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Can wheat germ have a beneficial effect on human health? A study protocol for a randomised crossover controlled trial to evaluate its health effects

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ABSTRACT

Introduction: Cardiovascular diseases (CVD) are the leading cause of mortality worldwide and diet is an important contributor to CVD risk. Thus, several food derivatives are being investigated for their beneficial impact on reducing cardiometabolic risk factors, either in risk groups or in healthy population as a preventive measure. Wheat germ is a food by-product with high nutritional value, especially as a concentrated source of dietary fibre and essential fatty acids, but its incorporation into the diet has been rare up to now. Previous studies do not clarify the hypothesised potential causal relationship between the consumption of wheat germ and benefits for human health.

Methods and analysis: We are conducting a randomised, double-blinded, crossover, placebo-controlled clinical trial designed to assess the physiological effects of daily consumption of wheat germ-enriched bread (containing 6 g of wheat germ) compared with non-enriched bread, over a 4-week period with a 15-week follow-up, in a healthy human population. A total of 55 participants (healthy volunteers, aged 18–60) have been recruited from the Porto metropolitan area in northern Portugal. Our aim is to evaluate the health effects of wheat germ on blood cholesterol and triglycerides, postprandial glycaemic response, gastrointestinal function and discomfort, and changes in intestinal microbiota and insulin resistance as secondary outcomes. The study follows the best practices for evaluating health claims in food according to the European Food Safety Authority (EFSA) scientific opinion, namely random allocation, double blinding, reporting methods to measure and maximise compliance, and validated outcomes with beneficial physiological effects as recommended by EFSA.

Ethics and dissemination: The study has been approved by the Health Ethics Committee of São João Hospital Centre (156-15) and the Ethics Committee of Faculty of Medicine of the University of Porto (PCEDCSS-FMUP07/2015). Results will be disseminated through peer-reviewed publications and presentations at international scientific meetings.

Trial registration number: NCT02405507; pre-results.

Strengths and limitations of this study

- The outcomes measured in this clinical trial are considered to be beneficial physiological effects, according to the European Food Safety Authority (EFSA) scientific opinion.
- The study follows the best practices for evaluating health claims in food, namely random allocation, double blinding, reporting methods to measure and maximise compliance, and validated outcomes.
- The proposed trial will clarify the role of wheat germ as a functional ingredient, namely whether there is any benefit to including it in a normal diet to reduce the symptoms associated with gastrointestinal discomfort and the management of hyperlipidaemia and glucose metabolism.
- Any statistically significant beneficial health could contribute to providing the scientific evidence needed for validation of health claims related to wheat germ.
- Compliance with self-reported questionnaires could decline over the 15-week follow-up.

INTRODUCTION

In recent decades, epidemiological studies and clinical trials have established an association between unhealthy dietary behaviours and chronic diseases such as cardiovascular diseases (CVD)[1–3] and type 2 diabetes,[4–6] which are leading causes of death in high-income countries. In turn, some studies have shown that populations can benefit from the incorporation of functional ingredients into their diets, proposing that functional food should be an integral part of public health programmes.[7 8] Accordingly, two recent systematic reviews confirmed that whole grain intake is associated with a reduced risk of CVD.[9 10]

At the same time, governmental authorities have limited health claims on food, allowing only those based on solid scientific evidence.

Examples of authorised health claims are the causal relationship between consumption of β-glucans from oats and blood cholesterol levels,[11 12] and a cause–effect relationship between walnut ingestion and improvement of endothelium-dependent vasodilation.[13]

Wheat germ is the main by-product of the flour milling industry, and although it is considered an excellent source of minerals, vitamins, fibre and essential fatty acids, it has been underused. Incorporation of wheat germ in the diet is almost exclusively restricted to animal feeding.[14–16] Even so, ingestion of raw wheat germ seems to reduce cholesterol and triglycerides (TG) in rats[17 18] and in human subjects with hypercholesterolaemia.[19 20] In turn, Matteuzzi *et al*[21] showed that ingestion of commercial wheat germ modifies the human colon microflora by lowering Gram-negative bacteria such as coliforms, while increasing potentially health-promoting bacteria, such as bifidobacteria and lactobacilli. However, existing studies reporting wheat germ are not sufficient to validate a causal relationship, especially due to the limited number of participants included.

Study aims

Our aim is to evaluate the impact of bread supplemented with wheat germ on blood cholesterol and TG, postprandial glycaemic response and insulin resistance; gastrointestinal discomfort will be evaluated in parallel. These outcomes are considered beneficial physiological effects by the European Food Safety Authority (EFSA), and therefore this randomised crossover controlled trial has been designed to provide scientific substantiation for health claims related to wheat germ. Bread was selected as the vehicle for wheat germ since it is a widely consumed food product and is therefore the ideal product to deliver functionality. Moreover, bread itself is the main contributor to the glycaemic index (GI) of the human diet[22] and since it is regularly consumed, a small change in its GI has been shown to have beneficial effects on health.[22–24] The percentage of wheat germ present in the enriched bread is 6%, a figure chosen because it offers the best ratio in terms of bread texture, volume and flavour.

METHODS

Design

This study is a consortium-initiated, randomised, double-blinded, crossover, placebo-controlled clinical trial designed to assess the physiological effects of daily consumption of wheat germ-enriched bread in a healthy human population. The trial has been implemented at the Faculty of Medicine of the University of Porto (Portugal)'s Centre for Health Technology and Services Research (CINTESIS).

Partners

The study is funded by the National Strategic Reference Framework (Portugal), and is part of the VALORINTEGRADOR project, jointly promoted by the Portuguese agro-food industry and three different Portuguese Research and Development (R&D) institutions (the Centre for Biological Engineering at the University of Minho, the College of Biotechnology at the Portuguese Catholic University and CINTESIS). The wheat bread supplemented with wheat germ was formulated by the two latter R&D institutions in partnership with GERM, South Africa, an agro-food company within the ambit of VALORINTEGRADOR.

Recruitment, randomisation and blinding

Volunteers were recruited through public advertisements in the university and faculty websites, and in online newspapers. After an initial phone contact, volunteers were invited to visit CINTESIS for a physical examination and a brief questionnaire about their medical history in order to check their eligibility to participate in the study. Study inclusion and exclusion criteria are described in table 1.

The full study protocol has been explained in depth to the participants, who were instructed not to change their normal diet during the study. A diary pack containing self-reported questionnaires and stool sampling kits is being delivered to each participant on enrolment.

Eligible volunteers have been randomised into two intervention groups (ratio 1:1) using a computer-generated allocation sequence, and concealed through sequentially numbered, opaque, sealed envelopes. The allocation sequence was generated by a statistician who had no involvement in recruitment and intervention delivery. Groups 1 and 2 will take, daily, wheat bread (100 g) named as A and B, respectively. Half of them will start with A and the other half with B. A and B are the intervention and placebo breads but the matching code will be blinded during the trial, in all stages until data analysis can be done. The participants, the principal investigator and the entire research team are blinded to the bread code; only the company contracted to produce the bread for the trial is aware of this code.

In this regard, we performed several tests and trials to define the final bread formula. Accordingly, we tested the best formula for white bread preparation that masks wheat germ supplementation regarding texture and colour. Subsequently, different bread preparations in which the percentage of wheat germ varied were tested. We found that the bread enriched with 6% of wheat germ has the best ratio in terms of bread texture, volume and flavour, being indistinguishable from the placebo. This finding was validated by a group of experts belonging to the VALORINTEGRADOR project. Moreover, the bread (A or B) has been delivered to each participant in opaque bags.

Intervention

An overview of the proposed study protocol is shown in figure 1. This is a 15-week parallel group randomised crossover trial. In the first stage, eligible participants will

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Adult men or women	1. Participant under prescription for medication for digestive symptoms such as antispasmodic, laxatives and antidiarrhoeic drugs or other digestive auxiliaries
2. Age 18–60 years	2. Relevant history, presence of any medical disorder or intake of medication/dietary supplements, potentially interfering with this trial at screening
3. Healthy volunteers free of chronic diseases with relevant effect on the gastrointestinal system or on visceral motility	3. Participants with stool frequency of ≤1 stool every 7 days
4. Without a diagnosis of any digestive disease including functional bowel disorders such as IBS	4. Participants not willing to avoid prebiotics and probiotics for the duration of the study
5. Non-diabetic, no gastric bypass surgery	5. Intake of antibiotics in the past 4 weeks and laxatives in the past 2 weeks
6. Fasting plasma glucose (finger-stick) <100 mg/dL (<5.5 mmol/L)	6. Current use of medication for lowering blood cholesterol or glucose
7. Non-smoker	7. Change of dietary habits within the 4 weeks prior to screening (for instance, start of a diet high in fibre)
8. Willing and able to provide written informed consent	8. Pregnant participant or participant planning to become pregnant during the study; breastfeeding participant
	9. Participants with a history of drug, alcohol or other substance abuse, or other factors limiting their ability to cooperate during the study
	10. Participants anticipating a change in their lifestyle or physical activity levels since this may also influence the results
	11. Known food intolerance or allergy
	12. Participant involved in any clinical or food study within the preceding month

IBS, irritable bowel syndrome.

undergo a 2-week run-in period. During this period, all participants will fill out a daily questionnaire to evaluate defaecation frequency and stool consistency (using the Bristol stool scale), as well as any gastrointestinal discomfort. During the run-in phase, participants do not consume any study product. The second stage corresponds to the randomised crossover phase. In this phase, participants are randomised to take daily wheat bread (100 g) supplemented with wheat germ (6 g/day; the intervention arm) or to consume daily wheat bread (100 g) without any supplementation (placebo; the control arm) for a 4-week period. This will be followed by a 5-week washout phase after which the participants will be crossed over for a second 4-week phase.

The participants will fill out a daily questionnaire to determine compliance to the study protocol (daily consumption of bread) and any changes in defaecation frequency and stool consistency. We will use diaries to assess compliance because there is no biomarker for wheat germ intake. Participants have been instructed to register and divulge any medication prescribed (not declared during recruitment) or adverse events during the study.

Follow-up and data collection

The evaluation will be performed at four different time points: initial visit (T1, at the end of the 2-week run-in period), intervention visit (T2, 4 weeks after T1), washout (T3, at the end of the 5-week washout phase), and final visit (T4; at the end of the intervention stage, after crossover). At each follow-up visit, a venous blood sample will be collected in fasting state from which capillary glycaemia will be measured using a glucose metre at 0, 30, 60 and 120 min after wheat bread (supplemented with wheat germ or placebo) intake. In addition, the participant will provide one stool sample from up to 72 hours beforehand, using the stool sampling kit provided by the research team. The self-reported questionnaires to score gastrointestinal discomfort and constipation-related quality of life, and to evaluate perceived stress during the run-in and intervention stages will be delivered on follow-up. Table 2 shows the schedule of visits and the variables under investigation in the present study.

In order to increase retention and complete follow-up, participants will receive a phone call 1-week before each visit, and a reminder text message on the day before. Self-collected and nurse-collected biological samples are labelled with the participant's study code assigned at enrolment. The samples will be used only for the purposes of this study, as stated in the protocol, and will be destroyed at the end. The diary packages containing self-reported questionnaires are also anonymised. Indeed, all collected data will be linked to the participant code

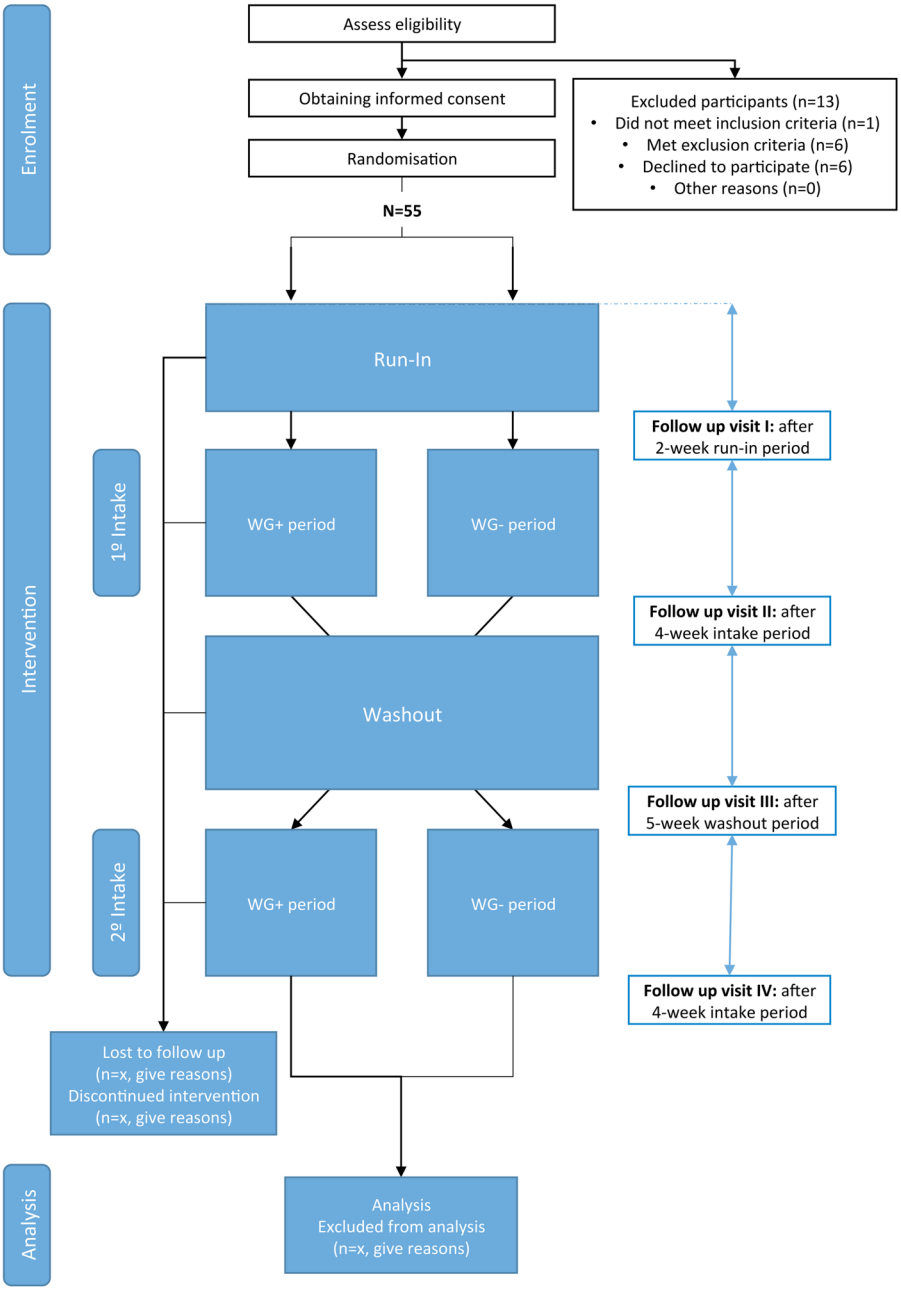

Figure 1 Trial flow chart. WG+ is wheat bread with wheat germ supplementation. WG− is wheat bread without wheat germ.

and only one member of the research team has access to participant information. Moreover, only the research coordinators will have access to all trial data.

Outcomes

Rationale for outcome measured

This randomised crossover controlled trial has been designed to provide scientific substantiation of health claims related to wheat germ, and therefore the primary outcomes measured can be considered beneficial physiological effects by the EFSA.

The outcomes measured are grouped into two categories: (1) risk factors for CVD and (2) gastrointestinal function. For cardiovascular health, changes in the blood lipid profile will be investigated by measuring low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and TG as primary outcomes. LDL cholesterol is a biomarker for CVD risk[25] and modification of one of these markers (LDL, HDL or TG) is considered a beneficial physiological effect by the EFSA.[26] Blood concentration of C reactive protein (CRP) will be also evaluated as a primary outcome since CRP could be related to long-term risk of CVD.[27] The EFSA considers that the maintenance of low plasma concentrations of CRP might be beneficial to human health.[28]

The hypoglycaemic effect of wheat germ will be also investigated. This food by-product

has a high content of dietary fibre (~18%),[29] which has been demonstrated to reduce blood glucose

Table 2 Schedule of assessments

	Follow-up							
	Run-in		1st intake		Washout		2nd intake	
Variables	1 week	2 weeks	4 weeks	6 weeks	9 weeks	11 weeks	13 weeks	15 weeks
Visit		x		x		x		x
Blood sample								
Total cholesterol		x_f		x_f		x_f		x_f
Triglycerides		x_f		x_f		x_f		x_f
HDL cholesterol		x_f		x_f		x_f		x_f
LDL cholesterol		x_f		x_f		x_f		x_f
C reactive protein		x_f		x_f		x_f		x_f
Insulin		x_f		x_f		x_f		x_f
Glucose		x_f		x_f		x_f		x_f
Capillary blood glucose		x_g		x_g		x_g		x_g
Stool sample								
Intestinal microbiota		x		x		x		x
Self-reported questionnaire								
Compliance (consumption of bread)	x_d	x_d	x_d	x_d	x_d	x_d	x_d	x_d
Stool frequency and consistency	x_d	x_d	x_d	x_d	x_d	x_d	x_d	x_d
PAC-SYM		x	x	x	x	x	x	x
PAC-QOL		x	x	x	x	x	x	x
PSS		x		x		x		x

HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAC-QOL, Patient Assessment of Constipation Quality of Life; PAC-SYM, Patient Assessment of Constipation Symptoms; PSS, Perceived Stress Scale; x_d, daily questionnaire; x_f, fasting venous blood sample; x_g, capillary blood glucose at 0, 30, 60 and 120 min after wheat bread (supplemented with wheat germ or placebo) intake.

response after carbohydrate intake (postprandial).[30] The intake of cereal fibre and whole grains as part of a diet with a low GI is a valuable strategy in the prevention of type 2 diabetes mellitus and CVD in the general population.[31] Besides the postprandial glucose response, the concentration of fasting plasma glucose and insulin will be measured as a secondary outcome to calculate homeostasis model assessment of insulin resistance (HOMA-IR). Several studies have demonstrated an association between insulin resistance, type 2 diabetes and CVD,[32 33] while wheat germ is rich in α-linolenic acid (nearly 0.5%),[29] a dietary precursor of omega-3, which has been reported to have beneficial effects on insulin resistance.[34]

Gastrointestinal discomfort will be assessed in the context of the maintenance or improvement of digestive function. Wheat germ modifies colonic microbiota[21] and it has previously been documented that these microbiota affect gut health in healthy individuals.[35 36] Gastrointestinal discomfort affects 16% of the healthy population and a reduction in its symptoms is considered to be an indicator of improved gastrointestinal function.[37 38] Patient-reported outcomes by means of self-reported validated questionnaires will be used for evaluation of gastrointestinal discomfort in this study. The use of these questionnaires as outcome variables for the scientific substantiation of health claims is accepted by the EFSA.[39] At the same time, the composition of colonic bacterial microbiota will be analysed as a secondary outcome. Decreasing potentially pathogenic gastrointestinal microorganisms might be a beneficial physiological effect, according to experts at the EFSA.[40 41]

Primary outcomes

The primary outcomes in this trial will be to demonstrate the difference (change) between wheat bread supplemented with wheat germ (6 g) and placebo in (1) serum TG and total cholesterol as well as LDL and HDL, (2) CRP, (3) postprandial blood glucose and (4) gastrointestinal discomfort associated with constipation.

Analysis of TG; total, LDL and HDL cholesterol; and CRP will be performed by an accredited outsourced laboratory. Constipation symptoms will be assessed using the validated Patient Assessment of Constipation Symptoms (PAC-SYM) questionnaire.[42 43] This self-reported questionnaire includes 12 constipation-related symptoms grouped into three subscales related to abdominal, stool and rectal symptoms. PAC-SYM is rated on a five-point scale, from 0 (no symptom) to 4 (very severe), aiming to measure the severity of gastrointestinal discomfort over the previous 2 weeks. A reduction in score (total or subscale) will reflect an improvement in symptoms. Cultural adaptation and linguistic validation of the PAC-SYM for Portugal was performed by the Mapi Research Trust (France).[44]

The change in blood glucose response will be calculated by computing the difference between the blood glucose concentration at 30, 60 and 120 min after bread intake and the baseline (t=0). Capillary finger-stick blood samples will be measured using a glucometre (Glucocard MX, ARKRAY Factory). The total blood

glucose response will be expressed as the incremental area under curve (AUC), ignoring the area under the baseline, and will be calculated geometrically using the trapezoidal rule.[45]

Secondary outcomes

The secondary outcomes in this trial will be to demonstrate the difference (change) between wheat bread supplemented with wheat germ (6 g) and placebo in (1) self-reported quality of life related to constipation, (2) stool frequency and consistency, (3) intestinal microbiota and (4) insulin resistance.

Participant's quality of life will be assessed using the Patient Assessment of Constipation Quality of Life (PAC-QOL) questionnaire.[46 47] The validated PAC-QOL is composed of 28 items grouped into four subscales related to physical discomfort, psychosocial discomfort, worries and concerns, and satisfaction. This self-reported questionnaire is rated on a five-point scale, from 0 to 4 (where 0=at no time/not at all, 4=all the time/extremely), and is used over a 2-week recall period. Cultural adaptation and linguistic validation of the PAC-QOL for Portugal was performed by the Mapi Research Trust (France).[44]

Secondary outcomes for stool frequency and consistency will be evaluated by means of self-reported questionnaires, while intestinal microbiota will be analysed by real-time quantitative PCR based on DNA extracted from stool samples.

HOMA-IR will be calculated to determine the degree of insulin resistance. The following formula will be used: HOMA-IR=(glucose×insulin)/405, where glucose is fasting glucose (mg/dL) and insulin is fasting insulin (mU/mL).[48]

Confounding variables

In addition to the randomisation protocol, the effect of wheat germ on gastrointestinal discomfort will be controlled for psychological stress, a confounding variable known to cause gastrointestinal symptoms. A psychometric assessment will be carried out using the Perceived Stress Scale (PSS). This 13-item self-reported questionnaire is designed to measure the degree to which situations in a person's life are appraised as stressful. Cultural adaptation and linguistic validation of the PSS for Portugal was implemented by Pais-Ribeiro and Marques.[49]

Sample size estimation

Sample size calculations have taken into account all the primary outcome variables. The sample size calculations determined that 40 participants were required to allow for an 80% power and 95% confidence level. Based on previous studies, the assumed difference in mean change from baseline and the respective SD were 5.0 and 11.0 mg/dL for total cholesterol;[50 51] 2.0 and 4.0 mg/dL for HDL cholesterol;[50 51] 5.0 and 11.0 mg/dL for LDL cholesterol;[50 51] 9.0 and 20.0 mg/dL for TG;[50 51] 0.035 and 0.080 mg/dL for CRP;[52] 35.0 and 80.0 mmol min/L for the incremental AUC of postprandial blood glucose;[53 54] and 0.35 and 0.75 for the PAC-SYM score.[55] Our aim was to enrol at least 50 participants to allow for dropouts.

Statistical analysis

Statistical analysis will be performed using SPSS V.23 software (SPSS, Chicago, Illinois, USA). Non-parametric tests will be used to analyse changes in PAC-SYM and PAC-QOL scores, since these data are not normally distributed. All other outcome parameters will be analysed using parametric approaches. Student's two-tailed unpaired t-tests will be used to compare baseline characteristics between participants. A Student's two-tailed paired t-test or repeated measures analysis of variance (ANOVA) will be used to analyse wheat germ supplementation effects within groups. The difference in the mean values of outcome variables between groups (intervention and control) will be measured using a Student's two-tailed unpaired t-test. All reported p values will be two-sided and tests will be performed with a 5% level of significance. Results will be summarised as descriptive statistics for baseline, and after 2-week and 4-week intake periods. Changes from baseline will be also evaluated for weeks 2 and 4.

The crossover design is only feasible in a setting where the conditions of interest remain stable during the study. If the conditions change during the study, they will affect the results, so it is very important to test for period effect.[56] Moreover, the study design includes a washout period between the two treatment periods. This washout period aims to minimise the risk of a carry-over effect from the intervention in period 1 into period 2. Therefore, to check for any residual carry-over effect, a test of interaction between treatment and period is also recommended.[56]

The statistical analysis will be performed applying an intention-to-treat (ITT) approach, Chichester, UK: John Wiley & Sons, with all randomised participants included in the analysis. It will make two main assumptions (in addition to the normality assumption for Student's paired and unpaired t-tests): no period effect and no carry-over effect (assessed by the treatment–period interaction). These two assumptions will be tested using the methods recommended by Pocock.[56] If the period effect is significant, the analysis will be adjusted in order to take the period effect into consideration.[56] Moreover, if the carry-over effect (interaction between treatment and period) is significant, only the first period will be used in the analysis, also following Pocock's recommendations.[56]

The PAC-SYM and PAC-QOL total score and subscale scores will be computed based on non-missing item responses. If more than 50% of items in the total scale/subscale are missing, the total scale/subscale score is not calculated and will be designated as 'missing'. For these previously described parameters, non-parametric tests will be used, as these data are not normally distributed.

Changes within and between groups will be checked with the Wilcoxon matched-pairs signed-rank test or the Mann-Whitney test, as appropriate.

ETHICS AND DISSEMINATION

All participants provided written informed consent. Any major amendments to the approved protocol will be resubmitted to the Ethics Committees. The trial has been conducted in accordance with the ethical principles of the Declaration of Helsinki, international law and Good Clinical Practice guidelines. Data monitoring has been performed by research coordinators along the study recruitment and follow-up an independent data monitoring committee has not been established for this study due to its minimal risk for human health. This trial was registered in ClinicalTrials.gov Database, reference NCT02405507, on 11 March 2015. The results from this study will be disseminated through peer-reviewed publications and presentations at international scientific meetings.

DISCUSSION

There is some evidence to suggest that wheat germ has a beneficial physiological effect in maintaining normal cholesterol levels[19 20] and in decreasing potentially pathogenic gastrointestinal microorganisms.[21] However, existing studies are scarce and have relevant design limitations, namely small sample size and insufficient information on study design (eg, sample size calculation, allocation concealment and blinding/masking). Thus, they are still inconclusive regarding the potential causal relationship between wheat germ intake and its alleged health benefits. Moreover, all these studies have used wheat germ as a supplement. Given that bread is one of the most widely consumed food products[57] and that the majority of consumers prefer whiter and softer bread formulations,[23 58] the aim was to create a wheat germ-enriched bread that preserved the taste and texture of refined white bread. Consequently, we expect that even a small health benefit effect, if observed, could lead to a major impact on public health.

To the best of our knowledge, this is the first clinical trial to assess the impact of wheat germ-enriched bread intake in healthy individuals. Furthermore, this study has used outcomes which are considered to be beneficial physiological effects, according to the EFSA. Hence, this randomised crossover controlled trial could have an important contribution to make for the research community, mainly for those who are facing challenges in providing scientific evidence for the physiological benefits of food derivatives for human health.

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Contributors AMR was partially responsible for study design, statistical analysis plan, carrying out the trial, protocol manuscript writing and final revision. HP was partially responsible for study design, carrying out the trial, manuscript writing and final revision. CC was responsible for the general coordination of the project, study design, protocol manuscript writing and final revision. LFA was responsible for the general coordination of the project, study design, statistical analysis plan, protocol manuscript writing and final revision.

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Disclaimer The funder had no role in study design and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Competing interests None declared.

Ethics approval Health Ethics Committee of São João Hospital Centre (reference 156-15) and the Ethics Committee of the Faculty of Medicine of the University of Porto (reference PCEDCSS-FMUP 07/2015).

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