist (cm)	Wheat	102.58 ± 0.95	100.76 ± 0.92	100.56 ± 1.99	1.11 (−0.15; 2.36)	1.90 (0.49; 3.30)	0.0834	**0.0083**
	Rye	103.16 ± 0.91	100.15 ± 0.88	99.19 ± 0.96				
Hip (cm)	Wheat	106.97 ± 0.67	105.91 ± 0.66	105.67 ± 0.72	0.94 (−0.19; 2.07)	1.65 (0.40; 2.89)	0.1013	**0.0098**
	Rye	106.21 ± 0.65	104.39 ± 0.63	103.39 ± 0.69				
Waist-to-hip ratio	Wheat	0.96 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.00 (−0.01; 0.01)	0.00 (−0.00; 0.01)	0.586	0.727
	Rye	0.97 ± 0.01	0.96 ± 0.01	0.96 ± 0.01				
Sagittal diameter (cm)	Wheat	22.05 ± 0.27	21.61 ± 0.26	21.45 ± 0.28	0.00 (−0.37; 0.37)	0.25 (−0.22; 0.73)	0.9932	0.2933
	Rye	22.37 ± 0.26	21.86 ± 0.25	21.46 ± 0.27				
Fat mass (kg)	Wheat	35.81 ± 0.69	34.56 ± 0.7	34.01 ± 0.73	0.42 (0.04; 0.80)	0.90 (0.25; 1.55)	**0.0319**	**0.0068**
	Rye	34.01 ± 0.66	32.35 ± 0.67	31.31 ± 0.7				
Lean mass (kg)	Wheat	49.47 ± 1.05	49.53 ± 1.07	49.65 ± 1.08	0.18 (−0.12; 0.48)	0.20 (−0.14; 0.55)	0.2468	0.2461
	Rye	50.94 ± 1.01	50.84 ± 1.02	50.96 ± 1.04				
Fat - gynoid (kg)	Wheat	6.37 ± 0.14	6.14 ± 0.14	6.04 ± 0.14	0.06 (−0.01; 0.13)	0.11 (0.00; 0.23)	0.1106	0.0554
	Rye	5.92 ± 0.14	5.65 ± 0.14	5.51 ± 0.14				
Fat - android (kg)	Wheat	3.57 ± 0.09	3.40 ± 0.09	3.35 ± 0.09	0.03 (−0.03; 0.08)	0.14 (0.06; 0.23)	0.3121	**0.0010**
	Rye	3.44 ± 0.09	3.25 ± 0.08	3.09 ± 0.09				

Data is 'mean ± SEM', 'mean (95%confidence interval)'. Significance level is p > 0.05, significant p-values are marked in bold font. n: rye = 108, wheat = 99.
[a] LSMEANS, not adjusted for baseline.
[b] Derived from baseline adjusted model, rye group is reference for confidence intervals.

Table 8
Secondary outcomes (complete case analysis).

		Week 0[a]	Week 6[a]	Week 12[a]	Δbetween groups wk 6[b]	Δbetween groups wk 12[b]	p wk 6[b]	p wk 12[b]
		Mean ± SEM			Mean difference (95% CI)			
Systolic BP (mmHg)	Wheat	123.82 ± 1.51	123.61 ± 1.53	123.08 ± 1.39	−0.64 (−2.88; 1.60)	−1.11 (−3.45; 1.23)	0.575	0.351
	Rye	126.26 ± 1.45	126.26 ± 1.47	125.81 ± 1.33				
Diastolic BP (mmHg)	Wheat	78.73 ± 0.87	78.47 ± 0.91	77.86 ± 0.86	−0.14 (−1.38; 1.11)	−0.33 (−1.63; 0.97)	0.827	0.615
	Rye	79.64 ± 0.84	79.39 ± 0.87	78.84 ± 0.82				
Pulse (bpm)	Wheat	62.67 ± 0.86	62.04 ± 0.84	61.95 ± 0.83	0.52 (−1.00; 2.04)	0.62 (−0.97; 2.22)	0.502	0.440
	Rye	61.96 ± 0.82	60.9 ± 0.80	60.72 ± 0.79				
Glucose (mmol/L)	Wheat	5.61 ± 0.06	5.56 ± 0.06	5.54 ± 0.06	0.03 (−0.07; 0.13)	0.04 (−0.05; 0.14)	0.609	0.355
	Rye	5.53 ± 0.05	5.46 ± 0.06	5.42 ± 0.06				
Total cholesterol (mmol/L)	Wheat	4.77 ± 0.09	4.88 ± 0.09	4.89 ± 0.09	0.09 (−0.04; 0.22)	0.04 (−0.08; 0.17)	0.177	0.502
	Rye	4.69 ± 0.09	4.71 ± 0.09	4.77 ± 0.09				
HDL cholesterol (mmol/L)	Wheat	1.43 ± 0.03	1.45 ± 0.03	1.49 ± 0.03	0.01 (−0.02; 0.05)	0.03 (0.00; 0.07)	0.424	0.070
	Rye	1.37 ± 0.03	1.38 ± 0.03	1.40 ± 0.03				
LDL cholesterol (mmol/L)	Wheat	3.07 ± 0.08	3.18 ± 0.08	3.17 ± 0.08	0.14 (0.03; 0.25)	0.10 (−0.02; 0.21)	**0.013**	0.095
	Rye	3.07 ± 0.08	3.04 ± 0.08	3.07 ± 0.08				
		Geometric mean (95% CI)[d]			Anti-log mean difference (95% CI)[d]			
Triglycerides (mmol/L)	Wheat	1.10 (1.03; 1.17)	1.11 (1.04; 1.19)	1.10 (1.03; 1.17)	0.98 (0.92; 1.04)	0.95 (0.89; 1.01)	0.545	0.091
	Rye	1.08 (1.02; 1.16)	1.12 (1.05; 1.19)	1.14 (1.07; 1.22)				
Insulin (mIU/L)	Wheat	9.83 (8.98; 10.75)	9.54 (8.69; 10.49)	9.11 (8.27; 10.04)	1.09 (1.00; 1.19)	1.04 (0.94; 1.14)	0.059	0.442
	Rye	9.05 (8.30; 9.86)	8.15 (7.45; 8.92)	8.18 (7.45; 8.97)				
CRP (mg/L)	Wheat	1.44 (1.19; 1.74)	1.48 (1.22; 1.8)	1.58 (1.29; 1.92)	1.27 (1.04; 1.55)	1.39 (1.15; 1.70)	**0.019**	**0.001**
	Rye	1.45 (1.21; 1.73)	1.16 (0.96; 1.39)	1.12 (0.93; 1.36)				
CRP[c] (mg/L)	Wheat	1.40 (1.17; 1.67)	1.38 (1.15; 1.64)	1.44 (1.19; 1.73)	1.21 (1.02; 1.44)	1.28 (1.07; 1.53)	**0.033**	**0.008**
	Rye	1.37 (1.16; 1.63)	1.10 (0.93; 1.31)	1.09 (0.92; 1.30)				
HOMA-IR	Wheat	2.44 (2.20; 2.69)	2.35 (2.11; 2.61)	2.23 (2.00; 2.48)	1.11 (1.00; 1.23)	1.06 (0.95; 1.18)	0.054	0.305
	Rye	2.21 (2.01; 2,44)	1.97 (1.78; 2.18)	1.96 (1.77; 2.18)				

Significance level is p > 0.05, significant p-values are marked in bold font. n: rye = 108, wheat = 99. Abbreviations: BP, blood pressure;; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment insulin resistance.
[a] LSMEANS, not adjusted for baseline or weight loss.
[b] Derived from model adjusted for baseline and change in bodyweight (week 0–12), rye group is reference for confidence intervals.
[c] Values above 10.00 mg/L has been omitted.
[d] Back transformed from natural logarithm scale.

r = 0.12, p = 0.08) and CRP (Week 6: r = 0.06, p = 0.36. Week 12: r = 0.12, p = 0.09) and the analyses were adjusted for change in body weight.

There were no differences between the groups for remaining clinical markers (Table 8). ITT analyses of clinical markers did not change the conclusions (Supplemental Tables 3–6).

3.5. Appetite

Figure 3 and Supplemental Fig. 2 shows the appetite response during the appetite assessments conducted at week 0, week 6 and week 12. There were no differences between the groups in week 0 and week 12. At week 6, the rye group reported lower desire to

eat, lower hunger and higher sensation of fullness, compared to the wheat group during the morning period. Over the rest of the day, and over the entire day, there were no differences between the groups at week 6.

4. Discussion

In this study we demonstrated that a hypocaloric diet rich in high fiber rye products providing approximately 30 g rye fiber/day lead to lower body weight, compared to refined wheat, during a 12-week dietary intervention study. We found that the weight reduction was primarily caused by loss in body fat mass. Additionally, we found reductions in low-grade inflammation in the rye group and indications of increased LDL cholesterol in the wheat group. The hypothesis that increased weight loss following consumption of rye products could be linked to improvements in appetite could not be confirmed in the present study, possibly due to methodological issues as discussed below.

This is one of the first studies investigating the effect of high-fiber rye products on body weight reduction in the context of a hypocaloric diet. Previous studies investigating the effect of whole grain rye products on body weight have not been conducted in the context of a hypocaloric diet, rather they have been *ad libitum* diets where the aim was to maintain a stable body weight [10,36]. Our analysis of body composition showed that the weight loss consisted of fat, while the lean mass remained stable. High fat mass has consistently been associated with increased disease risk and reducing fat mass is generally considered to improve the health status in overweight and obesity [37−39]. In some cases reductions in fat mass have been shown to be a greater predictor of improvements in health parameters than reductions in body weight, why reduction in fat mass during a weight loss is especially important [37−39]. Reductions in lean mass are generally considered a negative effect of weight loss and studies have shown that lean mass is regained to a lesser extent than fat mass during a weight regain, why it is especially important to preserve lean mass during weight loss [40,41]. More rapid weight loss has been shown

to be correlated with a greater proportion of the lost weight consisting of lean mass and it could be that the relatively slow weight loss pace in the present study (approx. 0.25 kg/week in the rye group) could have aided the preservation of lean mass [42]. Increased protein intake has been suggested to increase weight loss and preservation of lean mass [43,44]. While the protein intake increased slightly in the wheat group, it was stable in rye group, why the greater weight loss in the rye groups cannot be attributed to the potential effect of protein intake.

The energy intake was reduced approximately 100−200 kcal per day, which is less than the 500 kcal reduction that we were aiming for. A 100−200 kcal deficit per day corresponds to approximately a weight loss of approx. 0.2 kg/week, which is in line with the observed weight loss of our participants. The energy intake did not differ between the groups during the intervention; however, the fiber intake was substantially higher in the rye group due to the higher fiber content of the intervention products. It has been shown that increasing the cereal fiber intake in the diet can lead to increased fecal energy excretion due to binding of nutrients in the fiber matrix [45]. This could mean that while the participants in the two groups consumed a similar amount of energy, the higher fiber intake in the rye group reduced the energy uptake in the gastro-intestinal tract. However, as fecal energy excretion was not measured in the present study, this remains hypothetical.

It should be mentioned that dietary records are prone to measurement error, so the direct comparison between the participants reported energy intake and their weight reduction should be interpreted with care [46]. Satiety-inducing food products have been shown to correlate with body weight reduction and improved body weight management [17,47] and based on previous studies showing improved satiety response following consumption of rye-based products, compared to wheat-products, the difference in weight loss may also be explained by increased satiety and thereby decreased food intake in the rye group leading to an increased weight loss. However, other studies have indicated that differences in subjective appetite cannot be directly translated into difference in food intake [48].
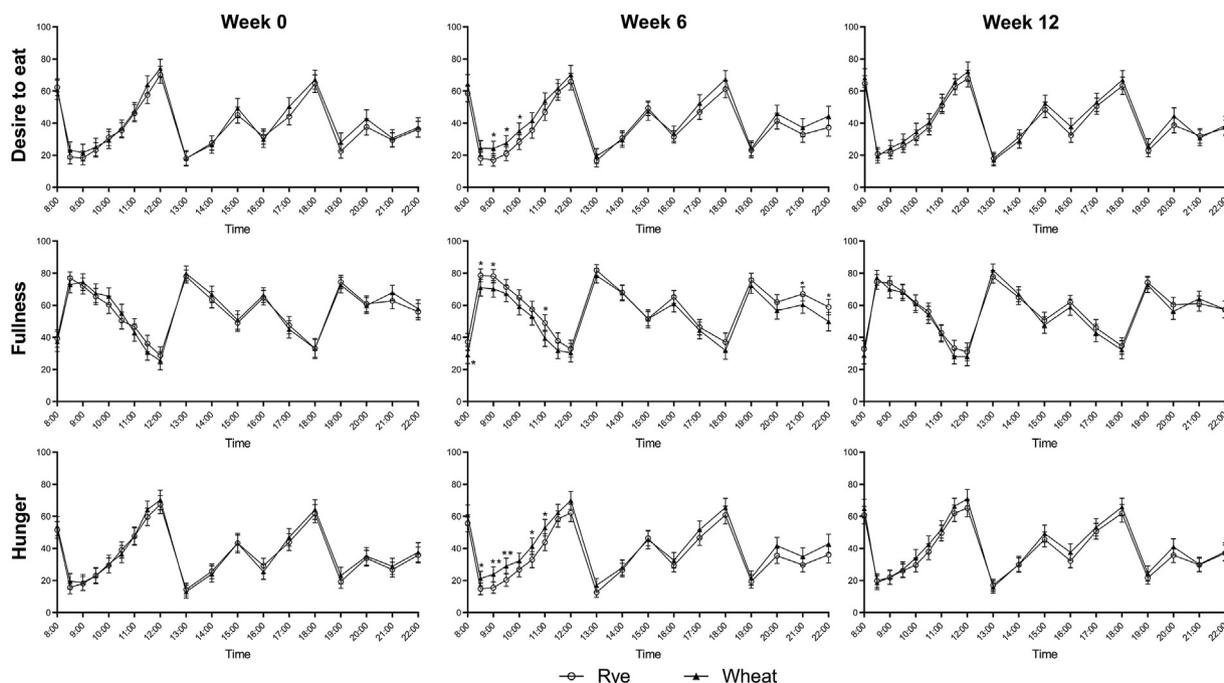

Fig. 3. Appetite response at week 0, week 6 and week 12. *p < 0.05, **p < 0.01. Data is mean and 95% confidence interval.

Previous studies have consistently shown a positive effect of rye products vs refined wheat control products on appetite response and validated by subsequent differences in *ad libitum* energy intakes [12—16]. However, these studies have been conducted in a clinical setting, where participants conducted most of the assessment in a research clinic under controlled conditions. Our present study is one of the first studies to conduct appetite assessment at home, in a completely free-living setting and over the course of a whole day and evening. This means that participants have likely been exposed to more outside stimuli, such as seeing or smelling foods not included in the study, talking about food with colleagues and family, and similar things that participants in clinical settings are isolated from. While this has likely led to more variation or "noise" in the data, it has also allowed us to evaluate the appetite response under more realistic, real-life conditions. However, it is important to remember that appetite responses obtained under clinical conditions, where participants are isolated from many stimuli that they would encounter in everyday life cannot necessarily be directly transferred to real-life conditions, and extrapolation of results should therefore be done with caution.

Low-grade inflammation was considerably lower in the rye group than in the refined wheat group, even after adjustment for change in body weight. This is line with finding from other studies showing reduced concentration of inflammatory markers following interventions with high cereal fiber intake, including whole grain rye [20,49—51]. However, low grade inflammation is associated with overweight and weight loss and the larger weight loss in the rye group may partially explain the reduction in inflammation [52], but other studies where subjects remained weight stable showed positive effects of rye, indicating and independent effect of rye [20,49]. LDL cholesterol increased in the wheat group, indicating an adverse effect of the wheat products. Generally, rye, and whole grain in general, have been shown to have a positive influence on LDL cholesterol [19,20] and weight loss is also associated with reductions in LDL cholesterol [53]. Based on this, we expected a difference between the groups to arise from a reduction in LDL cholesterol in one/both intervention groups, rather than an increase. It can be speculated that the increase is due to the fact that the study was conducted in a population with habitually high intake of whole grains [54] and the effect on LDL cholesterol is an effect of removing these whole grains from the diet of the participants in the wheat group.

A limitation of this study, as well as many other dietary intervention studies, is the lack of blinding [55]. The dieticians followed a standardized procedure when instructing the participants and had the initial meeting with the participants before randomization, furthermore the outcome assessors were blinded. However, it cannot be ruled out the participants awareness of their allocation may have affected their behavior during the study. The baseline sample collection was done after the 2-week run-in period and does not necessarily reflect the participants status before the intervention. Pre-run-in measurement of AR would have allowed us to substantiate our discussion regarding the participants habitual whole grain intake. The study duration is among the longest, compared to other studies of its kind [10,36], however long-term weight loss and weight maintenance is a major challenge in the treatment of overweight and obesity and further studies on the long-term effects is needed to draw conclusions. Nonetheless, weight loss over a 12-week period has been shown to predict long-term weight loss [56,57]. Furthermore, the dietary intervention induced in this study has the potential to be implemented in participants diet on a more permanent basis, for both weight loss and weight maintenance, as opposed to other weight loss strategies, such as very low calorie diets and pharmaceutical interventions, that is not always feasible in a long term perspective [58—60].

In conclusion, this study shows that replacing refined wheat products with high fiber rye products providing approximately 30 g fiber/day in the context of a hypocaloric diet induces a higher body weight and body fat reduction. Furthermore, participants in the rye group had a lower concentration of the inflammatory marker, CRP, after 12-week of intervention, compared to the participants in the wheat group. A link between weight loss and appetite response was not be shown in the present study but may be due to methodological issues and warrants further investigation.

Sources of support

Data availability

Data is available from the corresponding author upon reasonable request. As the data contains sensitive information the data will not be made publicly available.

Declaration of competing interest

RL is the founder of the Nordic Rye Forum, which is a research and dissemination platform for research related to rye and health that includes academic institutions as well as institutes and food industry with interest in rye across the Nordic region. The forum and its activities are funded by the industrial partners. RL is the PI of several projects funded by several cereal industrial companies. Such funding is used to carry out scientific studies. RL receives no salary, honorary, or by any other means has any personal economic benefits from industrial collaborations. Remaining authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2021.07.007.

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