STUDY PROTOCOL

Project HiREB ID: 4367

ClinicalTrials.gov Trial No: NCT03664531

Dietary triggers of gastrointestinal symptoms in IBS patients

RESPONSIBLE PERSONNEL

Principal Investigator	Name: **Premysl BERCIK** Title: MD, Associate Professor, Department of Medicine. Function: Principal Investigator. Institution: McMaster University Address: 1280 Main Street West, HSC 3N9, Hamilton, ON L8S4K1 Tel: +1 905 521 2100/ ext: 73495 Fax: +1 (905) 521 4958 bercikp@mcmaster.ca	……..…..…….… Date ..... Signature
Co-Investigator	Name: **Elena VERDU** Title: MD, Associate Professor Department of Medicine. Institution: McMaster University Address: 1280 Main Street West, HSC 3N8, Hamilton, ON L8S4K1 Tel: +1 905 527 4323/46043 Email: verdue@mcmaster.ca	…… Date ..... Signature
Co-Investigator	Name: **Stephen M. COLLINS** Title: MD, University Professor Department of Medicine Institution: McMaster Universtity Medical Centre Address: 1200 Main Street West, HSC 4W8, Hamilton, ON L8N 3Z5 Canada Tel: +1 905 525 2100/8452 Email: scollins@mcmaster.ca	…… Date ..... Signature
Co-Investigator	Name: **Paul MOAYYEDI** Title: MD, Professor Department of Medicine. Institution: McMaster University Health Sciences Centre Department of Medicine Hamilton, ON +1 905.525.2100 / 76764 Email: moayyep@mcmaster.ca	…… Date ..... Signature
Co-Investigator	Name: **M. Ines PINTO SANCHEZ** Title: Assistant Professor Department of Medicine Institution: McMaster Universtity Medical Centre Address: 1280 Main Street West, Hamilton, Canada. Tel: +1 905 525 9140/ 21875 Email: pintosm@mcmaster.ca	…… Date ..... Signature
Co-Investigator	Name: **Andrea NARDELLI** Title: MD, Research fellow	

	Department of Medicine Institution: McMaster University Medical Centre Address: 1280 Main Street West, Hamilton, Canada. Tel: +1 905 525 9140/ 21875 Email: nardela@mcmaster.ca	…… Date ..... Signature
Co-Investigator	Name: **Detlef SCHUPPAN** Title: MD, Professor Department of Medicine Institution: Johannes Gutenberg University Address: Langenbeckstrabe 1D, Mainz, Germany. Tel: +49 6131 17-7356 Email: detlef.schuppan@unimedizin-mainz.de	…… Date ..... Signature
Co-Investigator	Name: **Gaston RUEDA** Title: MD, Research Fellow Department of Medicine Institution: McMaster University Medical Centre Address: 1280 Main Street West, Hamilton, Canada. Tel: +1 905 525 9140/ 21267 Email: tongasrueda@gmail.com	…… Date ..... Signature
Co-Investigator	Name: **Pedro MIRANDA** Title: PhD, Research Fellow Department of Medicine Institution: McMaster University Medical Centre Address: 1200 Main Street West, Hamilton, Canada Tel: +1 (905) 515 6989 Email: mirandp@mcmaster.ca	…… Date ..... Signature
Co-Investigator	Name: **Rajka BOROJEVIC** Title: Research Assistant Department of Medicine Institution: McMaster University Medical Centre Address: 1280 Main Street West, Hamilton, Canada. Tel: +1 (905) 525 9149 Email: borojevi@mcmaster.ca	…… Date ..... Signature
Study Coordinator	Name: **Caroline SEILER** Title: PhD Candidate Department of Medical Sciences Institution: McMaster University Medical Centre	…… Date

	Address: 1280 Main Street West, Hamilton, Canada. Tel: +1 905 525 9140/ 21875 Email: seilercl@mcmaster.ca	..... Signature

SYNOPSIS

Dietary triggers of gastrointestinal symptoms in IBS patients
Principal Investigator: Premysl BERCIK
Study center: McMaster University Medical Centre, Hamilton, Canada.
Primary objective and outcome: The primary objective of this clinical trial is to evaluate the effect of two possible dietary triggers (whole wheat flour and non-purified gluten containing amylase trypsin inhibitors (ATIs) and purified gluten) on gastrointestinal symptoms in IBS patients who have been on a gluten-free diet for 3 or more weeks.
Secondary objectives and outcomes are to evaluate the effect of dietary triggers on ✓ IBS symptoms (IBS-SSS) ✓ Stool frequency and consistency (Bristol stool scale) ✓ General GI symptoms (PROMIS scales for bloating & gas, diarrhea, constipation, and belly pain) ✓ Anxiety, depression, and stress (DASS21) ✓ Somatic symptoms (PHQ-15) ✓ Gastrointestinal transit time (SHAPE study) ✓ Gut microbiota composition (16S rRNA) ✓ Systemic immune activation (isolated PBMC assay) ✓ Daily diet pictures (MealLogger) ✓ IBS symptom duration (IBS-SSS) ✓ Long-term gluten-free diet adherence ✓ Psychological and GI symptoms after learning diet triggers and genetic results
Design: This is a prospective, randomized, placebo-controlled, crossover design.
Number of patients (to be enrolled / to be analyzed): 40 patients will be enrolled such that the final number of patients analyzed will be at least 33 patients (+20% dropout)
Description of subjects and main criteria for inclusion: Patients aged 18+, diagnosed with IBS (Rome IV criteria) whose symptoms previously improved while on a gluten-free diet.
Intervention: Test product: muesli bars containing *1) whole wheat flour and non-purified gluten containing ATIs (10 g), 2) purified gluten (10 g)* Placebo: gluten-free, vegan, low FODMAPs muesli bars
Amount, dosage, route of administration, duration of treatment: Muesli bars will be administered daily for a period of 7 days, followed by a washout period of 14 days

Funding:
This study is supported by the Canadian Digestive Health Foundation and a Society for the Study of Celiac Disease (SSCD) grant for Non-Celiac Gluten/Wheat Sensitivity sponsored by the Nestle Research Center, Nestec SA to Drs. Bercik and Pinto-Sanchez.

LEGEND

Gluten free diet (GFD); wheat free diet (WFD); irritable bowel syndrome (IBS); gastrointestinal (GI); anti-gliadin antibodies IgG (AGA IgG); Fermentable, Oligo-, Di-, Monosaccharides And Polyols (FODMAP); gluten-related diseases (GRD)

BACKGROUND

Irritable bowel syndrome (IBS) is a common, poorly understood condition characterized by abdominal discomfort or pain that is associated with bowel activity changes[1,2]. Multiple factors, such as dietary, psychological, visceral hypersensitivity and/or motility disturbances, contribute to its pathology[3]. Various dietary approaches, including the gluten-free diet (GFD) and wheat-free diet (WFD), have been used on IBS patients with varying effects[4].

Gluten, a prolamin or storage protein of wheat, rye, and barley, is composed of the proteins gliadin and glutenin[5,6]. It is commonly consumed at around 10 g/d in Western countries and is implicated in various gluten related disorders, such as celiac disease or non-celiac gluten sensitivity [7–9].

Sensitivity to gluten has been proposed as one possible cause of functional gastrointestinal (GI) symptoms in patients diagnosed with IBS[10,11]. In fact, many IBS patients believe that certain foods, specifically gluten, trigger their symptoms[12,13], prompting them to start a GFD. Clinical studies have associated gluten to intestinal and extraintestinal symptoms, even without the autoimmune activity characteristic of celiac disease[14,15]. Another study found that a diarrhea-predominant subgroup of IBS patients who produce anti-gliadin antibodies (AGA) or are HLA DQ2 genotype carriers are more likely to respond to a GFD[16].

However, gluten cannot be considered in isolation when investigating IBS. Removal of dietary wheat clinically improves symptoms in some IBS patients, indicating that only a proportion of patients may truly be gluten sensitive[13,17]. One key component of wheat, also found in rye and barley, that may shed light on NCWS is the group of at least 11 mono-, di-, and tetrameric wheat proteins known as amylase trypsin inhibitors (ATIs)[18,19]. ATIs function by inhibiting amylase and trypsin enzymes in mammals and insects, indicating a protective effect in wheat[19]. They are found in higher quantities in modern hexaploidy wheat compared to

traditional spelt, emmer, einkorn, or durum wheat species[19]. Preclinical studies demonstrated that ATIs can cause intestinal inflammation by activating innate immune pathways, specifically through toll-like receptor 4 (TLR-4)[20]. On average, ATIs are consumed at 0.5-1.5 g per day[18]. It is unknown what amount is needed to provoke a significant immune reaction, thus the Salerno criterion of gluten flour with at least 0.3 g of ATIs/8 g of gluten will be used in this study[21].

There is a need for well-designed RCTs to disentangle the effects of these specific dietary components and better understand the role of gluten and ATIs in IBS pathophysiology. It is possible that gluten itself may not be responsible for IBS symptoms in some patients, and that instead ATIs act independently from or as an adjuvant to gluten to produce symptoms. Therefore, we designed a crossover RCT to investigate the effects of purified gluten versus non-purified gluten and whole wheat flour containing other wheat components on intestinal and extraintestinal symptoms in IBS patients.

RATIONALE

IBS is an extremely common condition affecting up to 30% of the general population. It is estimated that 12-14% of primary care visits and 28% of patients referred to gastroenterologists are diagnosed with IBS, making this illness a larger reason for consultancy than diabetes, hypertension, or asthma[22–24]. Not only is IBS associated with more visits to physicians, but patients also undergo more diagnostic tests, consume more medications, miss more workdays, and are hospitalized more frequently with higher overall financial and social costs than patients without IBS[22,25].

The GFD has gained considerable popularity in the general population[22,26]. Between 2004 and 2011 the market for gluten-free products grew at a compound annual growth rate of 28%, with annual sales expected to reach $4.7 billion by 2020[27] (Appendix 1). As of September 2017, Amazon.com listed 150 579 entries for the topic “gluten-free.” A Google

search at the same time for "gluten-free diet" produced more than 4.1 million results. The number-one reason consumers cite for buying gluten-free products is that they are perceived to be healthier than their gluten-containing counterparts[26].

Despite the perceived benefits of a GFD by IBS patients, the role of diet in this population is still unclear. Wheat-derived non-digestible carbohydrates have been reported to have beneficial effects like: a) decreased postprandial glycemia and insulinemia[28,29]; b) reduced fasting triglycerides[30] and blood pressure[31]; c) improved immune status[32] and vitamin and mineral absorption[33,34]; and d) beneficial changes in gut microbiota composition[35,36]. Thus, a GFD is questionable in people without celiac disease and is imprudent dietary advice for the general population[5]. Even for celiac patients who must follow a GFD indefinitely, it is difficult and is associated with social restrictions affecting their quality of life[37]. Additionally, avoidance diets can pose a significant socioeconomic challenge for non-celiac patients since the costs are not covered by government subsidy. Although food costs vary depending on the selection, overall gluten-free products were reported to be 240% more expensive compared to gluten-containing food[38]. Consequently, it is crucial to have a better understanding of the effects of the diet in patients suffering from IBS and to develop a rational approach to its treatment[39].

OBJECTIVES

Main objective:

To evaluate whether non-purified gluten and whole wheat flour (with ATIs) versus purified gluten worsen symptoms in IBS patients who previously improved with a GFD (Appendix 2).

Secondary objectives:

To evaluate the effects of non-purified gluten and whole wheat flour (with ATIs) and purified gluten on:

- ✓ IBS symptoms (IBS-SSS) (Appendix 2)

- ✓ General GI symptoms (PROMIS scales for belly pain, constipation, diarrhea, gas, and bloating) (Appendices 3-6)
- ✓ Stool frequency and consistency (Bristol stool scale) (Appendices 7 and 8)
- ✓ Anxiety, depression, and stress (DASS-21) (Appendix 9)
- ✓ Somatic symptoms (PHQ-15) (Appendix 10)
- ✓ Gastrointestinal transit time (SHAPE study) (Appendix 11)
- ✓ Gut microbiota composition (16S rRNA Illumina)
- ✓ Systemic immune reactivity (isolated PMBC assay) (Appendices 12 and 13)
- ✓ Diet composition (diet pictures using MealLogger app)
- ✓ IBS symptom duration (IBS-SSS)
- ✓ Long-term adherence to gluten-free diet
- ✓ Psychological and GI symptom impacts after learning genetic background and diet triggers (unblinding participants to diet triggers)

TRIAL DESIGN

This is a randomized controlled trial with a double-blind crossover design (Appendix 14). Crossover designs are most appropriate in studies where the effects of the treatment(s) are short-lived and reversible and are best suited to trials related to symptomatic but chronic conditions or diseases like IBS[40]. This type of design was considered appropriate since it estimates not only between groups, but within-patient differences in treatment effects. Because each patient receives all treatments, identical prognostic factors are expected, increasing the power of the results[41]. However, while considering this specific design it is important that the intervention during the first period must not carry over in the second period[42]. This design was implemented successfully in a similar study[13] and the carryover effect was not a concern after one week of washout. This study will use a two-week washout to ensure there is no carryover effect.

Subjects, groups, and centers

All in-person visits will be carried out at McMaster University Medical Centre, Hamilton, Canada. Due to the COVID-19 pandemic, however, McMaster University may or may not permit in-person research visits. If in-person research visits are not permitted, participants will have all study visits completed remotely via telephone, email, Signal, and/or the Zoom video conferencing platform. Patients will be required to be on a GFD 3 weeks prior to starting the study. Patients will receive two different dietary challenges, as well as placebo challenge, in a period of 7 weeks. The total duration of the study will be 8-9 weeks. According to previous experience, we expect that the study will be completed within a 2-year period.

Study population

Inclusion Criteria:

Adults aged 18+ with IBS diagnosis based on Rome IV criteria who previously improved while on a GFD, are able to comply with the study procedures (according to the investigator's own judgment), and have signed the Study Informed Consent will be considered for the study.

Exclusion Criteria:

Subjects representing one or more of the following criteria will be excluded from participation:

- Concurrent systemic disease and/or laboratory abnormalities considered by investigators to be risky or that could interfere with data collection.
- Concurrent organic benign GI pathology (i.e. celiac disease, inflammatory bowel disease, etc.) other than benign polyps, diverticulosis, hemorrhoids, lipoma, and melanosis coli.
- History of active cancer in the last 5 years, other than skin basal cells cancer.
- Pregnant or breastfeeding women.
- Patients currently participating or having participated in a trial within the past month.
- Patients currently receiving antibiotics or have received them 1 month before V0.

- Use of new medications 4 weeks or less prior to the study. Those on a consistent dose for over 4 weeks are acceptable.
- Major abdominal surgery with the exception of hernia repair, appendectomy, Caesarian section, tubal ligation and cholecystectomy, hysterectomy, and hemorrhoidectomy.
- *Note:* Participants who previously tested positive for COVID-19 may be allowed to participate in the study, but they will be required to wait at least two additional weeks after becoming asymptomatic or until they receive one negative COVID-19 test before participating in the study.

Intervention

All patients will be instructed to maintain a strict GFD in alignment with Canadian Celiac Association guidelines (Appendix 15)[43]. One session with the study coordinator at the beginning and the end of the study will be scheduled with this purpose. Habitual diet will be characterized by the study coordinator using a validated food frequency questionnaire[44,45] (Appendix 16). During the first visit (Study V1), patients will receive instructions to maintain a strict GFD and will be provided with a printed or PDF version of a list of allowed gluten free products and most common brand marks. GFD adherence will be reviewed at each of the following visits using a validated questionnaire[46] and will be categorized as Compliant ("Excellent", as expressed in the questionnaire), Partially Compliant ("good, fair, poor, very poor"), or Non-Compliant (Appendix 17)[46]. GFD compliance will also be assessed by measuring gluten immunogenic peptides in stool at each visit (iVYDAL GIP-S kit; Biomedal S.L., Sevilla, Spain).

Randomization

Participants will be randomized to 7-day dietary challenges of muesli bars containing either A, B, or C (commercial whole wheat flour and non-purified gluten containing ATIs, purified gluten, or a pure muesli bar (placebo)) separated by a washout period of 14 days. Participants

will be divided into six arms, so that they are randomized to have each of the three challenges (Table 1). A challenge period and washout were chosen based on previous studies involving a gluten challenge[47–50]. The sequence of the treatments will be randomly generated using a computer-based pseudo-random number generator (*randomizeBE* package in R version 3.5.0). Patients will receive one of challenges A, B, or C consisting of muesli bars (gluten-free, low FODMAPs, vegan) with either 10 g of commercial gluten and whole wheat flour containing ATIs (G5004-Sigma Lab, ON, Canada and Whole Wheat All-Purpose Flour, Robin Hood, ON, Canada), 10 g of purified gluten (the commercial gluten Sigma will be purified to remove 80-90% of the ATIs using the method from Schuppan in Appendix 18), or a pure muesli bar (placebo). The muesli bars will be made at McMaster University using a modified recipe by Monash University (Appendix 19)[51].

The identity of the specific product will be blinded to subjects, investigators, and statistician. All muesli bars will have the same appearance, taste, and smell to preserve blindness. Muesli bar production and packaging will be performed by the study coordinator and coded by a person otherwise uninvolved in the study. Allocation will be concealed for participants and investigators throughout the study.

Patients will be requested to take 1 bar per day (similar amount of gluten/ATIs normal consumption[7,8]) during the treatment period. Subjects will be asked to bring remaining muesli bars back to the investigator at the end of the period. Compliance will be calculated as a percentage and recorded in the Case Report Form (CRF). All products received and dispensed by the investigator will be inventoried and accounted for throughout the trial period. Unused product will be destroyed on site at the end of the study.

Table 1.

	Challenge 1	Challenge 2	Challenge 3
Arm 1	Gluten & whole wheat	Purified gluten	Placebo
Arm 2	Gluten & whole wheat	Placebo	Purified gluten
Arm 3	Purified	Gluten & whole wheat	Placebo

Arm 4	Purified	Placebo	Gluten & whole wheat
Arm 5	Placebo	Gluten & whole wheat	Purified gluten
Arm 6	Placebo	Purified gluten	Gluten & whole wheat

Post-study follow-up of participants

Due to restrictions from COVID-19 lockdowns, a large proportion of study participants were unable to complete the original study protocol including blood sampling for immune parameters and genetic susceptibility for celiac disease. Further, many study participants expressed interest in learning about their personal study results, including which diet challenges triggered symptoms and their celiac disease genetic susceptibility. However, it is unknown how disclosing diet challenge information impacts IBS patient behaviour and symptoms. Therefore, study participants who completed the study will be offered the opportunity for follow-up in which they will: 1) have the opportunity to come to McMaster to provide blood samples for genetic and immune assessments, if they completed the study virtually; and 2) receive their study results. Subjects who already completed blood sampling will not be invited to complete additional blood sampling but will only be asked whether they would like to receive their study results in this follow-up study.

Once we obtain consent for this follow-up, participants who completed the study virtually will have the option to come in-person to McMaster to provide blood samples for immune and genetic assessment. After all the blood samples are analyzed for anti-gliadin antibodies and celiac genotype, all previous study participants who completed the study at least 6 months previously will be asked to complete two online follow-up visits. At the first follow-up visit, we will ask follow-up questions on their diet adherence and practices after completing the study, as well as whether they had any significant changes in health since the study completion. We will also ask participants to complete the same surveys they completed during the study (including IBS-SSS, Bristol, PROMIS, DASS-21, and PHQ-15) at this baseline visit. Once these surveys are completed, we will send them a personalized study results form that

includes their clinical reactions to the study interventions, their blood anti-gliadin antibodies, and whether they have a celiac-prone genotype. Follow-up surveys would then be emailed to participants one month after disclosing this information to assess impacts on symptoms, diet adherence, and participant perceptions.

Concomitant diets and treatments

Any medication or treatment initiated during the course of the trial must be recorded in the clinical chart. Participants will be encouraged to maintain their dietary habits and medications. In case of any change in medication prescribed by the treating physician during the study period, the research team will discuss the decision to exclude them from the study. General dietary changes will be assessed by the study coordinator at each visit.

Unauthorized concomitant diets/treatments/medications

The following treatments may interfere with the primary outcome as considered by the investigator, but may be allowed if the dose is stable and has not been changed in the 4 weeks before the study commences:

a) Antidiarrheals, antispasmodics, laxatives, or antiemetic drugs (i.e. Prochlorperazine (Compazine), Promethazine (Phenergan), or Trimethobezamide (Tigan)); b) Anxiolytics, antidepressants, opioids (i.e.: morphine or codeine). These drugs will be only permitted if they are used in stable doses during the last 3 months and will not to be changed during the whole study period; c) Antibiotics should not be used in a 1-month period before V0 and during the whole study.

Outcomes and measurements

Primary outcome: Worsening of IBS symptoms of ≥ 50[52] points on the Irritable Bowel Syndrome Symptom Severity Score (IBS-SSS) (Appendix 2) after dietary challenges.

Secondary outcomes:

a) Increase in IBS symptoms. The IBS-SSS will also be evaluated as a continuous variable.

b) Increase in general GI symptoms (PROMIS scales): The Patient-Reported Outcomes Measurement Information System (PROMIS) is a standardized set of patient-reported outcomes (PROs) by the National Institutes of Health (NIH). This study will use the PROMIS scales to evaluate constipation (9 questions), diarrhea (6 questions), belly pain (5 questions), and gas and bloating (13 questions)[53].

c) Changes in GI Motility. 1) Orocecal Transit: The colonic SHAPE study[54] is a common technique used to measure colonic transit using radiopaque markers. Using a modified version of the Evans Method on the recommendation of the Department of Radiology, the patients will ingest one SITZMARKS capsule (each capsule contains 24 markers) 2 days (48 hours) prior to the study visit, at which point a plain abdominal film (x-ray) will be taken. The Evans Method is only able to detect delayed or normal gastrointestinal transit[55]. Therefore, this modified method will be used to detect delayed, normal, and expedited gastrointestinal transit. Fertile women will provide urine samples prior to the x-ray so that an instant pregnancy test can be done because x-rays pose a risk to developing fetuses. The urine pregnancy test will be performed by the study coordinator at the beginning of the study visit at McMaster University Medical Centre. Urine collection instructions are included in Appendix 20. If 20-24 (>83.3%) of the markers are passed by 48 hours, colonic transit is expedited. If 4-20 (16.7-83.3%) of the markers are passed by 48 hours, colonic transit is grossly normal. If 0-4 (<16.7%) of the markers are passed by 48 hours, the colonic transit is delayed. Paired statistical tests will be conducted to account for within-participant variation. More information is in Appendix 11. Due to the COVID-19 pandemic, in-person research visits may be restricted. Therefore, the colonic SHAPE study will only be conducted during in-person visits.

d) Determination of anti-gliadin antibodies (AGA IgA and IgG) and tissue transglutaminase IgA antibodies. Analyses will be done by the central laboratory at McMaster University Medical Centre for each participant visit (V1-V6). Due to the COVID-19 pandemic, in-person research visits may be restricted. Therefore, blood samples will only be taken at in-person

visits. If participants completed the study virtually, during follow-up before disclosing their study results, they will have the option to provide a blood sample for this assessment.

e) Blood chemistry and hematology will be done for the baseline visit (V1). CBC with automated differential, ESR, sodium, potassium, urea, fasting glucose, creatinine, total protein, AST, ALT, GGT, alkaline phosphatase, lactate dehydrogenase, total bilirubin, PT in INR, and PTT are the blood markers that will be measured. All analyses will be done by the central laboratory at McMaster University Medical Centre. Due to the COVID-19 pandemic, in-person research visits may be restricted. Blood samples will only be taken at in-person visits. If participants completed the study virtually, during follow-up before disclosing their study results, they will have the option to provide a blood sample for this assessment.

f) Changes in GI microbiota. To evaluate changes in gut microbiota as a possible mechanism of symptom generation, we will require that patients collect anaerobically one stool sample for each visit. All sample collection details will be explained to the patient during V1 and a written explanation will be also provided (Appendix 21). Analysis will be performed by 16S rRNA Illumina.

g) Genotype HLA DQ2/8. Will be performed using sequence-specific primer polymerase chain reaction (PCR) (DQ-CD Typing kit, BioDiaGene, s.r.l., Palermo, Italy)[56] at baseline. Due to the COVID-19 pandemic, in-person research visits may be restricted. Blood samples for the genetic test will only be taken at in-person visits. However, the research coordinator will provide the option of giving a blood sample for genetic testing after study completion if the participant was unable to provide a blood sample during the study but still would like to provide the sample at a later date.

h) IgG4 against wheat. IgG4 against wheat will be detected using a specific kit (Allergix IgG4 Food Antibodies - 90 Serum) at baseline. Values over 10 ng/mL will be considered as elevated[57].

Details of procedures at each visit are provided in Appendices 2-10.

i) Gluten immunogenic peptides (GIP). Anti-GIP immunochromatographic strips (iVYDAL GIP-S kit; Biomedal S.L., Sevilla, Spain) will be used to analyze the gluten content of participant stool samples to test for diet compliance. All stool collection details will be explained to the patient at V1 and a written explanation will also be provided (Appendix 21).

j) Changes in systemic immune reactivity will be measured by peripheral blood mononuclear cell (PBMC) stimulation. PBMC will be stimulated and levels of pro-inflammatory cytokines will be assessed (Appendices 12 and 13). Due to the COVID-19 pandemic, in-person research visits may be restricted. Blood samples will only be taken at in-person visits.

k) Diet composition. This study will include the use of the MealLogger app to take pictures of participant meals during the study. Participants will be asked to take pictures of their meals, and these pictures will be used by the investigators to categorize meal portion size and relative food types (i.e., whether participants have high protein, high fat, high carbohydrate, or high vegetable intakes or not).

l) Symptom duration. This study will evaluate symptom duration after the end of the diet challenge. Participants will be asked to complete the IBS-SSS for 7 days after they complete the diet challenge to determine how long their symptoms last.

m) Study results follow-up. At the end of the study when investigators are unblinded, the researchers will compile the study results (ex., worsening in IBS symptoms after gluten challenge) and provide this information to study participants. A dietary assessment and surveys used previously in the study will be sent to study participants immediately prior and one month after.

Early withdrawals

The following criteria are reasons for early discontinuation (dropouts) during the study: 1- Patient's decision; 2-Physician's decision in case of undue risk with continued participation; 3- Use of any unauthorized concomitant diets/treatments/medications; 4- Patient contracts COVID-19 during the study.

Medical care will be provided as directed by the study physician and/or the subject's physician. Subjects who withdraw their participation will be requested to return at the next visit for evaluation so that their data can be considered for the primary outcome in an Intention to Treat analysis.

If replacement of subjects is not feasible, 20% additional subjects will be recruited to compensate for attrition.

Patient recruitment

Website

This clinical study will be advertised on a McMaster webpage (healthsci.mcmaster.ca/diet-in-ibs/). The website will provide a description of the study, eligibility criteria, and the study contact information. The website will be advertised on Google ads (https://ads.google.com/) and social media outlets such as Facebook, Twitter, and Instagram. The website will also be posted on the Canadian Digestive Health Foundation's website (http://cdhf.ca/).

Posters

Posters will be used to advertise the study. They will be placed at McMaster University Medical Centre, in McMaster's 2F Clinic, and other locations around McMaster University campus. The posters will be placed at different hospitals and clinics affiliated with Hamilton Health Sciences, such at St. Joseph's Hospital and Allied Health Professional practices within a 15 km radius of McMaster or St. Joseph's Hospital. Posters will also be posted in pharmacies near McMaster University.

Screening Participants

The study coordinators will screen potential participants to determine whether they are eligible for the study. If the potential participants are registered at Hamilton Health Sciences, the study coordinators will ask to contact their physician and request a referral to determine whether they are eligible. If participants are not patients registered in Hamilton Health Sciences, they will be

asked for their verbal consent for the study coordinator to contact their medical practitioner to provide a referral or further information to assess their eligibility.

During their appointments, physicians at McMaster's Gastroenterology Outpatient 2F clinic may inform eligible patients about this study. If the patient is interested and consents to be contacted about research studies, the physician will inform the study coordinator so that they may discuss the study with the patient.

Data collection

Case report forms (CRFs) are an integral part of this protocol. All data captured by the investigators will directly be recorded on a computer database created using REDCap (Research Electronic Data Capture) Software, a secure web application for building and managing online surveys and database programs[58]. Data to be considered as sources in the CRFs are: a) Surveys and questionnaires (Appendices 2-10); b) Details of physical examination and clinical background; c) Study laboratory sheets; d) Pregnancy urine strips; e) Diet assessment: diet initiation, follow-up, and GFD compliance. All other data should be checked for accuracy against the patient record.

To preserve adequate data management, the database will be supervised and monitored by the Principal Investigator.

Videoconferences

All follow-up visits will be conducted online using either Zoom or telephone, depending on the participant's preference. The participants will be informed about the privacy risks involved in video conferencing in the informed consent form. Informed consent will be obtained from patients to communicate using Zoom, telephone, email, and/or the secure, encrypted messaging app Signal. Patients will be explained the risks related to unauthorized disclosures and/or interception of personal health information and steps they can take to help protect their information. A link to the Zoom privacy policy (https://zoom.us/privacy) will be included in the informed consent form for participants to learn more.

Signal messaging app

Participants will have the option of communicating with the study coordinators using Signal, a secure, encrypted messaging app. As mentioned in HiREB's *Video Conferencing and Consent Guidelines* (https://hireb.ca/wp-content/uploads/2020/04/Video-Conferencing-and-Consent-Guidelines.docx), Signal is "an encrypted voice calling or messaging service […] which has clear policies about the storing of logs of communication metadata". Signal does not store metadata or use the cloud to back up messages and uses end-to-end encryption by default. A link to Signal's privacy policy (https://signal.org/legal/#privacy-policy) will be provided to study participants to learn more. Additionally, the study coordinators will enable the 'Disappearing Messages' feature of Signal such that any text communications to participants will be deleted from both devices after 12 hours, as described here: https://support.signal.org/hc/en-us/articles/360007320771. Study participants will not be required to use Signal but may use it to communicate securely with the study coordinators if they prefer.

Communication with study participants

Study participants will have the option of communicating with the study coordinators via Zoom, phone calls, email, or the secure, end-to-end encrypted messaging platform Signal. They will be informed of the privacy risks associated with each method of communication.

Scripts to contact patients

If the study coordinators are contacted by a potential participant, then their first contact with the participant will include standardized scripts. Scripts for telephone and email contact are included in the HiREB application.

Privacy

This clinical study will include the use of electronic devices, including phones and computers, where study questionnaires and visits will be completed online. The study coordinators will use various measures to preserve participant privacy, including: 1) only storing participant

personal information on a secure, encrypted file in the McMaster server; 2) separating personal identifiers from study data; 3) communicating with participants on secure platforms, including Signal, for messaging or calling; and 4) informing participants of the privacy risks of using video conferencing platforms like Zoom.

Monitoring:

The Principal Investigator will serve as a monitor during the study. The strategy will involve monitoring trial oversight, data, and safety.

Since no higher risk is expected in any of the study measurements or treatments, a Data Safety monitoring board will not be required.

This clinical trial will be conducted following the principles of ICH (International Conference on Harmonization) for Good Clinical Practices and adherence with the applicable regulatory or legal requirements.

COVID-19 Standard Operating Procedures (SOPs)

Standard operating procedures applicable to clinical and basic research were developed to minimize the spread of the COVID-19 coronavirus. They can be found in Appendix 22.

All research personnel will complete the Ontario COVID-19 Self-Assessment (https://www.ontario.ca/page/2019-novel-coronavirus-covid-19-self-assessment) within 24 hours of going to McMaster University Medical Centre and when they pick up or drop off samples for patients. We will follow the guidelines for McMaster University's Return to Research (https://research.mcmaster.ca/phased-return-to-research-activity/), conducting research and study visits remotely or in-person depending on the current guidelines.

Pick-up & drop-off of samples for remote visits

Due to the COVID-19 pandemic, patients may or may not have in-person research visits at McMaster University. For remote visits, study coordinators will mail or drop off the specimen collection kit and study intervention to the participant before their study visit and will arrange a date and time to pick up the sample from their preferred address (i.e., home or workplace) after

it is collected. Specimens will be transported in a special biohazard container back to McMaster University Medical Centre for processing (Appendix 23). Study participants will complete the online COVID-19 screening tool (Ontario COVID-19 Self-Assessment) at the day of pick-up or drop-off. To coordinate pick-ups and drop-offs, the study coordinators and participants may call, email, or use end-to-end encrypted Signal messages (https://signal.org/en/), depending on participant preference, once they have been informed of the security levels associated with each platform.

The study coordinators will drive to the participant's preferred address to pick up the sample using a rental car from Enterprise (https://www.enterprise.ca/) and will be covered by Enterprise insurance in case of any accident. These samples will be kept in a special biohazard refrigerated container approved by Transport Canada to preserve the samples. There will be three containers used to ensure safe transportation of study samples: 1) sample collection containers with direct contact to participant samples; 2) a secondary container to hold the individual samples; and 3) a tertiary cooler with ice or ice packs to hold the secondary container. The study coordinator will follow the sample handling protocol in Appendix 24.

Statistical analysis

- **Sample size calculation**

According to the study design chosen, three independent groups with paired observations were considered for sample size calculations. The IBS-SSS is considered to have a minimal clinically important difference (MCID) at 50 points[52]. There is also a within-patient standard deviation in IBS-SSS overall score of 68.3 points in IBS-Constipation and 71.4 points in IBS-Diarrhea[59]. In order to reject the null hypothesis that there will be no difference in IBS-SSS between non-purified gluten and whole wheat flour, purified gluten, and placebo with a power of 0.80 and at a significance of $\alpha = 0.05$, 33 participants are required. 40 participants will be recruited to account for 20% attrition.

- **Statistical analysis**

Statistical testing[60] will be performed using the Statistical Package for Social Sciences for Windows software (SPSS 20.0; SPSS Inc., Chicago, IL) and R version 3.5.0. The study cohort will be characterized descriptively using simple proportions for categorical variables (e.g. gender) and means with SDs for parametric continuous variables (e.g. age). Groups will be compared using chi-squared tests. Parametric continuous variables will compare means and SDs using repeated measures ANOVA. The primary outcome, the IBS-SSS, will be used as a dichotomous variable and analyzed using a chi-squared test. Patients will be categorized as "worsened their symptoms" if they showed an increase of at least 50 points in overall score, and "not worsened" if the score was below that point when dichotomous data is required. The IBS-SSS will also be evaluated using repeated measures ANOVA. Baseline and post-treatment questionnaires (PROMIS scales, DASS21, etc.) will compare means and SDs using one-way ANOVA for between-group comparison or repeated measures ANOVA for within-group comparison[61]. For the secondary outcome (PROMIS scales), separated domain (diarrhea, constipation, gas and bloating, or belly pain) scores will also be analyzed using one-way ANOVA between groups and repeated measures ANOVA within groups. Secondary outcomes will also be adjusted for multiple comparisons using Tukey's post-hoc test and an overall p-value of 0.05 for significance.

A p-value of <0.05 will be considered statically significant. Biochemical measures (AGA and blood tests) will be reported as means and SDs if parametric or medians and IQRs if non-parametric. Correlation between changes in IBS-SSS and PROMIS scales and changes in intestinal microbiota will be tested using a Pearson's correlation coefficient if parametric, or a Spearman's correlation if non-parametric. Subgroup analysis of primary and secondary outcomes will be performed considering presence/absence of AGA, whether the patient has a celiac-compatible genotype (HLA DQ2/8 positive), and whether the participants are compliant vs non-compliant to the study protocol.

- **COVID Analyses**

Participants in our study will be asked whether they were tested for COVID-19 during the pandemic and whether they tested positive in a short questionnaire at each visit. Participants reporting a positive test will drop from the study and an early termination visit will be performed.

- **Biological Sample Processing**

Stool

Stool samples will be kept cold until further processing. On the day of collection, samples will be aliquoted into 6 different 2 mL cryovials in an anaerobic chamber. Aliquots will be stored at -80°C until the analysis.

Urine

Urine samples will be kept cold from collection until further processing, using an insulating bag with ice packs. The research coordinator will pick up the urine sample at the patient's home within the first 30-45 minutes after collection. On the day of collection, urine will be aliquoted into 0.5 mL sterile microtubes and stored at -80°C.

Blood

Blood samples will be collected in green-topped heparin-containing tubes, red-topped tubes with coagulant, and lavender-topped tubes with EDTA. Serum and plasma will then be separated by centrifugation within 1 hour after collection to prevent hemolysis. Plasma will be aliquoted into 1.5 mL tubes and stored at -80°C.

- **Analysis of the Microbiota: Molecular approach**

To understand the relationship between IBS states and the gut microbiota, we propose to analyze the microbial communities at two levels: (i) microbial diversity (16S rRNA gene analyses) and (ii) metabolic capabilities (metagenomics, or "bulk", DNA sequencing). Illumina sequencing of 16S rRNA genes will help us to comprehensively monitor the variation in microbial taxonomic representation of IBS patients over time. On the other hand, deep sequencing of bulk DNA will provide a picture of the genetic composition of samples, which will indicate the types of functions available to such microbial communities.

For 16S rRNA gene sequence analyses, we will use the QIIME software platform. This platform enables the following steps: aligning sequences using secondary-structure information, clustering sequences into operational taxonomic units (OTUs) by percent similarity, assigning taxonomic information to unique clusters, generating averaged accumulation (rarefaction) curves for each sample, and establishing estimates of total diversity.

Ethical aspects

The study protocol will be submitted by one of the investigators for approval by the Hamilton Integrated Research Ethics Board (HiREB). Commencement of the clinical trial is not permitted without written approval by the ethics committee. The HiREB must be notified of all subsequent additions or changes to the study protocol. Notification of the HiREB is also required in the event of a serious adverse event (SAE) during the clinical trial. This trial will be conducted according to the principles and rules laid down in the Declaration of Helsinki (Appendix 25) and its subsequent amendments.

Protection of the subject's confidentiality

Confidentiality of all study participants will be maintained; codes for subject identification (ID) will be utilized (i.e. participant, P, and number of enrolment; P001) and documents relating their identity to their ID will be kept separate from their study records.

Informed consent

When the investigator has determined that the subject is an appropriate candidate for the study,

the study will be described and explained by a researcher during an interview either in person, via phone, via end-to-end encrypted Signal calls, or on the online videoconferencing tool Zoom. Due to the COVID-19 pandemic, online visits were introduced using the online video conferencing platform Zoom. Subjects will be informed of the privacy risks of conducting online visits on Zoom before they consent to participating in this study. The investigator will fully answer all participant questions. A copy of the information letter and informed consent form will be provided to the subject. Informed consent will be obtained from each subject by the investigator prior to V1 and enrolment in the study, either in written format or as an e-consent. If consent is obtained in-person, the consent form will be signed and dated by the subject and the investigator. The consent form will be completed in two copies: the first copy is kept in the investigator's file and is checked by the monitor while the second is given to the patient. Participants will also have the option of having their informed consent form mailed to them to complete at home. Once the consent form is signed and dated, participants will mail the consent forms back using a pre-paid, pre-addressed envelope. If the consent is obtained online, participants will complete e-consent forms using either the REDCap E-Consent Framework or the DocuSign eSignature platform (https://www.docusign.ca/). The consent form will be electronically signed and dated by the participant and investigator. The Farncombe Institute's REDCap is hosted on a secure McMaster server and the e-consent forms would be collected and stored in a different REDCap project from the study CRFs to ensure that the participant ID is kept separate from any identifying information. Only REDCap users with 'full data set' privileges will be able to download archived e-consent forms. DocuSign is a secure eSignature platform that is commonly used at Hamilton Health Sciences and McMaster University Faculty of Health Sciences. As our lab already uses REDCap for our data collection, we will use REDCap in the interim but plan to collect consents using DocuSign if

we get a department, faculty, or institute-wide account.

No subject will undergo study-related procedures before completion of the informed consent.

Handling adverse events (AEs)

An AE is defined as any untoward occurrence in a patient or clinical investigation subject who has been administered an investigational product. It does not necessarily require a causal relationship with the treatment. AEs include abnormal laboratory findings, illnesses, signs, and/or symptoms that occur or worsen during the course of the study. They also include occasions when subjects contact the investigator or their private physician and are examined or given medical direction. They may or may not lead to the withdrawal of the subject from the study. AEs are generally classified as serious or non-serious. All AEs occurring during the study will be reported and recorded whether or not they are considered to be serious (SAEs), non-serious, and/or related to the treatment. The following information will be required in each case: subject ID and date, description of event, duration, frequency, intensity, seriousness, action taken, outcome and sequel, relationship to test product, and definitions. Documentation of a SAE that requires a separate form will be completed by the investigators in each case (Appendix 26). In case of any adverse event occurrence, adequate care will be provided by the research physicians.

Impact of the study

Traditional IBS management approaches often rely on a combination of dietary changes, antispasmodics, antidiarrheal, laxatives, antidepressants, and analgesics[62]. However, the efficacy, safety, and tolerability of these treatments have not been examined in well-controlled RCTs. A systematic review of the efficacy and safety of traditional therapies found that none of these agents demonstrated level I evidence to support their use in the treatment of IBS[63]. Furthermore, the adverse effects of some IBS treatments may outweigh the potential benefits. It is not surprising that IBS patients are often dissatisfied with the efficacy and tolerability of traditional treatment options. These treatments only address isolated symptoms rather than the

cause, thereby leading to repeated doctor visits, multiple medications, and frequent prescription changes. This is costly, contributing to a tremendous economic burden on both society and the health care system[64]. Additionally, symptoms experienced by IBS patients significantly impact their quality of life[65–67].

Adopting a GFD is becoming increasingly popular with IBS patients, as well as the general American population, despite the fact that it is 2-3 times costlier[68]. Several IBS patients either self-start or are recommended to start a GFD by their physicians in order to alleviate their symptoms, despite the fact there is little evidence gluten is a trigger. Thus, it is crucial to have a better understanding of the real effect of diet in IBS patients and that a rational approach to the prescription of a GFD is undertaken. The results of this study will contribute to greater knowledge of the potential mechanisms and effects of gluten and ATIs, leading to more effective future dietary treatments for the global IBS epidemic.

References

1. Boivin M. Socioeconomic impact of irritable bowel syndrome in Canada. *Can J Gastroenterol*. 2001;15 Suppl B:8B-11B. papers://5aecfcca-9729-4def-92fe-c46e5cd7cc81/Paper/p53689
2. Sanger GJ, Alpers DH. Development of drugs for gastrointestinal motor disorders: Translating science to clinical need. *Neurogastroenterol Motil*. 2008;20(3):177-184. doi:10.1111/j.1365-2982.2008.01084.x
3. Gibson PR, Shepherd SJ. Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach. *J Gastroenterol Hepatol*. 2010;25(2):252-258. doi:10.1111/j.1440-1746.2009.06149.x
4. McKenzie YA, Alder A, Anderson W, et al. British Dietetic Association evidence-based guidelines for the dietary management of irritable bowel syndrome in adults. *J Hum Nutr Diet*. 2012;25(3):260-274. doi:10.1111/j.1365-277X.2012.01242.x
5. Gaesser GA, Angadi SS. Gluten-free diet: Imprudent dietary advice for the general population? *J Acad Nutr Diet*. 2012;112(9):1330-1333. doi:10.1016/j.jand.2012.06.009
6. El-Chammas K, Danner E. Gluten-Free Diet in Nonceliac Disease. *Nutr Clin Pract*. 2011;26(3):294-299. doi:10.1177/0884533611405538
7. van Overbeek F, Uil-Dieterman I, Mol I, Köhler-Brands L, Heymans H, Mulder C. The daily gluten intake in relatives of patients with coeliac disease compared with that of the general Dutch population. *Eur J Gastroenterol Hepatol*. 1997;9(11):1097-1099.
8. Catassi C, Fabiani E, Iacono G, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am J Clin Nutr*. 2007;85(1):160-166. doi:85/1/160 [pii]
9. Lebwohl B, Granath F, Ekbom A, et al. Mucosal healing and mortality in coeliac disease. *Aliment Pharmacol Ther*. 2013;37(3):332-339. doi:10.1111/apt.12164

10. Ferch CC, Chey WD. Irritable bowel syndrome and gluten sensitivity without celiac disease: Separating the wheat from the chaff. *Gastroenterology*. 2012;142(3):664-666. doi:10.1053/j.gastro.2012.01.020

11. Verdu EF. Editorial: Can gluten contribute to irritable bowel syndrome? *Am J Gastroenterol*. 2011;106(3):516-518. doi:10.1038/ajg.2010.490

12. Eswaran S, Tack J, Chey WD. Food: the forgotten factor in the irritable bowel syndrome. *Gastroenterol Clin North Am*. 2011;40(1):141-162. doi:10.1016/j.gtc.2010.12.012

13. Carroccio A, Mansueto P, Iacono G, et al. Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. *Am J Gastroenterol*. 2012;107(12):1898-1906; quiz 1907. doi:10.1038/ajg.2012.236

14. Verdu EF, Huang X, Natividad J, et al. Gliadin-dependent neuromuscular and epithelial secretory responses in gluten-sensitive HLA-DQ8 transgenic mice. *Am J Physiol Gastrointest Liver Physiol*. 2008;294(1):217-225. doi:10.1152/ajpgi.00225.2007

15. Biesiekierski JR, Newnham ED, Irving PM, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. *Am J Gastroenterol*. 2011;106(3):508-514; quiz 515. doi:10.1038/ajg.2010.487

16. Wahnschaffe U, Schulzke JD, Zeitz M, Ullrich R. Predictors of clinical response to gluten-free diet in patients diagnosed with diarrhea-predominant irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2007;5(7):844-850; quiz 769. doi:10.1016/j.cgh.2007.03.021

17. Schuppan D, Pickert G, Ashfaq-Khan M, Zevallos V. Non-celiac wheat sensitivity: differential diagnosis, triggers and implications. *Best Pract Res Clin Gastroenterol*. 2015;29(3):469-476. doi:10.1016/j.bpg.2015.04.002

18. Zevallos VF, Raker V, Tenzer S, et al. Nutritional Wheat Amylase-Trypsin Inhibitors Promote Intestinal Inflammation via Activation of Myeloid Cells. *Gastroenterology*.

2017;152(5):1100-1113.e12. doi:10.1053/j.gastro.2016.12.006

19. Tatham AS, Shewry PR. Allergens to wheat and related cereals. *Clin Exp Allergy*. 2008;38(11):1712-1726. doi:10.1111/j.1365-2222.2008.03101.x

20. Junker Y, Zeissig S, Kim S-J, et al. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *J Exp Med*. 2012;209(13):2395-2408. doi:10.1084/jem.20102660

21. Catassi C, Elli L, Bonaz B, et al. Diagnosis of non-celiac gluten sensitivity (NCGS): The salerno experts' criteria. *Nutrients*. 2015;7(6):4966-4977. doi:10.3390/nu7064966

22. El-Salhy M, Ostgaard H, Gundersen D, Hatlebakk JG, Hausken T. The role of diet in the pathogenesis and management of irritable bowel syndrome (Review). *Int J Mol Med*. 2012;29(5):723-731. doi:10.3892/ijmm.2012.926

23. National Center for Health Statistics. Ambulatory Health Care Data. U.S. Department of Health & Human Services. Published 2017. Accessed September 17, 2017. https://www.cdc.gov/nchs/ahcd/index.htm

24. Mitchell CM, Drossman DA. Survey of the AGA membership relating to patients with functional gastrointestinal disorders. *Gastroenterology*. 1987;92(5 Pt 1):1282-1284. doi:10.1016/S0016-5085(87)91099-7

25. Pietzak M. Celiac disease, wheat allergy, and gluten sensitivity: when gluten free is not a fad. *JPEN J Parenter Enteral Nutr*. 2012;36(1 Suppl):68S-75S. doi:10.1177/0148607111426276

26. Marcason W. Is there evidence to support the claim that a gluten-free diet should be used for weight loss? *J Am Diet Assoc*. 2011;111(11):1786. doi:10.1016/j.jada.2011.09.030

27. Terazono E. Healthy appetites drive jump in sales of gluten-free foods. Financial Times. Published 2017. Accessed September 10, 2017. https://www.ft.com/content/4ec0f2f2-2c0a-11e7-9ec8-168383da43b7

28. Higgins JA. Whole grains, legumes, and the subsequent meal effect: implications for

blood glucose control and the role of fermentation. *J Nutr Metab*. 2012;2012:829238. doi:10.1155/2012/829238

29. Rosén LAH, Östman EM, Shewry PR, et al. Postprandial glycemia, insulinemia, and satiety responses in healthy subjects after whole grain rye bread made from different rye varieties. 1. *J Agric Food Chem*. 2011;59(22):12139-12148. doi:10.1021/jf2019825
30. Russo F, Riezzo G, Chiloiro M, et al. Metabolic effects of a diet with inulin-enriched pasta in healthy young volunteers. *Curr Pharm Des*. 2010;16(7):825-831. doi:10.2174/138161210790883570
31. Harris KA, Kris-Etherton PM. Effects of whole grains on coronary heart disease risk. *Curr Atheroscler Rep*. 2010;12(6):368-376. doi:10.1007/s11883-010-0136-1
32. Kelly G. Inulin-type prebiotics: a review. (Part 2). *Altern Med Rev*. 2009;14(1):36-55. http://www.ncbi.nlm.nih.gov/pubmed/19364192
33. Abrams SA, Griffin IJ, Hawthorne KM, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr*. 2005;82(2):471-476. doi:82/2/471 [pii]
34. Reddy BR. Noncaloric Benefits of Carbohydrates. *Nestle Nutr Inst Workshop Ser*. 2015;82:27-37. doi:10.1159/000381999
35. Tarini J, Wolever TMS. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Appl Physiol Nutr Metab*. 2010;35(1):9-16. doi:10.1139/H09-119
36. Neyrinck AM, Delzenne NM. Potential interest of gut microbial changes induced by non-digestible carbohydrates of wheat in the management of obesity and related disorders. *Curr Opin Clin Nutr Metab Care*. 2010;13(6):722-728. doi:10.1097/MCO.0b013e32833ec3fb
37. Lee A, Newman JM. Celiac diet: its impact on quality of life. *J Am Diet Assoc*. 2003;103(11):1533-1535. doi:10.1016/S0002

38. Lee AR, Ng DL, Zivin J, Green PHR. Economic burden of a gluten-free diet. *J Hum Nutr Diet*. 2007;20(5):423-430. doi:10.1111/j.1365-277X.2007.00763.x

39. Verdu EF, Armstrong D, Murray JA. Between celiac disease and irritable bowel syndrome: the "no man's land" of gluten sensitivity. *Am J Gastroenterol*. 2009;104(6):1587-1594. doi:10.1038/ajg.2009.188

40. Mills EJ, Chan A-W, Wu P, Vail A, Guyatt GH, Altman DG. Design, analysis, and presentation of crossover trials. *Trials*. 2009;10:27. doi:10.1186/1745-6215-10-27

41. Piantadosi S. *Clinical Trials: A Methodologic Perspective: Second Edition*. 2nd ed. (Shewhart WA, Wilks SS, eds.). John Wiley & Sons, Inc.; 2005. doi:10.1002/0471740136

42. Friedman LM, Furberg CD, DeMets DL, Reboussin DM, Granger CB. *Fundamentals of Clinical Trials*. 5th ed. Springer International Publishing; 2015. doi:10.1007/978-3-319-18539-2

43. Canadian Celiac Association and Dietitians of Canada. Gluten-Free Eating. Published 2011. Accessed September 17, 2017. http://www.celiac.ca/b/wp-content/uploads/2013/07/Gluten_Free_Eating_Apr_2011.pdf

44. Hodge A, Patterson AJ, Brown WJ, Ireland P, Giles G. The Anti Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation. *Aust N Z J Public Health*. 2000;24(6):576-583. doi:10.1111/j.1467-842X.2000.tb00520.x

45. Merchant AT, Dehghan M. Food composition database development for between country comparisons. *Nutr J*. 2006;5:2. doi:10.1186/1475-2891-5-2

46. Leffler DA, Dennis M, Edwards George JB, et al. A simple validated gluten-free diet adherence survey for adults with celiac disease. *Clin Gastroenterol Hepatol*. 2009;7(5):530-536, 536.e1-2. doi:10.1016/j.cgh.2008.12.032

47. Skodje GI, Sarna VK, Minelle IH, et al. Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-Reported Non-Celiac Gluten Sensitivity. *Gastroenterology*.

2018;154(3):529-539.e2. doi:10.1053/j.gastro.2017.10.040

48. Elli L, Tomba C, Branchi F, et al. Evidence for the Presence of Non-Celiac Gluten Sensitivity in Patients with Functional Gastrointestinal Symptoms: Results from a Multicenter Randomized Double-Blind Placebo-Controlled Gluten Challenge. *Nutrients*. 2016;8(2):84. doi:10.3390/nu8020084

49. Zanini B, Baschè R, Ferraresi A, et al. Randomised clinical study: gluten challenge induces symptom recurrence in only a minority of patients who meet clinical criteria for non-coeliac gluten sensitivity. *Aliment Pharmacol Ther*. 2015;42(8):968-976. doi:10.1111/apt.13372

50. 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. doi:10.1053/j.gastro.2013.04.051

51. Hill P. Low FODMAP Muesli Bars. Accessed March 24, 2018. https://www.monashfodmap.com/blog/low-fodmap-muesli-bars/

52. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther*. 1997;11(2):395-402. Accessed April 2, 2018. https://journals-scholarsportal-info.libaccess.lib.mcmaster.ca/pdf/02692813/v11i0002/395_tibsssibsaip.xml

53. Spiegel BMR, Hays RD, Bolus R, et al. Development of the NIH Patient-Reported Outcomes Measurement Information System (PROMIS) gastrointestinal symptom scales. *Am J Gastroenterol*. 2014;109(11):1804-1814. doi:10.1038/ajg.2014.237

54. Kim ER, Rhee P-L. How to interpret a functional or motility test - colon transit study. *J Neurogastroenterol Motil*. 2012;18(1):94-99. doi:10.5056/jnm.2012.18.1.94

55. Evans RC, Kamm MA, Hinton JM, Lennard-Jones JE. The normal range and a simple diagram for recording whole gut transit time. *Int J Colorectal Dis*. 1992;7(1):15-17.

doi:10.1007/BF01647654

56. Megiorni F, Mora B, Bonamico M, et al. A rapid and sensitive method to detect specific human lymphocyte antigen (HLA) class II alleles associated with celiac disease. *Clin Chem Lab Med*. 2008;46(2):193-196. doi:10.1515/CCLM.2008.049

57. Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. *Am J Gastroenterol*. 2005;100(7):1550-1557. doi:10.1111/j.1572-0241.2005.41348.x

58. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

59. Colucci R, Gambaccini D, Ghisu N, et al. Influence of the serotonin transporter 5HTTLPR polymorphism on symptom severity in irritable bowel syndrome. *PLoS One*. 2013;8(2):e54831. doi:10.1371/journal.pone.0054831

60. Dallal GE. The Computer-Aided Analysis of Crossover Studies. Gerard E. Dallal. Published 2012. Accessed September 17, 2017. http://www.jerrydallal.com/lhsp/crossovr.htm

61. Roalfe AK, Roberts LM, Wilson S. Evaluation of the Birmingham IBS symptom questionnaire. *BMC Gastroenterol*. 2008;8:30. doi:10.1186/1471-230X-8-30

62. Adam B, Liebregts T, Holtmann G. [Irritable bowel syndrome]. *Dtsch Med Wochenschr*. 2005;130(8):399-401. doi:10.1055/s-2005-863064

63. Fennerty MB. Traditional therapies for irritable bowel syndrome: an evidence-based appraisal. *Rev Gastroenterol Disord*. 2003;3 Suppl 2:S18-24. http://www.ncbi.nlm.nih.gov/pubmed/12775999

64. Johanson JF. Options for patients with irritable bowel syndrome: contrasting traditional and novel serotonergic therapies. *Neurogastroenterol Motil*. 2004;16(6):701-711.

doi:10.1111/j.1365-2982.2004.00550.x

65. Svedlund J, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci*. 1988;33(2):129-134. http://www.ncbi.nlm.nih.gov/pubmed/3123181

66. Patrick DL, Drossman DA, Frederick IO, DiCesare J, Puder KL. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. *Dig Dis Sci*. 1998;43(2):400-411. http://www.ncbi.nlm.nih.gov/pubmed/9512138

67. Drossman DA, Patrick DL, Whitehead WE, et al. Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire. *Am J Gastroenterol*. 2000;95(4):999-1007. doi:10.1111/j.1572-0241.2000.01941.x

68. Missbach B, Schwingshackl L, Billmann A, et al. Gluten-free food database: the nutritional quality and cost of packaged gluten-free foods. *PeerJ*. 2015;3:e1337. doi:10.7717/peerj.1337

69. Sapone A, Bai JC, Ciacci C, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med*. 2012;10:13. doi:10.1186/1741-7015-10-13

APPENDIX 1: Graphic of Dietary Trends in the US[69]

From:

BMC Med. 2012; 10: 13.

Published online 2012 February 7. doi: 10.1186/1741-7015-10-13

Figure 3

Click on image to zoom

Trend of three different diets (low carbohydrate diet, fat-free diet, and gluten-free diet), in the USA during the period 2004 to 2011. For the American general population, adopting a gluten-free diet is becoming an increasingly popular option. The market for gluten-free food and beverage products grew at a compound annual growth rate of 28% from 2004 to 2011, eclipsing the low carbohydrate diet and the fat-free diet in 2008, to finish with almost $1.6 billion in retail sales in 2010. By 2012 the market is expected to reach about $2.6 billion in sales. The fact that approximately three million Americans suffer from celiac disease and only a fraction of these patients have been diagnosed implies that patients suffering other forms of proven gluten reaction, including gluten sensitivity and wheat allergy, contribute to this market growth. The rest of the market is filled either by people who undertake the diet as occasional consumers (no medical necessity) or by individuals affected by maladies that have been claimed to be affected by gluten exposure, including autism spectrum disorder, attention deficit hyperactivity disorder, multiple sclerosis and irritable bowel syndrome, but for which there is no evidence of the effectiveness of the diet.

APPENDIX 2: Irritable Bowel Syndrome Symptom Severity Score (IBS-SSS)

Diagnosis and Management of IBS in Adults

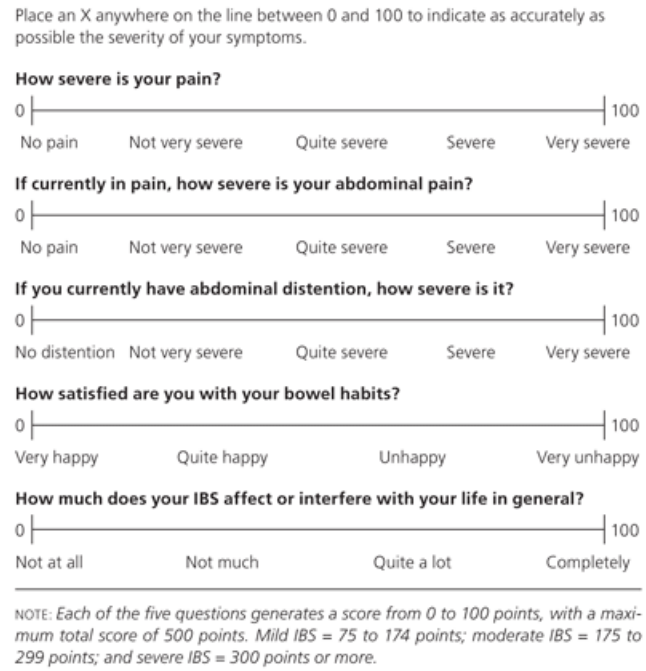


Figure 2.

Scoring system to assess the severity of irritable bowel syndrome (IBS) symptoms.

Adapted with permission from Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997;11(2):401.

APPENDIX 3: PROMIS Scale: Bloating & Gas

PROMIS Scale v1.0 – Gastrointestinal Gas and Bloating 13a

Gastrointestinal Gas and Bloating

Please respond to each question or statement by marking one box.

In the past 7 days…

1 GISX94 Did you have swelling in your belly?

- ☐ B No → **If No, go to #5**
- ☐ A Yes

2 GISX95 How bad did the swelling in your belly get?

- ☐ 1 Not bad at all
- ☐ 2 A little bad
- ☐ 3 Somewhat bad
- ☐ 4 Quite bad
- ☐ 5 Very bad

3 GISX96 How much did the swelling in your belly interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

Last Updated: 2 September 2016

PROMIS Scale v1.0 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

4 How much did having swelling in your belly bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

5 How often did you feel bloated?

- ☐ 1 Never → **If Never, go to #12**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

6 In general, how severe was your bloating?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

Last Updated: 2 September 2016

 Page 2 of 5

PROMIS Scale v1.0 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

7 At its worst, how severe was your bloating?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

8 In general, how severe did your bloating feel?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

9 How often did you know that you would feel bloated before it happened?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 2 September 2016

 Page 3 of 5

PROMIS Scale v1.0 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

10 How much did feeling bloated interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

11 How much did feeling bloated bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

12 How often did you pass gas?

- ☐ 1 Never→ **If Never, you are finished.**
- ☐ 2 Only once or twice a day
- ☐ 3 About every 3-4 hours
- ☐ 4 About every 2 hours
- ☐ 4 About every hour

Last Updated: 2 September 2016

 Page 4 of 5

PROMIS Scale v1.0 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

13	How often did you have gurgling or rumbling in your belly when you were not hungry?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 2 September 2016

 Page 5 of 5

APPENDIX 4: PROMIS Scale: Belly Pain

PROMIS Scale v1.0 – Gastrointestinal Belly Pain 5a

Belly Pain

Please respond to each question or statement by marking one box.

In the past 7 days…

1 How often did you have belly pain?

- ☐ 1 Never → **If Never, go to #5**
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

2 At its worst, how would you rate your belly pain?

- ☐ 1 Not bad at all
- ☐ 2 A little bad
- ☐ 3 Somewhat bad
- ☐ 4 Quite bad
- ☐ 5 Very bad

Last Updated: 1 September 2016

 Page 1 of 2

PROMIS Scale v1.0 – Gastrointestinal Belly Pain 5a

In the past 7 days…

3 How much did belly pain interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

4 How much did belly pain bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

5 How often did you have discomfort in your belly?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 1 September 2016

 Page 2 of 2

APPENDIX 5: PROMIS Scale: Diarrhea

Gastrointestinal Diarrhea

Please respond to each question or statement by marking one box.

In the past 7 days…

1 How many days did you have loose or watery stools?

- ☐ 1 No days → **If No Days, go to #4**
- ☐ 2 1 day
- ☐ 3 2 days
- ☐ 4 3-5 days
- ☐ 5 6-7 days

2 How much did having loose or watery stools interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

3 How much did having loose or watery stools bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

Last Updated: 1 September 2016

PROMIS Scale v1.0 – Gastrointestinal Diarrhea 6a

In the past 7 days…

4 How often did you feel like you needed to empty your bowels right away or else you would have an accident?

- ☐ 1 Never → **If Never, you are finished.**
- ☐ 2 One time during the past 7 days
- ☐ 3 2-6 times during the past 7 days
- ☐ 4 Often once a day
- ☐ 5 More than once a day

5 How much did feeling you needed to empty your bowels right away interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

6 How much did feeling you needed to empty your bowels right away bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

Last Updated: 1 September 2016

 Page 2 of 2

APPENDIX 6: PROMIS Scale: Constipation

PROMIS Scale v1.0 – Gastrointestinal Constipation 9a

Gastrointestinal Constipation

Please respond to each question or statement by marking one box.

In the past 7 days…

1 How often did you pass very hard or lumpy stools?

- ☐ 1 Never → **If Never, go to #3**
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

2 How much did hard or lumpy stools bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

3 How often did you strain while trying to have bowel movements?

- ☐ 1 Never → **If Never, go to #6**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 1 September 2016

 Page 1 of 3

PROMIS Scale v1.0 – Gastrointestinal Constipation 9a

In the past 7 days…

4 How much did you usually strain while trying to have a bowel movement?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

5 How much did straining during bowel movements bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

6 How often did you feel pain in your rectum or anus while trying to have bowel movements?

- ☐ 1 Never → **If Never, go to #8**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 1 September 2016

 Page 2 of 3

PROMIS Scale v1.0 – Gastrointestinal Constipation 9a

In the past 7 days…

7 At its worst, how would you rate the pain in your rectum or anus during bowel movements?

- ☐ 1 Not bad at all
- ☐ 2 A little bad
- ☐ 3 Somewhat bad
- ☐ 4 Quite bad
- ☐ 5 Very bad

8 How often after a bowel movement did you feel unfinished - that is, that you had not passed all your stool?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

9 How often did you use your finger or toilet paper to get out a stool?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 1 September 2016

 Page 3 of 3

APPENDIX 7: Stool consistency (Bristol Stool Form Scale): (all patients)

Please indicate the most common pattern of stool consistency that you had over the last seven days according to this scale:

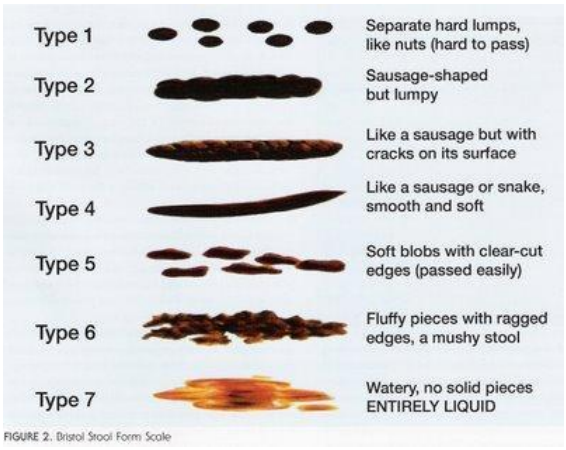


FIGURE 2. Bristol Stool Form Scale

Type 1: Separate hard lumps, like nuts (hard to pass)

Type 2: Sausage-shaped, but lumpy.

Type 3: Like a sausage but with cracks on its surface

Type 4: Like a sausage or snake, smooth and soft

Type 5: Soft blobs with clear cut edges (passed easily)

Type 6: Fluffy pieces with ragged edges, a mushy stool.

Type 7: Entirely liquid

APPENDIX 8: Stool frequency:

Please indicate the average number of bowel movements (i.e., number of times you have opened your bowels) *per day* in the last 7 days:

Average bowel movements *per day*: __

APPENDIX 9: DASS-21 Questionnaire

DASS21

Name: Date:

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you **over the past week**. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree or a good part of time
3 Applied to me very much or most of the time

1 (s)	I found it hard to wind down	0	1	2	3
2 (a)	I was aware of dryness of my mouth	0	1	2	3
3 (d)	I couldn't seem to experience any positive feeling at all	0	1	2	3
4 (a)	I experienced breathing difficulty (e.g. excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5 (d)	I found it difficult to work up the initiative to do things	0	1	2	3
6 (s)	I tended to over-react to situations	0	1	2	3
7 (a)	I experienced trembling (e.g. in the hands)	0	1	2	3
8 (s)	I felt that I was using a lot of nervous energy	0	1	2	3
9 (a)	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10 (d)	I felt that I had nothing to look forward to	0	1	2	3
11 (s)	I found myself getting agitated	0	1	2	3
12 (s)	I found it difficult to relax	0	1	2	3
13 (d)	I felt down-hearted and blue	0	1	2	3
14 (s)	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15 (a)	I felt I was close to panic	0	1	2	3
16 (d)	I was unable to become enthusiastic about anything	0	1	2	3
17 (d)	I felt I wasn't worth much as a person	0	1	2	3
18 (s)	I felt that I was rather touchy	0	1	2	3
19 (a)	I was aware of the action of my heart in the absence of physical exertion (e.g. sense of heart rate increase, heart missing a beat)	0	1	2	3
20 (a)	I felt scared without any good reason	0	1	2	3
21 (d)	I felt that life was meaningless	0	1	2	3

DASS-21 Scoring Instructions

The DASS-21 should not be used to replace a face to face clinical interview. If you are experiencing significant emotional difficulties you should contact your GP for a referral to a qualified professional.

Depression, Anxiety and Stress Scale - 21 Items (DASS-21)

The Depression, Anxiety and Stress Scale - 21 Items (DASS-21) is a set of three self-report scales designed to measure the emotional states of depression, anxiety and stress.

Each of the three DASS-21 scales contains 7 items, divided into subscales with similar content. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest / involvement, anhedonia and inertia. The anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset / agitated, irritable / over-reactive and impatient. Scores for depression, anxiety and stress are calculated by summing the scores for the relevant items.

The DASS-21 is based on a dimensional rather than a categorical conception of psychological disorder. The assumption on which the DASS-21 development was based (and which was confirmed by the research data) is that the differences between the depression, anxiety and the stress experienced by normal subjects and clinical populations are essentially differences of degree. The DASS-21 therefore has no direct implications for the allocation of patients to discrete diagnostic categories postulated in classificatory systems such as the DSM and ICD.

Recommended cut-off scores for conventional severity labels (normal, moderate, severe) are as follows:

NB Scores on the DASS-21 will need to be multiplied by 2 to calculate the final score.

	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34+

Lovibond, S.H. & Lovibond, P.F. (1995). Manual for the Depression Anxiety & Stress Scales. (2nd Ed.)Sydney: Psychology Foundation.

APPENDIX 10: Somatic Symptoms (PHQ-15)

PHYSICAL SYMPTOMS (PHQ-15)

During the past 4 weeks, how much have you been bothered by any of the following problems?

	Not bothered at all (0)	Bothered a little (1)	Bothered a lot (2)
a. Stomach pain	☐	☐	☐
b. Back pain	☐	☐	☐
c. Pain in your arms, legs, or joints (knees, hips, etc.)	☐	☐	☐
d. Menstrual cramps or other problems with your periods ***WOMEN ONLY***	☐	☐	☐
e. Headaches	☐	☐	☐
f. Chest pain	☐	☐	☐
g. Dizziness	☐	☐	☐
h. Fainting spells	☐	☐	☐
i. Feeling your heart pound or race	☐	☐	☐
j. Shortness of breath	☐	☐	☐
k. Pain or problems during sexual intercourse	☐	☐	☐
l. Constipation, loose bowels, or diarrhea	☐	☐	☐
m. Nausea, gas, or indigestion	☐	☐	☐
n. Feeling tired or having low energy	☐	☐	☐
o. Trouble sleeping	☐	☐	☐

(For office coding: Total Score T_____ = _____ + _____)

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

APPENDIX 11: SHAPE Method – Procedure

SITZMARKS diagnostic test helps physicians select the best therapeutic option, based on colon transit time, for adult patients with severe constipation who have otherwise negative GI evaluations.

- Convenient gelatin capsules
- Efficient pre-cut radiopaque rings
- Time saving and cost effective
- CPT code 99070
- 10 capsules per package; each capsule contains 24 Radiopaque polyvinyl chloride markers of 1 mm X 4.5 mm

SUGGESTED DIRECTIONS TO THE PHYSICIAN:

Simplified SITZMARKS Method:

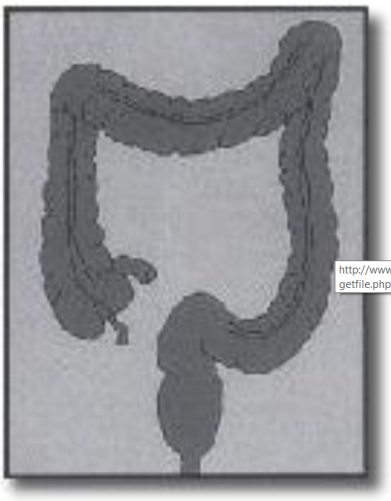
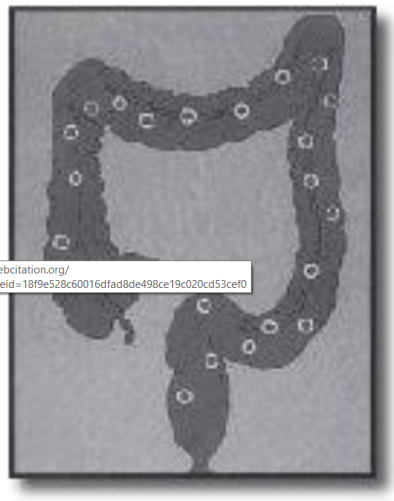
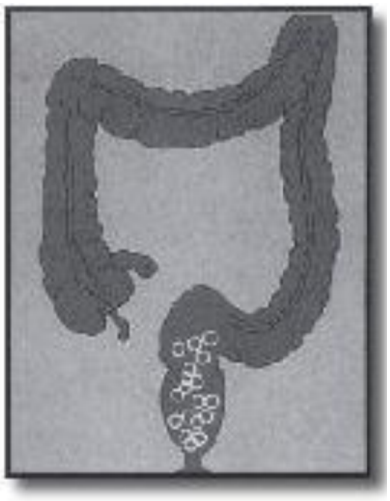
(1 capsule: each capsule contains 24 markers)

Step 1:

1. On day 0, direct patient to take SITZMARKS capsule by mouth with water, preferably with confirmation by office observation. Instruct patient to abstain from using laxatives, enemas or suppositories of any kind for 2 days.
2. Arrange a flat-plate abdominal X-ray on day 2 to determine the location and the extent of elimination of the radiopaque markers.
3. Patients who expel 4-20 (16.7-83.3%) markers have grossly normal colonic transit.
4. Patients who retain 6 or more markers may have follow-up abdominal X-rays within several days.
5. For patients whose markers accumulate in the rectosigmoid or when markers are retained diffusely, Step 2 may be warranted

Reading the Results:

If 80% of the markers are passed by day 5, colonic transit is grossly normal. If remaining markers are scattered about the colon, the condition is most likely hypomotilty or colonic inertia. If remaining markers are accumulated in the rectum or rectosigmoid, the condition is most likely functional outlet delay, e.g., internal rectal prolapse, anismus.

		
A. If 5-20 markers remain, patient has grossly normal colonic transit.	B. Most rings are scattered about the colon. Patient most likely has hypomotility or colonic inertia.	C. Most rings are gathered in the rectosigmoid. Patient has functional outlet obstruction.

APPENDIX 12: Isolation and Cryopreservation of Human PBMC from Whole Blood

(ValentinLEDUCQ, June 2014)

1. Principle.

Peripheral blood mononuclear cells (PBMC) are isolated from whole blood collected in heparin or citrate vacutainer tubes, by use of a density gradient centrifugation procedure. Then, PBMC can be frozen and stored into a cryoprotective media containing 10% DMSO and FBS, in order to have an acceptable yield and viability post-storage.

2. Materials and equipment.

a. PBMC isolation.

- Histopaque (Histopaque-1077, #10771 Sigma)
- 50 ml conical sterile centrifuge tubes
- 15 ml conical sterile centrifuge tubes
- PBS without Ca/Mg (PAA)
- RPMI-1640 complete (10%FBS, 10mM Penicillin Streptomycin, L-glutamine)
- Trypan blue 0.4%

b. PBMC cryopreservation.

- Sterile Cryovials 2 ml
- RPMI-1640 complete (10%FBS, 10mM Penicillin Streptomycin, L-glutamine)
- 2X freezing medium (20% DMSO in FBS)
- DMSO Anhydrous (LifeTechnologies #15250-061)
- PBS 1X without Ca/Mg (PAA)
- Hemocytometer
- Mr. Frosty freezing container
- -80°C freezer
- Liquid nitrogen freezer

3. Procedure.

a. PBMC isolation.

- After carefully checking the sample identification on the blood tubes, remove the whole blood from the heparin tubes and transfer to its corresponding 50ml centrifuge tubes.
- Mix the Histopaque-1077 prior to opening the bottle.
- Gently layer the blood onto an equal volume of Histopaque-1077, in a 15 ml conical centrifuge tube. Split the blood of one sample in as many tubes you need to use all the blood.
- Centrifuge at room temperature for 30 minutes at 400g, without brake.
- Remove the plasma and white cell interface and transfer to the new appropriately labeled 50ml conical tube. Try to retrieve all the cells from the interface without disturbing the red pellet at the bottom of the ficoll layer.
- Add RPMI w/o FBS to the new tube up to 50mls. Be sure to add at least an equal volume of RPMI. If this is not possible in a single tube, split into two 50 ml tubes.
- Centrifuge for 10 minutes at room temperature at 400g. Remove the supernatant from the pellet carefully and discard in an appropriate manner.

- Resuspend each pellet in a small volume of RPMI, and then fill each tube to 50ml with RPMI w/o FBS.
- Centrifuge for 10 minutes at room temperature at 400g. Remove the supernatant from the pellet carefully and discard in an appropriate manner.
- Resuspend cells for counting in 1ml complete RPMI at room temperature.
- Dilute 10µl of the cell suspension into 490µl PBS 1X + 500µl Trypan blue 0,4% (100X dilution factor).
- With a cover-slip in place, transfer 10µl into the chamber of the hemocytometer. Count cells in the 100 squares :

$$Cell/ml = cells \times 10^5$$

- Freeze the cells according to the Cryopreservation protocol, freezing about 10.10^6 cells per vial. Freeze at least 3 vials per sample. Thus, if there are less than 30.10^6 cells in a sample, split evenly into at least 2 vials.

b. PBMC cryopreservation.

- Label cryovials to freeze the sample (sample identification, number of cells).
- Take an appropriate volume of the sample to harvest 30.10^6 cells, and complete with cRPMI to a final volume of 1,5 ml. If there are less than 30.10^6 cells total, just complete the sample with cRPMI to a final volume of 1,5 ml.
- Add 1,5 ml 2X freezing medium at room temperature to the 1,5 ml cell suspension. Gently swirl the tube when adding the freezing medium and dispense 1ml per cryovial.
- Place the cryovials in a room temperature Mr. Frosty freezing container.
- Place the freezing container as soon as possible into the -80°C freezer.
- Transfer the cryovials to liquid nitrogen tank after 1 to 14 days for long-time conservation.

APPENDIX 13: PBMC isolation and preparation

Peripheral blood mononuclear cells (PBMC) are isolated from whole blood collected in heparin or citrate vacutainer tubes, by use of a density gradient centrifugation procedure (Ficoll).

PBMC are re-suspended at 10^6 cells/ml into complete RPMI and plated in a 96 wells culture plate (180µl of the cell suspension, $0.18x10^6$ cells per well). The cells then rest for 12 hours at 37°C, 5% CO_2 before any stimulation procedure.

PBMC stimulation.

PBMC are then stimulated (in duplicate) using the following stimuli for 24 hours at 37°C, 5% CO_2.

Stimuli	**Final Concentration**
MDP (invivogen, tlrl-mdp)	10 µg/ml
Curdlan (invivogen, tlrl-cura)	10 µg/ml
Heat Killed C. albicans	10^6 CFU/ml
Heat Killed E. Coli	10^6 CFU/ml
LPS e.coli (sigma, #12630)	10 ng/µl
PMA (invivogen, tlrl-pma)	500 ng/ml
Ionomycin (sigma, #I0634)	1 µg/ml
PBS 1X	/

After 24 hours, the supernatant is collected and stored at -80°C for further analysis (using multiplex beads array).

APPENDIX 14: Study Design

IC, PE (weight)	X		X	X	X	X	X
SHAPE Study		X	X		X		X
Stool-microbiota		X	X	X	X	X	X
Urine		X	X	X	X	X	X
Questionnaires	X		X	X	X	X	X
Daily Diet Pictures	X	X	X	X	X	X	X
General BW(CL)	X						
Blood (inflamm, AGA)	X		X		X		X
HLA DQ2/8	X						
Start challenge		X		X		X	

APPENDIX 15: GFD Instructions

Gluten-Free Eating

Gluten is a general name for the storage proteins (glutelins and prolamins) found in wheat, barley, rye and related cereal grains such as triticale, spelt and kamut. If you have either of these autoimmune diseases: celiac disease (CD) or dermatitis herpetiformis (DH), a skin form of celiac disease, even a small amount of gluten is harmful. In CD, gluten damages the small intestine resulting in poor absorption of nutrients including vitamins and minerals. In DH, gluten causes skin rashes and itching and can also damage the small intestine.

A strict gluten-free diet (GFD) is the only effective treatment for CD and DH and requires the lifelong elimination of all foods containing wheat, barley, rye, related cereal grains and regular commercial non-gluten-free oats.

A GFD will help your small intestine to heal and will eventually result in elimination of the signs and symptoms, which can include any or all:

- diarrhea
- constipation
- stomach pain/bloating/gas
- weight loss
- chronic fatigue/weakness
- low iron levels
- muscle cramps
- bone and joint pain

For a more complete list of symptoms see the Canadian Celiac Association website: https://www.celiac.ca/healthcare-professionals/diagnosis/.

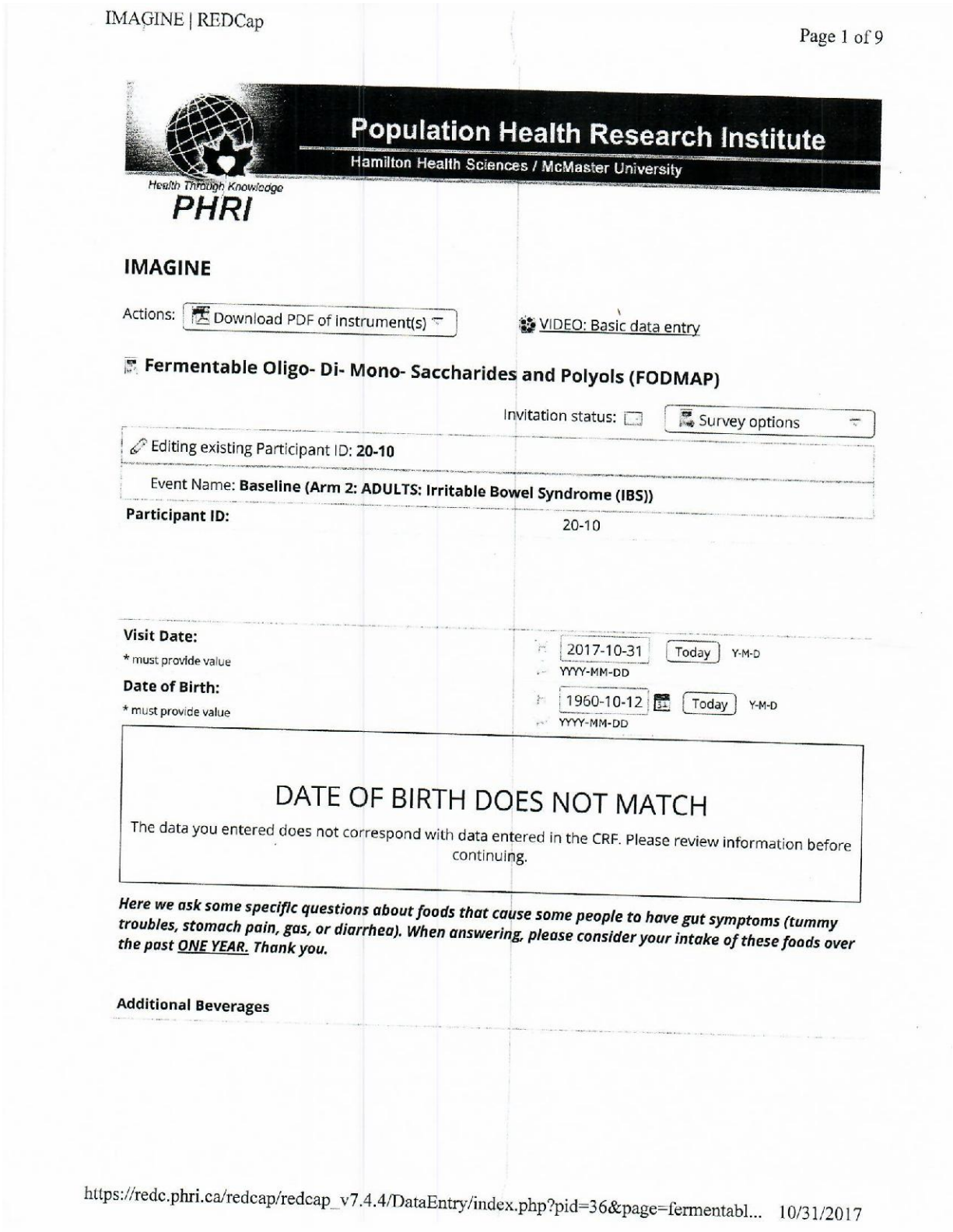
The diet also reduces the risk of developing osteoporosis, reduced fertility, lymphoma and potentially other autoimmune disorders. Even if you are symptom free, continuing with a strict gluten-free diet will reduce your risk of these long-term complications.

Getting Started on a Gluten-Free Diet

It is still essential that you meet with a registered dietitian with expertise in celiac disease who can help you to adapt to the gluten-free lifestyle, including any social and emotional aspects. A dietitian will also be able to help you identify and address any nutritional concerns as well as some of the practical issues of following a gluten-free diet. Some examples include: label reading, cross-contamination and eating away from home.

Updated: May 15, 2018

APPENDIX 16: Food Frequency Questionnaire (SPOR Study)



IMAGINE | REDCap

Page 1 of 9

Population Health Research Institute
Hamilton Health Sciences / McMaster University
Health Through Knowledge
PHRI

IMAGINE

Actions: Download PDF of instrument(s) VIDEO: Basic data entry

Fermentable Oligo- Di- Mono- Saccharides and Polyols (FODMAP)

Invitation status: Survey options

Editing existing Participant ID: **20-10**

Event Name: **Baseline (Arm 2: ADULTS: Irritable Bowel Syndrome (IBS))**

Participant ID: 20-10

Visit Date:
* must provide value
2017-10-31 Today Y-M-D
YYYY-MM-DD

Date of Birth:
* must provide value
1960-10-12 Today Y-M-D
YYYY-MM-DD

DATE OF BIRTH DOES NOT MATCH

The data you entered does not correspond with data entered in the CRF. Please review information before continuing.

Here we ask some specific questions about foods that cause some people to have gut symptoms (tummy troubles, stomach pain, gas, or diarrhea). When answering, please consider your intake of these foods over the past ONE YEAR. Thank you.

Additional Beverages

https://redc.phri.ca/redcap/redcap_v7.4.4/DataEntry/index.php?pid=36&page=fermentabl... 10/31/2017

APPENDIX 17: Expert Dietitian Evaluation of GFD Adherence

In general, how compliant do you believe the participant is with the GFD (include accidental and/or intentional ingestion of gluten in analysis)?

1. Excellent
- Participant eats gluten fewer than 3 times per year
- Uses only celiac-friendly restaurants or asks thorough questions when dining out
- Follows "when in doubt, leave it out" motto
- Review of diet reveals no obvious gluten sources
- Checks medications, supplements, and body care products that enter nose, eyes, and mouth
- Reads labels well
- Has eliminated cross-contamination potential in the kitchen

2. Good
- Participant eats gluten once per month
- Asks useful questions in restaurants
- Shows a high level of confidence in following the diet
- Review of diet may show minimal hidden gluten exposure
- Checks medications, supplements, and body care products
- Reads labels well
- Does not eat contaminated oats
- Has eliminated most, if not all, cross-contamination from kitchen

3. Fair
- Participant eats gluten 2 to 3 times per month
- Does not ask questions in restaurants or when dining out—guesses or takes chances
- Checks some but not all medications, supplements, and body care products
- Shows some confusion over label reading
- Review of diet shows some obvious gluten exposure
- Consumes gluten on occasion—intentionally or unintentionally—per diet/lifestyle recall
- May rely on partner/family member for some caregiving regarding diet
- Has not eliminated cross-contamination potential from kitchen
- May have mental or behavioral issues that make following the diet more difficult
- May have language skills that make dietary adherence more difficult

4. Poor
- Participant eats gluten 1 to 2 times per week
- Does not ask questions in restaurants or when dining out
- Does not check medications, supplements, or body care products
- Consumption of gluten containing foods for religious reasons (e.g., Communion/Passover)
- Requires repeated diet education on hidden gluten, and so forth
- Cheats regularly (1/wk) and intentionally
- May rely often/completely on partner/family member to answer my questions
- Has contaminated kitchen area
- May have mental or behavioral issues that make following the diet more difficult
- May have language skills that make dietary adherence more difficult

5. Very poor
- Participant eats gluten more than 2 times per week

● Does not ask questions in restaurants or when dining out
● Does not check medications, supplements, or body care products
● Takes Communion or Passover regularly
● Requires repeated diet education on hidden gluten, and so forth
● Cheats regularly (2/wk or more) and intentionally
● May rely often/completely on partner/family member to answer my questions
● Has contaminated kitchen area
● May have mental or behavioral issues that make following the diet more difficult
● May have language skills that make dietary adherence more difficult
● May show poor adherence/motivation as a result of denial, anger, or budgetary, family, or social concerns

6. No GFD
Participant does not follow the GFD

Food Label Quiz
Circle ingredients that participant incorrectly classifies.
(1) Dehydrated potatoes
(2) Oat gum
(3) Sugar
(4) Corn oil
(5) Partially hydrogenated corn oil
(6) Sea salt
(7) Soy lecithin
(8) Wheat flour
(9) Leavening (sodium bicarbonate)
(10) Natural flavors
(11) Sucrose
(12) Molasses
(13) Spices
(14) Wheat starch
(15) Tomato paste
(16) Dextrose
(17) Malt extract
(18) Maltodextrin
(19) Extracts of paprika
(20) Citric acid
(21) Beef fat
(22) Soy flour
(23) Corn syrup solids
(24) Barley malt flour
(25) Lactic acid
(26) Egg yolk
(27) Casein
(28) Peanut oil
(This ingredient list is a modified ingredient list for potato chips from a major manufacturer.)

Record Review/Recall
Review of 3-day food record or 24-hour diet recall

APPENDIX 18: Gluten Purification Protocol

1. Make sure the temperature of the room is 4-8 degrees Celsius.
2. Wash gluten with 5 mL/g of a solution of 50 mM ammonium bicarbonate and 1 M sodium chloride by soaking it in a bowl for **2 hours**.
3. After **2 hours**, strain the gluten in a strainer or cloth.
4. Wash the gluten with 5 mL/g of a solution of 50 mM ammonium bicarbonate and 1 M sodium chloride by soaking it in a bowl for **2 hours**.
5. After the **2 hours**, again strain the gluten in a strainer or cloth.
6. Wash the gluten with 5 mL/g of a solution of 50 mM ammonium bicarbonate and 1 M sodium chloride by soaking it in a bowl for **2 hours**.
7. After the **2 hours**, again strain the gluten in a strainer or cloth.
8. Wash the gluten with 5 mL/g of a solution of 50 mM ammonium bicarbonate and 1 M sodium chloride by soaking it in a bowl for **10 minutes**.
9. After the **10 minutes**, again strain the gluten in a strainer or cloth.
10. Wash the gluten with 5 mL/g of a solution of 50 mM ammonium bicarbonate and 1 M sodium chloride by soaking it in a bowl for **10 minutes**.
11. After the **10 minutes**, again strain the gluten in a strainer or cloth. This should be thorough, should de-enrich by 80-90%.
12. Lyophilize.
13. Store in container – any type (glass, plastic, etc).

APPENDIX 19: Granola Bars Recipe (Modified)

Makes 14 servings x 16 batches = 224 granola bars

Ingredients

375 g (4.5 cups) rolled oats x 16 batches = 6000 g of oats

140 g of gluten flour x 16 batches = 2240 g of gluten total

140 g of whole wheat flour x 16 batches = 2240 g of flour total

4.6 g (1 tsp) baking soda x 16 batches = 73.6 g of baking soda

4.33 g (1 tsp) vanilla extract x 16 batches = 73.6 g or 80 mL

157.8 g (2/3 cup) olive oil x 16 batches = 2667 mL olive oil

350 g (1 cup) rice malt syrup x 16 batches = 4000 mL

77.5 g (1/3 cup) packed brown sugar x 16 batches = 1240 g of brown sugar

177 g semisweet chocolate chips x **12** batches = 2142 g of chocolate chips

25 g pumpkin seeds (or, if chocolate-free, then 145 g pumpkin seeds)

Directions

1. Preheat oven to 325 degrees F (165 degrees C). Lightly grease one 9x13 inch pan.
2. In a large mixing bowl combine the oats, flour, baking soda, vanilla, oil, rice malt syrup and brown sugar. Stir in the chocolate chips and pumpkin seeds.
3. Lightly press mixture into the prepared pan. Bake at 325 degrees F (165 degrees C) for 18 to 22 minutes or until golden brown. Let cool for 10 minutes then cut into bars. Let bars cool completely in pan before removing or serving.

APPENDIX 20: Information Sheet for Urine Collection at Home

Dear study participant,

For the current study, we kindly ask you to collect your urine sample on the days that were communicated to you by the study staff. They will provide you with all necessary materials for collection purposes (plastic 50 mL container with orange top).

Collect your urine (Date: __/____/____)

Collect the **first morning urine** (mid-stream portion) directly into a plastic bottle provided by the investigators.

Write the **time and date** of urination on the urine container.

Preserve the Sample

You must put the bottle in the fridge (temperature 4-8C) in an upright position until you transfer it to the study staff.

Transfer to the study staff

Transport the urine sample in an upright position in a plastic bag (together with stool sample) as soon as possible.

APPENDIX 21: Information sheet for stool collection at home

Dear Sir or Madame,

Please read the following the instructions carefully.

For the current study, we kindly ask you to collect a stool sample before each visit. The study staff provided you with all necessary material for collection purposes (plastic plate, sample container, aluminum bag, AnaeroGen sachet, isotherm bag, ice block). You should put the ice block in the freezer as soon as possible.
Please collect your stool sample within 24 hours of your study visit by following the procedure below:

Collection of stool

- Defecate directly onto the plastic plate (or the cardboard plate, if provided)
- Transfer approximately 5 teaspoons (25 mL or just under 2 tbsp) of stool sample into the plastic container (white top container, 50 mL) using the provided spatula
- Close the lid but pay attention to **leave it a bit open** to allow the air to exchange
- **Note the date and time** of defecation on the container, as well as study ID or name

Preserve the stools

- Open the Oxoid sachet by tearing at the level of the notch
- Take the paper sachet that is inside and immediately place it into the smaller plastic bag
- Transfer the sample pot in the upright position in the smaller plastic bag (the pot lid must remain slightly opened to allow air exchange)
- Immediately seal the bag
- Place the smaller bag into the larger plastic bag and seal the larger bag
- After 30 minutes, put the bag in the FREEZER in the upright position until you transfer it to the study staff

Transfer to the STUDY Staff

- Transport the samples in an upright position in the isotherm bag after having added the ice block that was placed in advance in the freezer

APPENDIX 22: General rules for research coordinators and study participants

1. Prior to On-Site Arrival
   - Ensure that research coordinator and study participant have completed self-screen and have passed all screening criteria as required by MDCL and HHS sites
   - Ensure having a clean mask as required per current masking regulations for McMaster University/HHS site
   - Once on-site, a sign-in sheet will be available to document the research associate's name and arrival/departure time

2. Follow proper hygiene:
   - Wash hands thoroughly and regularly, following proper hand washing hygiene guidelines -- be sure to wash hands when entering and before leaving lab 3N59
   - Practice hand hygiene directly after contact with high touch areas
   - In areas with no proximity to a sink, use alcohol-based hand sanitizer (ensure hand sanitizer is available in the work area)
   - Avoid touching eyes, face, and mouth

3. Etiquette
   - Cover coughs and sneezes (cough/sneeze into upper sleeve, elbow, or tissue)
   - Turn away from others when coughing or sneezing
   - Respect and maintain 2 meters physical distancing

4. Masking
   - A mask must be worn in all public or shared spaces and at the time of contact with a study participant
   - When taking a new mask, ensure to wash your hands or apply hand sanitizer prior to reaching for fresh stock

5. Cleaning and Disinfection
   - At the start of work and prior to leaving, wipe down everything you have used with 70% ethanol (desktop, computer keyboard, telephone, computer screen, benchtops, fridge, freezer and lab equipment)
   - Use spray bottles of 70% ethanol and paper towels to disinfect common touchpoints (door handles, light switches, desk, etc.)
   - When traveling between rooms, please use paper towels to handle the doorknob and avoid contaminating your hands. Dispose of towel when you reach your destination.
   - Avoid sharing of work tools and equipment where possible

6. Shared Infrastructure
   - Common areas within the laboratory and office must adhere to physical distancing rules and be sanitized after use
   - Mechanisms will be in place to ensure common shared areas will not be congested

7. Physical Distancing
   - The research coordinator and study participant must maintain a distance of 2 meters from each other
   - You may chat with co-workers at break, but physical distancing rules must apply

8. Departure

- Please leave campus/HHS site promptly once essential tasks are completed

RESEARCH PLAN FOR LAB SPACE (3N59)

1. Research activities will be performed involving the work of Research Associates to support time-sensitive wet lab research which cannot occur from home – i.e. collection and processing of samples. These activities will be performed in room 3N59.
2. All staff are fully trained for the task they will execute and have adopted a "shift work" schedule to ensure that physical distancing is maintained. One (1) Research Associate from the team will be permitted on site at a time. Shifts will be tracked using a Google Calendar.
3. Self-monitoring of symptoms will continue to be vital. Anyone who feels unwell or may have been in contact with a possible COVID-19 case must not come to campus and should follow the advice on Ontario's COVID-19 self-screening website. The number of personnel in each room will be limited to ensure sufficient space to allow for appropriate physical distancing (2 meters).

CLEANING THE LAB AND OFFICE SPACE

1. Each day, research staff are responsible to regularly wipe down common surfaces/equipment using 70% ETOH. In general, every time a piece of equipment is used it should be wiped down BEFORE AND AFTER use with 70% ETOH. These areas include, but are not limited to: fridge/freezer door handles, centrifuge(s), door handles, drawer pulls, benchtops, faucets/taps, Lab Specimen Binder, computer, keyboard, desktop, printers, etc.
2. Housekeeping will increase the frequency of cleaning of highly touched surfaces, but you should avoid touching door handles wherever possible.
3. Keep all benches clean and tidy. All lab benches are to be wiped down with 70% ETOH before and after each use.
4. All shared equipment is to be wiped down with 70% ETOH before and after all processing.

ENTERING AND EXITING MCMASTER UNIVERSITY HSC/MUMC SITE

1. Entrance and exit procedures for the Health Sciences Centre is determined by the Faculty of Health Sciences. Please refer to the most recent notice in regard to entry requirements.
2. Consider the use of stairs to move between floors
3. Do not enter an elevator if 2 meters distancing is not possible
4. Everyone entering research workplaces must then proceed to wipe down both the external and internal door handles with 70% ETOH before engaging in any work. This ensures a clean door handle for the next person.
5. Wash hands or use hand sanitizer after touching high-touch surfaces
6. Upon exiting research workplaces ensure that all high touch areas including common lab benches, desks, keyboards, drawer handles, freezer/fridge handles door handles, light switches have been wiped down with 70% ETOH.

CHECKLIST FOR ENGAGING IN RESEARCH WORK IN OFFICE ROOMS

1. Enter room and immediately proceed to wipe down external and internal door handles with 70% ETOH with paper towels
2. Contain your personal belongings in a drawer at your desk. Use of high touch items such as a cell phone should be minimized
3. Ensure PPE is used as per guidelines
4. Wipe down your desk area, drawer handles, keyboard, mouse and all benches and lab equipment with 70% ETOH that are needed or used to complete your work
5. Complete your work in a timely fashion, do not hang around the office/lab unnecessarily
6. Physical distancing must be maintained during breaks. Remove all lab PPE before exiting the lab
7. When you have finished your lab work, wipe down all benches and equipment with 70% ETOH
8. Gather your things and do a final wipe down of your desk, drawer handles, and interior door handles with 70% ETOH. Leave the premises promptly after completing your daily tasks.

APPENDIX 23: Biohazard Container for Study Samples

SAF-T-PAK® STP-104 - 1.25L REUSABLE POLYPROPYLENE SECONDARY PRESSURE VESSEL, ORANGE LID, 6/CASE

180^{55}

SKU: 35154

Quantity

1

ADD TO CART

BUY IT NOW

FEATURES:

- For Category A or B, certified for use with the STP-100, STP-200, STP-320R and STP-340R.
- May be autoclaved for sterilization.

System Components:

- Polypropylene Container (1 ea.)
- Threaded Lid (1 ea.)
- O-Ring (1 ea.)
- Product Label (1 ea.)
- Box sealing tape

upport

APPENDIX 24: COVID-19 Measures for Patient Sample Handling

1. Preparing sample collection kits: Once the study coordinators thoroughly wash and sanitize their hands, they will prepare the stool and urine collection kits. The collection kit components are stored in a separated, low-traffic research staff room.
2. Mailing/drop off collection kits: Stool and urine collection kits will be placed in a package and will not be opened until delivered to the study participant's address.
3. Instructions for proper stool/urine collection:
    a. Participants will be instructed to wash their hands and wear gloves while collecting the samples.
    b. After collecting the sample, participants will clean the collection tube and place it in a double hermetic bag.
    c. The participants will then dispose of gloves and other used materials.
4. Instructions for pick up and transport: After the participant collects the sample, they will complete the online COVID-19 screening tool (Ontario COVID-19 Self-Assessment). If they pass the screening, they will coordinate a contactless pickup with the study coordinator where the sample will be placed in a specialized hermetic hazard container approved by Transport Canada.
5. Instructions for stool sample processing (aliquoting) and storage:
    a. Once samples are placed in the anaerobic chamber, the study coordinator will sanitize or wash their hands and put on gloves.
    b. Clean surfaces with 70% alcohol and make sure that other samples, cultures, and objects are far enough to prevent contamination if there are spills or splashes during sample aliquoting.
    c. Aliquot stool samples slowly from primary tube to smaller tubes placed in a clean plastic sample box.
    d. Once aliquoting is done, clean chamber surfaces and the closed sample box with alcohol 70%.
    e. Dispose of all used materials in a double biohazard bag. Remove the sample box and biohazard bags from the anaerobic chamber.
    f. Samples will be stored in a -80 freezer.

APPENDIX 25: Declaration of Helsinki

Declaration of Helsinki

World Medical Association Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964; amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. Introduction

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. Basic principles for all medical research

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the

APPENDIX 26: Serious Adverse Events Form

National Center for Complementary and Integrative Health

Serious Adverse Event (SAE) Report Form

STUDY NAME

Protocol Number:_____

Site Name:_____

Pt ID:_____

Date Participant Reported:

__ __ / __ __ __ / __ __ __ __
d d m m m y y y y

1. SAE onset date: ___ ___ / ___ ___ ___ / ___ ___ ___ ___
d d m m m y y y y

2. SAE stop date: ___ ___ / ___ ___ ___ / ___ ___ ___ ___
d d m m m y y y y

3. Location of SAE:_____

4. Was this an unexpected adverse event? ☐ Yes ☐ No

5. Brief description of participants with no personal identifiers:
   Sex: ☐ F ☐ M Age: _____
   Diagnosis for study participation:_____

6. Brief description of the nature of the SAE (attach description if more space is needed):

7. Category of the SAE:
   - ☐ Date of death ___/_____/_____ (dd/mmm/yyyy)
   - ☐ Life threatening
   - ☐ Hospitalization – initial or prolonged
   - ☐ Disability/incapacity
   - ☐ Congenital anomaly/birth defect
   - ☐ Required intervention to prevent permanent impairment
   - ☐ Other:_____

8. Intervention type:
   - ☐ Medication or nutritional supplement (specify):_____
   - ☐ Device (specify):_____
   - ☐ Surgery (specify):_____
   - ☐ Behavioral/lifestyle (specify):_____

9. Relationship of event to intervention:
   - ☐ Unrelated (clearly not related to the intervention)
   - ☐ Possible (may be related to intervention)
   - ☐ Definite (clearly related to intervention)

10. Was study intervention discontinued due to event? ☐ Yes ☐ No

11. What medications or other steps were taken to treat the SAE?

    _____

12. List any relevant tests, laboratory data, and history, including preexisting medical conditions:

    _____

13. Type of report:
    - ☐ Initial
    - ☐ Follow-up
    - ☐ Final

Signature of principal investigator: _____ Date: _____