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A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes – The CarbHealth study

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A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes – The CarbHealth study

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Abstract

Introduction In 2012, the estimated global prevalence of pre-diabetes was 280 million, and the prevalence is expected to rise to 400 million by 2030. Oat-based foods are a good source of beta-glucans, which have been shown to lower postprandial blood glucose. Studies to evaluate the effectiveness of the long-term intake of beta-glucan-enriched bread as part of a habitual diet among individuals with prediabetes are needed. Therefore, we designed a multicenter intervention study in adults with pre-diabetes to investigate the effects of consumption of an oat-derived beta-glucan-enriched bread as part of a normal diet on HbA1c in comparison to consumption of a wholegrain wheat bread.

Method and analysis The CarbHealth trial is a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants 40-70 years of age with a BMI≥27 kg/m^2 and HbA1c 35-50 mmol/mol. The study is conducted at four universities located in Norway, Sweden and Germany and uses intervention breads specifically designed for the trial by Nofima AS. The aim is to recruit 250 participants. The primary outcome is the difference in HbA1c between the intervention and the control group. The main analysis will include intervention group, study center, and baseline HbA1c as independent variables in an ANCOVA-model.

Ethics and dissemination The study protocol was approved by respective ethic authorities in participating countries. The results of the study will be communicated through publication in international scientific journals and presentations at (inter)national conferences.

Trial registration number Clinical trials: NCT04994327.

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Article summary

Strengths and limitations of this study

- The multicenter design allows us to recruit the required 250 participants, as it would be a difficult undertaking for one study center alone.
- The multicenter study also takes advantage of the expertise of different groups, thus adding microbiota research, chronotype and continuous glucose measurements as well as consumer acceptance to the study outcomes.
- Furthermore, collaboration with food technologists that were able to design, produce and extensively characterize a beta-glucan-enriched bread is an additional strength of the multicenter study.
- The intervention bread contains > 4 g beta-glucan per 30 g available carbohydrate and qualifies for an EFSA health claim on reduction of post-prandial glycaemic response.
- Due to logistics, breads had to be provided frozen, which is known to reduce bread quality and could lower consumer acceptance.

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Introduction

The prevalence of type 2 diabetes mellitus (T2D) has increased drastically over the last 35 years and is expected to continue to rise (1, 2). Impaired glucose tolerance (IGT) and impaired fasting glycemia (IGF) are intermediate conditions between normal glucose metabolism and T2D and are often referred to as pre-diabetes. In 2012, the International Diabetes Federation estimated the global prevalence of pre-diabetes to 280 million, which is expected to rise to 400 million by 2030 (3). Persons with pre-diabetes are at high risk of developing T2D, and it is estimated that 70% of those with pre-diabetes may develop T2D within 10 years (3, 4). Glycated hemoglobin (HbA1c) is used as a measure of glycemic control since HbA1c reflects average plasma glucose over the previous eight to twelve weeks. Common diagnostic criteria of pre-diabetes is an intermediate HbA1c of 42-47 mmol/mol (5). The causes and etiology of IGT and IGF are not fully understood, but there are strong links to obesity, age, ethnicity as well as heredity, and nutrition (6-8). Cereal grain products, especially bread, are staple foods in European diets and cereals are the main source of carbohydrate, plant protein, dietary fiber, and total energy world-wide (9). High whole grain and cereal fiber intake have consistently been associated with lower risks of T2D (10). Hence, replacing refined grains with dietary fiber-rich whole grains is regarded as a major strategy to improve public health (11). Oat- or barley-based foods are a good source of mixed-linkage beta-glucans, i.e., viscous forming dietary fiber, which have been shown to improve postprandial blood glucose. This has been endorsed through authorized health claims by the European Food Safety Authority (EFSA). However, few studies have investigated the long-term effect of breads enriched with beta-glucans on HbA1c and thereby the risk of developing T2D (12-14). The existing studies are of small sample sizes and uses a high amount of test food (8 servings per day = 320 g bread), thus not reflecting average consumption conditions. There is a need for studies to evaluate the effectiveness of the long-term intake of feasible amounts of bread enriched in beta-glucan as part of a habitual diet on diabetes risk factors particularly among individuals at elevated risk.

Therefore, we designed a multicenter intervention study in adults with a moderate to high risk of developing T2D i.e., persons with pre-diabetes to evaluate the long-term effects of regular consumption of an oat-derived beta-glucan-enriched bread, as part of a normal diet on HbA1c, in comparison to consumption of a wholegrain wheat bread. Furthermore,

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exploratory analysis will be performed assessing effects on fasting blood glucose and serum lipid profile, body weight, hepatic steatosis markers, 24 h glucose profiles, gastric emptying, changes in microbiota but also consumer acceptance and attrition rates.

Methods/design

The CarbHealth trial is a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants with high normal HbA1c concentrations. The study is conducted at four University centers at i) University of Bergen- Bergen, Norway, ii) Chalmers University of Technology- Gothenburg, Sweden, iii) Paderborn University- Paderborn, Germany, and iv) Leipzig University- Leipzig, Germany. Intervention breads were specifically produced for the study by Nofima (Ås, Norway). This study was initiated in July 2019 and the recruitment started in July 2021. The trial is expected to be finalized by December 2022.

Ethics and dissemination

The study protocol was approved by the respective ethic authorities (Swedish Ethical Review Authority, Sweden (Protocol DNR 2021-02584), Ethical committee of Paderborn University, Paderborn (approved 13 July 2021), Ethic Committee of the Medical Faculty of the University of Leipzig, Leipzig (316/21-ek), Regional Committees for Medical and Health Research Ethics, Norway (REC Nord, ref. 106931)). The study is registered in the public trial registry *Clinicaltrials.gov* (NCT04994327). The results of the study will be communicated through publication in international scientific journals and presentations at (inter)national conferences.

Patient and public involvement

Participants and public were not involved in designing this study. Results will be presented to participants at the end of the trial. Participants will receive information on allocated group, HbA1c, blood lipids and body composition at the end of the trial.

Experimental design

Prior to the intervention, potential participants take part in a pre-screening evaluation to

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assess eligibility for inclusion, either by phone, or using an online questionnaire. If eligible, a screening visit is booked. At the subsequent screening visit, non-fasting blood samples are drawn and analyzed locally for HbA1c, liver enzymes and safety markers. Furthermore, height, body weight, waist circumference, and blood pressure are measured. Participants complete a medical history questionnaire including assessment of prescribed and non-prescribed medication in relation to exclusion criteria. If enrolled, clinical visits take place in weeks 0, 8 and 16. During the baseline visit, the intermediate visit at 8 weeks and the final visit at 16 weeks, measurements of body weight, body composition, waist circumference and blood pressure are made, fasting blood samples are drawn, and participants provide frozen fecal samples. In two centers (Gothenburg and Paderborn), continuous glucose monitoring (CGM) measurements are performed covering one week at baseline and at the end of the study. Participants are asked to maintain habitual diets and levels of physical activity during the study period. This is monitored by 6 in-study 24 h dietary recalls which are not pre-announced to the participants and by physical activity questionnaires at the study visits. An overview of the study design is presented in **Figure 1**. During the intervention period, participants are instructed to replace their usually consumed bread with the study breads. The participants are asked to consume at least 3 slices of the pre-sliced intervention bread or the pre-sliced control bread on at least 6 days per week for 16 weeks.

Figure 1. Flowchart over the study visits in CarbHealth multicenter study.

Study bread

The ingredients for the breads are shown in **Table 1** and the calculated nutrient composition in **Table 2**. The two breads were matched for starch and fat content on a slice basis (**Table 2**). The daily portion of three slices of the beta-glucan enriched bread provide 286 kcal, 16.6 g dietary fiber and 6 g of beta-glucan. Three slices of the wheat bread provide 244 kcal, 5 g dietary fiber, and 0 g beta-glucan per day (**Table 2**). Both breads were developed and distributed by Nofima AS, Norway. The breads were baked at Åpent Bakeri, Oslo, Norway. The bread is provided frozen in vacuum packs of 6 slices and free of charge to the participants.

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Table 1: Ingredients for beta-glucan and control bread.

Ingredients	Supplier	Beta-glucan bread (%)	Control bread (%)
Rapeseed oil	Idun Industri AS, Norway	0.7	4.7
Dry yeast	Idun Industri AS, Norway	0.7	0.6
Salt	GC Rieber AS, Norway	1.0	1.0
Sieved white wheat flour	Lantmännen Cerealia, Norway	21.9	18.7
Wholegrain wheat flour	Lantmännen Cerealia, Norway	0	37.5
Water	Oslo kommune, Norway	53.8	37.5
SWEOAT® Bran BG14 Bakery	Swedish Oat fiber, Sweden	21.9	0
Coatec sorbic acid (E200)	RAPS GmbH Co. KG, Germany	0.05	0.05

Table 2: -Macronutrient composition of test breads

Beta-glucan bread (intervention)			
	g/slice	g/day (3 slices)	kcal/day
starch	12.2	36.7	146.7
fat	2.1	6.3	56.8
beta-glucan	2.0	6.0	
protein	4.1	12.4	49.4
fiber	5.5	16.5	32.9
salt	0.6	1.8	
Moisture	24.8	74.4	
Total	50.7	152.1	285.9

Whole grain Wheat bread (Control)			
	g/slice	g/day (3 slices)	kcal/day
starch	12.3	36.9	148.0
fat	2.1	6.2	56.0
beta-glucan	0.02	0.06	
protein	2.5	7.5	29.9
fiber	1.6	4.8	9.8
salt	0.4	1.20	
Moisture	11.4		
Total	30.4	91.2	243.7

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Eligibility criteria

The eligibility criteria were designed to reach people with pre-diabetes; hence, we invite men and women with BMI ≥ 27 kg/m^2, aged 40-70 years. Additional inclusion criteria: HbA1c 35-50 mmol/mol, signed informed consent, regular bread eater and having freezer capacity for at least 2 loafs of bread. Exclusion criteria are: type 1 diabetes mellitus or pharmacologically treated type 2 diabetes mellitus, non-fasting blood glucose > 11.1 mmol/l, urine glucose ≥ 180 mg/dl, or protein excretion as indicated by dipstick (+++, Combur 10 test strips (Roche Diagnostics)), food allergies or intolerances preventing consumption of the study breads, pregnancy, lactation or planning a pregnancy during the study period, systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg at screening (15), history of stomach or gastrointestinal conditions (i.e. inflammatory bowel disease, Crohn's disease) history of myocardial infarction, heart failure, stroke, heart attack or cancer within 3 years prior to screening, use of anti-diabetic agents or insulin, history of alcohol abuse. Use of other medications or over-the-counter drugs or dietary supplements are allowed if the dose has been stable for a minimum of 3 months prior to the study. Any medication used will be recorded as will any changes of use. Initiation of medication to treat diabetes during the study is a reason for withdrawal.

Recruitment

In general, participants are recruited via leaflets, press releases, newspaper ads, ads in social media and blackboard flyers. Study specific websites are developed in Bergen and Paderborn, with the possibility for online registration. If a potential participant is interested, pre-screening will be conducted over the phone or using an online questionnaire to assess eligibility, and if eligible, a screening visit is scheduled.

Informed consent procedure

All participants screened for eligibility are provided written information about the study prior to the first study visit. At the first study visit, the participants receive additional oral information on the study and are given the opportunity to ask questions to the research personnel before signing written informed consent. The study will not carry on and no

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samples will be drawn until the participant gives consent in writing. The participants have the right to withdraw their consent at any time, and to request that their biological samples and data will be destroyed.

Randomization

Participants are randomized into one of the two intervention groups (1:1 allocation) using block randomization with random block lengths, stratified by sex. The web-based randomization is configured by the Biostatistics and Data Management Group of the Clinical Trials Unit at the University Medical Center Göttingen, Germany, a third party not otherwise involved in the clinical trial. To reduce performance bias, at each of the four sites, a person, who is not member of the research team, is responsible for the randomization. All other research personnel at the sites are blinded for the allocation group.

Dietary assessment

The dietary assessment follows the principle of 24 h dietary recalls using country-specific food composition data. Practical conduction differs slightly between centers.

Bergen, Paderborn, and Leipzig: Dietary intakes are assessed by six 24-hour recalls (24HR) at the beginning (weeks 0-2), middle (weeks 7-9) and end of the study (weeks 15-16) using the validated tool “myfood24” (https://www.myfood24.org/). The 24HR are performed at unannounced times to attenuate the observer effect. The German version of myfood24 is based on German Food Code and Nutrient Database (Bundeslebensmittelschlüssel (BLS) version 3.02) for generic food items and the database of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study for branded food items (16, 17). The Norwegian version is based on the Norwegian Food Composition Table for generic food items and supplemented with food composition data for missing Norwegian dishes from other sources (18).

Gothenburg: As Myfood24© is not available based on Swedish food composition data, dietary intake is assessed by six 24HR on study site and over phone. Three 24HR are performed at site by a trained dietitian, using images of portion sizes from The Swedish Food Agency. Additionally, three 24HR are performed over phone when the participants estimate portion sizes using a standard kitchen measure (e.g., dl measure and slices). To determine the nutritional composition of the intake, the DietistNet Pro software

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(Kost och Näringsdata AB, Bromma, Sweden) is used which is based on Swedish Food Composition Database.

Clinical assessments

Visits include collection of blood and urine samples, assessment of blood pressure, and anthropometric data including waist circumference, body weight and height, as well as body composition (described under "anthropometric measurements"). Participants are instructed to not eat or drink (except maximum 0.5L of non-carbonated water) 10 hours prior to the visits. Additionally, participants are instructed to avoid alcohol consumption, smoking and use of other tobacco products, and vigorous physical activity 12 h prior to the clinical visit.

Consumer acceptance

Participants evaluate the bread on day 1 and on week 8 of the study. The questionnaire is adapted from a previously established method (19). Participants rate their hunger, acceptability, and expected satiation in a 9-point scale. The participants rate expected satiety on a 6-point scale. Participants describe the bread via a check-all-that-apply (CATA) question (19, 20), "Choose all the attributes/terms that you think apply to this bread", using 28 hedonic and descriptive attributes and 16 usage & attitude attributes. Terms are randomized within groups and across participants. They answer two consumption questions:" In which meals do you consume bread?" and" How many bread slices do you eat on a typical day".

Data for Norway and Sweden were collected through online forms in EyeQuestion (Logic8 BV, The Netherlands) and stored in a secure server owned by Nofima. Participants from Germany filled in the questionnaire on paper.

Blood collection and analysis

During study visits, fasting blood samples are taken from an antecubital vein and placed in tubes containing either a clot activator or lithium heparin (Becton-Dickinson, Eysins, Switzerland) to obtain serum or plasma. Serum tubes are stored at room temperature for 30 minutes and then centrifuged at 1300g at 4°C for 10 minutes. Plasma tubes are immediately centrifuged except for one tube for HbA1c measurements. EDTA-plasma, serum and Li-Heparin plasma are immediately refrigerated/kept on ice, processed, and aliquoted into

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microtubes. Whole blood, plasma and serum aliquots are frozen at -20°C within 2 h of sample collection and transferred into -80°C within 24h. Blood samples are sent on dry ice to the Department of Medical Biochemistry and Pharmacology at Haukeland University Hospital, Bergen, every 6 weeks for analyses of HbA1c and secondary outcomes.

Analytical methods

Blood glucose is measured by a validated, portable system at room temperature (HemoCue© Glucose 201 RT system (HemoCue AB, Ängelholm, Sweden) at all centers. All other blood or serum measurements will be done at Haukeland University Hospital, Bergen (certified laboratory NSEN-ISO 15189), in frozen samples stored at -80 °C. HbA1c is measured in EDTA whole blood samples which have been stored at maximum for 8 weeks by HPLC (BioRad, Hercules, CA). Liver enzymes and plasma lipids are measured using standard methods on a Cobas c702 autoanalyzer. Liver enzymes are measured photometrically according to the IFCC method (Roche Diagnostics, Mannheim, Germany). Serum triacylglycerides and total cholesterol are measured with an enzymatic colorimetric method. LDL cholesterol is measured photometrically, and HDL cholesterol is measured by a homogeneous enzymatic colorimetric method.

Dietary compliance

Compliance is assessed based on the evaluation of the 24HR and a pre-coded compliance journal kept by the participant during the study. Participants are instructed to tick off the number of slices consumed on each day. Compliance is a secondary outcome and sensitivity analysis will be performed based on compliance journals and 24HR.

Anthropometric assessments

Body weight is measured during all study visits including screening, with the participants wearing light clothing (e.g., underwear and t-shirt) and no shoes. Body weight is noted to the nearest 0.1 kg in the case report form (CRF). Height is measured using a Seca Stadiometer, model 217 or using the mBCA 515 integrated stadiometer. Height is measured once at screening without shoes to the nearest 0.1 cm. Waist circumference is measured twice on each occasion with a Seca 201 cm tape measurer to the nearest 0.1 cm according to WHO standards. The average of the two measurements are used for data analysis. Body

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composition (whole body mass, fat mass and lean body mass) is measured at all study visits by Bio-electrical Impedance Analysis using Seca mBCA 515 (in Bergen, Paderborn, and Leipzig). The measurements are performed in the morning after a 12 hour fast and in accordance with a standardized protocol.

Gothenburg: Body composition is measured using a Dual Energy X-ray absorptiometry, DEXA (iDXA, GE Medical Systems, Madison, WI, USA) using the software Core version 18.0. The DEXA-scan is conducted in the morning after an overnight fast with the participants wearing light clothing.

Twenty-four-hour continuous glucose monitoring

A continuous glucose monitoring device (CGM) is used (Gothenburg and Paderborn) to obtain 24-h continuous interstitial glucose concentration data on two occasions for 7 days each between baseline and week 1 and between week 15 and week 16. CGM data is used to calculate 24-h interstitial glucose peak, mean, coefficient of variation (CV) and total area under the curve (AUC). In addition, glucose response to bread consumption is evaluated separately for morning and evening meals.

The devices differ between centers and the specific devises are described below.

In Paderborn: A Dexcom G6 CGM (DexCom Inc., San Diego, US) is used. The glucose oxidase sensor is inserted into the upper part of the non-dominant arm or the abdominal area at least 5 cm away from the umbilicus to obtain an interstitial glucose measurement. Self-monitoring glucose readings (finger sticks) are performed with a blood sugar monitoring device (CONTOUR®NEXT ONE, Ascencia Diabetes Care, US) twice per day (morning and evening). The sensor does not need to be calibrated.

In Gothenburg: An Abbott FreeStyle Libre Pro iQ CGM (Chicago, Illinois, US) is used. The glucose oxidase sensor is inserted into the back of the upper part of the non-dominant arm. Participants can wear the sensor for up to 14 days and it does not need to be calibrated.

Fecal samples

Spot fecal samples are collected at baseline, week 8 and 16. Collection is voluntary, and non-delivery of fecal samples is not a reason for exclusion. The samples will be collected in specific devises (Easy Sampler Collection Kit, GP Medical Devices ApS, Denmark). The participants are

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instructed to collect the fecal sample within 72 h of the clinical visit and are instructed to keep the sample in a household freezer until delivery to the study center. The participants are instructed to keep the sample in a freezer bag with cooling blocks during transportation to the study center. At the study center the samples are transferred to -80°C within 24 h. Samples are analyzed for the composition of the gut microbiota to provide possible mechanistic explanations underlying differential responses of participants of selected outcomes. All fecal microbiota analysis will be performed at Chalmers University of Technology (Gothenburg, Sweden).

2.10 Questionnaires

Participants complete several questionnaires during the study period. All questionnaires have been validated and are available in respective languages:

For physical activity assessment, participants complete the International Physical Activity Questionnaire (IPAQ) at baseline, week 8 and 16. The IPAQ is used to estimate daily inactivity and physical activity and has been shown to have adequate validity and reliability in various nationalities (21).

For chronotype, the Munich Chronotype Questionnaire (MCTQ) is administered at baseline, week 8 and 16 to estimate chronotype and sleep behavior during the trial. Chronotype is defined as the midpoint of sleep on free days corrected for sleep debt on workdays (MSFsc). Chronotype is also verified by use of an accelerometer in week 1 and 16 (Paderborn) (22).

For subjective health and well-being assessment, the 12-item Short Form Health Survey (RAND SF-12) is used at baseline, week 8 and 16. The questionnaire is used to evaluate perceived physical and emotional well-being.

For alcohol abuse, the four-question validated CAGE (Cut down, Annoyed, Guilty and Eye opener) questionnaire will be administrated at screening visit to assess the risk of alcohol abuse.

2.14. Study outcomes

The primary outcome is the difference in glycemic control measured by HbA1c after 16 weeks between the intervention and control group. The trial has defined a number of exploratory secondary outcomes : I) difference in fasting capillary blood glucose after 16

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weeks, II) difference in fat mass (kg) and lean body mass (kg), III) difference in blood lipids (LDL-C, HDL-C, triglycerides), IV) difference in fatty liver index (based on serum liver enzyme activities (ALAT, ASAT)), changes in fecal microbiota composition, V) consumer acceptance at baseline and 8 weeks, VI) difference in postprandial response to morning and evening meals with the two breads based on CGM-data (Paderborn and Gothenburg only), VII) if the individual chronotype of a person influences bread consumption and metabolic health, VIII) analysis of plasma and fecal metabolome and short chain fatty acids in fecal and blood samples, and IX) sensitivity analysis of compliance to the protocol measured with 24 h recalls and compliance journal.

2.15. Data analysis and sample size estimates

Intention-to-treat analysis will be performed following a statistical analysis plan that was set up a priori.

The primary outcome is the difference in HbA1c between the intervention and the control group at the end of the study, and the main analysis will include intervention group, study center, and baseline HbA1c as independent variables in a linear regression model. The results will be presented as the mean difference (95% confidence intervals), with corresponding p-values. Missing data will be handled with flexible imputation of missing data. The main analysis will be accompanied by complete case analysis. A best-worst and worst-best sensitivity analyses will be performed to evaluate the theoretical range of uncertainty due to missing outcome data (23). The best-worst-case scenario will be constructed by assuming all dropouts in the intervention group to have an HbA1c at 16 weeks of the study center specific intervention group mean -2SD, and all dropouts in the control group to have an Hba1c of control group mean +2SD, and vice versa. The same analysis plan will apply to secondary outcome variables. Exploratory post-hoc analysis will include stratified analyses by sex, by BMI (27-30, >30 kg/m^2), by chronotype (based on MCTQ), and by center to explore potential country- and center-specific differences.

The statistical power calculation is based on the difference in HbA1c between the two groups (intervention and control) at the end of the study, as the estimated effect. We expect the starting HbA1c concentration to be approximately 41 mmol/mol with a standard deviation of 6 mmol/mol and expect a reduction to 38 mmol/mol in the intervention group with small

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changes in the control group. Power calculation is based on this between-group difference in the HbA1c concentrations, with a standard deviation of 6 mmol/mol (assuming similar standard deviations in both groups) and at a power of 90% and at a significance level of 0.05. To allow for 45% dropouts and ensure the conditions met, the aim was to recruit 250 participants into the entire multicenter study: 125 in each treatment group. Assumptions and estimates for the power-calculation were taken from dietary intervention studies (14, 24) and the calculation was using the R software package (power t-test). The effect size was chosen to be moderate as this is a single-food intervention study.

2. Discussion

The CarbHealth trial aims to evaluate effectiveness of a beta-glucan-enriched bread i.e., whether habitual consumption of a bread containing beta-glucan (>4 g beta-glucan per 30 g available carbohydrate) vs a control wheat bread with 66% whole grain wheat under everyday conditions will affect long term glycemic control among persons at risk for T2D over a period of 16 weeks.

The CarbHealth trial is a pragmatic trial investigating food typically consumed. Participants replace their habitual bread with study bread, instead of adding or removing food items. Studies have shown that people are conservative regarding dietary changes (25), thus swapping a healthier alternative for a habitually consumed foods may be easier than changing food habits. A bread with 66% wholegrain wheat was chosen as the control bread instead of refined wheat bread to reflect dietary habits in Northern European countries. Since the quality of bread and the associated metabolic effects varies substantially (26) use of a medium to low glycemic index-bread rich in beta-glucan may substantially benefit metabolic health. Whilst complex interventions have been found to substantially reduce progression to diabetes among persons with pre-diabetes (27), many patients may not be prepared for such complex changes; thus exchange of bread with a healthier alternative may be more feasible.

Nutrition studies often lack statistical power hampering firm conclusions (28, 29). Multicenter studies may offer a strategy to overcome this shortcoming. Recruitment of the required 250 participants would be difficult for one study center alone. Central production and distribution

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of the study breads by a third research partner (Nofima) enables this approach. Furthermore, the multicenter design employed in CarbHealth allows to study the metabolic effects and the effectiveness of bread against the background of different bread consumption patterns in the participating countries and to analyze differences in general dietary practices and potential acceptability. The multicenter study also takes advantage of the expertise of different groups, thus adding microbiota research, chronotype and continuous glucose measurements as well as consumer acceptance to the study outcomes. Furthermore, collaboration with food technologists that were able to produce a beta-glucan enriched bread is an additional strength of the multicenter study. Additional strengths of the CarbHealth-study include strong design features such as randomization, double-blinding, and provision of bread. One limitation of the study is that, due to logistics, breads had to be provided frozen, which is known to reduce bread quality and could lower consumer acceptance which could result in a lower compliance.

There may be important implications from this research regardless of the findings. If a beneficial effect was supported by evidence of a positive effect on long term blood glucose levels among persons with pre-diabetes, public health efforts should be taken to make beta-glucan-enriched bread available in European countries. This should be accompanied by efforts to increase the awareness, particularly among persons at risk of T2D, of a simple and effective replacement. Conversely, if a beneficial effect was not supported this could suggest that the bread is either not sufficiently enriched with beta-glucans, the beta-glucan has sub-optimal physiological characteristics e.g. solubility, pre-frozen bread may not be the optimal matrix for beta-glucan or that the reduction in post-prandial glycemic response achieved with a similar beta-glucan-enriched bread (30) does not translate into strong long term benefits for blood glucose control compared to a wholegrain wheat bread under every-day conditions. Notwithstanding, exploratory sub-group analyses will allow insights into factors determining responsiveness (e.g., compliance, meal context, consumer acceptance in different countries/center, sex). Similarly, secondary analyses will inform whether any potential effect or lack of effectiveness extends to other metabolic parameters.

Hence, the results of the CarbHealth study will provide important information on the public health relevance of a beta-glucan-enriched bread for reduction of post-prandial glycemic

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response in persons with pre-diabetes.

Ethics statements

Patient consent for publication

Not applicable.

Declaration of competing interest

All authors state no competing interests.

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Contributors

TH wrote the first the first draft of the manuscript. JD is the chief investigator of this trial. JD along with RL, AB, AK, and SB: research question, study design, acquisition of data, obtaining the funding, implementation of the study protocol, critical revision, and final approval of the manuscript. TH, HRR, IR, LT, AS, US, KM, KP, AR, VL, PV: feedback of study design, implementation of study protocol, acquisition of data, critical revision, and final approval of the manuscript.

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Figure Legend

Figure 1. Flowchart over the study visits in CarbHealth multicenter study.

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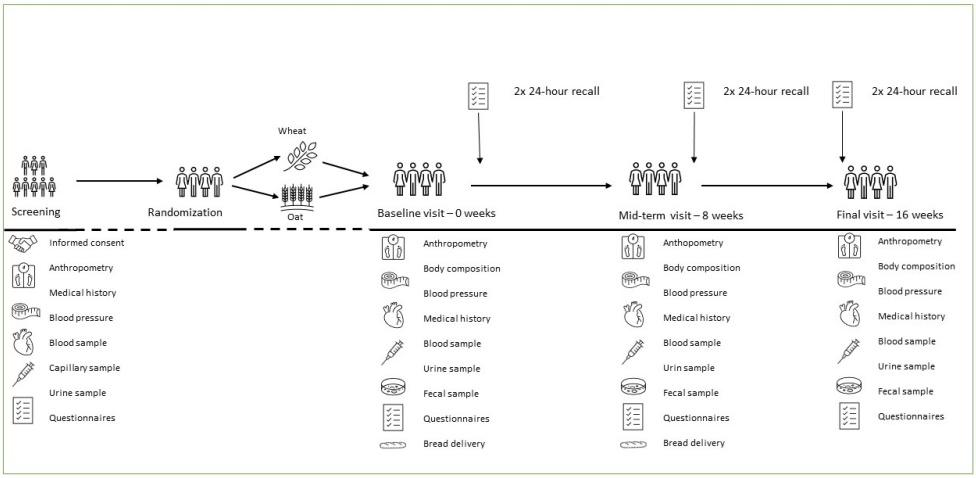

Figure 1. Flowchart over the study visits in CarbHealth multicenter study.

338x190mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

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Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 , 17-18
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	17
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	N/A

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		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6

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Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14

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Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6-7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	9

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		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-15

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Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	N/A
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further	N/A

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		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	6
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A

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Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	N/A
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A

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Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

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BMJ Open

A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes- a study protocol of The CarbHealth study

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Secondary Subject Heading:	Global health, Health policy, Nutrition and metabolism
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A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes: a study protocol of The CarbHealth study

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Abstract

Introduction In 2012, the estimated global prevalence of pre-diabetes was 280 million, and the prevalence is expected to rise to 400 million by 2030. Oat-based foods are a good source of beta-glucans, which have been shown to lower postprandial blood glucose. Studies to evaluate the effectiveness of the long-term intake of beta-glucan-enriched bread as part of a habitual diet among individuals with prediabetes are needed. Therefore, we designed a multicenter intervention study in adults with pre-diabetes to investigate the effects of consumption of an oat-derived beta-glucan-enriched bread as part of a normal diet on HbA1c in comparison to consumption of a wholegrain wheat bread.

Method and analysis The CarbHealth trial is a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants 40-70 years of age with a BMI≥27 kg/m^2 and HbA1c 35-50 mmol/mol. The study is conducted at four universities located in Norway, Sweden and Germany and uses intervention breads specifically designed for the trial by Nofima AS. The aim is to recruit 250 participants. The primary outcome is the difference in HbA1c between the intervention and the control group. The main analysis will include intervention group, study center, and baseline HbA1c as independent variables in an ANCOVA-model.

Ethics and dissemination The study protocol was approved by respective ethic authorities in participating countries. The results of the study will be communicated through publication in international scientific journals and presentations at (inter)national conferences.

Trial registration number Clinical trials: NCT04994327.

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Article summary

Strengths and limitations of this study

- The multicenter study takes advantage of the expertise of different groups, thus adding microbiota research, chronotype and continuous glucose measurements as well as consumer acceptance to the study outcomes.
- Furthermore, collaboration with food technologists that were able to design, produce and extensively characterize a beta-glucan-enriched bread is an additional strength of the multicenter study.
- The intervention bread contains > 4 g beta-glucan per 30 g available carbohydrate and qualifies for an EFSA health claim on reduction of post-prandial glycaemic response.
- Due to logistics, breads had to be provided frozen, which is known to reduce bread quality and could lower consumer acceptance.

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Keywords

Hyperglycemia

Beta-glucan

Pre-diabetes

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Introduction

The prevalence of type 2 diabetes mellitus (T2D) has increased drastically over the last 35 years and is expected to continue to rise (1, 2). Impaired glucose tolerance (IGT) and impaired fasting glycemia (IGF) are intermediate conditions between normal glucose metabolism and T2D and are often referred to as pre-diabetes. In 2012, the International Diabetes Federation estimated the global prevalence of pre-diabetes to 280 million, which is expected to rise to 400 million by 2030 (3). Persons with pre-diabetes are at high risk of developing T2D, and it is estimated that 70% of those with pre-diabetes may develop T2D within 10 years (3, 4). Glycated hemoglobin (HbA1c) is used as a measure of glycemic control since HbA1c reflects average plasma glucose over the previous eight to twelve weeks. Common diagnostic criteria of pre-diabetes is an intermediate HbA1c of 42-47 mmol/mol (5). The causes and etiology of IGT and IGF are not fully understood, but there are strong links to obesity, age, ethnicity as well as heredity, and nutrition (6-8). Cereal grain products, especially bread, are staple foods in European diets and cereals are the main source of carbohydrate, plant protein, dietary fiber, and total energy world-wide (9). High whole grain and cereal fiber intake have consistently been associated with lower risks of T2D (10). Hence, replacing refined grains with dietary fiber-rich whole grains is regarded as a major strategy to improve public health (11). Oat- or barley-based foods are a good source of mixed-linkage beta-glucans, i.e., viscous forming dietary fiber, which have been shown to improve postprandial blood glucose. This has been endorsed through authorized health claims by the European Food Safety Authority (EFSA). However, few studies have investigated the long-term effect of breads enriched with beta-glucans on HbA1c and thereby the risk of developing T2D (12-14). The existing studies are of small sample sizes and uses a high amount of test food (8 servings per day = 320 g bread), thus not reflecting average consumption conditions. A recent study investigated the efficacy of beta-glucans on blood lipid profile and fasting plasma glucose in a cross-over study on 83 subjects, showing significant effects on blood lipid profile but not on fasting blood glucose. However, the participants were euglycemic and the dose of beta-glucan was comparably low (3 g/d) and provided under strictly controlled conditions (15). Hence, there is a need for studies to evaluate the effectiveness of the long-term intake of feasible amounts of bread enriched in beta-glucan as part of a habitual diet on diabetes risk factors particularly among individuals at elevated risk.

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Therefore, we designed a multicenter intervention study in adults with a moderate to high risk of developing T2D i.e., persons with pre-diabetes to evaluate the long-term effects of regular consumption of an oat-derived beta-glucan-enriched bread, as part of a normal diet on HbA1c, in comparison to consumption of a wholegrain wheat bread. Furthermore, exploratory analysis will be performed assessing effects on fasting blood glucose and serum lipid profile, body weight, hepatic steatosis markers, 24 h glucose profiles, gastric emptying, changes in microbiota but also consumer acceptance and attrition rates.

Methods/design

The CarbHealth trial is a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants with high normal HbA1c concentrations. The study is conducted at four University centers at i) University of Bergen- Bergen, Norway, ii) Chalmers University of Technology- Gothenburg, Sweden, iii) Paderborn University- Paderborn, Germany, and iv) Leipzig University- Leipzig, Germany. Intervention breads were specifically produced for the study by Nofima (Ås, Norway). This study was initiated in July 2019 and the recruitment started in July 2021. The trial is expected to be finalized by summer 2023.

Ethics and dissemination

The study protocol was approved by the respective ethic authorities (Swedish Ethical Review Authority, Sweden (Protocol DNR 2021-02584), Ethical committee of Paderborn University, Paderborn (approved 13 July 2021), Ethic Committee of the Medical Faculty of the University of Leipzig, Leipzig (316/21-ek), Regional Committees for Medical and Health Research Ethics, Norway (REC Nord, ref. 106931)). The study is registered in the public trial registry *Clinicaltrials.gov* (NCT04994327). The results of the study will be communicated through publication in international scientific journals and presentations at (inter)national conferences.

Patient and public involvement

Participants and public were not involved in designing this study. Results will be presented to participants at the end of the trial. Participants will receive information on allocated group, HbA1c, blood lipids and body composition.

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Experimental design

Prior to the intervention, potential participants take part in a pre-screening evaluation to assess eligibility for inclusion, either by phone, or using an online questionnaire. If eligible, a screening visit is booked. At the subsequent screening visit, non-fasting blood samples are drawn and analyzed locally for HbA1c, liver enzymes and safety markers. Furthermore, height, body weight, waist circumference, and blood pressure are measured. Participants complete a medical history questionnaire including assessment of prescribed and non-prescribed medication in relation to exclusion criteria. If enrolled, clinical visits take place in weeks 0, 8 and 16. During the baseline visit, the intermediate visit at 8 weeks and the final visit at 16 weeks, measurements of body weight, body composition, waist circumference and blood pressure are made, fasting blood samples are drawn, and participants provide frozen fecal samples. In two centers (Gothenburg and Paderborn), continuous glucose monitoring (CGM) measurements are performed covering one week at baseline and at the end of the study. Participants are asked to maintain habitual diets and levels of physical activity during the study period. This is monitored by 6 in-study 24 h dietary recalls which are not pre-announced to the participants and by physical activity questionnaires at the study visits. An overview of the study design is presented in **Figure 1**. During the intervention period, participants are instructed to replace their usually consumed bread with the study breads. The participants are asked to consume at least 3 slices of the pre-sliced intervention bread or the pre-sliced control bread on at least 6 days per week for 16 weeks.

Figure 1. Flowchart over the study visits in CarbHealth multicenter study.

Study bread

The ingredients for the breads are shown in **Table 1** and the calculated nutrient composition in **Table 2**. The two breads were matched for starch and fat content on a slice basis (**Table 2**). The daily portion of three slices of the beta-glucan enriched bread provide 286 kcal, 16.6 g dietary fiber and 6 g of beta-glucan. Three slices of the wheat bread provide 244 kcal, 5 g dietary fiber, and 0.02 g beta-glucan per day (**Table 2**). Both breads were developed and distributed by Nofima AS, Norway. The breads were baked at Åpent Bakeri, Oslo, Norway. The

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bread is provided frozen in vacuum packs of 6 slices and free of charge to the participants.

Table 1: Ingredients for beta-glucan and control bread.

Ingredients	Supplier	Beta-glucan bread (%)	Control bread (%)
Rapeseed oil	Idun Industri AS, Norway	0.7	4.7
Dry yeast	Idun Industri AS, Norway	0.7	0.6
Salt	GC Rieber AS, Norway	1.0	1.0
Sieved white wheat flour	Lantmännen Cerealia, Norway	21.9	18.7
Wholegrain wheat flour	Lantmännen Cerealia, Norway	0	37.5
Water	Oslo kommune, Norway	53.8	37.5
SWEOAT® Bran BG14 Bakery	Swedish Oat fiber, Sweden	21.9	0
Coatec sorbic acid (E200)	RAPS GmbH Co. KG, Germany	0.05	0.05

Table 2: -Macronutrient composition of test breads

Beta-glucan bread (intervention)			
	g/slice	g/day (3 slices)	kcal/day
starch	12.2	36.7	146.7
fat	2.1	6.3	56.8
beta-glucan	2.0	6.0	
protein	4.1	12.4	49.4
fiber	5.5	16.5	32.9
salt	0.6	1.8	
Moisture	24.8	74.4	
Total	50.7	152.1	285.9

Whole grain Wheat bread (Control)			
	g/slice	g/day (3 slices)	kcal/day
starch	12.3	36.9	148.0
fat	2.1	6.2	56.0
beta-glucan	0.02	0.06	
protein	2.5	7.5	29.9

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fiber	1.6	4.8	9.8
salt	0.4	1.20	
Moisture	11.4		
Total	30.4	91.2	243.7

Eligibility criteria

The eligibility criteria were designed to reach people with pre-diabetes; hence, we invite men and women with BMI ≥ 27 kg/m^2, aged 40-70 years. Additional inclusion criteria: HbA1c 35-50 mmol/mol, signed informed consent, regular bread eater and having freezer capacity for at least 2 loafs of bread. Exclusion criteria are: type 1 diabetes mellitus or pharmacologically treated type 2 diabetes mellitus, non-fasting blood glucose > 11.1 mmol/l, urine glucose ≥ 180 mg/dl, or protein excretion as indicated by dipstick (+++, Combur 10 test strips (Roche Diagnostics)), food allergies or intolerances preventing consumption of the study breads, pregnancy, lactation or planning a pregnancy during the study period, systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg at screening (16), history of stomach or gastrointestinal conditions (i.e. inflammatory bowel disease, Crohn's disease) history of myocardial infarction, heart failure, stroke, heart attack or cancer within 3 years prior to screening, use of anti-diabetic agents or insulin, history of alcohol abuse. Use of other medications or over-the-counter drugs or dietary supplements are allowed if the dose has been stable for a minimum of 3 months prior to the study. Any medication used will be recorded as will any changes of use. Initiation of medication to treat diabetes during the study is a reason for withdrawal.

Recruitment

In general, participants are recruited via leaflets, press releases, newspaper ads, ads in social media and blackboard flyers. Study specific websites are developed in Bergen and Paderborn, with the possibility for online registration. If a potential participant is interested, pre-screening will be conducted over the phone or using an online questionnaire to assess eligibility, and if eligible, a screening visit is scheduled.

Informed consent procedure

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All participants screened for eligibility are provided written information about the study prior to the first study visit. At the first study visit, the participants receive additional oral information on the study and are given the opportunity to ask questions to the research personnel before signing written informed consent. The study will not carry on and no samples will be drawn until the participant gives consent in writing. The participants have the right to withdraw their consent at any time, and to request that their biological samples and data will be destroyed.

Randomization

Participants are randomized into one of the two intervention groups (1:1 allocation) using block randomization with random block lengths, stratified by sex. The web-based randomization is configured by the Biostatistics and Data Management Group of the Clinical Trials Unit at the University Medical Center Göttingen, Germany, a third party not otherwise involved in the clinical trial. To reduce performance bias, at each of the four sites, a person, who is not member of the research team, is responsible for the randomization. All other research personnel at the sites are blinded for the allocation group.

Dietary assessment

The dietary assessment follows the principle of 24 h dietary recalls using country-specific food composition data. Practical conduction differs slightly between centers.

Bergen, Paderborn, and Leipzig: Dietary intakes are assessed by six 24-hour recalls (24HR) at the beginning (weeks 0-2), middle (weeks 7-9) and end of the study (weeks 15-16) using the validated tool “myfood24” (https://www.myfood24.org/). The 24HR are performed at unannounced times to attenuate the observer effect. The German version of myfood24 is based on German Food Code and Nutrient Database (Bundeslebensmittelschlüssel (BLS) version 3.02) for generic food items and the database of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study for branded food items (17, 18). The Norwegian version is based on the Norwegian Food Composition Table for generic food items and supplemented with food composition data for missing Norwegian dishes from other sources (19).

Gothenburg: As Myfood24© is not available based on Swedish food composition data, dietary intake is assessed by six 24HR on study site and over phone. Three

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24HR are performed at site by a trained dietitian, using images of portion sizes from The Swedish Food Agency. Additionally, three 24HR are performed over phone when the participants estimate portion sizes using a standard kitchen measure (e.g., dl measure and slices). To determine the nutritional composition of the intake, the DietistNet Pro software (Kost och Näringsdata AB, Bromma, Sweden) is used which is based on Swedish Food Composition Database.

Clinical assessments

Visits include collection of blood and urine samples, assessment of blood pressure, and anthropometric data including waist circumference, body weight and height, as well as body composition (described under "anthropometric measurements"). Participants are instructed to not eat or drink (except maximum 0.5L of non-carbonated water) 10 hours prior to the visits. Additionally, participants are instructed to avoid alcohol consumption, smoking and use of other tobacco products, and vigorous physical activity 12 h prior to the clinical visit.

Consumer acceptance

Participants evaluate the bread on day 1 and on week 8 of the study. The questionnaire is adapted from a previously established method (20). Participants rate their hunger, acceptability, and expected satiation in a 9-point scale. The participants rate expected satiety on a 6-point scale. Participants describe the bread via a check-all-that-apply (CATA) question (20, 21), "Choose all the attributes/terms that you think apply to this bread", using 28 hedonic and descriptive attributes and 16 usage & attitude attributes. Terms are randomized within groups and across participants. They answer two consumption questions:" In which meals do you consume bread?" and" How many bread slices do you eat on a typical day".

Data for Norway and Sweden were collected through online forms in EyeQuestion (Logic8 BV, The Netherlands) and stored in a secure server owned by Nofima. Participants from Germany filled in the questionnaire on paper.

Blood collection and analysis

During study visits, fasting blood samples are taken from an antecubital vein and placed in tubes containing either a clot activator or lithium heparin (Becton-Dickinson, Eysins,

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Switzerland) to obtain serum or plasma. Serum tubes are stored at room temperature for 30 minutes and then centrifuged at 1300g at 4°C for 10 minutes. Plasma tubes are immediately centrifuged except for one tube for HbA1c measurements. EDTA-plasma, serum and Li-Heparin plasma are immediately refrigerated/kept on ice, processed, and aliquoted into microtubes. Whole blood, plasma and serum aliquots are frozen at -20°C within 2 h of sample collection and transferred into -80°C within 24h. Blood samples are sent on dry ice to the Department of Medical Biochemistry and Pharmacology at Haukeland University Hospital, Bergen, every 6 weeks for analyses of HbA1c and secondary outcomes.

Analytical methods

Blood glucose is measured by a validated, portable system at room temperature (HemoCue© Glucose 201 RT system (HemoCue AB, Ängelholm, Sweden) at all centers. All other blood or serum measurements will be done at Haukeland University Hospital, Bergen (certified laboratory NSEN-ISO 15189), in frozen samples stored at -80°C. HbA1c is measured in EDTA whole blood samples which have been stored at maximum for 8 weeks by HPLC (BioRad, Hercules, CA). Liver enzymes and plasma lipids are measured using standard methods on a Cobas c702 autoanalyzer. Liver enzymes are measured photometrically according to the IFCC method (Roche Diagnostics, Mannheim, Germany). Serum triacylglycerides and total cholesterol are measured with an enzymatic colorimetric method. LDL cholesterol is measured photometrically, and HDL cholesterol is measured by a homogeneous enzymatic colorimetric method.

Dietary compliance

Compliance is assessed based on the evaluation of the 24HR and a pre-coded compliance journal kept by the participant during the study. Participants are instructed to tick off the number of slices consumed on each day. Compliance is a secondary outcome and sensitivity analysis will be performed based on compliance journals and 24HR.

Anthropometric assessments

Body weight is measured during all study visits including screening, with the participants wearing light clothing (e.g., underwear and t-shirt) and no shoes. Body weight is noted to the nearest 0.1 kg in the case report form (CRF). Height is measured using a Seca Stadiometer,

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model 217 or using the mBCA 515 integrated stadiometer. Height is measured once at screening without shoes to the nearest 0.1 cm. Waist circumference is measured twice on each occasion with a Seca 201 cm tape measurer to the nearest 0.1 cm according to WHO standards. The average of the two measurements are used for data analysis. Body composition (whole body mass, fat mass and lean body mass) is measured at all study visits by Bio-electrical Impedance Analysis using Seca mBCA 515 (in Bergen, Paderborn, and Leipzig). The measurements are performed in the morning after a 10 hour fast and in accordance with a standardized protocol.

Gothenburg: Body composition is measured using a Dual Energy X-ray absorptiometry, DEXA (iDXA, GE Medical Systems, Madison, WI, USA) using the software Core version 18.0. The DEXA-scan is conducted in the morning after an overnight fast with the participants wearing light clothing.

Twenty-four-hour continuous glucose monitoring

A continuous glucose monitoring device (CGM) is used (Gothenburg and Paderborn) to obtain 24-h continuous interstitial glucose concentration data on two occasions for 7 days each between baseline and week 1 and between week 15 and week 16. CGM data is used to calculate 24-h interstitial glucose peak, mean, coefficient of variation (CV) and total area under the curve (AUC). In addition, glucose response to bread consumption is evaluated separately for morning and evening meals.

The devices differ between centers and the specific devises are described below.

In Paderborn: A Dexcom G6 CGM (DexCom Inc., San Diego, US) is used. The glucose oxidase sensor is inserted into the upper part of the non-dominant arm or the abdominal area at least 5 cm away from the umbilicus to obtain an interstitial glucose measurement. Self-monitoring glucose readings (finger sticks) are performed with a blood sugar monitoring device (CONTOUR®NEXT ONE, Ascencia Diabetes Care, US) twice per day (morning and evening). The sensor does not need to be calibrated.

In Gothenburg: An Abbott FreeStyle Libre Pro iQ CGM (Chicago, Illinois, US) is used. The glucose oxidase sensor is inserted into the back of the upper part of the non-dominant arm. Participants can wear the sensor for up to 14 days and it does not need to be calibrated.

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Fecal samples

Spot fecal samples are collected at baseline, week 8 and 16. Collection is voluntary, and non-delivery of fecal samples is not a reason for exclusion. The samples will be collected in specific devises (Easy Sampler Collection Kit, GP Medical Devices ApS, Denmark). The participants are instructed to collect the fecal sample within 72 h of the clinical visit and are instructed to keep the sample in a household freezer until delivery to the study center. The participants are instructed to keep the sample in a freezer bag with cooling blocks during transportation to the study center. At the study center the samples are transferred to -80°C within 24 h. Samples are analyzed for the composition of the gut microbiota to provide possible mechanistic explanations underlying differential responses of participants of selected outcomes. All fecal microbiota analysis will be performed at Chalmers University of Technology (Gothenburg, Sweden).

2.10 Questionnaires

Participants complete several questionnaires during the study period. All questionnaires have been validated and are available in respective languages:

For physical activity assessment, participants complete the International Physical Activity Questionnaire (IPAQ) at baseline, week 8 and 16. The IPAQ is used to estimate daily inactivity and physical activity and has been shown to have adequate validity and reliability in various nationalities (22).

For chronotype, the Munich Chronotype Questionnaire (MCTQ) is administered at baseline, week 8 and 16 to estimate chronotype and sleep behavior during the trial. Chronotype is defined as the midpoint of sleep on free days corrected for sleep debt on workdays (MSFsc). Chronotype is also verified by use of an accelerometer in week 1 and 16 (Paderborn) (23).

For subjective health and well-being assessment, the 12-item Short Form Health Survey (RAND SF-12) is used at baseline, week 8 and 16. The questionnaire is used to evaluate perceived physical and emotional well-being.

For alcohol abuse, the four-question validated CAGE (Cut down, Annoyed, Guilty and Eye opener) questionnaire will be administrated at screening visit to assess the risk of alcohol abuse.

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2.14. Study outcomes

The primary outcome is the difference in glycemic control measured by HbA1c after 16 weeks between the intervention and control group. The trial has defined the following secondary outcomes: I) difference in fasting capillary blood glucose after 16 weeks, II) difference in fat mass (kg) and lean body mass (kg), III) difference in blood lipids (LDL-C, HDL-C, triglycerides), IV) difference in fatty liver index (based on serum liver enzyme activities (ALT, AST)), changes in fecal microbiota composition, V) consumer acceptance at baseline and 8 weeks. Furthermore, the following exploratory investigations will be undertaken: I) difference in postprandial response to morning and evening meals with the two breads based on CGM-data (Paderborn and Gothenburg only), II) if the individual chronotype of a person influences bread consumption and metabolic health, III) analysis of plasma and fecal metabolome and short chain fatty acids in fecal and blood samples, and IV) sensitivity analysis of compliance to the protocol measured with 24 h recalls and compliance journal.

2.15. Data analysis and sample size estimates

Intention-to-treat analysis will be performed following a statistical analysis plan that was set up a priori.

The primary outcome is the difference in HbA1c between the intervention and the control group at the end of the study. The main analysis will be a linear regression model adjusted for study center and baseline HbA1c. The results will be presented as the mean difference in HbA1c (95% confidence intervals) between groups at the end of study, with corresponding p-values. Missing data will be handled with multiple imputation methods, and the analysis will be accompanied by a complete case analysis. To evaluate the theoretical range of uncertainty due to missing outcome data, a best-worst and worst-best sensitivity analyses will be performed (24). The best-worst-case scenario will be constructed by assuming all dropouts in the intervention group to have an HbA1c at 16 weeks of the study center specific intervention group mean -2SD, and all dropouts in the control group to have an Hba1c of study center specific control group mean +2SD, and vice versa. The same analysis plan will apply to secondary outcome variables. Exploratory post-hoc analysis will include stratified analyses by sex, by BMI (27-30, >30 kg/m^2), by chronotype (based on MCTQ), and by center to explore potential country- and center-specific differences. Product terms between the

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intervention and the stratification variable will be included in the models to evaluate potential interactions. Statistical analyses will be performed using R, and the linear regression models will be fitted with the lm() function.

The statistical power calculation is based on the difference in HbA1c between the two groups (intervention and control) at the end of the study, as the estimated effect. We expect the starting HbA1c concentration to be approximately 41 mmol/mol with a SD of 6 mmol/mol and expect a reduction to 38 mmol/mol in the intervention group with small changes in the control group. Power calculation is based on this between-group difference in the HbA1c concentrations, with a SD of 6 mmol/mol (assuming similar standard deviations in both groups) and at a power of 90% and at a significance level of 0.05. To allow for 45% dropouts and ensure the conditions met, the aim was to recruit 250 participants into the entire multicenter study: 125 in each treatment group. Assumptions and estimates for the power-calculation were taken from dietary intervention studies (14, 25) and the calculation was made with a paired-t test using the power.t.test () function from R. The effect size was chosen to be moderate as this is a single-food intervention study.

2. Discussion

The CarbHealth trial aims to evaluate effectiveness of a beta-glucan-enriched bread i.e., whether habitual consumption of a bread containing beta-glucan (>4 g oat beta-glucan per 30 g available carbohydrate) vs a control wheat bread with 66% whole grain wheat under everyday conditions will affect long term glycemic control among persons at risk for T2D over a period of 16 weeks.

The CarbHealth trial is a pragmatic trial investigating food typically consumed. Participants replace their habitual bread with study bread, instead of adding or removing food items. Studies have shown that people are conservative regarding dietary changes (26), thus swapping a healthier alternative for a habitually consumed foods may be easier than changing food habits. A bread with 66% wholegrain wheat was chosen as the control bread instead of refined wheat bread to reflect dietary habits in Northern European countries. Since the quality of bread and the associated metabolic effects varies substantially (27) use of a medium

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to low glycemic index-bread rich in beta-glucan may substantially benefit metabolic health. Whilst complex interventions have been found to substantially reduce progression to diabetes among persons with pre-diabetes (28), many patients may not be prepared for such complex changes; thus exchange of bread with a healthier alternative may be more feasible.

Nutrition studies often lack statistical power hampering firm conclusions (29, 30). Multicenter studies may offer a strategy to overcome this shortcoming. Central production and distribution of the study breads by a third research partner (Nofima) enables this approach. Furthermore, the multicenter design employed in CarbHealth allows to study the metabolic effects and the effectiveness of bread against the background of different bread consumption patterns in the participating countries and to analyze differences in general dietary practices and potential acceptability. The multicenter study also takes advantage of the expertise of different groups, thus adding microbiota research, chronotype and continuous glucose measurements as well as consumer acceptance to the study outcomes. Furthermore, collaboration with food technologists that were able to produce a beta-glucan enriched bread is an additional strength of the multicenter study. Additional strengths of the CarbHealth-study include strong design features such as randomization, double-blinding, and provision of bread. One limitation of the study is that, due to logistics, breads had to be provided frozen, which is known to reduce bread quality and could lower consumer acceptance which could result in a lower compliance.

There may be important implications from this research regardless of the findings. If a beneficial effect was supported by evidence of a positive effect on long term blood glucose levels among persons with pre-diabetes, public health efforts should be taken to make beta-glucan-enriched bread available in European countries. This should be accompanied by efforts to increase the awareness, particularly among persons at risk of T2D, of a simple and effective replacement. Conversely, if a beneficial effect was not supported this could suggest that the bread is either not sufficiently enriched with beta-glucans, the beta-glucan has sub-optimal physiological characteristics e.g. solubility, pre-frozen bread may not be the optimal matrix for beta-glucan or that the reduction in post-prandial glycemic response achieved with a similar beta-glucan-enriched bread (31) does not translate into strong long term benefits for blood glucose control compared to a wholegrain wheat bread under every-day conditions.

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Notwithstanding, exploratory sub-group analyses will allow insights into factors determining responsiveness (e.g., compliance, meal context, consumer acceptance in different countries/center, sex). Similarly, secondary analyses will inform whether any potential effect or lack of effectiveness extends to other metabolic parameters.

Hence, the results of the CarbHealth study will provide important information on the public health relevance of a beta-glucan-enriched bread for reduction of post-prandial glycemic response in persons with pre-diabetes.

Patient consent for publication

Not applicable.

Declaration of competing interest

All authors state no competing interests.

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Contributors

TH wrote the first the first draft of the manuscript. JD is the chief investigator of this trial. JD along with RL, AB, AK, and SB: research question, study design, acquisition of data, obtaining the funding, implementation of the study protocol, critical revision, and final approval of the manuscript. TH, HRR, IR, LT, AS, US, KM, KP, AR, VL, PV: feedback of study design, implementation of study protocol, acquisition of data, critical revision, and final approval of the manuscript.

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Figure Legend

Figure 1. Flowchart over the study visits in CarbHealth multicenter study.

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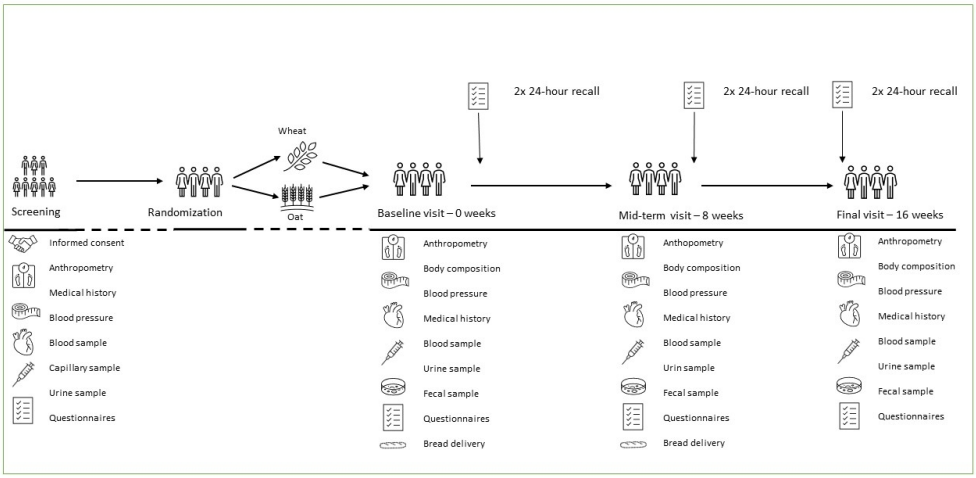

Figure 1. Flowchart over the study visits in CarbHealth multicenter study.

338x190mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 , 17-18
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	17
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	N/A

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		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6

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Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14

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Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6-7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	9

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		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-15

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Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	N/A
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further	N/A

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		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	6
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A

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Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	N/A
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A

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Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

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