RESEARCH PROTOCOL (December 2020)

The effects of bread consumption on intestinal and extra-intestinal symptoms in non-coeliac gluten sensitivity.

Version number: 3.0, date 16-12-2020

PROTOCOL TITLE "The effects of bread consumption on intestinal and extra-intestinal symptoms in non-coeliac gluten sensitivity."

Short title	Gluten and (extra-)intestinal symptom in NCGS
Version	3.0
Date	16-12-2020
Coordinating investigator/project leader	Prof. dr. D.M.A.E. Jonkers Division Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University T: +31 (0) 43 388 42 66 E: d.jonkers@maastrichtuniversity.nl Maastricht, The Netherlands
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	Prof. dr. D.M.A.E. Jonkers Division Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University T: +31 (0) 43 388 42 66 E: d.jonkers@maastrichtuniversity.nl Maastricht, The Netherlands
Primary investigator Amsterdam UMC – location VUmc	Prof. dr. G. Bouma Dept. of Gastroenterology, Amsterdam UMC - location VUmc T: +31 (0) 20 444 06 04 E: g.bouma@amsterdamumc.nl Amsterdam, The Netherlands
Primary investigator Wageningen University	Wageningen Universiteit Prof. dr. B.J.M. Witteman Dept. of Gastroenterology and Hepatology, Gelderse Vallei Hospital / Division of Human Nutrition, Wageningen University E: wittemanb@zgv.nl Ede / Wageningen, The Netherlands
Sponsor (in Dutch: verrichter/opdrachtgever)	Maastricht University Maastricht, The Netherlands

Subsidising party	TKI- Top Knowledge Institute
Independent expert	Drs. T.Y. Fung Dept. of Internal Medicine; Nephrology Maastricht University Medical Center+ T: +31 (0) 43 387 11 07 E: tszyeung.fung@mumc.nl
Project team members	Prof. dr. Fred Brouns Emeritus Professor Health Food Innovation Faculty of Health, Medicine and Lifesciences Maastricht University, The Netherlands Prof. dr. Daisy Jonkers Division Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University, The Netherlands Prof. dr. Ad Masclee Division Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University Medical Center+, The Netherlands Prof. dr. C. Rob Markus, Neuropsychology & Psychopharmacology Faculty of Psychology and Neuroscience Maastricht University, The Netherlands Dr. Daniel Keszthelyi Division Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University Medical Center+, The Netherlands Prof. dr. Louise Dye, Nutrition and Behaviour Human Appetite Research Unit, School of Psychology, University of Leeds, UK

	Dr. Clare Lawton, Nutrition and Behaviour Human Appetite Research Unit, School of Psychology, University of Leeds, UK Marlijne de Graaf, MSc Division Gastroenterology- Hepatology Dept. of Internal Medicine Maastricht University, The Netherlands Prof. dr. G. Bouma Dept. of Gastroenterology, Amsterdam UMC - location VUmc, The Netherlands Prof. dr. B.J.M. Witteman Dept. of Gastroenterology and hepatology, Gelderse Vallei Hospital / Division of Human Nutrition, Wageningen University, Ede / Wageningen, The Netherlands

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Principle investigator: Prof. dr. D.M.A.E. Jonkers Division Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University **Head of Department:** Prof. dr. A. Masclee Div. Gastroenterology-Hepatology Dept. of Internal Medicine Maastricht University Medical Center+		

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, "ABR = Algemene Beoordeling en Registratie")**
AE	**Adverse Event**
Amsterdam UMC – location VUmc	**Amsterdam University Medical Center – location Vrije Universiteit medical center**
AR	**Adverse Reaction**
AVG	**General Data Protection Regulation (in Dutch: Algemene Verordening Gegevensbescherming)**
BSS	**Bristol Stool Scale**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: "Centrale Commissie Mensgebonden Onderzoek"**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
E-	**Expectancy of getting gluten-free bread**
E+	**Expectancy of getting gluten-containing bread**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
FD	**Functional Dyspepsia**
FDA	**Food and Drug Administration**
FODMAPs	**Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols**
G	**Gram**
G-	**Actual gluten-free bread**
G+	**Actual gluten-containing bread**
GCP	**Good Clinical Practice**
GFD	**Gluten-Free Diet**
GI	**Gastrointestinal**
GMP	**Good Manufacturing Practice**
GP	**General Practitioner**
h	**Hour**

IB	**Investigator's Brochure**
IBS	**Irritable Bowel Syndrome**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
kcal	**Kilocalories**
Kg	**Kilogram**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
mg	**Milligram**
min	**Minutes**
Mm	**Millimetre**
MUMC+	**Maastricht University Medical Center+**
NA	**Non-Applicable**
NCGS	**Non-coeliac gluten sensitivity**
NCWS	**Non-coeliac wheat sensitivity**
NSAIDs	**Nonsteroidal anti-inflammatory drugs**
PANAS	**Positive and Negative Affective Schedule**
PIF	**Participant Information Sheet (in Dutch: "Proefpersoon informatie formulier")**
(S)AE	**(Serious) Adverse Event**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
VAS	**Visual Analogue Scale**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**
WUR	**Wageningen University & Research**

SUMMARY

Rationale: Although wheat- and gluten-containing food products are generally considered to be healthy, a large number of individuals in the general population reduce or limit their intake because of possible symptoms as can be read in the social media and popular books. Some individuals may be prone to developing non-coeliac gluten sensitivity (NCGS) which is accompanied by a range of gastrointestinal (GI) (*e.g.* abdominal discomfort, bloating, diarrhoea, rumbling, pain) and/or extra-intestinal complaints (*e.g.* headache, lethargy, depression, anxiety) soon after consuming wheat or gluten, which improve after gluten/wheat withdrawal (14). Evidence for a biological rationale is however limited. Increasing evidence indicates that the expectation that a person may suffer from NCGS may also be fuelled by psychosocial factors, in part by articles in the lay press and social media. Psychological factors and expectation mediate placebo and nocebo effects by influencing GI sensory and motor functions along the bidirectional brain-gut axis. However, few studies have examined the contribution of a negative expectation leading to symptoms when consuming gluten. Therefore, we aim to examine, in a placebo-controlled randomized study, whether expectancy of consuming gluten will impact overall GI symptoms in individuals with NCGS. We hypothesize that expected gluten intake via bread (as influenced by verbal information), but not the actual bread composition (containing gluten or not) affects subjective GI and extra-intestinal symptoms in individuals with NCGS.

Objective: The primary objective of this study is to investigate the effects of consumer expectancy, related to either gluten-containing or gluten-free oat bread, on short-term (within 8 hours) overall symptoms in individuals with NCGS. Secondary objectives include the effects of the actual intake of gluten on overall symptoms and the effect of expected gluten intake and actual gluten intake on individual GI and extra-intestinal symptoms as well as the impact of psychological factors on overall, intestinal and extra-intestinal symptoms after expected or actual gluten intake in individuals with NCGS.

Study design: The study conforms to a randomized, double-blind, controlled intervention study.

Study population: People in the general population with NCGS, aged between 18-70 years old, will be recruited to participate in this study.

Intervention: Participants will first be randomized into two different 'expectancy' groups; either being told that they have to consume "gluten-free" (E-), "gluten-containing" (E+) oat bread for breakfast and lunch. In fact, each expectancy group will be divided into 2 groups that receive either gluten-free (G-) or gluten-containing (G+) oat bread as the actual intervention for breakfast and lunch during the test day. This will lead to 4 groups:

1. Participants with the expectation of receiving gluten-free bread but actually receiving gluten-containing oat bread during the test day. (E-, G+)

2. Participants with the expectation of receiving gluten-free bread and actually receiving gluten-free oat bread during the test day. (E-, G-)
3. Participants with the expectation of receiving gluten-containing bread and actually receiving gluten-containing oat bread during the test day. (E+, G+)
4. Participants with the expectation of receiving gluten-containing bread, but actually receiving gluten-free oat bread during the test day. (E+, G-)

Main study parameters/endpoints: The main study parameter is overall GI symptom score, measured by a 100-mm visual analogue scale (VAS). Secondary study parameters are individual intestinal symptoms (*i.e.* abdominal pain, abdominal discomfort, belching, bloating, flatulence, diarrhoea, constipation, nausea, and urge to empty bowel and fullness) and extra-intestinal symptoms (*i.e.* tiredness, headache, foggy mind) measured on a 100-mm VAS. Average stool frequency and consistency will be measured by the Bristol Stool Scale (BSS) before and after intervention. Changes in mood will be measured by Positive and Negative Affect Schedule (PANAS) questionnaire.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participants may experience some burden during this study. Study participants will be required to visit the nearest study site on two separate occasions; a 1-hour screening visit and one test day visit which takes almost the entire day (from 8.00AM – 4.30PM) for the bread (i.e. gluten-free or gluten-containing) intervention. The study oat breads are commercially available in supermarkets in the Netherlands. Bread itself is considered to be a harmless food product, consumed on a daily basis by the general population worldwide. The participants may or may not experience mild GI symptoms after the consumption of the study bread, since the included participants suffer from self-reported gluten sensitivity. Such symptoms typically improve or disappear shortly (mostly within hours) after gluten is withdrawn from the diet. During the test day, participants will be asked to report their GI symptoms, extra-intestinal symptoms, stool characteristics every hour. They will also be asked to complete these self-assessments at the end of the test day and at the end of the next 2 days (which takes about 5 minutes in total each day). Moreover, in a subgroup of participants (i.e. those who have not previously been tested for coeliac disease, and who still consume a low level of gluten in their diet) a blood sample will be taken (by venepuncture) to exclude coeliac disease by means of serological tests on anti-tTG IgA. This requires participants to make an additional 20-minute visit to the nearest study site prior to the screening visit, may lead to minor discomfort and can cause small and transitory hematoma/bruises to appear.

1. INTRODUCTION AND RATIONALE

Wheat has been used for millennia as a raw material for basic foods and is generally known as a health promoting cereal grain, providing an important contribution to daily energy intake and nutrients worldwide. This is partly due to its favourable nutritional composition which provides a good source of dietary fibres as well as vitamins and minerals (1). The evidence about the health benefits of consuming whole grain foods made from wheat, oat, barley and rye is rather consistent. It is associated with reduced risk of type 2 diabetes, cardiovascular disease, obesity as well as cancer (2-7). However, gluten-containing cereals may also elicit adverse physical reactions (*e.g.* coeliac disease, wheat allergy) in susceptible individuals. Coeliac disease is a chronic, small-intestinal immune-mediated enteropathy initiated by exposure to dietary gluten in genetically predisposed individuals, with a prevalence of 0.5-1% in the general population (8). Wheat allergy is a distinct entity, characterized by an IgE-mediated allergic response to wheat with a prevalence of 0.2% (9). In addition, there is a large proportion of the general population (0.5-30%) that avoid or reduce gluten-containing products, despite the fact that *e.g.* coeliac disease and/or allergy have been ruled out (10-14). This phenomenon is referred to as non-coeliac gluten sensitivity (NCGS) or non-coeliac wheat sensitivity (NCWS) and manifests as gastrointestinal (GI) (*e.g.* abdominal discomfort, bloating, diarrhoea, rumbling, pain) and/or extra-intestinal complaints (*e.g.* headache, lethargy, depression, anxiety) soon after consuming wheat or gluten, which improve after gluten/wheat withdrawal (14). The biological rationale leading to symptoms in NCGS or NCWS is limited. Increasing evidence indicates that NCGS may also be affected by psychosocial factors, in part fuelled by articles in the lay press and social media. Information is emerging that gluten-containing products can cause medical and psychological symptoms, with a link to the belief that our body has not sufficiently evolved to adapt to grains in our diet. As a consequence, more and more people embrace a gluten-free diet, especially with regard to the consumption of (wheat/gluten-containing) bread. Although some replace wheat-based food products with oat products that do not contain gluten but are rich in fibers and micronutrients, others adjust their diet without adequate replacement, which may lead to unbalanced dietary intake and nutrient deficiency (15).

So far, studies on eliminating wheat or gluten-containing products from the diet showed some beneficial effects, but are mostly based on uncontrolled designs. The review of Catassi *et al*. (16) indicates that the absence of a clear biological mechanism of action in which psychological factors may also play a role, have immersed NCGS into even deeper controversy. A double-blind placebo-controlled crossover study by Biesiekierski *et al.* (17) showed a significant worsening of overall symptoms and pain irrespective of the diet (*i.e.* placebo, low-gluten or high-gluten diet) and markedly, the symptom scores were highest with the first treatment they

received. It is also remarkable that all the participants resumed to a gluten-free diet (GFD) at the end of the trial as they “report feeling better”. These findings indicate that psychological factors and expectation mediate placebo and nocebo effects by influencing GI sensory and motor functions along the bidirectional brain-gut axis (18). However, studies examining the contribution of a negative expectation leading to symptoms when consuming gluten has never been investigated. Therefore, we aim to examine, in a randomized, double-blind, controlled study, whether expectancy of consuming gluten will impact on overall intestinal and extra-intestinal symptoms in individuals with NCGS. With this study we will gain important insights in the effects of different types of bread on GI health, which may highlight the importance of considering the nocebo effect and eventually will help to make dietary recommendations in NCGS.

2. OBJECTIVES

Primary Objective:

In this randomized, double-blind, controlled study, we aim to investigate the effects of consumer expectancy, related to either gluten-containing or gluten-free oat bread on short-term (within 8 hours) overall GI symptoms in individuals with NCGS.

Hypothesis:

We hypothesize that expected gluten intake via bread consumption (as influenced by verbal information), but not the actual gluten intake increases subjective overall GI symptom score in individuals with NCGS. (see Figure 1, comparison E+G- vs. E-G-).

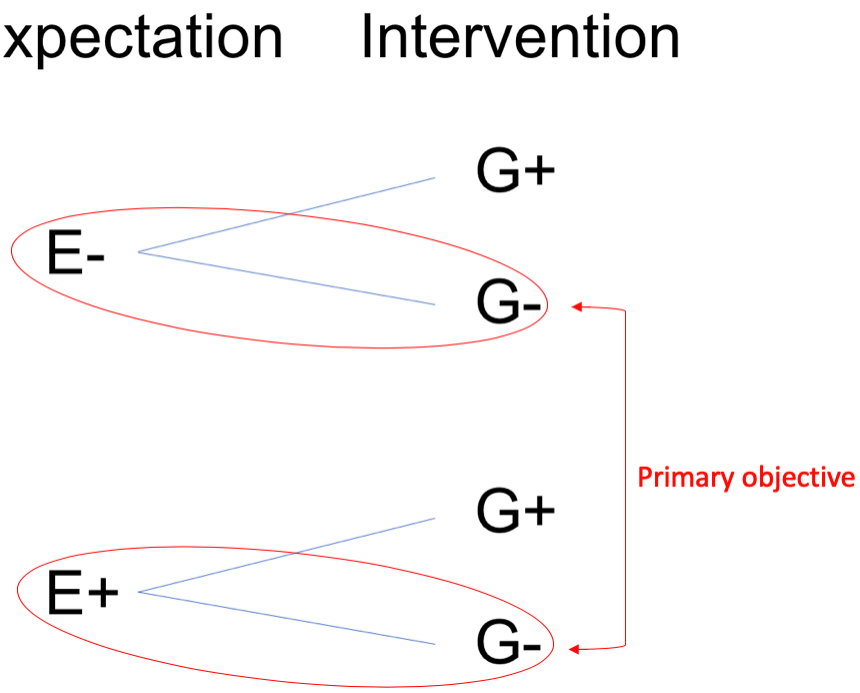

Figure 1. Overview of group comparison related to the primary objective (*i.e.* comparing E-G- vs. E+G- groups). (E- = Expectancy of getting gluten-free bread, E+ = Expectancy of getting gluten-containing bread, G + = actual gluten-containing bread, G- = actual gluten-free bread).

Secondary Objective(s):

- To investigate the effects of the actual intake of gluten on overall symptoms in individuals with NCGS.

  Hypothesis:

  We hypothesize that expected bread consumption (E+ or E-) (as influenced by verbal information), but not the actual bread composition (G+ or G-) affects subjective GI and extra-intestinal symptoms in individuals with NCGS.

- To investigate the effects of expected gluten intake and actual gluten intake on individual GI and extra-intestinal symptoms in individuals with NCGS.

  Hypothesis:

  We hypothesise that in participants who expect to receive gluten-containing bread, there will be an increase in GI and extra-intestinal symptoms regardless of the actual bread composition (G+ or G-) in individuals with NCGS.

- To study the impact of emotional well-being on overall, intestinal and extra-intestinal symptoms after expected or actual gluten intake in individuals with NCGS.

  Hypothesis:

  We hypothesize that individuals with decreased emotional well-being (as measured by PHQ-9, PHQ-15 and GAD-7) are more vulnerable to expectation in relation to gluten intake (as influenced by verbal information) leading to more symptoms.

- To investigate the effects of expectation and/or actual intervention on mood (affective) changes in individuals with NCGS.

  Hypothesis:

  We hypothesize that expectation and/or actual intervention leads to symptom relief/impairment which affects mood (affective) changes in individuals with NCGS. More frequent and more severe symptoms will most likely lead to a more negative mood while fewer symptoms will lead to a more positive mood, measured by the PANAS.

3. STUDY DESIGN

The negative expectancy of consuming gluten on overall symptoms in individuals with NCGS will be investigated in a multicentre, randomized, double-blind, controlled intervention study. Participants will first be randomized into a group told that they will receive gluten-containing bread (E+) or gluten-free oat bread (E-). Subsequently, within each arm, participants will be randomized to gluten-containing oat bread (G+) or gluten-free oat bread (G-), which they will receive during the test day (see also Figure 2). An intervention of 1-day has been chosen as most symptoms develop within 6 hours in 72% of NCGS subjects (see also inclusion criteria) (14). In this respect, an 8 hours follow-up is sufficient to pick up changes in acute overall symptoms and to keep the study protocol feasible for participants. Additionally, the risk of a single consumption of gluten-containing bread for breakfast and lunch is negligible. Moreover, longer treatment periods would increase the burden for the participants and might lead to higher non-adherence rates. Nonetheless, a follow-up period of 2 days is included to monitor the effects of the 1-day bread intervention and the disappearance of the symptoms during these days.

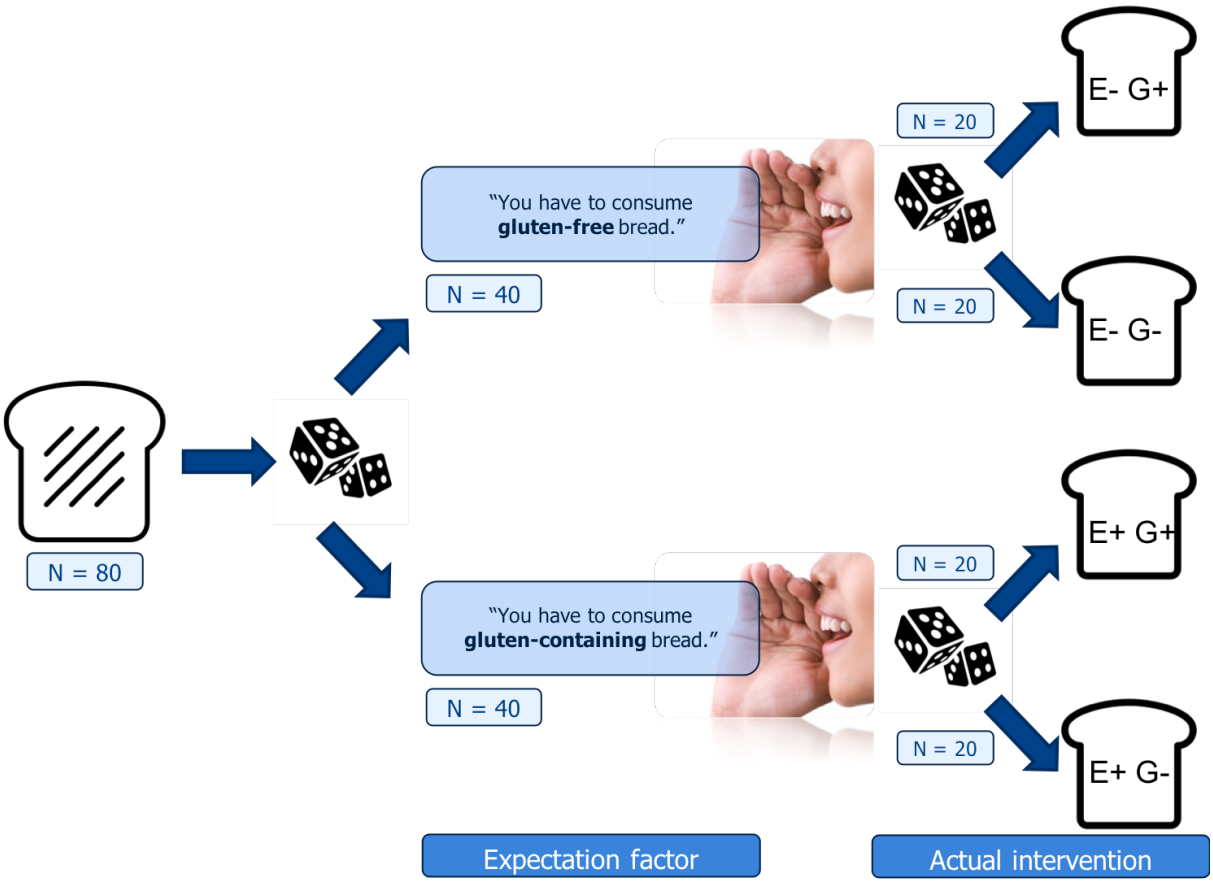

Figure 2. Study design. (E- = Expectancy of getting gluten-free bread, E+ = Expectancy of getting gluten-containing bread, G+ = actual gluten-containing bread, G- = actual gluten-free bread).

Participant recruitment will start after the Medical Research Ethics Committee (METC) of Maastricht University Medical Center+ (MUMC+) has approved the study according to the revised version of the Guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The study will be executed according to International Conference on Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines.

Recruitment (and test days) will take place via MUMC+, Amsterdam University Medical Center (UMC) location VUmc and Wageningen University & Research (WUR). Participants will be recruited via the outpatient clinic (including the “stadspoli” or local GP practices) of the gastroenterology department and of the dietetics department of the participating study sites. Eligible individuals with NCGS will be given the patient information brochure by their treating physician or dietitian during their visit to the outpatient clinic. Furthermore, volunteers will be recruited by means of advertisements on the website of the “Prikkelbare Darmsyndroom Belangenvereniging”, “Coeliakie vereniging”, local and faculty newspapers, and social media, as well as by using posters in publicly accessible sites such as the hospital, university, health centres, pharmacies, gyms, bakeries and relevant shops (such as “the Bisschopsmolen” in Maastricht (http://www.bisschopsmolen.nl) and *e.g.* restaurants serving gluten-free meals), and the website www.onderzoeksmachine.nl (“see also document “E3_Advertentietekst_NL64850.068.18_Versie2.0_02-10-2019”).
Lists available in the participating centres, with potential eligible subjects (*e.g.* IBS, NCGS/NCWS and controls) who have previously indicated that they can be contacted about future studies, will also receive one single mailing containing the advertisement text with the study information. This mail will be sent by the local administrator of the database or local researcher of the participating centre. Potentially interested volunteers will contact the coordinating researcher by e-mail or telephone and will subsequently receive the written information brochure by e-mail or regular mail.
Furthermore, the recruitment website Proefpersonen.nl (https://www.proefpersonen.nl), which is facilitated by the company Link2Trials, will be used for recruiting and pre-screening eligible subjects. Volunteers enrolled in their database will be pre-screened by Link2Trials based on age. If they match these criteria, they will receive the advertisement text from Link2Trials. Furthermore, the advertisement text will be placed on their website, which is accessible to both volunteers already registered at Link2Trials and unregistered volunteers. Potentially interested volunteers will contact the coordinating researcher by e-mail or telephone, and will subsequently receive the written information brochure by e-mail or regular mail. Enrolment in the Link2Trials database is not necessary to obtain the researchers contact information, but is voluntary if subjects are interested in receiving information about other research.

In the participant information sheet (PIF), participants will be informed that they will be randomized to receive either the gluten-free or gluten-containing bread. On the test day, we will inform them that they will be allocated to one of the groups (to create expectancy), but they will not be informed that thereafter they will be randomized to receive gluten-free or gluten-containing bread as that would affect our primary outcome. The PIF, including the informed consent form is enclosed as separate document to the study protocol (see document "E1+E2_Proefpersoneninformatie_NL64850.068.18_Versie3.0_16-12-2020").

A time period of one week (minimum) is provided to decide whether the volunteer would like to participate. If the volunteer is interested and willing to participate, an informed consent form will be signed (co-signed by the investigator). After obtaining informed consent, a screening visit will be performed to assess the eligibility of the volunteer to participate in this study (based on the in- and exclusion criteria). However, when coeliac disease has not been excluded previously, a blood sample will be taken in an additional visit prior to the screening visit, but after signing informed consent. After inclusion, the total duration of the study will be 3 days. A total of 80 participants will complete the study.

The location of the test day will be planned together with the participants.
The study consists of (See Figure 3; Time line):

1. Screening visit (at MUMC+, Amsterdam UMC or WUR)
    a. Coeliac disease test (at MUMC+, Amsterdam UMC or WUR)
2. Test day – Day 1 (at MUMC+, Amsterdam UMC, WUR or otherwise (in consultation))
    a. Randomization and treatment allocation
3. Follow-up questionnaires – End of day 1, day 2 and 3
4. Debriefing (by mail), when all 80 participants have completed the study.

3.1 Screening visit

A screening visit will be performed to assess the eligibility of the volunteers to participate in this study. First, in- and exclusion criteria will be checked. Participants must have an overall symptom score for gastro-intestinal symptoms below or equal to 30mm in VAS-score over the last week (see "F1.1_Screeningsvragenlijst_WoWstudy_Versie1.1_04-04-2018") in order to participate in this study, after a gluten-free or gluten-restricted diet for at least one week. Thereafter, several baseline parameters will be assessed by means of questionnaires to obtain information about general health (*e.g.* personal history of medical and psychiatric illness (*e.g.* pregnancy), lifestyle, weight and height, adherence to gluten-free diet and general health (see paragraph 7.3)).

Furthermore, participants will be asked to give permission to contact their general practitioner (GP) for previous serologic examination(s) to exclude coeliac disease (*e.g.* anti-tTG IgA test or upper GI endoscopy). Serological tests (*i.e.* anti-tTG IgA) to rule out coeliac disease will be carried out during a visit prior to the screening visit, if tests to exclude coeliac disease have not been done before and if participants still consume traces or very low amount of gluten (as defined by the Biagi questionnaire) in their current diet. Volunteers will only be eligible when coeliac disease is sufficiently ruled out by means of serologic tests and if they meet the in/exclusion criteria. The blood sample (via venepuncture) for anti-tTG IgA will be taken prior to the screening visit and the results can take up to approximately 7 days. In the exceptional case of a positive test, the participant cannot participate in this study and their GP will be informed (see also IC form). To prevent us from collecting additional data from a non-eligible participant, it is preferred to have the test results available before the screening visit.

The screening visit will require approximately 60 minutes and will take place at the MUMC+, Amsterdam UMC or WUR, The Netherlands. The serological test visit requires an additional visit of 20 minutes.

3.2 Test day (Day 1)

On the test day, upon arrival in MUMC+, Amsterdam UMC or WUR, the participants will be informed by the researcher(s) that they have been allocated to a specific group (*i.e.* E+ or E-). They will then receive either gluten-containing (G+) or gluten-free (G-) oat bread. The participant and the investigator will both be blind to the actual study intervention the participant will receive, *i.e.* the gluten-containing (G+) or gluten-free bread (G-) within each of the expectancy groups (See Figure 2).

This randomization results in 4 groups:

Group 1: Participants with the expectation of receiving gluten-free bread but actually receiving gluten-containing bread during the test day (E-, G+).

Group 2: Participants with the expectation of receiving gluten-free bread and actually receiving gluten-free bread during the test day (E-, G-).

Group 3: Participants with the expectation of receiving gluten-containing bread and actually receiving gluten-containing bread during the test day (E+, G+).

Group 4: Participants with the expectation of receiving gluten-containing bread but actually receiving gluten-free bread during the test day (E+, G-).

During the test day, participants are asked to come to the MUMC+, Amsterdam UMC or WUR in a fasted state at 8.00AM. Before the participants are informed about which group they are assigned to, they will first have to fill in the symptom questionnaire and the positive and negative affective scale (PANAS) as baseline measurements. Then (± 8.15 AM) they will be informed that they have been allocated to the gluten-containing or gluten–free groups (i.e. creating expectancy, E- or E+) and at 8.30AM they have to consume 2 slices of study bread for breakfast and at 12.30PM a further 2 slices for lunch. All slices can be prepared with margarine plus cooked ham, or cheese, or marmalade (one standardised portion of one topping per slice, all gluten-free). Furthermore, they will be allowed to drink coffee, tea or water (ad libitum) during the test day. Other foods or drinks are not permitted during the test day until 4.30PM. During the test day, the participants are free to do what they want to (watching TV, reading, working, possibly take a walk), provided that they complete the questionnaires every hour.
Frozen packages of bread portions for the test day will be provided by European Bakery Innovation Centre, Papendrecht, The Netherlands, with a label that refers to the "expectancy" arm, but the actual treatment is blinded. Participants will be asked to report their GI symptoms, extra-intestinal symptoms, stool characteristics, and the PANAS in an electronic diary at baseline (at 8.00AM) and every hour till 4.30PM. After 4.30PM, participants will go home and will be allowed to eat freely as normal. During the study visits and telephonic contacts, the presence of serious adverse events will be checked. Participants will also be instructed to contact the researcher if their health status changes.

If participants are unable to stay at the MUMC+, Amsterdam UMC or WUR the entire day, they will receive the breakfast and the baseline questionnaire at the study location and can do the follow-up (*i.e.* completing the hourly questionnaire and consuming the study lunch) at home or at work, provided that all hourly questionnaires will be completed on the same tablet or notebook. If necessary, a study tablet will be provided for the test day. Subjects will receive a text message 5 minutes before each timepoint they have to fill in a questionnaire and will be informed that time of completion will be logged. Furthermore, participants have to adhere to the dietary restrictions. If possible, participants consume the study lunch in the presence of the researcher. Alternatively, participants are asked to provide pictures as proof of completely consuming the study lunch. The starting time of the test day can be flexible (before 9.00AM) and results in a shift of all time points, therefore it does not affect the structure of the test day. Any alterations in the test day (e.g. location of completing the hourly questionnaires) will be logged and taken into account during analyses.

3.3 Follow-up (end of day 1, day 2 and 3)

During the follow-up days participants will resume to their normal dietary habits, which is their usual gluten or wheat restricted/gluten-free diet, or the gluten-free/gluten-restricted diet they started at the beginning of the study. Furthermore, participants will be asked to complete the end of the day questionnaire (between 10.00-12.00PM), consisting of the daily symptom questionnaire, stool diary, and the PANAS.

3.4 Debriefing

On completion of the study, participants will be debriefed, but to avoid spread of information and bias in the data, the deception associated with the intervention and expectation as well as the aim of the study will only be disclosed after all participants (n=80) have completed the study. If there are any questions after the debriefing, participants can contact the research team at any time (contact details will be given via the debriefing mail). The debriefing information is available in document (see "E4_Debriefing_NL64850.068.18_Versie1.2_12-03-2019").

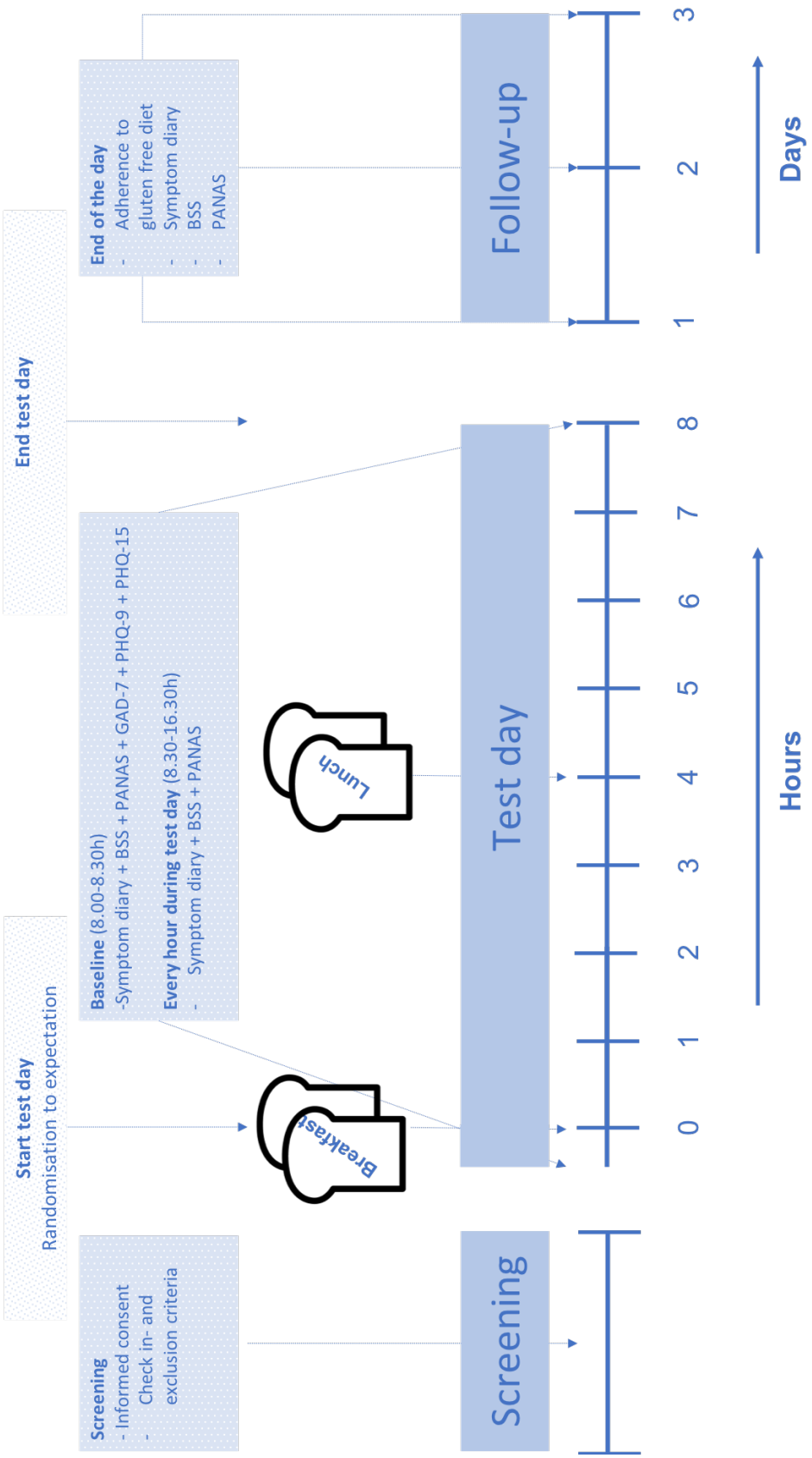

Figure 3. Time line study

4. STUDY POPULATION

4.1 Population (base)

People in the general population with NCGS will be recruited to participate in this study.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Develops self-reported GI symptoms within 8 hours after a single intake of gluten-containing products, without a diagnosis of an organic GI disease;
- Aged between 18-70 years;
- Following of willing to follow a gluten-free or gluten-restricting diet (based on a gluten-free dietary compliance questionnaire of Biagi *et al.* (only group 2, 3 and 4 will be eligible), see also "F1.2_Biagi vragenlijst naleving glutenvrij dieet_WoWstudy_Versie1.1_28-03-2018") for at least 1 week prior to study participation and willing to continue with this throughout the study;
- Asymptomatic or only mildly symptomatic (overall (GI) symptom score with VAS $\leq$ 30mm) while on the gluten-free diet;
- Willing to participate in a study in which they have to consume 4 slices of gluten-containing or gluten-free bread for breakfast and lunch during the test day;
- Willing and able to give written informed consent and to understand, participate and comply with the research project requirements.

4.3 Exclusion criteria

Participants who meet any of the following criteria will be excluded from participation in this study:

- Coeliac disease;
- Wheat allergy;
- Presence of an organic GI disease (such as inflammatory bowel disease) or other disease which may interfere with NCGS symptoms (upon judgment of the physician-clinical investigator (Dr. Keszthelyi, Gastroenterologist MUMC+, and/or in consultation with clinicians/PI prof. Bouma or prof. Witteman, when applicable));
- Previous major abdominal surgery or radiotherapy interfering with GI function:
    - Uncomplicated appendectomy, cholecystectomy and hysterectomy allowed unless within the past 6 months;

    - Other surgery may be allowed based upon judgment of the physician-clinical investigator (Dr. Keszthelyi, Gastroenterologist MUMC+), who will decide on in- or exclusion based upon the surgery applied;
- Use of medication potentially influencing GI function and/or NCGS symptoms is allowed, provided that dosing has been stable for > 6 weeks before enrolment;
- Administration of antibiotics, probiotic or prebiotic supplements, investigational drugs or participation in any scientific intervention study, which may interfere with this study (to be decided by the principle investigator), in the 14 days prior to the study
- Excessive use of alcohol (>15 alcoholic units per week), or other drugs;
- Plan to lose weight or follow a specific diet within the study period;
- Any malignancy;
- Pregnancy or breastfeeding;
- Insufficient fluency of the Dutch language.

PM: Non-postmenopausal women will not be planned for the test day during menstrual bleeding.

4.4 Sample size calculation

Sample size calculation is based on our primary outcome measure, the overall symptom score, using a visual analogue scale (VAS). Since no data of overall symptom score based on expectancy in an NCGS population are available, we based our sample size calculations on a well-designed study most comparable to the present study by Biesiekierski *et al.* (2013) (17). In that study, the effects of gluten on GI symptoms in patients with NCGS after dietary reduction of fermentable, poorly absorbed, short chain carbohydrates (17) were examined. They reported an increase in mean overall symptom score after the ingestion of either 16g gluten/day (high-gluten arm) of 10.3mm in VAS-score with a standard deviation of 12.8mm (data obtained by requesting raw data from author (17)). In the study of Biesiekierski *et al.* (2013) a change of 20mm in VAS of overall symptom score was considered clinically relevant. Since our study has a different study design in which expectancy may play a major role and symptom scores may increase less, we deem an overall symptom difference in VAS-score of 15mm to be clinically relevant. Using a difference in overall mean symptoms of 15mm with a standard deviation of 12.8mm, power of 80% and two-sided alpha of 0.05, a sample size of 20 participants per group are required. So, a total sample size of 80 participants is required.

In addition, in order to account for potential drop outs due to drop-out after randomisation and during the test day for a reason other than intolerable symptoms, we ask permission to include max 5% (*i.e.* 4) extra participants, resulting in maximal 84 participants in total. Power analysis was conducted in the statistical power analysis program “G*power, version 3.1 for Macintosh” (19).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The investigational products in this study are two different types of bread, *i.e.* gluten-free oat bread and gluten-containing oat bread. Oat itself does not contain gluten. This gluten-free oat bread is commercially available at the Dutch supermarket “Jumbo”, the bread is called “Yam” and made from 100% food-grade ingredients suitable for human consumption. The gluten-containing oat-bread is the same bread, but with the addition of 9.3% gluten (*i.e.* ~4 g per slice, resulting in a minimal intake of 16g per day). No improvers, preservatives, sweeteners, flavours and no colours from artificial sources will be used in this bread recipe. Both breads are similar with regard to texture, taste and appearance and will be baked, labelled and provided in kind by the European Bakery Innovation Centre, Papendrecht, The Netherlands. For more information on the nutritional value of the product, see section 6.1.

5.2 Use of co-intervention (if applicable)

Volunteers reporting a gluten-free or gluten-restricting diet should have been on this for at least one week prior to the study and continue with this throughout the study. To minimalize the influence of altered food and beverage intake on outcome parameters during the study period, the test day will take place at the MUMC+, Amsterdam UMC or WUR in a controlled setting under supervision of the researcher. If part of the test day is carried out outside these study sites, the participants will be given clear instructions on the (un)allowed intake and will be asked to record their beverage intake during the day. Subsequently, we will ask participants to maintain their dietary habits (or the gluten-free/gluten-restricted diet they started prior to the study) as much as possible during the follow-up period. There will be no further dietary restrictions, apart from the test day. During the test day, the participants are free to do what they want to (watching TV, reading, working, possibly take a walk), provided they complete the questionnaires every hour.

5.3 Escape medication (if applicable)

We do not expect that the use of escape medication will be necessary. However, participants are permitted to use paracetamol if necessary. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are not allowed during the test day. The number of tablets, dose, and the reason for the medication use have to be reported in the electronic diary. If paracetamol does not work adequately and participants experience intolerable symptoms during the study, the involved gastroenterologists in this study (Dr. Keszthelyi) will advise on whether or not to stop the intervention. In that case, VAS-scores for the remaining questionnaires were replenished using the highest VAS-score achieved thus far.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

For this randomized, double-blind, controlled trial two different types of bread will be manufactured by the European Bakery Innovation Centre (Papendrecht, The Netherlands), *i.e.* gluten-free oat bread and gluten-containing oat bread. Both breads will be made using the same baker's oat flour and are prepared using food-grade ingredients suitable for human consumption. The gluten-free oat bread will be baked under gluten-free conditions. The gluten-containing oat-bread consists of oat flour with gluten as additive (see also product information sheet, D2). The amount of added gluten does not exceed the amount of gluten in commercially available bread (*i.e.* 9.3%, resulting in about 4g of gluten per slice). The baking process has been optimised so that both breads are similar with regard to texture, taste and appearance (see Figure 4), confirmed with preliminary testing in 10 healthy volunteers. The nutrient composition of the study bread has been analysed by European Bakery Innovation Centre (Papendrecht, The Netherlands), see Table 1.

Table 1. Nutritional composition of the study breads per slice of bread

Nutrient composition per portion (46 gram)	**Gluten-free oatbread**	**Gluten-containing oatbread**
Energy (kJ)	435.6	433.6
Energy (kcal)	103.3	101.4
Gluten (g), (%)	0.0 (0.0)	4 (9.3)
Total Protein (g)	3.1	6.8
Animal protein (g)	0.0	0.0
Plant protein (g)	3.1	6.8
Total Fat (g)	1.7	1.4
Saturated fat (g)	0.2	0.2
Monosaturated fatty acids (g)	0.8	0.7
Polysaturated fatty acids (g)	0.6	0.5
Linoleic acid (g)	0.1	0.1
Carbohydrates (g)	18.0	14.1
Mono/disaccharides (g)	1.1	0.9
Polysaccharides (g)	16.4	13.2
Dietary fibre (g)	3.0	2.5
NaCl (g)	0.6	0.4

There are no known health risks concerning the consumption of these study breads, as all products will be made with ingredients that are commercially available on the market. The

risk of consuming gluten for 1 day is considered to be very low, especially considering the fact that coeliac patients are excluded.

Figure 4. Study breads. 1. Gluten-free oat bread, 2. Gluten-containing oat bread

6.2 Summary of findings from non-clinical studies

Not applicable.

6.3 Summary of findings from clinical studies

The biological feasibility of gluten inducing symptoms would be greatly enhanced if mechanisms could be defined. However, the aetiology of NCGS is under debate, since clear biological mechanisms of action and specific biomarkers are lacking (20). So far, studies examining the contribution of consumer perceptions and expectation in symptom development are scarce. A well-designed placebo controlled, cross-over study of Biesiekierski *et al.* (2013) (17), found no evidence of specific or dose-dependent effects of gluten in patients with NCGS placed diets low in FODMAPs. Overall symptoms and pain significantly worsened compared with mean scores during the last week of each dietary treatment period, irrespective of the diet (*i.e.* placebo, low-gluten or high-gluten diet). Furthermore, it is striking that the symptom scores were highest with the first treatment they received, pointing to a nocebo effect and indicating that a strong anticipatory symptomatic response was present independently of the nature of the challenge protein.

These findings indicate that a negative expectation (*i.e.* nocebo effect) and psychological factors may play a role in the experience of symptoms. Based on this study we hypothesize

that the effects of gluten or wheat bread are conditional on believing that one has or has not consumed gluten-containing foods. So, by telling self-reported gluten sensitive people that they will be eating study bread containing gluten, they may experience gluten induced GI-symptoms, irrespective of the actual bread composition.

6.4 Summary of known and potential risks and benefits

The study oat breads are commercially available. Bread itself is a harmless food product, which is widely consumed on a daily basis by the general population worldwide. The volunteers may or may not experience mild GI symptoms after the consumption of the study bread, since the included participants suffer from self-reported gluten sensitivity. It should be noted that the consumption of gluten by non-coeliac gluten sensitive participants does not lead to coeliac-specific antibodies and villous atrophy or any allergy related processes (21). Typically, symptoms improve or disappear shortly (mostly within hours) after gluten is withdrawn from the diet (14, 22). The provided study breads contain no more gluten than is present in normal commercially available bread (9.3%) consuming 4 slices of the study bread will not exceed the average amount of gluten that is normally consumed per day (23). Participating in this study does not lead to direct benefits for the individual participants, but may give the participants some insights into the effects of gluten and wheat on their symptoms and well-being. The results of this study will be used to better understand why some types of bread can lead to certain symptoms and who is most vulnerable to experience these symptoms.

Although the risk of consuming gluten for 1 day is considered to be very low, we still would like to limit any potential risk by excluding participants with coeliac disease. Furthermore, the inclusion of such patients may lead to a bias in the study and the expected effects. Therefore, volunteers will only be eligible when coeliac disease is ruled out by means of serologic examination or previous upper GI endoscopy. Participants reporting wheat allergy during the screening visit will also be excluded.

6.5 Description and justification of route of administration and dosage

Participants are requested to consume the study breads during one day: *i.e.* 2 slices for breakfast and 2 slices for lunch (for more details see section 3.2).

6.6 Dosages, dosage modifications and method of administration

When baseline measurements are completed, participants will start the intake of the provided study bread during the test day for breakfast and lunch, *i.e.* gluten-free oat bread

or gluten-containing oat bread (containing 9.3% gluten) made from 100% food-grade ingredients suitable for human consumption. Participants will be asked to consume 4 slices of study bread that day, *i.e.* 2 slices for breakfast and 2 slices for lunch with the choice to prepare their bread meal with margarine plus cheese, cooked ham or jam (one standardised portion of one topping per slice, all gluten-free). It is important that this is the only dietary change the participants make during the test day. For more detailed information, see paragraph 3.2.

6.7 Preparation and labelling of the Intervention Product

Study bread products will be packed and labelled at European Bakery Innovation Centre, Papendrecht, The Netherlands. Blinding is ensured by the fact that the study breads are similar in texture, taste, and appearance, confirmed with preliminary testing in 15 healthy volunteers. Frozen packages of daily bread portions for the test day will be provided with a label that refers to the "expectancy" arm only, thus the actual treatment alignment is blinded for both the participants and researcher(s).

The packages will contain the following information:

- Study name (WoW study)
- Subject code ("WOW{000}").
- Packing date (*e.g.* 04-12-2017)
- Expiration date (*e.g.* 08-12-2019)

6.8 Study bread accountability

The study breads will be shipped by delivery courier to the MUMC+. Products will be labelled according to the randomization scheme, see paragraph 7.2. The products will be preserved in the freezer with a temperature of -18°C until needed at the central kitchen of the MUMC+, this freezer is only used to store food products. Participants will consume 2 slices study breads for breakfast and 2 slices for lunch.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Overall symptom score in the short term (within 8 hours)

The primary outcome is the overall GI symptom score after manipulation of information giving a certain expectation about the bread treatment. Overall (GI) symptom score will be measured using VAS (0 to 100 mm) anchored at the low end (score of 0) with the absence of overalls symptoms and at the other end with extreme of severity of symptoms, the worst it has ever been (score of 100) (see also addendum "*F2_Klachtendagboek_WoWstudy_ Versie1.0_05-03-2018"*). A relevant change is defined as at least a 15 mm increase in self-reported overall symptoms compared with baseline.

7.1.2 Secondary study parameters/endpoints

- Abdominal pain - Abdominal discomfort - Belching - Bloating - Flatulence - Diarrhoea - Constipation - Urge to empty bowel - Fullness	Intestinal symptoms, measured on 100-mm VAS. (See "*F2_Klachtendagboek_WoWstudy_ Versie1.0_05-03-2018"*)
- Nausea - Tiredness - Headache - Foggy Mind	Extra-intestinal symptoms, measured on 100-mm VAS. (See "*F2_Klachtendagboek_WoWstudy_ Versie1.0_05-03-2018"*)

- Average stool frequency and consistency (measured by the Bristol Stool Scale)
- Changes in mood measured by PANAS (*e.g.* interested, alert, excited and distressed, ashamed, nervous).

7.1.3 Tertiary study parameters/endpoints

- Participant characteristics in relation to NCGS (demographics, psychological parameters)

7.2 Randomisation, blinding and treatment allocation

After screening and subsequent inclusion in the study, each participant will be assigned to a unique participant identifier code. Participants will be randomly assigned to one of the following experimental conditions:

1. Gluten-free expectation and actually receiving gluten-free oat bread (E-, G-).
2. Gluten-free expectation but actually receiving gluten-containing oat bread (E-, G+).
3. Gluten-containing expectation but actually receiving gluten-free oat bread (E+, G-).
4. Gluten-containing expectation and actually receiving gluten-containing oat bread (E+, G+).

The randomisation lists will be generated by an independent co-worker from the same department as the principal investigator who is not further involved in the study, using a publicly available procedure through the internet (http://randomizer.org). This random number generator performs balanced permutations, resulting in an equal number of participants per treatment order followed. Participants will be randomly allocated to the treatment conditions and there will be a stratification on gender using block randomisation (block sizes of 8). The randomisation scheme will be up to n=90, this to be sure in case of dropouts, to randomize potential (new) participants to a new randomization number.

The investigator has no access to the randomisation list that conceals the intervention code. However, since expectation may play a role in the experience of symptoms, the investigator is unblinded to the expectation arm since the investigator has to inform the participant about allocation to the gluten-free or gluten-containing expectation group. Of course, the investigator is blinded to the actual treatment. The randomisation list will be kept in a sealed envelope by the head of the department (prof. dr. Masclee), to reveal the treatment in case of medical emergency. Primary investigators of all participating centres will receive the contact details of prof. dr. Masclee. The condition numbers as allocated by the independent colleague, who will carry out the randomisation, are registered by the investigator in the screening and enrolment log. Furthermore, the same co-worker will keep the randomisation lists. The “randomization key” will be provided to the investigator only after all experimental and statistical procedures have been completed.

In general, there should be no need to unblind the allocated product. Unblinding should only be done in those rare cases when a participant suffers from a serious adverse event (SAE) and the investigator or independent physician believes that clinical management depends on whether the participant received gluten-free oat bread or gluten-containing oat bread. In

case of emergencies, the co-worker has been provided with the concealment list and will be able to de-blind.

Since this study focuses on the role of expectation on overall symptoms in people with self-reported gluten sensitivity, we cannot give the complete and correct information about the actual intervention to the study participants in advance. This is part of the design of this study. Participants are however informed in the PIF that they will be randomized to receive gluten-free or gluten-containing bread (paragraph 3.2). Only one single person will be scheduled during a test day, so there is no contact between the participants. At the test day, we will give participants a certain expectation by informing participants that they have been allocated to receive the gluten-free bread or gluten-containing bread. In fact, within each of these arm, half of the participants will then be assigned to the actual treatment. The actual treatment cannot be revealed during the study and is blinded for both the investigator as well as the participant. On completion of the study, participants will be debriefed, but to avoid spread of information and bias in the data, the deception associated with the intervention and expectation as well as the aim of the study will only be disclosed after all participants completed the study. If there are any questions after the debriefing, participants can contact the research team at any time (contact details will be given via the debriefing email/mail).

7.3 Study procedures

Questionnaires

All questionnaires will be completed in a secured, electronic environment (e.g. Castor_ that can be accessed at different time via the internet by electronic devices (telephone, tablet, or computer).

Participants will be asked to fill out the following questionnaires:

- Demographic characteristics, medical history and comorbidities

  See "F1.1_Screeningsvragenlijst_WoWstudy_Versie1.1_04-04-2018"

  Participants will fill out this questionnaire regarding baseline characteristics, demographics, life-style (including diet) and medical history.
- Gluten-free dietary compliance questionnaire (Biagi questionnaire)

  See "F1.2_Biagi vragenlijst naleving glutenvrij dieet_WoWstudy_Versie1.1_28-03-2018"

  This questionnaire of Biagi *et al. (24)* consists of a few questions that can be administered in a few minutes even by non-expert personnel. The numerical result makes it possible to monitor the strictness of GFD compliance by non-expert personnel. The questionnaire provides a final score in five levels (0–4), which can

be grouped into three levels. Patients with scores of 0 or 1 are in fact those who do not follow a strict GFD. Patients with scores of 2, on the other hand, follow a GFD but with important errors that require correction. Patients with scores of 3 and 4 follow a strict GFD.

- Rome criteria for IBS and Functional Dyspepsia

  See "F1.3_Rome IBS_WoWstudy_Versie1.1_28-03-2018"

  Irritable Bowel Syndrome (IBS) and Functional Dyspepsia (FD) will be diagnosed according to the Rome criteria (III and IV for IBS and IV for FD). The Rome criteria will be completed at screening as part of the medical history, since there is a big overlap between IBS and NCGS symptoms.

- Positive and Negative Affective Schedule (PANAS)

  See "F1.4_PANAS_WoWstudy_Versie1.1_28-03-2018"

  The Positive and Negative Affect Schedule (25) will be used to assess mood/emotional wellbeing (using the Dutch version; see Peeters *et al.*, 1996 (26); Engelen *et al.*, 2006 (27)) during the test day (every hour) and at the end of day 1, 2, 3.. The PANAS consists of a list of 20 emotions, including 10 positive emotions (Positive Affect (PA), *e.g.* excited, positive, happy) and 10 negative emotions (Negative Affect (NA), *e.g.* nervous, depression, sadness). Participants will be instructed to rate to what extend each emotion had been experienced during that day (as opposed to only considering their current emotional state), using a scale from 1 ('not at all') to 5 ('extremely').

- Generalized Anxiety Disorder assessment (GAD-7)

  See "F1.5_GAD7_WoWstudy_Versie1.1_28-03-2018"

  The GAD-7 is a validated 9-item questionnaire which has been demonstrated to be an efficient tool for screening for generalized anxiety disorder and assessing its severity in clinical practice and research (29). The GAD-7 will be completed at home between the screening and the test day by means of electronic questionnaires.

- Patient Health Questionnaire-9 (PHQ-9)

  See "F1.6_PHQ9_WoWstudy_Versie1.1_28-03-2018"

  The PHQ-9 is a validated multipurpose instrument for screening, diagnosing, monitoring and measuring the severity of depression. It incorporates DSM-IV depression diagnostic criteria with other leading major depressive symptoms into a brief self-report tool. The PHQ-9 can also be administered repeatedly, which can reflect improvement or worsening of depression in response to an intervention (28). The PHQ-9 will be completed at home between the screening and the test day by means of electronic questionnaires.

- Patient Health Questionnaire-15 (PHQ-15)

  See "F1.7_PHQ15_WoWstudy_Versie1.1_28-03-2018"

  The PHQ-15 is a brief, self-administered questionnaire that is useful in screening for somatization and in monitoring somatic symptom severity in clinical practice and research (30). The PHQ-15 will be completed at home between the screening and the test day by means of electronic questionnaires.

- Symptom diary and Bristol Stool Scale

  See "F2_Klachten Dagboek_WoWstudy_Versie1.0_05-03-2018"

  Participants will be asked to rate their daily symptoms on 100-mm VAS scale as well as rating their stool pattern during each hour (only after bowel movement) at the test day and at the end of day 1, 2, 3. The diary includes six sections: 1. Stool frequency and consistency - Bristol Stool Scale, 2. GI Symptoms, 3. Overall GI symptoms, 4. Other symptoms, 5. What did you eat today?, 6. Medication. The questionnaire can be completed at the MUMC+ or at home (or work), provided that the same tool is used to complete all the hourly questionnaires.

Blood sampling to exclude coeliac disease

Serological tests (*i.e.* anti-tTG) to rule out coeliac disease will be carried out during screening, if tests to exclude coeliac have not been done before and if participants still consume traces or very low amounts of gluten in their current diet (according to the Biagi questionnaire). This is estimated to be in about 20% of the participants who indicate interests to participate in the study. In this subgroup of participants, a venepuncture (10mL blood sample) will be done to exclude coeliac disease by means of serological tests on anti-tTG IgA. Blood samples will be collected from an antecubital vein in the fore-arm. This his may lead to minor discomfort and can cause small and transitory hematoma/bruises to appear.

7.4 Withdrawal of individual subjects

Participants can leave the study at any time without having to provide a reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.4.1 Specific criteria for withdrawal (if applicable)

The investigator can decide to withdraw a participant from the study for urgent medical reasons.

7.5 Replacement of individual subjects after withdrawal

We do not expect dropouts, because the study is not very burdensome in a physical way and so, is not very difficult to complete. When participants withdrawn from the study after signing informed consent but prior to the test day, or prove to be unsuitable on the basis of the screening and therefore cannot participate in the study, an alternative candidate may be selected, in order to ultimately meet the calculated sample size/power. If participants withdraw from the study before the test day, these results will be destroyed immediately after completion of the study.

When participants withdrawn from the study at the test day (*i.e.* after randomisation), before or after receiving and consuming the study breads because of intolerable symptoms, there will be no replacement of participants. These participants are included in the intention-to-treat analysis, because this is seen as important regarding tolerance of the intervention. In that case, missing VAS-scores for the remaining days of the study period will be imputed with the last observation carried forward.

7.6 Follow-up of subjects withdrawn from treatment

After withdrawal, no follow-up of participants will take place. In case of withdrawal due to medical complications, participants will be referred to a general physician.

7.7 Premature termination of the study

There are no expected reasons for premature termination of the study. However, if certain urgent medical problems occur during study (for example SAEs that result in death or are life threatening) the investigator (if necessary together with the medical committee) can decide to halt the study to check whether it is safe to proceed or if termination is preferred.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there are sufficient grounds that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all participants are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the food intervention (*i.e.* study breads). All adverse events reported spontaneously by the participants or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The coordinating investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the SAE by the concerning investigator. Additionally, the sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.3 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9. STATISTICAL ANALYSIS

Statistical analyses will be carried out using IBM SPSS statistics 23.0 for Macintosh (Chicago IL, USA) and GraphPad Prism 6.0 for Macintosh (La Jolla, CA, USA). Baseline characteristics (including the outcomes of the questionnaires at baseline) will be analysed and summarized using descriptive statistics. Dichotomous variables will be presented as percentages and will be analysed using the Chi-square test. A Fisher exact test will be performed if necessary. Normality of continuous or ordinal variables will be evaluated by the Kolmogorov-Smirnov test and histograms for normality of the distribution. These variables will be analysed with the Wilcoxon signed-ranked test, when the distribution of the variables is non-parametric. If the variables are normally distributed a paired t-test will be performed. The independent variables will be analysed with the Mann-Whitney test if the distribution of the variables is non-parametric. If the variables are normally distributed an independent sample t-test will be done. Correlations will be assessed according to Spearman or Pearson and regression analyses will be done. An intention-to-treat analysis (ITT) will be conducted for all outcomes. All data will first be examined for accuracy of data-entry and missing values. A p-value < 0.05 is considered to be statistically significant. A similar study will be performed at the University of Leeds (United Kingdom), which will eventually provide us with the opportunity to compare the outcomes. Data will be analysed for each study site separately and also together using study site as a factor in the analysis.

9.1 Primary and secondary study parameter(s)

The main research question will be analysed by means of repeated measures univariate analyses of variance (ANOVA) with Expectancy (“gluten-containing” vs. “gluten-free”) and Gluten (“gluten-containing bread” vs. “gluten-free bread”) as between-subjects factors and Time (baseline and after intervention) as repeated measure factors on the several dependent measures including changes in overall (GI) symptom score, gastro- and extra-intestinal symptoms, positive and negative affective parameters, and well-being. Only significant main- or interaction effects revealed by these procedures will be further examined by post-hoc univariate tests, corrected for multiple comparisons.

9.2 Other study parameters

Baseline characteristics will be described as means and standard deviations (in case of normal distribution) or medians and interquartile ranges (in absence of a normal distribution) for continuous variables and frequencies for categorical variables.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study has to be approved by the Medical Ethical Committee of Maastricht University (METC). The study will be conducted according to the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), the Medical Research Involving Human Subjects Act (WMO) and the FDA guidelines for the conduct of clinical trials. The general principles of informed consent, ethics review and data management will be in line with Good Clinical Practice (GCP).

10.2 Recruitment and consent

After approval of the study by the METC of MUMC+, recruitment will take place via MUMC+, Amsterdam UMC and WUR. Participants will be recruited via the outpatient clinic of the gastroenterology department and of the dietetics department of the participating study sites. Eligible individuals with NCGS will be informed by their treating physician during their visit to the outpatient clinic or “stadspoli”, or local GP practices. The treating physicians will give oral and written information to eligible individuals with NCGS and will inform them that a researcher will contact them once if they are interested in receiving further information regarding the study. Further information will be provided verbally or via email by the researcher and the written information brochure will be sent by regular mail or email. A time period of one week (minimum) is provided to decide whether the participant would like to participate. The participant is asked to contact the investigator by telephone or email if he or she would like to participate. If the participant does not contact the researcher however, the researcher will contact the participant once (at the earliest 7 days after sending the written information brochure, and only if the participant has given permission to do so during the first conversation). Thereafter, an appointment with the researcher will be made to sign an informed consent.

Lists available in the participating centres, with potential eligible subjects (*e.g.* IBS, NCGS/NCWS and controls) who have previously indicated that they can be contacted about future studies, will also receive one single mailing with containing the advertisement text with the study information. This mail will be sent by the local administrator of the database or local researcher of the participating centre. Furthermore, volunteers will be recruited by means of advertisements on the website of the “Prikkelbare Darmsyndroom Belangenvereniging”, “Coeliakie vereniging”, local and faculty newspapers, and social media, as well as by using posters in publicly accessible sites such as the hospital, university, health centres, pharmacies, gyms, bakeries and relevant shops (such as “the

Bisschopsmolen" in Maastricht (http://www.bisschopsmolen.nl) and *e.g.* restaurants serving gluten-free meals), and the website www.onderzoeksmachine.nl ("see also document "E3_Advertentietekst_NL64850.068.18_Versie2.0_02-10-2019"). Additionally, the recruitment website Proefpersonen.nl (https://www.proefpersonen.nl), which is facilitated by the company Link2Trials, will be used for recruiting and pre-screening eligible subjects. Volunteers enrolled in their database will be pre-screened by Link2Trials based on age. If they match these criteria, they will receive the advertisement text from Link2Trials. Furthermore, the advertisement text will be placed on their website, which is accessible to both volunteers already registered at Link2Trials and unregistered volunteers. Potentially interested volunteers will contact the coordinating researcher by e-mail or telephone, and will subsequently receive the written information brochure by e-mail or regular mail. Enrolment in the Link2Trials database is not necessary to obtain the researchers contact information, but is voluntary if subjects are interested in receiving information about other research.
If a volunteer is interested in participation in this study, he or she can contact the research team by e-mail or telephone, as given on the advertisement.

Participants can always contact the researcher or independent medical doctor if any question will arise and will be referred to the VWS brochure "General information for research participants" that contains general information about medical-scientific research. The researcher will contact the interested individuals by e-mail or telephone and send them the PIF via e-mail or general mail. Participation will be on voluntary basis. Before the start of the study, volunteers will be given written information about the study, its aim and the measurements. A time period of one week (minimum) is provided to decide whether the participant is willing to participate. The participant is asked to contact the investigator by telephone or email if he or she would like to participate. If the participant does not contact the researcher however, the researcher will contact the participant once (at the earliest 7 days after sending the written information brochure, and only if the participant has given permission to do so during the first conversation). Permission to contact the patient by telephone will be logged in the subject screening and enrolment log. Thereafter, an appointment with the researcher will be made to sign an informed consent. All participants will have to sign an informed consent prior to the screening in duplicate. The informed consent form has to be filled out completely. Both the participant and researcher have to sign the form at the same time. Date of assignment is necessary. The participant will directly receive a copy of the signed informed consent form.
Due to COVID-19, informed consent can also be obtained without an on-site appointment. After the researcher provided verbal information over the phone, participants will receive

the PIF plus attached informed consent form in duplicate via general mail. They will be asked to sign one copy and return this via regular mail. Upon receivement, the researcher will also sign the informed consent form. Only after obtaining the informed consent the screening will be performed via a (video)call.

Privacy of the participants will be protected, which means that the study results will be related to an identification code. Codes and related participant information will not be available to individuals others than the investigator and the project leader and will be stored in a password-protected file. Participants can, at any time, make an appeal to the independent doctor (Drs. T.Y. Fung of MUMC+) that has been appointed to this study. Participants will be informed that their decision to participate is totally voluntary and they can withdraw at any time without giving a reason. Participants have the opportunity to be informed about the group results at the end of the study.

10.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

10.4 Benefits and risks assessment

Participants will not receive any direct benefits as a result of participating in this study. The screening visit does, however, include a general health check, which would provide beneficial information for the participant. An indirect benefit of this study will be the insights into the effects of expectation of consuming gluten on overall symptoms and thus the tolerability of gluten in non-coeliac gluten sensitivity. Moreover, this study will provide information on which bread type leads to symptoms in this specific population of people with non-coeliac gluten sensitivity. There are some small burdens participants can experience during this study. First, the time that they will invest at home by filling out questionnaires (which takes about 5 minutes in total each day). Secondly, participants have to visit the MUMC+ on 2 occasions for: a 1-hour screening visit and one test day visit which takes almost the entire day (from 8.00AM – 4.30PM) for the bread (*i.e.* gluten-free or gluten-containing) intervention. Moreover, in a subgroup of participants a blood test (by venepuncture) will be taken to exclude coeliac disease by means of serological tests on anti-tTG IgA. This requires participants to make an additional 20-minute visit to the MUMC+ prior to the screening visit, may lead to minor discomfort and can cause small and transitory hematoma/bruises to appear.

Bread intervention

Participants will record in daily diaries any signs of illness, medication used, any deviations from the protocol and adverse events. The study breads are safe and commercially available. Bread itself is a harmless food product, which is widely consumed daily by the general population worldwide.

Gluten-containing oat bread

Participants may experience mild GI symptoms after the consumption of the study bread, since the included participants suffer from self-reported gluten sensitivity. It should be noted that the consumption of gluten by non-coeliac gluten sensitive participants does not lead to coeliac-specific antibodies and villous atrophy or any allergy related processes in participants with NCGS and exclusion of coeliac disease (21). The gluten-containing study bread contains an amount of gluten comparable to that is present in normal commercially available bread and consumption of 4 slices of this bread does not exceed the average amount of gluten that is normally consumed per day. Moreover, the risk of consuming gluten for 1 day is considered to be very low, especially considering the fact that coeliac patients are excluded.

Oat bread

The included participants follow a gluten-free/gluten-restricted diet because of the experience of symptoms induced by wheat- or gluten-containing food products. These gluten proteins do not occur in oats, which means that oats can be used to create safe food products for people with coeliac disease and non-coeliac gluten sensitivity (31).

10.5 Compensation for injury

The sponsor/investigator has a liability insurance, which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance, which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research participants through injury or death caused by the study.

- € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 5.000.000,-- (i.e. five million Euro) for death or injury for all participants who participate in the Research;
- € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the

Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives (if applicable)

In compensation for participation, participants will receive a financial compensation of 60 EURO in case of completing the study. Furthermore, travel expenses will be compensated. Proportional amounts will be given in case of incomplete participation. Participants will not receive payment if they only complete the screening visit, but will receive a compensation for travel expenses (if applicable). The screening visit does, however, include a general health check, which would provide beneficial information for the participant. People who started a one week gluten-free diet for the study, will be given an additional 15 EURO compensation to buy some gluten-free products.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Data will be handled confidentially (coded) and the privacy of the participants will be guaranteed, according to the Dutch Personal Data Protection Act ("Algemene Verordening Gegevensbescherming" (AVG)). All electronic Case Report Forms (eCRFs in *e.g.* Castor) will be coded in such a way that no personal information about the participant can be derived.

All samples and data will be coded in such a way that no personal information about the participant can be derived. Each participant will be allocated to an individual code, which is preceded by an abbreviation to identify the inclusion site:

- Maastricht University Medical Center+: MUMC.S00xx
- Amsterdam UMC – location VUmc: VUMC.S00xx
- Wageningen University & Research: WUR.S00xx

These individual codes will be visible on biological samples. Furthermore, the label of each blood sample will contain the date of collection. The principal investigator will keep the key of the code in a locked cabinet, to which only the principle investigator and a co-worker has access. Together with the members of the project team, assigned monitors from the CTCM, IGZ and members of the medical ethical committee will have access to the research data. All primary documents and data shall be kept for 15 years after the end of the experimental phase of the study for possible inspection. The electronic CRF, electronic diary and electronic questionnaires will be developed taking into account current regulations.

In the informed consent, participants indicate whether they give consent for collecting human material (*i.e.* blood samples) that will be used for exclusion of coeliac disease. These blood samples will be drawn by the research physician of this project. All samples will be coded in such a way that no personal information about the participant can be derived. The analysis of the coded blood samples will be carried out by Medische Laboratoria Dr. Stein & Collegae. The analyzed blood samples will be destroyed immediately after analysis.

Subjects recruited via Link2Trials can voluntarily give permission to the website to save their contact details for further recruitment for other research by giving consent to data storage according to the privacy statement listed on the web page https://www.proefpersonen.nl/privacy-statement.html. If they do not consent, their data will be deleted after completion of the recruitment for the WoW study. Link2Trials adheres to the AVG.

11.2 Monitoring and Quality Assurance

A qualified monitor of the CTCM will monitor the conduct of the study. A specific monitoring plan will be created after classification of the risk of this study in line with local guidelines.

11.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of participants included and numbers of participants that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (premature) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as when the last participant has completed the end of day questionnaire on day 3. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.6 Public disclosure and publication policy

Publication policy is in agreement with the Centrale Commissie Mensgebonden Onderzoek (CCMO-statement publicatie beleid, 2002) publication statement. The results of the study will be published in peer-reviewed scientific journals. Both positive and negative results of the study will be disclosed. The authorship of the article shall be determined in appropriate consultation based on a considerable contribution to the set-up and execution of the study and an active participation in publication. This study will be registered in a public trial registry before the first volunteer is recruited.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The “Expectancy Theory”, proposed in the context of social learning, is a mixture of learning and subjective mental–neural processes (32). The theory explains behaviour through individuals’ expectancies of the rewarding effects of their action toward a desired outcome.

Therefore, information shapes the beliefs and, thereby, the actions of individuals. Beliefs trigger neurological activities (33) similar to psychoactive agents. Positive beliefs can induce placebo effects, negative beliefs result in debilitating nocebo effects. Furthermore, the bidirectional communication between the brain and the gut axis, is known to contribute to GI symptom development and perception, as involved for example in the pathophysiology of irritable bowel syndrome (34). Thereby, the interplay between gluten and expectation, may elicit a wide array of both intestinal and extra-intestinal symptoms in individuals with NCGS.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

The study breads are commercially available. Bread itself is a harmless food product which is widely consumed daily by the general population worldwide. Oats and oat-based products are well recognised among consumers, due to their good nutritional properties. Oats are widely consumed by people suffering from gluten intolerance and non-coeliac gluten sensitivity. Furthermore, the study of Catassi *et al.* (2013) showed that the consumption of gluten by non-coeliac gluten sensitive participants does not lead to coeliac-specific antibodies and villous atrophy or any allergy related symptoms. In this study, the provided study breads contain no more gluten than is present in normal commercially available bread and consuming 4 slices of the gluten-containing bread will not exceed the amount of gluten that is normally consumed per day (21). The short-term intervention (1 day) with gluten can lead to temporary more symptoms but these should disappear within a few hours after gluten has been eliminated from the diet.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

No, in this study, the effects of gluten-containing bread consumption on gastro-intestinal complaints and wellbeing will be investigated in non-diagnosed gluten-sensitive participants *in vivo* due to the manipulation of expectancy.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Not relevant.

e. Analysis of potential effect

The amount of gluten present in the gluten-containing study bread is at a dose normally present in commercially available bread (i.e. about 4g per slice). The bread is safe for human use and is also safe to consume in this specific (NCGS) study population. Bread is a natural product and has been on the food market for decades where it has a long history of apparent safe use. The short-term intervention (1 day) with gluten can lead to temporary GI symptoms but these should disappear within a few hours after gluten has been eliminated from the diet.

f. Pharmacokinetic considerations

Not relevant.

g. Study population

Volunteers (18-70 years) with self-reported gluten sensitivity (without other GI diseases) will be recruited. In particular, we will screen for coeliac disease and people diagnosed with coeliac disease will be excluded from the study.

h. Interaction with other products

No interaction with other products, posing a health risk to the individual of any kind, are expected.

i. Predictability of effect

Previous studies suggest that a nocebo effect plays a role in the experience of GI symptoms after gluten consumption in individuals with NCGS since a clear basis for underlying biological mechanisms is lacking. Telling self-reported gluten sensitive people that they will be eating gluten-containing study bread containing gluten, is therefore hypothesized to induce (extra)intestinal-symptoms regardless of whether or not the bread actually contains gluten.

j. Can effects be managed?

The risk associated with participating in the intervention is low since the expected side effects are only mild and transient. Moreover, the intervention comprises only 1 day (breakfast and lunch) and the amount of gluten in the gluten-containing bread does not

exceed the amount of gluten in commercially available bread. Participants will be asked to report their symptoms and side effects daily (in the electronic diary). If participants are unable to continue the consumption of the study breads due to intolerable symptoms, they can stop consuming the study breads.

12.2 Synthesis

There are no significant risks expected regarding the test products in this study. The bread products are commercially available and impose no risk to non-coeliac gluten sensitive people apart from temporary symptom induction. Overall, any side effects are expected to be mild and transient.

13. REFERENCES

1. Shewry PR, Hey SJ. The contribution of wheat to human diet and health. Food Energy Secur. 2015;4(3):178-202.
2. Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. BMJ. 2016;353:i2716.
3. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev. 2010;23(1):65-134.
4. Wu H, Flint AJ, Qi Q, van Dam RM, Sampson LA, Rimm EB, et al. Association between dietary whole grain intake and risk of mortality: two large prospective studies in US men and women. JAMA Intern Med. 2015;175(3):373-84.
5. Huang T, Xu M, Lee A, Cho S, Qi L. Consumption of whole grains and cereal fiber and total and cause-specific mortality: prospective analysis of 367,442 individuals. BMC Med. 2015;13:59.
6. Benisi-Kohansal S, Saneei P, Salehi-Marzijarani M, Larijani B, Esmaillzadeh A. Whole-Grain Intake and Mortality from All Causes, Cardiovascular Disease, and Cancer: A Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies. Adv Nutr. 2016;7(6):1052-65.
7. Chen GC, Tong X, Xu JY, Han SF, Wan ZX, Qin JB, et al. Whole-grain intake and total, cardiovascular, and cancer mortality: a systematic review and meta-analysis of prospective studies. Am J Clin Nutr. 2016;104(1):164-72.
8. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol. 2012;18(42):6036-59.
9. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, et al. The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol. 2008;121(5):1210-8 e4.
10. Cabrera-Chavez F, Granda-Restrepo DM, Aramburo-Galvez JG, Franco-Aguilar A, Magana-Ordorica D, Vergara-Jimenez Mde J, et al. Self-Reported Prevalence of Gluten-Related Disorders and Adherence to Gluten-Free Diet in Colombian Adult Population. Gastroenterol Res Pract. 2016;2016:4704309.
11. Di Sabatino A, Corazza GR. Nonceliac gluten sensitivity: sense or sensibility? Ann Intern Med. 2012;156(4):309-11.
12. Molina-Infante J, Santolaria S, Sanders DS, Fernandez-Banares F. Systematic review: noncoeliac gluten sensitivity. Aliment Pharmacol Ther. 2015;41(9):807-20.
13. Ontiveros N, Lopez-Gallardo JA, Vergara-Jimenez MJ, Cabrera-Chavez F. Self-Reported Prevalence of Symptomatic Adverse Reactions to Gluten and Adherence to Gluten-Free Diet in an Adult Mexican Population. Nutrients. 2015;7(7):6000-15.
14. van Gils T, Nijeboer P, CE IJ, Sanders DS, Mulder CJ, Bouma G. Prevalence and Characterization of Self-Reported Gluten Sensitivity in The Netherlands. Nutrients. 2016;8(11).
15. Mariani P, Viti MG, Montuori M, La Vecchia A, Cipolletta E, Calvani L, et al. The gluten-free diet: a nutritional risk factor for adolescents with celiac disease? J Pediatr Gastroenterol Nutr. 1998;27(5):519-23.
16. Catassi C, Alaedini A, Bojarski C, Bonaz B, Bouma G, Carroccio A, et al. The Overlapping Area of Non-Celiac Gluten Sensitivity (NCGS) and Wheat-Sensitive Irritable Bowel Syndrome (IBS): An Update. Nutrients. 2017;9(11).
17. Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. Gastroenterology. 2013;145(2):320-8 e1-3.
18. Elsenbruch S, Enck P. Placebo effects and their determinants in gastrointestinal disorders. Nat Rev Gastroenterol Hepatol. 2015;12(8):472-85.
19. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175-91.

20. Gibson PR, Skodje GI, Lundin KE. Non-coeliac gluten sensitivity. J Gastroenterol Hepatol. 2017;32 Suppl 1:86-9.
21. Catassi C, Bai JC, Bonaz B, Bouma G, Calabro A, Carroccio A, et al. Non-Celiac Gluten sensitivity: the new frontier of gluten related disorders. Nutrients. 2013;5(10):3839-53.
22. Volta U, De Giorgio R. New understanding of gluten sensitivity. Nat Rev Gastroenterol Hepatol. 2012;9(5):295-9.
23. Khan K. Wheat: chemistry and technology.: Elsevier; 2016; p. 233.
24. Biagi F, Andrealli A, Bianchi PI, Marchese A, Klersy C, Corazza GR. A gluten-free diet score to evaluate dietary compliance in patients with coeliac disease. Br J Nutr. 2009;102(6):882-7.
25. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol. 1988;54(6):1063-70.
26. Peeters FPMIP, R.W.H.M.; Vermeeren, M.T.G.;. Affectiviteit en zelfbeoordeling van depressie en angst. Tijdschrift voor Psychiatrie. 1996;38:240-50.
27. Engelen UDP, S.; Victoir, A. Verdere validering van de “Positive and Negative Affect Schedule” (PANAS) en vergelijking van twee Nederlandstalige versie. Gedrag en Gezondheid. 2006;34:89-102.
28. Stafford L, Berk M, Jackson HJ. Validity of the Hospital Anxiety and Depression Scale and Patient Health Questionnaire-9 to screen for depression in patients with coronary artery disease. Gen Hosp Psychiatry. 2007;29(5):417-24.
29. Spitzer RL, Kroenke K, Williams JB, Lowe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. Arch Intern Med. 2006;166(10):1092-7.
30. Kroenke K, Spitzer RL, Williams JB. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. Psychosom Med. 2002;64(2):258-66.
31. Kaukinen K, Collin P, Huhtala H, Maki M. Long-term consumption of oats in adult celiac disease patients. Nutrients. 2013;5(11):4380-9.
32. Bandura A, Adams NE, Beyer J. Cognitive processes mediating behavioral change. J Pers Soc Psychol. 1977;35(3):125-39.
33. Meissner K, Bingel U, Colloca L, Wager TD, Watson A, Flaten MA. The placebo effect: advances from different methodological approaches. J Neurosci. 2011;31(45):16117-24.
34. Kinsinger SW. Cognitive-behavioral therapy for patients with irritable bowel syndrome: current insights. Psychol Res Behav Manag. 2017;10:231-7.