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The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota (PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a Randomized Controlled Trial

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Manuscripts

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3 1 **The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota**
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5 2 **(PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a**
6
7 3 **Randomized Controlled Trial**
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22 **ABSTRACT**

23 *Introduction:* Excess body weight is associated with a state of low-grade chronic inflammation
24 and alterations of the gut microbiome. Powdered meal replacements (PMR) have been shown
25 to be an effective strategy for weight management; however, their effect on inflammation and
26 the gut microbiome remains unclear. The aim of this 12-week randomized control clinical trial
27 is to investigate the effects of PMR consumption on inflammation, gut microbiome, and overall
28 metabolism in individuals with excessive body weight.

29 *Methods and analysis:* Healthy adults with excess body weight (n=88) are being recruited and
30 randomly assigned to one of the following groups: a) Control group (CON): maintaining usual
31 diet for 12 weeks, or b) PMR group: replacing morning and afternoon snacks daily with a PMR
32 for 12 weeks. Participants are asked to maintain body weight throughout the study and fill out
33 a journal with information about PMR consumption, body weight, food intake, appetite
34 sensations, and medications. Three study visits are required: baseline, week 6, and week 12.
35 Outcome measures include systemic inflammatory biomarkers, gut microbiome composition,
36 metabolic blood markers, host energy metabolism, body composition, appetite sensations, and
37 host gene expression profile.

38 *Ethics and dissemination:* This research protocol was approved by the University of Alberta
39 Ethics Board (Pro00070712) and adheres to the Canadian Tri-Council Policy statement on the
40 use of human participants in research. Procedures and potential risks are fully discussed with
41 participants. Study findings will be disseminated in peer-reviewed journals, conference
42 presentations, and social media.

43 *Registration details:* ClinicalTrials.gov identifier: NCT03235804.

44
45 **Keywords:** Powdered meal replacement; obesity; inflammation; gut microbiome.

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3 47 **ARTICLE SUMMARY**
4

5 48 **Strengths and Limitations of this study**
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- 7
8 49 • This will be the first 12-week randomized controlled clinical trial to investigate the
9
10 50 effects of a powdered meal replacement on inflammation, gut microbiome, metabolism,
11
12 51 and gene expression profile in weight-stable individuals with excess body weight.
13
14
15 52 • Methodological strengths include the rigorous study design, the use of state-of-the-art
16
17 53 technology such as the metabolic chamber for indirect calorimetry, dual-energy X-ray
18
19 54 absorptiometry, and up-to-date methods for gut microbiome analysis, gene expression,
20
21 55 and genetic polymorphisms.
22
23
24 56 • Additional strengths regarding study design include stable body weight throughout the
25
26 57 study period, regular assessments and follow-ups, and a comprehensive variety of
27
28 58 outcomes assessed.
29
30
31 59 • The main limitation is the lack of placebo and the study is not double-blinded. The gut
32
33 60 microbiome analysis relies on fecal samples; thus, it may not capture changes in gut
34
35 61 microbiome composition of more proximal parts of gastrointestinal tract.
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40 63 **Word-count:** 4241 words.
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64 INTRODUCTION

65 Excess body weight can be defined as a body mass index (BMI) ≥ 25.0 kg/m² (1). This
66 condition has been associated with a state of systemic low-grade chronic inflammation, which
67 is characterized by a persistent activation of immune and non-immune cells and production of
68 cytokines, chemokines, and acute phase proteins (2, 3). Those inflammatory biomarkers
69 include interleukins (IL), such as IL-6 and IL-8, tumor necrosis factor- α (TNF- α), and C-
70 reactive protein (CRP) (2). Systemic low-grade chronic inflammation causes tissue and organ
71 damage, which can, in turn, lead to the onset and progression of chronic diseases, such as
72 diabetes mellitus, cancer, metabolic syndrome, and cardiovascular diseases (2).

73 In individuals with excessive body weight, the state of systemic low-grade chronic
74 inflammation can be mediated by increased adiposity, as well as by mechanisms through the
75 gut microbiota (2). Increased adipocyte size (i.e., hypertrophy) is associated with cellular
76 dysfunction and distress (4, 5). Hypertrophic adipocytes secrete an increased number of pro-
77 inflammatory chemokines, such as TNF- α , IL-6, IL-8, and monocyte chemoattractant protein
78 1 (MCP-1) (3-5). The increased size of adipocytes and cytokine production lead to adipose
79 tissue hypoxia and death, as well as local and systemic inflammation (3-5).

80 Excess body weight is associated with altered gut microbiome composition and reduced
81 microbiome diversity, which might cause metabolic aberrations and enrich for opportunistic
82 pathogens (e.g. at the epithelial interface) that contribute to inflammation (6, 7). Individuals
83 with excessive body weight usually present with altered gut permeability, which elevates
84 systemic levels of endotoxins (i.e., lipopolysaccharides) (2). When in the bloodstream,
85 lipopolysaccharides binds to toll-like receptor 4 leading to activation of nuclear factor kappa B
86 and consequently production of pro-inflammatory cytokines, including IL-6 and TNF- α (7).

87 Considering the numerous negative health outcomes associated with excess body
88 weight, much effort has been made to develop effective weight management strategies. Among

1
2
3 89 those are meal replacements, which are food products fortified with vitamins and minerals used
4
5 90 to replace one or more meals per day. Meal replacements are commonly used in association
6
7 91 with calorie restriction. Research has shown that the consumption of meal replacements leads
8
9 92 to greater weight loss when compared to reduced-calorie diets alone (8, 9). Improvement in
10
11 93 metabolic parameters is generally observed with weight loss, including improvement in
12
13 94 glucose metabolism, reduction of triacylglycerol, low-density lipoprotein cholesterol (LDL-C),
14
15 95 systolic blood pressure (8, 10, 11), and the inflammatory markers CRP and IL-6 (12).
16
17 96 Considering the positive health effects of weight loss in individuals with excessive body weight
18
19 97 (13-15) and the beneficial health effects of meal replacements (8, 10), it is important to
20
21 98 differentiate the effects of meal replacements from that of weight loss on overall health, which
22
23 99 have not been investigated so far. Therefore, the aim of this study is to compare the effects of
24
25 100 a 12-week consumption of a powdered meal replacement (PMR group) versus usual diet
26
27 101 (control group, CON) on inflammation, gut microbiome, overall metabolic health, gene
28
29 102 expression profile, and genetic background in individuals with excessive body weight who are
30
31 103 in weight maintenance.
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40 105 **METHODS**

41 106 **Study design and ethical procedures**

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44 107 This study is a randomized, controlled, parallel group, clinical trial conducted at the
45
46 108 Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada). The
47
48 109 corresponding research protocol fulfils the requirements of the Standard Protocol Items:
49
50 110 Recommendations for Interventional Trials (SPIRIT) checklist (16). This research protocol was
51
52 111 approved by the University of Alberta Ethics Board (HREB, identifier Pro00070712) and
53
54 112 complies with the standards established by the Canadian Tri-Council Policy statement on the
55
56 113 use of human participants in research. Procedures and potential risks involved in the study are
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114 discussed with participants prior to obtaining informed consent. This protocol is registered on
 115 ClinicalTrials.gov (NCT03235804), and recruitment started on April 2019 and is expected to
 116 finish in November 2023 (**Table 1**).

117 **Table 1.** World Health Organization trial registration dataset

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03235804
Date of registration in primary registry	August 1, 2017
Secondary identifying numbers	University of Alberta Research Ethics Board # Pro00070712
Source(s) of monetary or material support	Almased Wellness-GmbH (Bienenbüttel, Germany)
Primary sponsor	Almased Wellness-GmbH (Bienenbüttel, Germany)
Secondary sponsor(s)	N/A
Contact for public queries	Dr Carla Prado +1 (780) 492-9555 carla.prado@ualberta.ca and Jens Walter +353 (0)21 490-1773 jenswalter@ucc.ie
Contact for scientific queries	Dr Carla Prado +1 (780) 492-9555 carla.prado@ualberta.ca and Jens Walter +353 (0)21 490-1773 jenswalter@ucc.ie
Public title	The impact of a powdered meal replacement on metabolism and gut microbiota (Premium Study)
Scientific title	The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight (The PREMIUM Study)
Countries of recruitment	Canada
Health condition(s) or problem(s) studied	Overweight and obesity
Intervention(s)	Powdered meal replacement
Key inclusion and exclusion criteria	Inclusion Criteria: (a) female/male aged 18 to 50 years; (b) non-smoker; (c) body mass index (BMI) between 25 and 37 kg/m ² ; (d) weight stable; (e) fat mass ≥20% for men and ≥25% for women; (f) stable physical activity level. Exclusion Criteria: (a) diagnosis of chronic diseases or acute infections; (b) taking any medication that may alter study outcomes; (c) taking pre- and probiotics; (d) use of antibiotics

	in the past two months; (e) females that are pregnant or lactating.
Study type	Randomized controlled trial
Date of first enrolment	April 1, 2019
Sample size	88
Recruitment status	Actively recruiting
Primary outcome(s)	Interleukin-6
Key secondary outcomes	Gut microbiota
Ethics review	University of Alberta Research Ethics Board # Pro00070712
Completion date	N/A
Summary results	N/A
Individual Participant Data (IPD) sharing statement	De-identified data will be shared with the participant upon completion of the study (publication)

118

119 Outcome measures

120 The primary study outcome is change in IL-6 concentration over time (within groups) and
 121 between the PMR and CON groups. Secondary outcome is change in gut microbiome
 122 composition over time (within groups) and between the PMR and CON groups. Exploratory
 123 outcomes include:

- 124 • Change in markers of systemic inflammation (high-sensitivity CRP [hs-CRP], IL-8, and
 125 TNF- α) and immune modulation (IL-10) over time (within groups) and between the
 126 PMR and CON groups.
- 127 • Change in concentrations of metabolic blood markers (glucose, insulin, total
 128 cholesterol, LDL-C, high-density lipoprotein cholesterol [HDL-C], triglycerides,
 129 peptide tyrosine-tyrosine [PYY], glucagon-like peptide-1 [GLP-1], ghrelin,
 130 adiponectin, leptin, free glycerol, free fatty acids, and thyroid stimulating hormone
 131 [TSH]) over time (within groups) and between the PMR and CON groups.

- 1
2
3 132 • Change in resting energy expenditure (REE) and respiratory exchange ratio (RER) over
4
5 133 time (within groups) and between the PMR and CON groups.
6
7
8 134 • Change in body composition (fat mass [FM] and lean soft tissue [LST]) over time
9
10 135 (within groups) and between the PMR and CON groups.
11
12
13 136 • Change in appetite sensations (hunger, satiety, fullness, and prospective food
14
15 137 consumption) over time within the PMR group.
16
17 138 • Differences in the responses to the intervention according to genetic polymorphisms
18
19 139 over time.
20
21
22 140 • Changes in inflammation and excess body weight-related gene expression profile over
23
24 141 time and between the PMR and CON groups.
25
26
27 142

143 **Research participants**

144 Inclusion criteria are as follows: male or female; non-smoker; between 18 and 50 years
145 of age; BMI between 25.0 and 37.0 kg/m²; with a stable body weight 6 months prior to study
146 initiation (i.e., variation <5 kg); fat mass ≥20% for males and ≥25% for females; willingness
147 to maintain stable physical activity level throughout the study; and females must use effective
148 birth control methods.

149 Exclusion criteria includes participation in >3 hours per week of vigorous physical
150 activity; pregnancy or lactation; diagnosis of any chronic or acute diseases (except for excess
151 body weight); use of any medication that impacts study outcomes, except for antidepressants,
152 anxiolytic, and/or thyroid replacement therapy in a stable dose 3 months prior to study initiation
153 and throughout the study period; use of antibiotics 2 months prior to study initiation; use of
154 protein supplements 1 month prior to study initiation; allergy to PMR ingredients (soy, honey,
155 and yogurt); allergy or intolerance to soy, gluten, and/or lactose; following a vegetarian, vegan,
156 or any other restrictive dietary pattern; claustrophobia; or being unable to comprehend and

1
2
3 157 complete the required questionnaires. Participants consuming supplements or food items that
4
5 158 contain pre- or probiotics (e.g., kefir or kombucha) before being enrolled in the study will be
6
7 159 asked to discontinue the use of these products and wait 1 month before starting the study. The
8
9 160 use of other nutritional supplements, such as multivitamins and vitamin D₃ will be allowed if
10
11 161 on a stable dose.
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17 163 **Recruitment, randomization, and intervention**

18
19 164 Study advertisement is done using flyers displayed at the University of Alberta
20
21 165 campuses, surrounding communities, other post-secondary education institutions in Edmonton
22
23 166 (AB, Canada), and health care centres in the city. The study is also advertised in University of
24
25 167 Alberta email lists, newspapers, classrooms presentations, and on social media (e.g., Kijiji,
26
27 168 Facebook, and Twitter). Additionally, a personalized website (premium.ualberta.ca) was
28
29 169 created.
30
31
32

33 170 Individuals interested in being part of the study will be invited to attend a screening
34
35 171 visit at the HNRU. This visit will include anthropometric measurements (i.e., height, weight,
36
37 172 and waist circumference), body composition assessment (bioelectrical impedance analysis
38
39 173 [BIA]), blood tests (i.e., creatinine, estimated glomerular filtration rate [eGFR], albumin,
40
41 174 aspartate transaminase [AST], alanine transaminase [ALT], sodium, potassium, chloride, and
42
43 175 TSH), review of medical history, and completion of a physical activity questionnaire. If deemed
44
45 176 eligible, participants are randomly assigned into either the CON or PMR group. Randomization
46
47 177 is stratified by sex using a Microsoft Office Excel[®] spreadsheet.
48
49
50

51 178 Participants assigned to the CON group are asked to maintain their usual diet for 12
52
53 179 weeks. The ones in the PMR group are asked to replace their morning and afternoon snacks
54
55 180 using a powdered meal replacement (Almased USA, Inc., St. Petersburg, FL, USA) and
56
57 181 otherwise maintain their usual diet for 12 weeks. Each snack is replaced by 50 grams of powder
58
59
60

182 mixed with 250 mL of water. The nutritional information of the meal replacement is displayed
 183 in **Table 2**.

184 **Table 2.** Nutritional information of the powdered meal replacement (PMR)

Nutrient	50 g of Product (PMR)
Calories (kcal)	180
Total fat (g)	1.0
<i>Saturated fat (g)</i>	0.5
<i>Trans fat (g)</i>	0
<i>Polyunsaturated fat (g)</i>	0.1
<i>Monounsaturated fat (g)</i>	0.4
Cholesterol (mg)	3
Total carbohydrates (g)	15
<i>Dietary fiber (g)</i>	0.5
<i>Sugars (g)</i>	15
Protein (g)	27
Sodium (mg)	340
Potassium (mg)	500
Vitamin A (IU)	794
Vitamin C (mg)	16
Vitamin E (IU)	6
Thiamin (Vitamin B1) (mg)	5
Riboflavin (Vitamin B2) (mg)	6
Vitamin B6 (mg)	7
Calcium (mg)	215

Iron (mg)	4.9
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186

187 **Experimental protocol**

188 The study design is illustrated in **Figure 1**. The schedule of enrollment, interventions,
189 and assessments are shown in **Figure 2**. Following the screening visit and randomization
190 process, enrolled participants are invited to attend 3 study visits: baseline, week 6, and week
191 12. Assessments during each of these visits include: 1-hour resting metabolic rate (RMR),
192 blood draw, body composition, and physical activity questionnaire. They additionally receive
193 stool collection kits and instructions for fecal sample collection. During the baseline visit,
194 participants receive a scale and a journal to record the following information daily: body
195 weight, date and time of meal replacement intake (PMR group only), and medication intake (if
196 any). They are also asked to record on a weekly basis a 24h dietary recall (both groups) and fill
197 out appetite sensation questionnaires (PMR group only). Instructions on how to fill out the
198 journal and dietary records are given. Additionally, participants assigned to the PMR group
199 receive 84 packages of the PMR during visits at baseline and week 6, as well as instructions on
200 how to prepare it. Those assigned to the PMR group start consuming the supplement the day
201 after the first stool sample collection. Study materials (study journal and scale) are returned on
202 week 12.

203 A member of the study team contacts participants weekly to verify adherence to the
204 dietary intervention and potential adverse events. Their body weight is also discussed at that
205 time. If a body weight change greater than $\pm 2\%$ of their initial body weight is noticed, a
206 nutrition consult with a Registered Dietitian is scheduled to provide instructions on how to
207 increase or decrease food intake and physical activity levels to return to baseline body weight.

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3 209 *Anthropometry and body composition*
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5 210 At the screening visit, anthropometric measurements are taken twice, and the average
6
7 211 is used for data analysis. Height is measured using a digital stadiometer (235 Heightronic™,
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9 212 Concepts, Quick Medical, Snoqualmie, WA, USA) to the nearest 0.1 cm. Body weight is
10
11 213 measured to the nearest 0.1 kg using a calibrated digital scale (Health-o-meter® Professional
12
13 214 Remote Display, Sunbeam Products Inc., FL, USA). Waist circumference is measured using a
14
15 215 measuring tape at the level of participant's belly button, as per standard procedure (17).

16
17 216 A digital scale (HD-314 TANITA Corporation, Tokyo, Japan) is provided to
18
19 217 participants during the baseline visit, which is returned at the study completion. Body weight
20
21 218 is recorded daily in the morning in a fasting state, and with an empty bladder.

22
23 219 Body composition is assessed using dual energy X-ray absorptiometry (DXA, GE
24
25 220 Lunar iDXA, General Electric Company, Madison, USA), air displacement plethysmography
26
27 221 (ADP, Bod Pod 1SB-060M, Life Measurement Instruments, Concord, CA, USA), and BIA
28
29 222 (Seca mBCA525, Seca GmbH & Co, Hamburg, Germany). A number of techniques is being
30
31 223 used to explore potential changes in body composition using multicompartment modeling (18,
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33 224 19).

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37 226 *Resting energy expenditure*
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41 227 Resting energy expenditure is assessed by indirect calorimetry using an open-circuit
42
43 228 metabolic chamber, which measures the volume of oxygen (O₂) and carbon dioxide (CO₂) from
44
45 229 participant's respiration. Participants lie down in a relaxed position without falling asleep and
46
47 230 breathe normally for 60 minutes. Mixed air with the expired CO₂ is drawn from the chamber
48
49 231 at a constant flow rate (60 ± 2 L/min) while fresh air with constant O₂ is passively drawn into
50
51 232 the chamber. The first 30 minutes of the test are considered time for acclimatization and hence
52
53 233 removed from analysis. Gas exchange (volume of CO₂ and O₂) is analysed minute-by-minute
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234 by the Advance Optima AO2000 Series CO₂ analyser (ABB Automation GmbH, Frankfurt,
 235 Germany) and the Oxymat 6 O₂ analyser (Siemens AG, Munich, Germany). Data is transferred
 236 from those analysers to a computer (Acer Aspire AM3910-E3122, Acer Inc., New Taipei City,
 237 Taiwan) via the National Instruments NI USB-6221 device (National Instruments Corporation,
 238 Austin, Tex., USA) using the PMCSS Software version 1.8 (Pennington Metabolic Chamber
 239 Software Suite, Pennington Biomedical Research Center, La., USA). Resting energy
 240 expenditure (kcal/day) is calculated using the average kcal/min multiplied by 1440.

241

242 *Blood analysis*

243 Blood is sampled from participants by venipuncture after an overnight fast during the
 244 screening visit and at baseline, week 6, and week 12. Evaluated biomarkers are listed in **Table**
 245 **3**.

246 **Table 3.** Blood parameters, sample, and laboratory responsible for blood analysis

Parameter	Screening	Baseline	Week 6	Week 12	Sample	Laboratory
Albumin	x				Serum	External
Creatinine/eGFR	x				Serum	External
ALT	x				Serum	External
AST	x				Serum	External
Electrolytes ^a	x				Serum	External
TSH	x	x	x	x	Serum	External
hs-CRP		x	x	x	Serum	External
Glucose		x	x	x	Serum	External
Insulin		x	x	x	Serum	External
Lipid panel ^b		x	x	x	Serum	External
Leptin		x	x	x	Serum	On site

Free glycerol	x	x	x	Serum	On site
Free fatty acids	x	x	x	Serum	On site
Interleukins ^c	x	x	x	Plasma	On site
TNF- α	x	x	x	Plasma	On site
Adiponectin	x	x	x	Plasma	On site
PYY	x	x	x	Plasma	On site
GLP-1	x	x	x	Plasma	On site
Ghrelin	x	x	x	Plasma	On site
Polymorphisms	x			Whole blood	On site
Gene expression	x		x	Whole blood	On site

^a Electrolytes include chloride, sodium, and potassium. ^b Lipid panel include triglycerides, total cholesterol, LDL-C, and HDL-C. ^c Interleukins include IL-6, IL-8, and IL-10. Abbreviations: ALT alanine aminotransferase; AST aspartate aminotransferase; eGFR estimated glomerular filtration rate; GLP-1 glucagon like peptide; hs-CRP high-sensitivity C-reactive protein; PYY peptide tyrosine-tyrosine; TNF- α tumor necrosis factor α ; TSH thyroid stimulating hormone.

Blood samples are collected using BD Vacutainer[®] tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Tubes containing silica and a polymer are used for serum separation, tubes containing K2-ethylenediaminetetraacetic acid (EDTA) are used for plasma separation, and tubes containing K2EDTA and protease inhibitors (dipotassium and tacrine, BD P800) are used for GLP-1 and ghrelin analysis.

Creatinine, eGFR, albumin, AST, ALT, sodium, potassium, chloride, and TSH are analysed by an external lab (DynaLIFE Medical Labs, Edmonton, AB, Canada) at the screening visit prior to enrollment. Glucose, insulin, lipid panel (triglycerides, total cholesterol, LDL-C, and HDL-C), TSH, and hs-CRP will be analysed by DynaLIFE Medical Labs (Edmonton, AB,

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3 262 Canada). Interleukin 6, IL-8, IL-10, TNF- α , PYY, GLP-1, ghrelin, adiponectin, leptin, free
4
5 263 glycerol, and free fatty acids will be analysed in our laboratory (University of Alberta, AB,
6
7
8 264 Canada).

9
10 265 An additional blood draw is requested the day following each study visit for hs-CRP
11
12 266 analysis due to this being a sensitive marker which can vary substantially within hours of
13
14 267 collection for several reasons (20). Therefore, the average of CRP measured on two consecutive
15
16 268 days will be taken in case they are similar. If a participant is in an infectious state (i.e., CRP
17
18 269 >10 mg/L or significant changes between the two days measurement) the highest value will be
19
20
21 270 excluded from analysis.

22
23
24 271 For gene expression profile, ribonucleic acids (RNAs) will be sequenced at baseline
25
26 272 and week 12. Whole blood (500 μ L) is aliquoted into an RNase-free microfuge tube and added
27
28 273 1.3 mL of RNAlater stabilization solution (Thermo Fisher Scientific, Waltham, MA, USA).
29
30 274 Total RNA will be extracted from whole blood using the RiboPure™ Blood Kit (Thermo Fisher
31
32 275 Scientific, Waltham, USA). The RNA purity will be determined by measuring the 260/280 nm
33
34 276 ratio (ideal ratio ~2.0) and the 260/230 nm ratio (ideal ratio 2.0-2.2) using a spectrophotometer.
35
36 277 The quality of RNA samples will be evaluated prior to library preparation for RNA-Seq, using
37
38 278 a bioanalyzer and an RNA Integrity Number (RIN) ≥ 7 will be accepted. Samples of high purity
39
40 279 and quality RNA will be prepared with the TruSeq RNA Sample Prep kit (Illumina, San Diego,
41
42 280 USA). The sequencing will be performed by an external company using the platform Illumina
43
44 281 HiSeq 4000 (Illumina, San Diego, USA), in the paired-end mode, in which the 2 ends will be
45
46 282 sequenced with a length of 100 base pairs (bp) (2 x 100 bp). At the end, 2 files in the 'fastq'
47
48 283 format will be generated for each of the evaluated samples. The evaluation of the quality of the
49
50 284 sequences will be performed with the FastQC tool. The Trimmomatic software (21) will be
51
52 285 used to remove low-quality strings and adapters. Then, the libraries will be evaluated again in
53
54 286 the FastQC software, for proper verification. The RNA sequencing data will be subjected to
55
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1
2
3 287 analysis by RNA-seq using the protocol described in Trapnell, Roberts (22). The functional
4
5 288 annotation of differentially expressed genes will be carried out through the GeneOntology
6
7 289 platform (<http://geneontology.org>). Analyses to identify differentially expressed metabolic
8
9
10 290 pathways will be performed using the fgsea package of the R software.

11
12 291 Genetic polymorphisms will be analysed at baseline. Genomic deoxyribonucleic acid
13
14 292 (gDNA) will be extracted with the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) from
15
16
17 293 leukocytes in peripheral blood. The gDNA purity will be verified in a spectrophotometer at
18
19 294 260 and 280 nm. The samples will be considered of good quality if the ratio between
20
21 295 absorbances is between 1.7 and 2.0. The gDNA concentration will be measured on a
22
23 296 fluorimeter. For genotyping, a customized Infinium Global Screening Array-24 + v3.0 Kit
24
25 297 (Illumina, San Diego, USA) will be used. Inflammation and excess body weight-related genetic
26
27 298 polymorphisms will be analyzed, which will be selected from the results of the differential
28
29 299 expression of genes, and will be evaluated with the R package 'argyle' (23).

30
31
32
33 300

34 35 301 *Fecal sample collection and gut microbiome sequencing*

36
37 302 A total of three fecal samples are collected at baseline, week 6, and week 12. Fecal
38
39 303 samples are either collected at the HNRU the day of the study visits, or at home, kept at room
40
41 304 temperature, and delivered to the HNRU as soon as possible. During the baseline visit,
42
43 305 participants are instructed on how to collect fecal samples using the provided collection kits.
44
45 306 The fecal collection tubes (DNA/RNA Shield, Zymo Research, Irvine, CA, USA) preserve
46
47 307 nucleic acids in the sample and maintain stability at room temperature. Once delivered to the
48
49 308 lab, the fecal sample tubes are frozen at -80°C until processing and analysis.

50
51 309 The microbial DNA will be extracted from all samples including positive and negative
52
53 310 controls, using QIAamp Fast DNA Stool Mini Kit as previously described (24), packed with
54
55 311 dried-ice, and shipped to University of Minnesota Genomic Center (Minnesota, US) for
56
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60

1
2
3 312 sequencing. Shipping will adhere the regulation of Environment, Health and Safety
4
5 313 Department, University of Alberta. MiSeq Illumina technology (300 bp pair-end) will be used
6
7
8 314 to sequence 16S ribosomal ribonucleic acid (rRNA) targeting V5-V6 region to characterize the
9
10 315 fecal microbiome composition using primer pair 784F [5'-RGGATTAGATACCC -3'] and
11
12 316 1064R [5'-CGACRRCCATGCANACCT-3'].

317

318 *Physical activity questionnaire*

319 The Godin-Shephard leisure-time physical activity questionnaire will be completed at
320 baseline, week 6, and week 12 to estimate physical activity levels (25, 26). In this questionnaire,
321 participants answer how often they perform strenuous, moderate, and light exercise for more
322 than 15 minutes in one week. A physical activity score is calculated based on intensity = $(9 \times$
323 $\text{strenuous}) + (5 \times \text{moderate}) + (3 \times \text{light})$ (26, 27). This will be used to classify participants as
324 insufficiently active (<14 units), moderately active (≥ 14 and <24 units), or active (≥ 24 units)
325 (27).

326

327 *Dietary intake*

328 The dietary intake will be assessed using the online Automated Self-Administered 24-
329 hour Recall (ASA24[®]) Canada (28). A paper-based version is available per individual
330 participant and is returned to the study team weekly by email or fax. Dietary information is
331 entered in ASA24[®] to ensure consistency. Three 24h recalls are completed at weeks 1, 6, and
332 12 (two weekdays and one weekend day) and one 24h recall per week on the remaining weeks
333 of the study period (one weekday). Energy, macronutrients, and micronutrients intake will be
334 obtained using ASA24[®] automated coding based on the amount of each food consumed.

335

336 *Appetite sensations*

1
2
3 337 To assess how the PMR affects appetite, participants assigned to the PMR group rate
4
5 338 their appetite sensations using the study journals once a week and at five timepoints: 1)
6
7 339 immediately after waking up/fasting, 2) immediately before the morning PMR consumption,
8
9 340 3) 30 minutes after the morning PMR consumption, 4) immediately before the afternoon PMR
10
11 341 consumption, and 5) 30 minutes after the afternoon PMR consumption. Hunger, satiety,
12
13 342 fullness, and prospective food consumption will be assessed using a paper-and-pen 100-mm
14
15 343 visual analogue scale (29). They are instructed to make a single vertical mark between 2
16
17 344 anchors to indicate the intensity of their subjective states regarding each element, on a scale
18
19 345 from 0 to 100 mm. The following questions are asked: How hungry do you feel? (I am not
20
21 346 hungry at all – I have never been more hungry); How satisfied do you feel? (I am completely
22
23 347 empty – I cannot eat another bite); How full do you feel? (not at all full – totally full); How
24
25 348 much do you think you can eat? (nothing at all – a lot).

30
31 349

32 350 *Adherence and withdraw/discontinuation*

33
34
35 351 Participants are immediately withdrawn from the study if they: 1) have significant
36
37 352 variation in body weight ($>\pm 2\%$ of baseline body weight) that does not return to baseline 2
38
39 353 weeks after the nutrition consult; 2) become pregnant; 3) start or change medications or
40
41 354 supplement intake listed in the eligibility criteria; 4) no longer meet the inclusion criteria.
42
43 355 Participants assigned to the PMR group are asked to return all supplement bags (empty or not)
44
45 356 to the visits on week 6 and 12. These are weighted, and participants are excluded from the
46
47 357 study if the PMR have not been consumed twice daily during the 12 weeks or if there is $>20\%$
48
49 358 of product left inside the bags. In addition, participants can withdraw from the study at any
50
51 359 time.

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54 360

55 361 **Statistical analyses**

1
2
3 362 *Sample size estimate*
4

5 363 A total of 74 participants (37 in each group) will be needed to detect a medium effect
6 size of 0.669. The effect size was calculated based on a previously published study (30), in
7
8 364 which the mean percent change in IL-6 from baseline to 12 months was -6.76 ± 36.95 pg/mL
9
10 365 in a group receiving soy protein versus 17.62 ± 35.92 pg/mL in the control group. Accounting
11
12 366 for a 20% attrition rate, the total sample size of 88 participants (44 in each group) will have a
13
14 367 power of 80% with a significance level of 5%. The sample size calculation was done using
15
16 368 G*Power version 3.1.9.2. Interim analysis will be conducted (n=44), and sample size will be
17
18 369 adjusted accordingly.
19
20
21
22
23

24 371

25
26 372 *Data analysis*
27

28 373 Normality of the study variables will be assessed by the Shapiro-Wilk W-test. By
29
30 374 inspecting boxplots, values >1.5 box-lengths from the edge of the box will be considered as
31
32 375 outliers and may be excluded from analysis. Baseline characteristics between groups will be
33
34 376 assessed by the independent t-test or Mann–Whitney test, according to data distribution.
35
36 377 Differences between groups of nominal variables will be analysed by Pearson's χ^2 test or
37
38 378 Fisher's exact test. Both group effect and time effect will be analyzed using a two-way mixed
39
40 379 analysis of variance (ANOVA) or analysis of covariance (ANCOVA) as appropriate.
41
42 380 Assumption of homogeneity of variances will be tested using Levene's test of equality of
43
44 381 variances. Correlation between variables will be assessed by Pearson's correlation. If
45
46 382 significant correlations between nutrients and energy intake are noticed, the residual method
47
48 383 will be applied in order to describe the relationship between aspects of food intake and
49
50 384 biochemical characteristics independent of energy intake (31). All analyses will be performed
51
52 385 using IBM® SPSS® Statistics version 24 (International Business Machines Corporation),
53
54 386 considering a critical significance value of 5%, unless otherwise stated.
55
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1
2
3 387 Regarding genetic polymorphisms analysis, adherence to the Hardy-Weinberg
4
5 388 equilibrium will be checked using the χ^2 -square test. To verify whether the results differ among
6
7 389 the genotypes, the dominant model (major allele x heterozygous + minor allele) will be applied.
8
9
10 390 Effect size (ES) will be assessed by Cohen's d-test and multivariate analysis (MANOVA) will
11
12 391 be applied, including time and genotype as the two independent variables. For post-hoc
13
14 392 analysis, the Stell Dwass test ($p < 0.05$) will be applied. Statistical analysis of the gene
15
16 393 expression profile will be performed with bioinformatic tools.

17
18
19 394 For the gut microbiome analysis, raw sequencing data will be undergone multiple
20
21 395 quality control steps including primer removal, trimming, chimera removal as previously
22
23 396 described (32, 33). Sequences will be classified using classify-sklearn algorithm (34) against
24
25 397 Silva database v.138 generated for given primers by RESCRIPT (35). To decontamination, non-
26
27 398 target sequences will be removed such as mitochondria, chloroplast and archaea. Possible
28
29 399 contamination detected by positive and negative controls and sequences with raw count < 3 and
30
31 400 present in $< 10\%$ of samples will be removed. Sample with an extremely low number of reads
32
33 401 (< 2000 after filtering) will not be considered in microbiome analysis. Filtered ASV table in
34
35 402 count data will be converted to relative abundant data for visualisation, then centered log-ratio
36
37 403 (CLR) transformed. The indices of α -diversity (e.g., observed species, Shannon, Phylogenetic
38
39 404 Diversity, Inver Simpson) and β -diversity (e.g., Bray-Curtis and Aitchison distance) will be
40
41 405 calculated using Phyloseq (36) and microbiome (37) R packages. Bray-Curtis distance will be
42
43 406 used to generate non-parametric multidimensional scaling ordination plots for β -diversity
44
45 407 metrics with scaled and centered results. Stability over time will also be assessed based on
46
47 408 Bray-Curtis distances. The adonis2 function in R package vegan will be used for permutational
48
49 409 multivariate analysis of variance (PERMANOVA). Aitchison distance will be used for
50
51 410 Principal component analysis (PCA) in mapping microbiome and metabolic markers data to
52
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1
2
3 411 exploring multidimensional association (24). False discovery rate (FDR) will be used to adjust
4
5 412 p values, and $FDR < 0.05$ will be considered as statistically significant.
6
7

8 413

9
10 414 **Patient and public involvement:**

11
12 415 None.
13
14

15 416

16
17 417 **ETHICS AND DISSEMINATION**

18
19 418 This study is approved by the University of Alberta's HREB (Pro00070712) and is
20
21 419 registered on ClinicalTrials.gov (NCT03235804). This research adheres to the standards as set
22
23 420 out in the Canadian Tri-Council Policy statement on the use of human participants in research.
24
25 421 This study is regulated by Health Canada. Amendments will be submitted to the HREB and
26
27 422 Health Canada review and approval prior implementations. ClinicalTrials.gov will be updated
28
29 423 accordingly.
30
31

32
33 424 All personal information is kept private, and participation is anonymous. Participants
34
35 425 are assigned a study ID, which is kept separated from any personal information collected. A
36
37 426 master list with identifiable information and study IDs is cryptographically protected and stored
38
39 427 at the HNRU. All personal information will be kept in a locked cabinet for 5 years after the
40
41 428 completion of the study. If participants withdraw consent, they are asked for permission to use
42
43 429 the data collected until that point; however, if they deny it, their data is destroyed. Absence of
44
45 430 answer is considered as permission to use the data. The Quality Management in Clinical
46
47 431 Research (QMCR) Department at the University of Alberta is independent of investigators and
48
49 432 sponsor. The QMCR is responsible for monitoring the study data and will conduct yearly
50
51 433 auditing.
52
53

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56 434 Following data collection, analysis, and review of findings, manuscripts will be
57
58 435 prepared for submission to peer-reviewed journals and results presented in national and
59
60

1
2
3 436 international conferences. Study findings will also be disseminated through social media. Data
4
5 437 will be published regardless of outcomes and the University of Alberta retains the right to
6
7 438 publish. Authorship eligibility will adhere to the International Committee of Medical Journal
8
9 439 Editors' recommended guidelines (38). As a mandate of completing a registered trial, the
10
11 440 results must be published within 12 months of the completion of the trial. Dataset and statistical
12
13 441 code may be provided upon request.
14
15
16
17 442

18
19 443 **Figure legends:**

20
21 444 **Figure 1.** Experimental protocol. Abbreviations: CON control group, PMR powdered meal
22
23 replacement group.
24
25

26 446 **Figure 2.** Schedule of enrollment, interventions, and assessments (SPIRIT figure).
27
28 447 Abbreviations: CON control group, PMR powdered meal replacement group.
29
30

31 448

32
33 449 **Author contributions:**

34
35 450 All authors were involved in the design of the study. JM, CLPO, AMS, JW, and CMP wrote
36
37 451 the study protocol. All authors participated in drafting and revising the manuscript. All authors
38
39 452 read and approved the final manuscript.
40
41
42

43 453

44
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46
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48
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50
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54
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56
57
58
59
60

1
2
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4
5 461 USA Inc.
6
7
8 462

9
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11
12 464 receiving honoraria and/or paid consultancy from Abbott and AMRA Medical Inc. outside the
13
14 465 scope of this work. AB received research support for their departments and consultant or
15
16 466 speakers' honoraria from the Almased-Wellness-GmbH. AMS reports receiving honoraria
17
18 467 and/or paid consultancy from Novo Nordisk, Johnson & Johnson, Boehringer Ingelheim, and
19
20 468 Xeno Biosciences outside the scope of this work. PDC is inventor on patent applications
21
22 469 dealing with the use of specific bacteria and components in the treatment of different diseases.
23
24 470 PDC was co-founder of The Akkermansia Company SA and of Enterosys S.A. JW has received
25
26 471 research funding and consulting fees from industry sources involved in the manufacture and
27
28 472 marketing of dietary fibers, prebiotics, and probiotics. JW is further a co-owner of Synbiotics
29
30 473 Health, a developer of synbiotic products. CMP reports receiving honoraria and/or paid
31
32 474 consultancy from Abbott Nutrition, Nutricia, Nestle Health Science, Fresenius Kabi, Pfizer,
33
34 475 and AMRA medical outside the scope of this work. Other authors declare no conflict of interest.
35
36
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40 476

41 42 477 **References**

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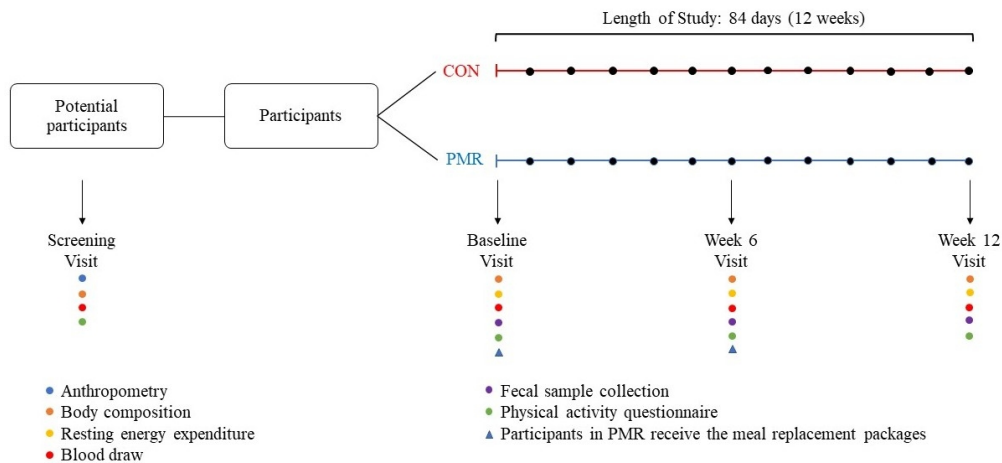


Figure 1. Experimental protocol. Abbreviations: CON control group, PMR powdered meal replacement group.

315x148mm (96 x 96 DPI)

TIMEPOINT		STUDY PERIOD					
		Enrollment	Allocation	Post-Allocation			
		Screening visit	Post-screening	Visit 1 (week 1)	Visit 2 (week 6)	Visit 3 (week 12)	Weekly
ENROLLMENT	Eligibility screen	X					
	Informed consent	X					
	Anthropometry	X					
	Body composition	X					
	Blood tests	X					
	Questionnaires	X					
	Allocation		X				
INTERVENTIONS	CON			●	●		
	PMR			●	●		
ASSESSMENTS	Gut microbiome			X	X	X	
	Systemic inflammatory biomarkers			X	X	X	
	Metabolic blood markers			X	X	X	
	Gene expression			X		X	
	Gene polymorphisms			X			
	Energy metabolism			X	X	X	
	Body composition			X	X	X	
	Appetite sensation						X
	Physical activity questionnaire			X	X	X	
Dietary intake						X	

Figure 2. Schedule of enrollment, interventions, and assessments (SPIRIT figure). Abbreviations: CON control group, PMR powdered meal replacement group.

205x139mm (150 x 150 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2, 6, 17
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	6
Protocol version	#3	Date and version identifier	2, 5, 17
Funding	#4	Sources and types of financial, material, and other support	18-19
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 18

1	Roles and	#5b	Name and contact information for the trial sponsor	18
2	responsibilities:			
3	sponsor contact			
4	information			
5				
6				
7				
8	Roles and	#5c	Role of study sponsor and funders, if any, in study design;	18
9	responsibilities:		collection, management, analysis, and interpretation of data;	
10	sponsor and funder		writing of the report; and the decision to submit the report for	
11			publication, including whether they will have ultimate authority	
12			over any of these activities	
13				
14				
15				
16	Roles and	#5d	Composition, roles, and responsibilities of the coordinating centre,	18
17	responsibilities:		steering committee, endpoint adjudication committee, data	
18	committees		management team, and other individuals or groups overseeing the	
19			trial, if applicable (see Item 21a for data monitoring committee)	
20				
21				
22				
23	Introduction			
24				
25	Background and	#6a	Description of research question and justification for undertaking	4-5
26	rationale		the trial, including summary of relevant studies (published and	
27			unpublished) examining benefits and harms for each intervention	
28				
29				
30	Background and	#6b	Explanation for choice of comparators	5
31	rationale: choice of			
32	comparators			
33				
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35				
36	Objectives	#7	Specific objectives or hypotheses	5
37				
38	Trial design	#8	Description of trial design including type of trial (eg, parallel	5-6
39			group, crossover, factorial, single group), allocation ratio, and	
40			framework (eg, superiority, equivalence, non-inferiority,	
41			exploratory)	
42				
43				
44				
45	Methods:			
46	Participants,			
47	interventions, and			
48	outcomes			
49				
50				
51	Study setting	#9	Description of study settings (eg, community clinic, academic	5
52			hospital) and list of countries where data will be collected.	
53			Reference to where list of study sites can be obtained	
54				
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57	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable,	7
58			eligibility criteria for study centres and individuals who will	
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60				

		perform the interventions (eg, surgeons, psychotherapists)	
1			
2	Interventions:	#11a Interventions for each group with sufficient detail to allow	8
3	description	replication, including how and when they will be administered	
4			
5	Interventions:	#11b Criteria for discontinuing or modifying allocated interventions for a	15
6	modifications	given trial participant (eg, drug dose change in response to harms,	
7		participant request, or improving / worsening disease)	
8			
9	Interventions:	#11c Strategies to improve adherence to intervention protocols, and any	15
10	adherence	procedures for monitoring adherence (eg, drug tablet return;	
11		laboratory tests)	
12	Interventions:	#11d Relevant concomitant care and interventions that are permitted or	15
13	concomitant care	prohibited during the trial	
14			
15	Outcomes	#12 Primary, secondary, and other outcomes, including the specific	6-7
16		measurement variable (eg, systolic blood pressure), analysis metric	
17		(eg, change from baseline, final value, time to event), method of	
18		aggregation (eg, median, proportion), and time point for each	
19		outcome. Explanation of the clinical relevance of chosen efficacy	
20		and harm outcomes is strongly recommended	
21	Participant timeline	#13 Time schedule of enrolment, interventions (including any run-ins	8-9
22		and washouts), assessments, and visits for participants. A	
23		schematic diagram is highly recommended (see Figure)	
24			
25	Sample size	#14 Estimated number of participants needed to achieve study	15
26		objectives and how it was determined, including clinical and	
27		statistical assumptions supporting any sample size calculations	
28			
29	Recruitment	#15 Strategies for achieving adequate participant enrolment to reach	8
30		target sample size	
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45	Methods: Assignment		
46	of interventions (for		
47	controlled trials)		
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49			
50	Allocation: sequence	#16a Method of generating the allocation sequence (eg, computer-	8
51	generation	generated random numbers), and list of any factors for	
52		stratification. To reduce predictability of a random sequence,	
53		details of any planned restriction (eg, blocking) should be provided	
54		in a separate document that is unavailable to those who enrol	
55		participants or assign interventions	
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1	Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central	8
2	mechanism		telephone; sequentially numbered, opaque, sealed envelopes),	
3			describing any steps to conceal the sequence until interventions are	
4			assigned	
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8	Allocation:	#16c	Who will generate the allocation sequence, who will enrol	17-18
9	implementation		participants, and who will assign participants to interventions	
10				
11	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial	3
12			participants, care providers, outcome assessors, data analysts), and	
13			how	
14				
15				
16				
17	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible,	n/a
18	emergency unblinding		and procedure for revealing a participant's allocated intervention	
19			during the trial	
20				
21				
22	Methods: Data			
23	collection,			
24	management, and			
25	analysis			
26				
27				
28				
29	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other	8-15
30			trial data, including any related processes to promote data quality	
31			(eg, duplicate measurements, training of assessors) and a	
32			description of study instruments (eg, questionnaires, laboratory	
33			tests) along with their reliability and validity, if known. Reference	
34			to where data collection forms can be found, if not in the protocol	
35				
36				
37				
38				
39	Data collection plan:	#18b	Plans to promote participant retention and complete follow-up,	9/ 15
40	retention		including list of any outcome data to be collected for participants	
41			who discontinue or deviate from intervention protocols	
42				
43				
44	Data management	#19	Plans for data entry, coding, security, and storage, including any	17-18
45			related processes to promote data quality (eg, double data entry;	
46			range checks for data values). Reference to where details of data	
47			management procedures can be found, if not in the protocol	
48				
49				
50				
51	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes.	15-17
52			Reference to where other details of the statistical analysis plan can	
53			be found, if not in the protocol	
54				
55				
56	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and adjusted	n/a
57	analyses		analyses)	
58				
59				
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1	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	16-17
2	population and missing		adherence (eg, as randomised analysis), and any statistical methods	
3	data		to handle missing data (eg, multiple imputation)	
4				
5				
6	Methods: Monitoring			
7				
8	Data monitoring:	#21a	Composition of data monitoring committee (DMC); summary of its	18
9	formal committee		role and reporting structure; statement of whether it is independent	
10			from the sponsor and competing interests; and reference to where	
11			further details about its charter can be found, if not in the protocol.	
12			Alternatively, an explanation of why a DMC is not needed	
13				
14	Data monitoring:	#21b	Description of any interim analyses and stopping guidelines,	17-18
15	interim analysis		including who will have access to these interim results and make	
16			the final decision to terminate the trial	
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22	Harms	#22	Plans for collecting, assessing, reporting, and managing solicited	9
23			and spontaneously reported adverse events and other unintended	
24			effects of trial interventions or trial conduct	
25				
26				
27	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and	18
28			whether the process will be independent from investigators and the	
29			sponsor	
30				
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33	Ethics and			
34	dissemination			
35				
36				
37	Research ethics	#24	Plans for seeking research ethics committee / institutional review	17-18
38	approval		board (REC / IRB) approval	
39				
40				
41	Protocol amendments	#25	Plans for communicating important protocol modifications (eg,	17
42			changes to eligibility criteria, outcomes, analyses) to relevant	
43			parties (eg, investigators, REC / IRBs, trial participants, trial	
44			registries, journals, regulators)	
45				
46				
47	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial	5-6
48			participants or authorised surrogates, and how (see Item 32)	
49				
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51	Consent or assent:	#26b	Additional consent provisions for collection and use of participant	n/a
52	ancillary studies		data and biological specimens in ancillary studies, if applicable	
53				
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55	Confidentiality	#27	How personal information about potential and enrolled participants	17-18
56			will be collected, shared, and maintained in order to protect	
57			confidentiality before, during, and after the trial	
58				
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1	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
2				
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5	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17-19
6				
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10	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
11				
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14	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17-18
15				
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21	Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	18
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24	Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
25				
26				
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28	Appendices			
29				
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31	Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
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35	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	11-13
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 42 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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BMJ Open

The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota (PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a Randomized Controlled Trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-070027.R1
Article Type:	Protocol
Date Submitted by the Author:	24-May-2023
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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Genetics and genomics, Immunology (including allergy), Research methods
Keywords:	NUTRITION & DIETETICS, MICROBIOLOGY, IMMUNOLOGY, Obesity

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3 1 **The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota**
4
5 2 **(PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a**
6
7 3 **Randomized Controlled Trial**
8
9

10 4 Julia Montenegro^{1*}, Camila L. P. Oliveira^{1*}, Anissa M. Armet¹, Aloys Berg², Arya M.
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12 5 Sharma³, Laurie Mereu³, Cristiane Cominetti⁴, Sunita Ghosh⁵, Caroline Richard¹, Nguyen K.
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14 6 Nguyen^{6,7}, Patrice D. Cani^{6,7}, Jens Walter^{1,8*}, Carla M. Prado^{1*}
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22 ABSTRACT

23 *Introduction:* Excess body weight is associated with a state of low-grade chronic inflammation
24 and alterations of the gut microbiome. Powdered meal replacements (PMR) have been shown
25 to be an effective strategy for weight management; however, their effect on inflammation and
26 the gut microbiome remains unclear. The aim of this 12-week randomized control clinical trial
27 is to investigate the effects of PMR consumption, here given as a soy-yogurt-honey formula,
28 on inflammation, gut microbiome, and overall metabolism in individuals with excessive body
29 weight.

30 *Methods and analysis:* Healthy adults with excess body weight (n=88) are being recruited and
31 randomly assigned to one of the following groups: a) Control group (CON): maintaining usual
32 diet for 12 weeks, or b) PMR group: replacing morning and afternoon snacks daily with a PMR
33 for 12 weeks. Participants are asked to maintain body weight throughout the study and fill out
34 a journal with information about PMR consumption, body weight, food intake, appetite
35 sensations, and medications. Three study visits are required: baseline, week 6, and week 12.
36 Outcome measures include systemic inflammatory biomarkers, gut microbiome composition,
37 metabolic blood markers, host energy metabolism, body composition, appetite sensations, and
38 host gene expression profile.

39 *Ethics and dissemination:* This research protocol was approved by the University of Alberta
40 Ethics Board (Pro00070712) and adheres to the Canadian Tri-Council Policy statement on the
41 use of human participants in research. Procedures and potential risks are fully discussed with
42 participants. Study findings will be disseminated in peer-reviewed journals, conference
43 presentations, and social media.

44 *Registration details:* ClinicalTrials.gov identifier: NCT03235804.

46 **Keywords:** Powdered meal replacement; obesity; inflammation; gut microbiome.

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6 48 **ARTICLE SUMMARY**7
8 49 **Strengths and Limitations of this study**

- 9
- 10 50 • The randomized controlled clinical trial design, coupled with regular assessments and
- 11 follow-up sessions, as well as a comprehensive range of evaluated outcomes,
- 12 effectively reduces biases and confounding factors.
- 13 51
- 14 52
- 15 53 • Cutting edge technology, such as the metabolic chamber and dual-energy X-ray
- 16 absorptiometry, enables precise outcome measures.
- 17 54
- 18 55 • The multi-omics approach, incorporating gut microbiome, gene expression, and genetic
- 19 polymorphisms, supports the progress of precision nutrition and facilitates the
- 20 examination of causal relationships and underlying mechanisms .
- 21 56
- 22 57
- 23 58 • A primary limitation of the study is the absence of a placebo group and the fact it is not
- 24 not double-blinded.
- 25 59
- 26 60 • Since the gut microbiome analysis depends on fecal samples, it might not fully
- 27 represent changes in the gut microbiome composition occurring in more proximal parts
- 28 of the gastrointestinal tract.
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- 43 64 **Word-count:** 5072 words.
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65 INTRODUCTION

66 Excess body weight can be defined as a body mass index (BMI) ≥ 25.0 kg/m² (1), which
67 encompasses both the overweight and obesity categories (2). This condition has been
68 associated with a state of systemic low-grade chronic inflammation, which is characterized by
69 a persistent activation of immune and non-immune cells and production of cytokines,
70 chemokines, and acute phase proteins (3, 4). Those inflammatory biomarkers include
71 interleukins (IL), such as IL-6 and IL-8, tumor necrosis factor- α (TNF- α), and C-reactive
72 protein (CRP) (3). Systemic low-grade chronic inflammation causes tissue and organ damage,
73 which can, in turn, lead to the onset and progression of chronic diseases, such as diabetes
74 mellitus, cancer, metabolic syndrome, and cardiovascular diseases (3).

75 In individuals with excessive body weight, the state of systemic low-grade chronic
76 inflammation can be mediated by increased adiposity, as well as by mechanisms through the
77 gut microbiota (3). Increased adipocyte size (i.e., hypertrophy) is associated with cellular
78 dysfunction and distress (5, 6). Hypertrophic adipocytes secrete an increased number of pro-
79 inflammatory chemokines, such as TNF- α , IL-6, IL-8, and monocyte chemoattractant protein
80 1 (MCP-1) (4-6). The increased size of adipocytes and cytokine production lead to adipose
81 tissue hypoxia and death, as well as local and systemic inflammation (4-6).

82 Excess body weight is associated with altered gut microbiome composition and reduced
83 microbiome diversity, which might cause metabolic aberrations and enrich for opportunistic
84 pathogens (e.g. at the epithelial interface) that contribute to inflammation (7, 8). Individuals
85 with excessive body weight usually present with altered gut permeability, which elevates
86 systemic levels of endotoxins (i.e., lipopolysaccharides) (3). When in the bloodstream,
87 lipopolysaccharides binds to toll-like receptor 4 leading to activation of nuclear factor kappa B
88 and consequently production of pro-inflammatory cytokines, including IL-6 and TNF- α (8).

1
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3 89 Considering the numerous negative health outcomes associated with excess body
4
5 90 weight, much effort has been made to develop effective weight management strategies. Among
6
7 91 those are meal replacements, which are food products fortified with vitamins and minerals used
8
9 92 to replace one or more meals per day. Meal replacements are commonly used in association
10
11 93 with calorie restriction. Research has shown that the consumption of meal replacements leads
12
13 94 to greater weight loss when compared to reduced-calorie diets alone (9, 10). Improvement in
14
15 95 metabolic parameters is generally observed with weight loss, including improvement in
16
17 96 glucose metabolism, reduction of triacylglycerol, low-density lipoprotein cholesterol (LDL-C),
18
19 97 systolic blood pressure (9, 11, 12), and the inflammatory markers CRP and IL-6 (13).
20
21 98 Considering the positive health effects of weight loss in individuals with excessive body weight
22
23 99 (14-16) and the beneficial health effects of meal replacements (9, 11), it is important to
24
25 100 differentiate the effects of meal replacements from that of weight loss on overall health, which
26
27 101 have not been investigated so far. Therefore, the aim of this study is to compare the effects of
28
29 102 a 12-week consumption of a powdered meal replacement (PMR group), given as a soy-yogurt-
30
31 103 honey formula (17), versus usual diet (control group, CON) on inflammation, gut microbiome,
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33 104 overall metabolic health, gene expression profile, and genetic background in individuals with
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35 105 excessive body weight who are in weight maintenance.
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107 **METHODS**

108 **Study design and ethical procedures**

109 This study is a randomized, controlled, parallel group, clinical trial conducted at the
110 Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada). The
111 study is an Investigator Initialized Trial sponsored by the Almased Wellness Comp.,
112 Bienenbüttel, Germany. The corresponding research protocol fulfils the requirements of the
113 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist (18).

1
2
3 114 This research protocol was approved by the University of Alberta Ethics Board (HREB,
4
5 115 identifier Pro00070712) and complies with the standards established by the Canadian Tri-
6
7
8 116 Council Policy statement on the use of human participants in research. Procedures and potential
9
10 117 risks involved in the study are discussed with participants prior to obtaining informed consent
11
12 118 (supplementary material). This protocol is registered on ClinicalTrials.gov (NCT03235804),
13
14
15 119 and recruitment started on April 2019 and is expected to finish in November 2023 (**Table 1**).

16
17 **Table 1.** World Health Organization trial registration dataset

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03235804
Date of registration in primary registry	August 1, 2017
Secondary identifying numbers	University of Alberta Research Ethics Board # Pro00070712
Source(s) of monetary or material support	Almased Wellness-GmbH (Bienenbüttel, Germany)
Primary sponsor	Almased Wellness-GmbH (Bienenbüttel, Germany)
Secondary sponsor(s)	N/A
Contact for public queries	Dr Carla Prado +1 (780) 492-9555 carla.prado@ualberta.ca and Jens Walter +353 (0)21 490-1773 jenswalter@ucc.ie
Contact for scientific queries	Dr Carla Prado +1 (780) 492-9555 carla.prado@ualberta.ca and Jens Walter +353 (0)21 490-1773 jenswalter@ucc.ie
Public title	The impact of a powdered meal replacement on metabolism and gut microbiota (Premium Study)
Scientific title	The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight (The PREMIUM Study)
Countries of recruitment	Canada
Health condition(s) or problem(s) studied	Overweight and obesity
Intervention(s)	Powdered meal replacement

Key inclusion and exclusion criteria	Inclusion Criteria: (a) female/male aged 18 to 50 years; (b) non-smoker; (c) body mass index (BMI) between 25 and 37 kg/m ² ; (d) weight stable; (e) fat mass $\geq 20\%$ for men and $\geq 25\%$ for women; (f) stable physical activity level. Exclusion Criteria: (a) diagnosis of chronic diseases or acute infections; (b) taking any medication that may alter study outcomes; (c) taking pre- and probiotics; (d) use of antibiotics in the past two months; (e) females that are pregnant or lactating.
Study type	Randomized controlled trial
Date of first enrolment	April 1, 2019
Sample size	88
Recruitment status	Actively recruiting
Primary outcome(s)	Interleukin-6
Key secondary outcomes	Gut microbiota
Ethics review	University of Alberta Research Ethics Board # Pro00070712
Completion date	N/A
Summary results	N/A
Individual Participant Data (IPD) sharing statement	De-identified data will be shared with the participant upon completion of the study (publication)

121

122 Outcome measures

123 The primary study outcome is to compare changes in IL-6 concentration over time
 124 (within groups) and between the PMR and CON groups. Secondary outcome is to examine
 125 shifts in in gut microbiome composition over time (within groups) and between the PMR and
 126 CON groups, such as diversity indices and relative abundances of bacteria at different
 127 taxonomic levels (i.e., phylum, family, genus, and amplicon sequence variant [ASV]), as
 128 assessed by 16S rRNA sequencing. Exploratory outcomes include:

- 1
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3 129 • Change in markers of systemic inflammation (high-sensitivity CRP [hs-CRP], IL-8, and
4
5 130 TNF- α) and immune modulation (IL-10) over time (within groups) and between the
6
7 131 PMR and CON groups.
8
9
10 132 • Change in concentrations of metabolic blood markers (glucose, insulin, total
11
12 133 cholesterol, LDL-C, high-density lipoprotein cholesterol [HDL-C], triglycerides,
13
14 134 peptide tyrosine-tyrosine [PYY], glucagon-like peptide-1 [GLP-1], ghrelin,
15
16 135 adiponectin, leptin, free glycerol, free fatty acids, and thyroid stimulating hormone
17
18 136 [TSH]) over time (within groups) and between the PMR and CON groups.
19
20
21 137 • Change in resting energy expenditure (REE) and respiratory exchange ratio (RER) over
22
23 138 time (within groups) and between the PMR and CON groups.
24
25
26 139 • Change in body composition (fat mass [FM] and lean soft tissue [LST]) over time
27
28 140 (within groups) and between the PMR and CON groups.
29
30
31 141 • Change in appetite sensations (hunger, satiety, fullness, and prospective food
32
33 142 consumption) over time within the PMR group.
34
35
36 143 • Differences in the responses to the intervention according to genetic polymorphisms
37
38 144 over time.
39
40
41 145 • Changes in inflammation and excess body weight-related gene expression profile over
42
43 146 time and between the PMR and CON groups.
44
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48

148 **Research participants**

149 Inclusion criteria are as follows: male or female; non-smoker; between 18 and 50 years
150 of age; BMI between 25.0 and 37.0 kg/m²; with a stable body weight 6 months prior to study
151 initiation (i.e., variation <5 kg); fat mass $\geq 20\%$ for males and $\geq 25\%$ for females; willingness
152 to maintain stable physical activity level throughout the study; and females must use effective
153 birth control methods.
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3 154 Exclusion criteria includes participation in >3 hours per week of vigorous physical
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5 155 activity; pregnancy or lactation; diagnosis of any chronic or acute diseases (except for excess
6
7 156 body weight); use of any medication that impacts study outcomes, except for antidepressants,
8
9 157 anxiolytic, and/or thyroid replacement therapy in a stable dose 3 months prior to study initiation
10
11 158 and throughout the study period; use of antibiotics 2 months prior to study initiation; use of
12
13 159 protein supplements 1 month prior to study initiation; allergy to PMR ingredients (soy, honey,
14
15 160 and yogurt); allergy or intolerance to soy, gluten, and/or lactose; following a vegetarian, vegan,
16
17 161 or any other restrictive dietary pattern; claustrophobia; or being unable to comprehend and
18
19 162 complete the required questionnaires. Participants consuming supplements or food items that
20
21 163 contain pre- or probiotics (e.g., kefir or kombucha) before being enrolled in the study will be
22
23 164 asked to discontinue the use of these products and wait 1 month before starting the study. The
24
25 165 use of other nutritional supplements, such as multivitamins and vitamin D₃ will be allowed if
26
27 166 on a stable dose.
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167

168 **Recruitment, randomization, and intervention**

169 Study advertisement is done using flyers displayed at the University of Alberta
170 campuses, surrounding communities, other post-secondary education institutions in Edmonton
171 (AB, Canada), and health care centres in the city. The study is also advertised in University of
172 Alberta email lists, newspapers, classrooms presentations, and on social media (e.g., Kijiji,
173 Facebook, and Twitter). Additionally, a personalized website (premium.ualberta.ca) was
174 created.

175 Individuals interested in being part of the study will be invited to attend a screening
176 visit at the HNRU. This visit will include anthropometric measurements (i.e., height, weight,
177 and waist circumference), body composition assessment (bioelectrical impedance analysis
178 [BIA]), blood tests (i.e., creatinine, estimated glomerular filtration rate [eGFR], albumin,

179 aspartate transaminase [AST], alanine transaminase [ALT], sodium, potassium, chloride, and
 180 TSH), review of medical history, and completion of a physical activity questionnaire. Although
 181 glucose, insulin, or lipid panel tests are not conducted during the screening visit, individuals
 182 who exhibit symptoms of or are taking medications for chronic diseases (e.g., diabetes,
 183 hypertension, and dyslipidemia) are deemed ineligible for participation.

184 If deemed eligible, participants are randomly assigned into either the CON or PMR
 185 group. Randomization is stratified by sex using a Microsoft Office Excel® spreadsheet.

186 To guarantee impartial allocation of participants to the groups, a study team member
 187 created a list of random numbers and assigns them to each group in a concealed and systematic
 188 manner. Subsequently, a second investigator follows the predetermined order of numbers and
 189 assigns participants to their respective groups based on the order of their screening.

190 Participants assigned to the CON group are asked to maintain their usual diet for 12
 191 weeks. The ones in the PMR group are asked to replace their morning and afternoon snacks
 192 using a powdered meal replacement (Almased Wellness Comp., Bienenbüttel, Germany) and
 193 otherwise maintain their usual diet for 12 weeks. Each snack is replaced by 50 grams of powder
 194 mixed with 250 mL of water. The nutritional information of the meal replacement is displayed
 195 in **Table 2**.

196 **Table 2.** Nutritional information of the tested soy-honey-yogurt formula, a powdered meal
 197 replacement (PMR)

Nutrient	50 g of Product (PMR)
Calories (kcal)	180
Total fat (g)	1.0
<i>Saturated fat (g)</i>	0.5
<i>Trans fat (g)</i>	0
<i>Polyunsaturated fat (g)</i>	0.1

<i>Monounsaturated fat (g)</i>	0.4
Cholesterol (mg)	3
Total carbohydrates (g)	15
<i>Dietary fiber (g)</i>	0.5
<i>Sugars (g)</i>	15
Protein (g)	27
Sodium (mg)	340
Potassium (mg)	500
Vitamin A (IU)	794
Vitamin C (mg)	16
Vitamin E (IU)	6
Thiamin (Vitamin B1) (mg)	5
Riboflavin (Vitamin B2) (mg)	6
Vitamin B6 (mg)	7
Calcium (mg)	215
Iron (mg)	4.9

198

199

200 **Experimental protocol**

201 The study design is illustrated in **Figure 1**. The schedule of enrollment, interventions,
 202 and assessments are shown in **Figure 2**. Following the screening visit and randomization
 203 process, enrolled participants are invited to attend 3 study visits: baseline, week 6, and week
 204 12. Assessments during each of these visits include: 1-hour resting metabolic rate (RMR),
 205 blood draw, body composition, and physical activity questionnaire. They additionally receive
 206 stool collection kits and instructions for fecal sample collection. During the baseline visit,

1
2
3 207 participants receive a scale and a journal to record the following information daily: body
4
5 208 weight, date and time of meal replacement intake (PMR group only), and medication intake (if
6
7 209 any). They are also asked to record on a weekly basis a 24h dietary recall (both groups) and fill
8
9 210 out appetite sensation questionnaires (PMR group only). Instructions on how to fill out the
10
11 211 journal and dietary records are given. Additionally, participants assigned to the PMR group
12
13 212 receive 84 packages of the PMR during visits at baseline and week 6, as well as instructions on
14
15 213 how to prepare it. Those assigned to the PMR group start consuming the supplement the day
16
17 214 after the first stool sample collection. Study materials (study journal and scale) are returned on
18
19 215 week 12.

20
21
22
23
24 216 A member of the study team contacts participants weekly to verify adherence to the
25
26 217 dietary intervention and potential adverse events. Their body weight is also discussed at that
27
28 218 time. If a body weight change greater than $\pm 2\%$ of their initial body weight is noticed, a
29
30 219 nutrition consult with a Registered Dietitian is scheduled to provide instructions on how to
31
32 220 increase or decrease food intake and physical activity levels to return to baseline body weight.

33 34 35 221 36 37 222 *Anthropometry and body composition*

38
39
40 223 At the screening visit, anthropometric measurements are taken twice, and the average
41
42 224 is used for data analysis. Height is measured using a digital stadiometer (235 Heightronic™,
43
44 225 Concepts, Quick Medical, Snoqualmie, WA, USA) to the nearest 0.1 cm. Body weight is
45
46 226 measured to the nearest 0.1 kg using a calibrated digital scale (Health-o-meter® Professional
47
48 227 Remote Display, Sunbeam Products Inc., FL, USA). Waist circumference is measured using a
49
50 228 measuring tape at the level of participant's belly button, as per standard procedure (19).

51
52
53 229 A digital scale (HD-314 TANITA Corporation, Tokyo, Japan) is provided to
54
55 230 participants during the baseline visit, which is returned at the study completion. Body weight
56
57 231 is recorded daily in the morning in a fasting state, and with an empty bladder.

1
2
3 232 Body composition is assessed using dual energy X-ray absorptiometry (DXA, GE
4
5 233 Lunar iDXA, General Electric Company, Madison, USA), air displacement plethysmography
6
7 234 (ADP, Bod Pod 1SB-060M, Life Measurement Instruments, Concord, CA, USA), and BIA
8
9 235 (Seca mBCA525, Seca GmbH & Co, Hamburg, Germany). A number of techniques is being
10
11 236 used to explore potential changes in body composition using multicompartiment modeling:
12
13 237 DXA for bone mineral content, BIA for total body water, ADP for body density, which is used
14
15 238 to calculate the remaining compartment: adipose tissue and residues (i.e., dry LST) (20, 21).
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239

240 *Resting energy expenditure*

241 Resting energy expenditure is assessed by indirect calorimetry using an open-circuit
242 metabolic chamber, which measures the volume of oxygen (O₂) and carbon dioxide (CO₂) from
243 participant's respiration. Participants lie down in a relaxed position without falling asleep and
244 breathe normally for 60 minutes. Mixed air with the expired CO₂ is drawn from the chamber
245 at a constant flow rate (60 ± 2 L/min) while fresh air with constant O₂ is passively drawn into
246 the chamber. The first 30 minutes of the test are considered time for acclimatization and hence
247 removed from analysis. Gas exchange (volume of CO₂ and O₂) is analysed minute-by-minute
248 by the Advance Optima AO2000 Series CO₂ analyser (ABB Automation GmbH, Frankfurt,
249 Germany) and the Oxymat 6 O₂ analyser (Siemens AG, Munich, Germany). Data is transferred
250 from those analysers to a computer (Acer Aspire AM3910-E3122, Acer Inc., New Taipei City,
251 Taiwan) via the National Instruments NI USB-6221 device (National Instruments Corporation,
252 Austin, Tex., USA) using the PMCSS Software version 1.8 (Pennington Metabolic Chamber
253 Software Suite, Pennington Biomedical Research Center, La., USA). Resting energy
254 expenditure (kcal/day) is calculated using the average kcal/min multiplied by 1440.

255

256 *Blood analysis*

257 Blood is sampled from participants by venipuncture after an overnight fast during the
 258 screening visit and at baseline, week 6, and week 12. Evaluated biomarkers are listed in **Table**
 259 **3**.

260 **Table 3.** Blood parameters, sample, and laboratory responsible for blood analysis

Parameter	Screening	Baseline	Week 6	Week 12	Sample	Laboratory
Albumin	x				Serum	External
Creatinine/eGFR	x				Serum	External
ALT	x				Serum	External
AST	x				Serum	External
Electrolytes ^a	x				Serum	External
TSH	x	x	x	x	Serum	External
hs-CRP		x	x	x	Serum	External
Glucose		x	x	x	Serum	External
Lipid panel ^b		x	x	x	Serum	External
Free glycerol		x	x	x	Serum	On site
Free fatty acids		x	x	x	Serum	On site
Interleukins ^c		x	x	x	Plasma	On site
TNF- α		x	x	x	Plasma	On site
Insulin		x	x	x	Plasma	On site
Leptin		x	x	x	Plasma	On site
Adiponectin		x	x	x	Plasma	On site
PYY		x	x	x	Plasma	On site
GLP-1		x	x	x	Plasma	On site
Ghrelin		x	x	x	Plasma	On site
Polymorphisms		x			Whole blood	On site

Gene expression	x	x	Whole blood	On site
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261 ^aElectrolytes include chloride, sodium, and potassium. ^bLipid panel include triglycerides, total
 262 cholesterol, LDL-C, and HDL-C. ^c Interleukins include IL-6, IL-8, and IL-10. Abbreviations:
 263 ALT alanine aminotransferase; AST aspartate aminotransferase; eGFR estimated glomerular
 264 filtration rate; GLP-1 glucagon like peptide; hs-CRP high-sensitivity C-reactive protein; PYY
 265 peptide tyrosine-tyrosine; TNF- α tumor necrosis factor α ; TSH thyroid stimulating hormone.

267 Blood samples are collected using BD Vacutainer[®] tubes (Becton, Dickinson and
 268 Company, Franklin Lakes, NJ, USA). Tubes containing silica and a polymer are used for serum
 269 separation, tubes containing K2-ethylenediaminetetraacetic acid (EDTA) are used for plasma
 270 separation, and tubes containing K2EDTA and protease inhibitors (dipotassium and tacrine,
 271 BD P800) are used for GLP-1 and ghrelin analysis.

272 Creatinine, eGFR, albumin, AST, ALT, sodium, potassium, chloride, and TSH are
 273 analysed by an external lab (DynaLIFE Medical Labs, Edmonton, AB, Canada) at the screening
 274 visit prior to enrollment. Glucose, lipid panel (triglycerides, total cholesterol, LDL-C, and
 275 HDL-C), TSH, and hs-CRP will be analysed by DynaLIFE Medical Labs (Edmonton, AB,
 276 Canada). Interleukin 6, IL-8, IL-10, TNF- α , insulin, PYY, GLP-1, ghrelin, adiponectin, leptin,
 277 free glycerol, and free fatty acids will be analysed in our laboratory (University of Alberta, AB,
 278 Canada). Interleukin 6, IL-8, IL-10, TNF- α , insulin, PYY, GLP-1, ghrelin, adiponectin, and
 279 leptin will be analysed by electrochemiluminescence immunoassay (MesoScale Discovery[®],
 280 Maryland, USA).

281 An additional blood draw is requested the day following each study visit for hs-CRP
 282 analysis due to this being a sensitive marker which can vary substantially within hours of
 283 collection for several reasons (22). Therefore, the average of CRP measured on two consecutive
 284 days will be taken in case they are similar. If a participant is in an infectious state (i.e., CRP

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3 285 >10 mg/L or significant changes between the two days measurement) the highest value will be
4
5 286 excluded from analysis.

7 287 For gene expression profile, ribonucleic acids (RNAs) will be sequenced at baseline
8
9 288 and week 12. Whole blood (500 μ L) is aliquoted into an RNase-free microfuge tube and added
10
11 289 1.3 mL of RNAlater stabilization solution (Thermo Fisher Scientific, Waltham, MA, USA).
12
13 290 Total RNA will be extracted from whole blood using the RiboPure™ Blood Kit (Thermo Fisher
14
15 291 Scientific, Waltham, USA). The RNA purity will be determined by measuring the 260/280 nm
16
17 292 ratio (ideal ratio ~2.0) and the 260/230 nm ratio (ideal ratio 2.0-2.2) using a spectrophotometer.
18
19 293 The quality of RNA samples will be evaluated prior to library preparation for RNA-Seq, using
20
21 294 a bioanalyzer and an RNA Integrity Number (RIN) ≥ 7 will be accepted. Samples of high purity
22
23 295 and quality RNA will be prepared with the TruSeq RNA Sample Prep kit (Illumina, San Diego,
24
25 296 USA). The sequencing will be performed by an external company using the platform Illumina
26
27 297 HiSeq 4000 (Illumina, San Diego, USA), in the paired-end mode, in which the 2 ends will be
28
29 298 sequenced with a length of 100 base pairs (bp) (2 x 100 bp). At the end, 2 files in the 'fastq'
30
31 299 format will be generated for each of the evaluated samples.

32
33 300 We will select a set of candidate genes that are known to be involved in the regulation
34
35 301 of inflammation and/or excess body weight and are differentially expressed after the
36
37 302 intervention. From these genes, we will analyze the most extensively studied polymorphisms.
38
39 303 The reasoning behind this approach is that genetic variations in these candidate genes could
40
41 304 potentially impact the expression and/or function of the proteins they encode, ultimately
42
43 305 influencing the response to the intervention. Genetic polymorphisms will be analysed at
44
45 306 baseline. Genomic deoxyribonucleic acid (gDNA) will be extracted with the QIAamp DNA
46
47 307 Micro Kit (Qiagen, Hilden, Germany) from leukocytes in peripheral blood. The gDNA purity
48
49 308 will be verified in a spectrophotometer at 260 and 280 nm. The samples will be considered of
50
51 309 good quality if the ratio between absorbances is between 1.7 and 2.0. The gDNA concentration
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3 310 will be measured on a fluorimeter. For genotyping, a customized Infinium Global Screening
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5 311 Array-24 + v3.0 Kit (Illumina, San Diego, USA) will be used.
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9
10 313 *Fecal sample collection and gut microbiome sequencing*
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12 314 A total of three fecal samples are collected at baseline, week 6, and week 12. Fecal
13
14 315 samples are either collected at the HNRU the day of the study visits, or at home, kept at room
15
16 316 temperature, and delivered to the HNRU as soon as possible. During the baseline visit,
17
18 317 participants are instructed on how to collect fecal samples using the provided collection kits.
19
20 318 The fecal collection tubes (DNA/RNA Shield, Zymo Research, Irvine, CA, USA) preserve
21
22 319 nucleic acids in the sample and maintain stability at room temperature. Once delivered to the
23
24 320 lab, the fecal sample tubes are frozen at -80°C until processing and analysis.
25
26
27

28 321 The microbial DNA will be extracted from all samples including positive and negative
29
30 322 controls, using QIAamp Fast DNA Stool Mini Kit as previously described (23), packed with
31
32 323 dried-ice, and shipped to University of Minnesota Genomic Center (Minnesota, US) for
33
34 324 sequencing. Shipping will adhere the regulation of Environment, Health and Safety
35
36 325 Department, University of Alberta. MiSeq Illumina technology (300 bp pair-end) will be used
37
38 326 to sequence 16S ribosomal ribonucleic acid (rRNA) targeting V5-V6 region to characterize the
39
40 327 fecal microbiome composition using primer pair 784F [5'-RGGATTAGATACCC -3'] and
41
42 328 1064R [5'-CGACRRCCATGCANACCT-3'].
43
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50 330 *Physical activity questionnaire*
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52 331 The Godin-Shephard leisure-time physical activity questionnaire will be completed at
53
54 332 baseline, week 6, and week 12 to estimate physical activity levels (24, 25). In this questionnaire,
55
56 333 participants answer how often they perform strenuous, moderate, and light exercise for more
57
58 334 than 15 minutes in one week. A physical activity score is calculated based on intensity = $(9 \times$
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60

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2
3 335 strenuous) + (5 × moderate) + (3 × light) (25, 26). This will be used to classify participants as
4
5 336 insufficiently active (<14 units), moderately active (≥14 and <24 units), or active (≥24 units)
6
7
8 337 (26).
9

10 338

11 339 *Dietary intake*

12
13
14 340 The dietary intake will be assessed using the online Automated Self-Administered 24-
15
16 341 hour Recall (ASA24®) Canada (27). A paper-based version is available per individual
17
18 342 participant and is returned to the study team weekly by email or fax. Dietary information is
19
20 343 entered in ASA24® to ensure consistency. Three 24h recalls are completed at weeks 1, 6, and
21
22 344 12 (two weekdays and one weekend day) and one 24h recall per week on the remaining weeks
23
24 345 of the study period (one weekday). Energy, macronutrients, and micronutrients intake will be
25
26 346 obtained using ASA24® automated coding based on the amount of each food consumed.
27
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30 347

31 348 *Appetite sensations*

32
33 349 To assess how the PMR affects appetite, participants assigned to the PMR group rate
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35 350 their appetite sensations using the study journals once a week and at five timepoints: 1)
36
37 351 immediately after waking up/fasting, 2) immediately before the morning PMR consumption,
38
39 352 3) 30 minutes after the morning PMR consumption, 4) immediately before the afternoon PMR
40
41 353 consumption, and 5) 30 minutes after the afternoon PMR consumption. Hunger, satiety,
42
43 354 fullness, and prospective food consumption will be assessed using a paper-and-pen 100-mm
44
45 355 visual analogue scale (28). They are instructed to make a single vertical mark between 2
46
47 356 anchors to indicate the intensity of their subjective states regarding each element, on a scale
48
49 357 from 0 to 100 mm. The following questions are asked: How hungry do you feel? (I am not
50
51 358 hungry at all – I have never been more hungry); How satisfied do you feel? (I am completely
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3 359 empty – I cannot eat another bite); How full do you feel? (not at all full – totally full); How
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5 360 much do you think you can eat? (nothing at all – a lot).
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10 362 *Adherence and withdraw/discontinuation*

11
12 363 Participants are immediately withdrawn from the study if they: 1) have significant
13
14 364 variation in body weight ($>\pm 3\%$ of baseline body weight (29)) that does not return to baseline
15
16 365 2 weeks after the nutrition consult; 2) become pregnant; 3) start or change medications or
17
18 366 supplement intake listed in the eligibility criteria; 4) no longer meet the inclusion criteria.
19
20 367 Participants assigned to the PMR group are asked to return all supplement bags (empty or not)
21
22 368 to the visits on week 6 and 12. These are weighted, and participants are excluded from the
23
24 369 study if the PMR have not been consumed twice daily during the 12 weeks or if there is $>20\%$
25
26 370 of product left inside the bags. In addition, participants can withdraw from the study at any
27
28 371 time.
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34
35 373 **Statistical analyses**

36
37 374 *Sample size estimate*

38
39 375 A total of 74 participants (37 in each group) will be needed to detect a medium effect
40
41 376 size of 0.669. The effect size was calculated based on a previously published study (30), in
42
43 377 which the mean percent change in IL-6 from baseline to 12 months was -6.76 ± 36.95 pg/mL
44
45 378 in a group receiving soy protein versus 17.62 ± 35.92 pg/mL in the control group. Accounting
46
47 379 for a 20% attrition rate, the total sample size of 88 participants (44 in each group) will have a
48
49 380 power of 80% with a significance level of 5%. The sample size calculation was done using
50
51 381 G*Power version 3.1.9.2.
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58 383 *Data analysis*
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3 384 Normality of the study variables will be assessed by the Shapiro-Wilk W-test. By
4
5 385 inspecting boxplots, values >1.5 box-lengths from the edge of the box will be considered as
6
7
8 386 outliers and may be excluded from analysis. Differences between groups of nominal variables
9
10 387 will be analysed by Pearson's χ^2 test or Fisher's exact test. Both group effect and time effect
11
12 388 will be analyzed using a two-way mixed analysis of variance (ANOVA) or analysis of
13
14 389 covariance (ANCOVA) as appropriate. Assumption of homogeneity of variances will be tested
15
16
17 390 using Levene's test of equality of variances. Correlation between variables will be assessed by
18
19 391 Pearson's correlation. If significant correlations between nutrients and energy intake are
20
21 392 noticed, the residual method will be applied in order to describe the relationship between
22
23 393 aspects of food intake and biochemical characteristics independent of energy intake (31). All
24
25 394 analyses will be performed using IBM® SPSS® Statistics version 24 (International Business
26
27
28 395 Machines Corporation), considering a critical significance value of 5%, unless otherwise
29
30 396 stated.

31
32
33 397 Regarding genetic polymorphisms analysis, adherence to the Hardy-Weinberg
34
35 398 equilibrium will be checked using the χ^2 -square test. The R package 'argyle' will be used to
36
37 399 analyze the genotype data and assess the potential impact on the responses to the intervention
38
39 400 (32). To verify whether the results differ among the genotypes, the dominant model (major
40
41 401 allele x heterozygous + minor allele) will be applied. Effect size (ES) will be assessed by
42
43 402 Cohen's d-test and multivariate analysis (MANOVA) will be applied, including time and
44
45 403 genotype as the two independent variables. For post-hoc analysis, the Stoll Dwass test ($p < 0.05$)
46
47 404 will be applied. Statistical analysis of the gene expression profile will include the evaluation
48
49 405 of the quality of the sequences with the FastQC tool. The Trimmomatic software (33) will be
50
51 406 used to remove low-quality strings and adapters. Then, the libraries will be evaluated again in
52
53 407 the FastQC software, for proper verification. The RNA sequencing data will be subjected to
54
55 408 analysis by RNA-seq using the protocol described in Trapnell, Roberts (34). The functional
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3 409 annotation of differentially expressed genes will be carried out through the GeneOntology
4
5 410 platform (<http://geneontology.org>). Analyses to identify differentially expressed metabolic
6
7 411 pathways will be performed using the fgsea package of the R software.
8
9

10 412 For the gut microbiome analysis, raw sequencing data will be undergone multiple
11
12 413 quality control steps including primer removal, trimming, chimera removal as previously
13
14 414 described (35, 36). Sequences will be classified using classify-sklearn algorithm (37) against
15
16 415 Silva database v.138 generated for given primers by RESCRIPT (38). To decontamination, non-
17
18 416 target sequences will be removed such as mitochondria, chloroplast and archaea. Possible
19
20 417 contamination detected by positive and negative controls and sequences with raw count <3 and
21
22 418 present in <10% of samples will be removed. Sample with an extremely low number of reads
23
24 419 (< 2000 after filtering) will not be considered in microbiome analysis. Filtered ASV table in
25
26 420 count data will be converted to relative abundant data for visualisation, then centered log-ratio
27
28 421 (CLR) transformed. The indices of α -diversity (e.g., observed species, Shannon, Phylogenetic
29
30 422 Diversity, Inver Simpson) and β -diversity (e.g., Bray-Curtis and Aitchison distance) will be
31
32 423 calculated using Phyloseq (39) and microbiome (40) R packages. Bray-Curtis distance will be
33
34 424 used to generate non-parametric multidimensional scaling ordination plots for β -diversity
35
36 425 metrics with scaled and centered results. Stability over time will also be assessed based on
37
38 426 Bray-Curtis distances. The adonis2 function in R package vegan will be used for permutational
39
40 427 multivariate analysis of variance (PERMANOVA). Aitchison distance will be used for
41
42 428 Principal component analysis (PCA) in mapping microbiome and metabolic markers data to
43
44 429 exploring multidimensional association (23). False discovery rate (FDR) will be used to adjust
45
46 430 p values, and FDR<0.05 will be considered as statistically significant.
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56 432 **Patient and public involvement:**

57
58 433 None.
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5 435 **ETHICS AND DISSEMINATION**

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7
8 436 This study is approved by the University of Alberta's HREB (Pro00070712) and is
9
10 437 registered on ClinicalTrials.gov (NCT03235804). This research adheres to the standards as set
11
12 438 out in the Canadian Tri-Council Policy statement on the use of human participants in research.
13
14 439 This study is regulated by Health Canada. Amendments will be submitted to the HREB and
15
16 440 Health Canada review and approval prior implementations. ClinicalTrials.gov will be updated
17
18 441 accordingly.

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21 442 All personal information is kept private, and participation is anonymous. Participants
22
23 443 are assigned a study ID, which is kept separated from any personal information collected. A
24
25 444 master list with identifiable information and study IDs is cryptographically protected and stored
26
27 445 at the HNRU. The study information will be kept for 15 years after the completion of the study.
28
29 446 If participants withdraw consent, they are asked for permission to use the data collected until
30
31 447 that point; however, if they deny it, their data is destroyed. Absence of answer is considered as
32
33 448 permission to use the data. The Quality Management in Clinical Research (QMCR) Department
34
35 449 at the University of Alberta is independent of investigators and sponsor. The QMCR is
36
37 450 responsible for monitoring the study data and will conduct yearly auditing.

38
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40 451 Following data collection, analysis, and review of findings, manuscripts will be
41
42 452 prepared for submission to peer-reviewed journals and results presented in national and
43
44 453 international conferences. Study findings will also be disseminated through social media. Data
45
46 454 will be published regardless of outcomes and the University of Alberta retains the right to
47
48 455 publish. Authorship eligibility will adhere to the International Committee of Medical Journal
49
50 456 Editors' recommended guidelines (41). As a mandate of completing a registered trial, the
51
52 457 results must be published within 12 months of the completion of the trial. Dataset and statistical
53
54 458 code may be provided upon request.

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Figure legends:

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8 461 **Figure 1.** Experimental protocol. Abbreviations: CON control group, PMR powdered meal
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10 462 replacement group.

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12 463 **Figure 2.** Schedule of enrollment, interventions, and assessments (SPIRIT figure).
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14
15 464 Abbreviations: CON control group, PMR powdered meal replacement group.
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20 466 **Author contributions:**

21
22 467 JM, CLPO, AMA, AB, AMS, LM, CC, SG, CR, NKN, PDC, JW, and CMP were involved in
23
24 468 the design of the study. JM, CLPO, AMS, JW, and CMP wrote the study protocol. JM, CLPO,
25
26 469 AMA, AB, AMS, LM, CC, SG, CR, NKN, PDC, JW, and CMP participated in drafting and
27
28 470 revising the manuscript. JM, CLPO, AMA, AB, AMS, LM, CC, SG, CR, NKN, PDC, JW, and
29
30 471 CMP read and approved the final manuscript.
31
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57 482 **Competing interests' statement:** In addition to what is noted under "Funding", CLPO reports
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59 483 receiving honoraria and/or paid consultancy from Abbott and AMRA Medical Inc. outside the
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11
12 488 dealing with the use of specific bacteria and components in the treatment of different diseases.
13
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17
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19
20 492 Health, a developer of synbiotic products. CMP reports receiving honoraria and/or paid
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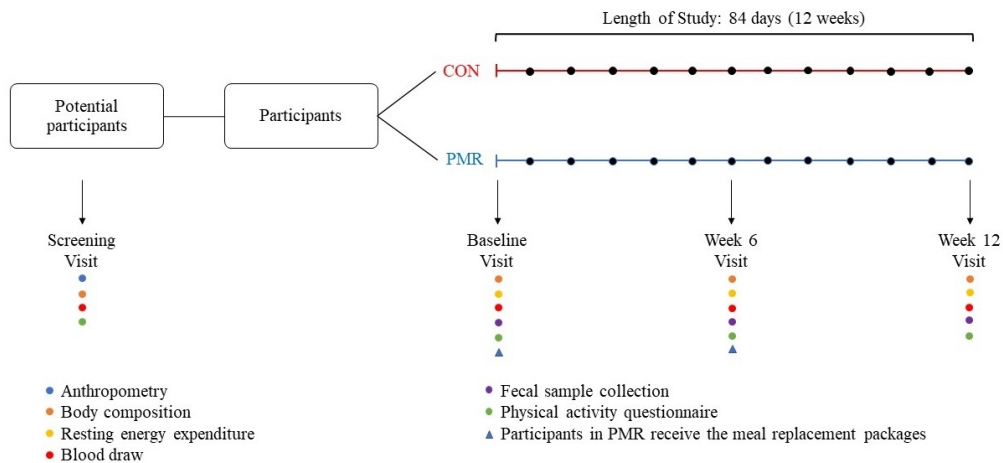


Figure 1. Experimental protocol. Abbreviations: CON control group, PMR powdered meal replacement group.

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TIMEPOINT		STUDY PERIOD					
		Enrollment	Allocation	Post-Allocation			
		Screening visit	Post-screening	Visit 1 (week 1)	Visit 2 (week 6)	Visit 3 (week 12)	Weekly
ENROLLMENT	Eligibility screen	X					
	Informed consent	X					
	Anthropometry	X					
	Body composition	X					
	Blood tests	X					
	Questionnaires	X					
	Allocation		X				
INTERVENTIONS	CON			●	●		
	PMR			●	●		
ASSESSMENTS	Gut microbiome			X	X	X	
	Systemic inflammatory biomarkers			X	X	X	
	Metabolic blood markers			X	X	X	
	Gene expression			X		X	
	Gene polymorphisms			X			
	Energy metabolism			X	X	X	
	Body composition			X	X	X	
	Appetite sensation						X
	Physical activity questionnaire			X	X	X	
Dietary intake						X	

Figure 2. Schedule of enrollment, interventions, and assessments (SPIRIT figure). Abbreviations: CON control group, PMR powdered meal replacement group.

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INFORMED CONSENT FORM

Title of Study: The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight (The PREMIUM Study).

Principal Investigators:

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Background

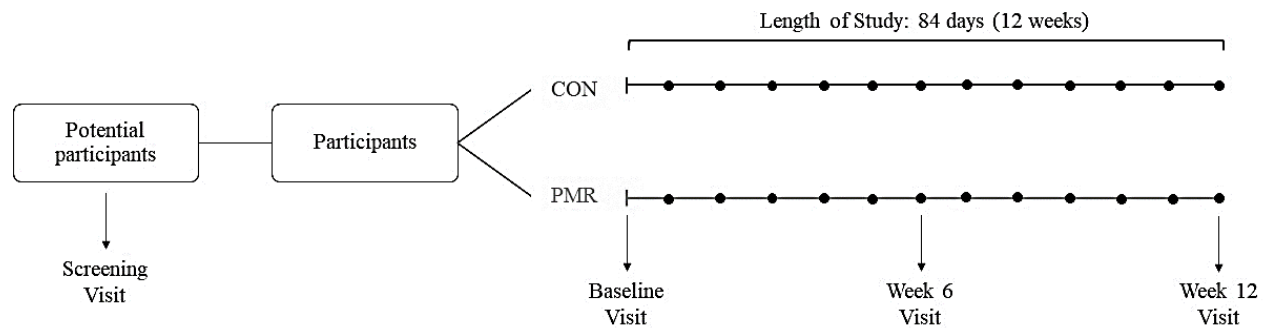
Meal replacements are nutritionally complete formula foods used to substitute a meal. They can be a drink, bar, or soup. These products have been gaining popularity because they can help individuals lose weight. In addition, depending on its ingredients, meal replacements may affect our health. For this reason, meal replacements have been studied for health benefits. However, how meal replacements affect the microbes living in our gut, inflammation, and our genes is not known. Therefore, our study will investigate how a meal replacement affects these factors, as well as metabolism, the amount of fat and muscle of our bodies, and appetite. The powdered meal replacement used in this study is not investigational and is available for purchase by the public. However, because we are using it in this study to see the effect on gut microbes, this meal replacement is considered investigational by Health Canada, which has approved this study.

What will happen in the study?

Participants will attend at least 4 clinic visits over a 14-week period. The day and time of your visits will be decided by you and the study coordinator. We will first collect a blood sample to determine if you are eligible for the study. If eligible, you will be randomly assigned to a powdered meal replacement group or control group. Participants in the powdered meal replacement group will add the meal replacement to their diets twice daily for 12 weeks. Participants in the control group will maintain their usual food intake. The meal replacement is neutral tasting powder which you will add to water and drink. The study coordinator will show you how to do this. Besides from taking the meal replacement, no other lifestyle changes are needed and maintain your normal medication regime and physical activity level is required. You must inform study staff if you make any changes to your current medication or nutritional supplement use. You cannot participate in the study if you take any natural health products which may



alter inflammation, gut microbiome, energy metabolism, body weight and composition, or hormone levels. You should also continue to eat your normal diet. You will weigh yourself daily during the study using a scale we will give you. It is important that you do not lose or gain weight during the study. If this happens you will meet with a registered dietitian to adjust your food intake. Over 12 weeks we will collect three blood samples to measure the level of inflammation, different hormones, genes related to nutrient metabolism and gene expression, fat, and sugar in your blood. We will also collect three stool samples to study the microbes living in your gut and three urine samples to study the substances you consumed with the meal replacement (only if you are in the powdered meal replacement group). You will also complete different questionnaires that ask you about your food intake and level of physical activity. Moreover, for the powdered meal replacement group, you will answer questionnaires about how hungry or full you feel.



Screening visit: Sign “Informed Consent Form”, fasting blood draw, complete questionnaires (personal information, health status, and physical activity), anthropometric and body composition assessments.

Visits at baseline and week 6: Participants in PMR group collect nutritional supplement packages.

Intervention visits (baseline, week 6, and week 12): 1-hour WBCU test, fasting blood draw, urine (PMR group) and stool sample collection, body composition assessment, physical activity questionnaire.

• **Weekly:** Phone call to verify compliance, participants send 1-day dietary record, body weight and appetite sensation (PMR group). On the weeks 1, 6 and 12 there will be 3-day dietary record instead of 1-day dietary record.

Figure: Study design.

Visit 1 – Screening (about 1 hour):

This visit will happen in the morning. For this visit you should not consume alcohol for 3 days and not eat or drink for 12 hours before the visit. You may drink water. First, the study coordinator will explain the study to you. If you are interested in participating, you will be asked to sign this consent form. Then trained personnel will collect 10 mL of your blood (2 teaspoons). This blood sample will help us determine if you are eligible to participate based on your liver, kidney and thyroid function, and hydration status. If one or more of these tests is outside the reference values for a healthy person, you will be notified and considered not eligible to participate in our study. You may not participate in this study if you are pregnant, the blood test will tell us if you are currently pregnant. During the study, those who are of childbearing potential, must practice adequate methods of birth control (e.g., total abstinence, hormonal birth control methods (oral, injectable, transdermal, or intra-vaginal), intrauterine devices, confirmed successful vasectomy of partner etc.). If you become pregnant over the duration of the study, you must stop taking



the product, and immediately inform the study investigators. You will also answer questions related to personal characteristics, health status and physical activity. We will measure your height, weight, and waist circumference. We will also measure the fat in your body using bioelectrical impedance analysis (BIA). You will lie down on a bed and eight self-adhesive electrodes will be connected to both of your hands and feet. You will not feel anything, and the procedure will take 1 to 2 minutes. Or you will be asked to stand on a scale with the ball and the heel of each foot in contact with four metal electrodes. This measurement will take no more than 15 seconds. At the end of this visit we will offer to you a snack and a beverage. Once we test your blood, we will contact you to let you know if you are eligible. If so, you will be randomly assigned to a powdered meal replacement group or control group and we will schedule your second visit.

Visit 2 (about 3 hours):

This visit will also happen in the morning. For this visit, you should not consume alcohol for 3 days and not eat or drink for 12 hours before the visit. You may drink water. You should not exercise 24 hours before this visit and do as little activity as possible before the visit. For example, taking the elevator instead of the stairs.

At the beginning of this visit we will estimate how many calories you burn during a day using the whole-body calorimetry unit (WBCU). The WBCU is similar to a small hotel room. It has a bed, armchair, table, sink, toilet, television, computer/internet and treadmill. You will lie in a relaxed position on a bed. You will breathe regularly and relax without falling asleep. The test will take about 1 hour.

Then, we will measure the fat and muscle in your body using 3 safe and routine techniques. The first technique is a dual energy X-ray absorptiometry [DXA] scan, which may happen in a different day than the visit but in the same week or as close as possible. Before the scan we will make sure it is safe for you by asking some questions. If you are a woman, we will test your blood sample to see if you are pregnant. Pregnant women will not be allowed to participate in this study. During the scan you will lie down on a bed and the technician will position you correctly. The equipment “arm” will pass over your body and it will not cause any discomfort to you. It will take about 20 minutes to finish this test. The second technique is air displacement plethysmography (ADP). During this test you will sit comfortably inside a chamber and stay relaxed and quiet for 1 minute. This will happen twice. The entire test will take about 5 minutes. For this test, you will need to wear minimal, form-fitting clothing and a swim-cap. The last technique is the BIA, you will lie down on a bed and eight electrodes will be connected to both of your hands and feet. You will not feel anything, and the procedure will take 1 to 2 minutes.

After these assessments, certified personnel will collect 24 mL of your blood (5 teaspoons) to assess your level of inflammation, hormones, the interaction between your genes and the diet, fat, and sugar in your blood. If you are in the powdered meal replacement group, you will also collect your urine (~1 cup) to assess the level of substances contained in the meal replacement.

Then, we will give you a snack to eat. You will then answer a physical activity questionnaire. The study coordinator will explain how to monitor your body weight and complete questionnaires for the study. These questionnaires will ask about how hungry and full you feel and the foods you eat.

We will also explain how to collect a stool sample at home using the kit provided. You can bring this stool sample back later the same day or the next day. Once we receive your first stool sample, the research coordinator will give to participants assigned to the powdered meal replacement group the



1
2
3 packages with the product and explain how to use it. The product will be mixed with water and taken
4 twice daily as snacks in the morning and afternoon for 12 weeks (84 days). Lastly, the study coordinator
5 will provide you with another stool collection kit.
6
7

8 *Visit 3 – Day 42 (about 3 hours):*

9
10 This visit will happen in the morning and is similar to Visit 2, except genetic analyses, that will
11 not be assessed. This means that the amount of blood we will collect will be 20 mL (4 teaspoons). For this
12 visit, you should not consume alcohol for 3 days and not eat or drink for 12 hours before the visit. You
13 may drink water. You should not exercise 24 hours before this visit and do as little activity as possible
14 before the visit. During this visit we will complete the same assessments as Visit 2. You will also bring
15 your stool sample to this visit. If you are unable to bring it to this visit, you can bring on the day before or
16 after your scheduled appointment.
17
18

19 The study coordinator will check if your weight has changed from the beginning of the study and
20 review your study journal. If your weight has changed the dietitian will work with you to adjust your diet
21 to make sure your weight remains stable. At the end of the visit, the study coordinator will provide you
22 with another stool collection kit. Participants in the powdered meal replacement group should keep taking
23 the meal replacement mixed with water twice daily as snacks in the morning and afternoon until Visit 4.
24 Participants in the control group will maintain their usual food intake.
25
26
27

28 *Visit 4 – Day 84 (about 3 hours):*

29
30 For this visit you will prepare as you did for Visit 2. This will be the last visit you will attend. For
31 this visit you should bring back the scale provided during the study period and your final stool sample. As
32 with the other stool sample collections, you are able to bring the sample to the visit, or the day before or
33 after the scheduled visit. The study will have been completed at the end of this visit.
34
35

36 *Weekly Assessment / Communication:*

37
38 At the beginning of the study, we will provide a study journal. This provides reminders of what
39 needs to be done every day throughout the study. Daily and weekly tasks include weighing yourself and
40 completing questionnaires. These questionnaires will be about how hungry and full you feel (only for
41 participants assigned to the powdered meal replacement group) and the foods you eat. Before Visits 2, 3,
42 and 4 you will complete an online questionnaire about the food you ate three days during the weeks of
43 these study visits. This will include one weekend day and one weekday. For the other weeks of the study,
44 you will complete one of these questionnaires each week. The study coordinator will call or email you
45 each week. This is to make sure you are following the recommendations provided. You will also be asked
46 about your daily weight measurements and reminded to complete your study journal.
47
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51 *Results:*

52 You will learn how many calories you burn in a day. You will also receive information about the
53 amount of fat, bone, and lean soft tissue (i.e., everything else, but fat and bone) in your body.
54
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Information about Investigational Product:

Serving Size: 8 tablespoons (50g)

Servings Per Container: 10

Amount Per Serving	% Daily Value*
Calories 180	
Calories from Fat 9	
Total Fat 1.0g*	1.5%
Saturated Fat 0.5g*	2.5%
Trans Fat 0g	**
Polyunsaturated Fat 0.1g	**
Monounsaturated Fat 0.4g	**
Cholesterol 3mg	1%
Sodium 340mg	15%
Potassium 500mg	14%
Total Carbohydrates 15g*	6%
Dietary Fiber 0.5g*	2%
Sugars 15g	**
Protein 27g*	54%

Vitamin A 794 IU	16%
Vitamin C 16mg	27%
Vitamin E 6 IU	20%
Thiamin (Vitamin B1) .5mg	33%
Riboflavin (Vitamin B2) 6mg	350%
Vitamin B6 .7mg	35%
Calcium 215mg	22%
Iron 4.9mg	27%

* Percent Daily Values are based on a 2,000 calorie diet.

** Daily Value not established.

Essential and Potentially Essential

Amino Acid Content of Protein Ingredients

Amino Acid		Per Serving 50g	
L Tyrosine	950mg	L Leucine	2300mg
L Methionine	400mg	L Isoleucine	1400mg
L Cystine	300mg	L Valine	1400mg
L Lysine	1550mg	L Histidine	700mg
L Threonine	950mg	L Arginine	1800mg
L Tryptophan	400mg	L Phenylalanine	1300mg

Ingredients: Soy Protein Isolate, Honey, Skim Milk Yogurt Powder, Potassium Chloride, Magnesium Carbonate, Calcium Citrate, Vitamin C, Niacin, Color Additive: Riboflavin (Vitamin B2), Vitamin E, Zinc Oxide, Ferrous Fumarate, Manganese Sulfate, Calcium Pantothenate, Vitamin B2, Vitamin B6, Vitamin B1, Vitamin A, Folic Acid, Potassium Iodide, Sodium Selenite, Biotin, Vitamin D3, Vitamin B12



What are the risks and discomforts?

There are no known risks of eating the meal replacement, there may be unknown risk with taking this investigational natural health product and potential side effects may include liver related drug adverse events. It is very unlikely to cause you any discomfort. The blood draws are a routine procedure performed by trained personnel. A needle will be inserted into a vein and blood will be withdrawn for lab tests. It is possible you may experience mild pain, fainting, bleeding, and bruising, and or an infection at the insertion site. Bruising is common, but usually goes away after a few days. Infection, dizziness, and fainting are rare during this procedure. There are no risks of having the genetic analysis, only potential general genetic linkages with metabolism of nutrients will be identified. You may also feel uncomfortable being alone in the WBCU. However, the tests will take only 1 hour and there will always be research staff close by and there is an intercom system to talk to them.

The X-ray dose associated with DXA scan is very low and not believed to have any long-term bad effects on your health. Pregnant women are excluded as a precaution. Having a DXA scan does not make it unsafe for you to have other X-rays in the future.

The BIA test is a risk for you only if you have a pacemaker or other internal electrical medical device. This is due to the risk of device malfunction from the weak electrical signal. Individuals with pacemakers or internal medical devices will not be able to participate in this study.

Dr. Laurie Mereu is a member of our research team and medical doctor. She will review your blood tests. If there are abnormal results Dr. Mereu will provide suggestions on how to proceed.

Risk of exposure to COVID-19 with your participation include exposure to others (research personnel and other participants) and increased time within our research unit. Measures undertaken to reduce this risk include ensuring all personnel and participants wear a mask, and frequent hand washing. All hard surfaces and common touched areas are disinfected before and after each visit. One-way traffic and physical distancing of 2 meters are encouraged at all times.

What happens if I am injured because of this research?

If you become ill or injured as a result of being in this study, you will still be able to receive necessary medical treatment. This will occur at no additional cost to you. By signing this consent form, you are not releasing the investigators, institution, or sponsors from their legal and professional duties.

What are the benefits to me?

There are no direct benefits to you for participating in this study. We hope the study will give us more information about how our bodies use the powdered meal replacement.

Do I have to participate?

No. Taking part in this study is your choice. You may stop participating in the study at any time. You can withdraw by contacting a study coordinator. Phone number: (780) 492-9010.

Will I be paid to be in the research?

After you complete the study, we will compensate your time with a \$300 honorarium. We will also give to you a parking pass in case you need to park your car in front of our clinic. There is no cost associated with participating.



Will my information be kept private?

During the study we will collect your health information. This will be kept private. We will not release information containing your name outside of the study investigators office. It will not be listed in the research when published. By law, we may have to release your information with your name so we cannot guarantee absolute privacy. However, we will make every legal effort to make sure that your health information is kept private.

During research studies it is important that the data we get is accurate. For this reason, your health data and name may be looked at by people from the University of Alberta auditors or members of the Research Ethics Board. By signing this consent form, you are giving permission for the study staff to collect your health information and use it for research purposes.

After the study is done, we will securely store your health data that was collected as part of the study. As per Health Canada requirements, your data will be stored and kept confidential for 15 years. If you leave the study, we will ask permission to keep your data. If you do not respond, we will use your data that had been collected so far.

If you leave the study, we will not collect any new information from you. However, we will keep the data that we have already collected, unless you specifically request it to be destroyed.

What if I have questions?

If you have any questions about this research, please contact the principal investigator (Dr. Carla Prado at 780-492-7934) or the study coordinator (Julia Montenegro at 780-492-9010).

If you suffer a research related injury, please contact the study coordinator at this number as well.

If you have any questions about your rights as a research participant, you may contact the Health Research Ethics Board at 780-492-2615. This office is independent of the study investigators.

The study is being sponsored by the ALMASED WELLNESS GMBH, the company that makes the powdered meal replacement. If you need, you can request any details about this product from the Principal Investigator.



CONSENT

Title of Study: The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight.

Principal Investigators:

Dr. Carla Prado	Phone: (780) 492-7934	E-mail: carla.prado@ualberta.ca
Dr. Jens Walter		E-mail: jwalter1@ualberta.ca
Dr. Arya Sharma		E-mail: amsharm@ualberta.ca

Qualified Investigator:

Dr. Laurie Mereu	Phone: (780) 492-3626	E-mail: laurie.mereu@ualberta.ca
------------------	-----------------------	----------------------------------

Study Manager:

Camila Oliveira, Post-doctoral Fellow	Phone: (780) 492-9010	E-mail: premium@ualberta.ca
---------------------------------------	-----------------------	-----------------------------

Study Coordinator:

Julia Montenegro, PhD Student	Phone: (780) 492-9010	E-mail: premium@ualberta.ca
-------------------------------	-----------------------	-----------------------------

By signing below, you understand:

- That you have read the above information and have had anything that you do not understand explained to you to your satisfaction.
- That you will be taking part in a research study.
- That you read and received a copy of the attached Information Sheet.
- The benefits and risks involved in taking part in this research study.
- That you are free to leave the study at any time, without having to give a reason and without affecting your future medical care.
- Who will have access to your records, including personally identifiable health information.

Who explained this study to you? _____

Signature of Research Participant _____

(Printed Name) _____

Date: _____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator or Designee _____ Date _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A SIGNED COPY GIVEN TO THE RESEARCH PARTICIPANT.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

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

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet	2, 6, 17

1		registered, name of intended registry	
2			
3			
4	Trial registration:	#2b All items from the World Health Organization Trial	6
5			
6	data set	Registration Data Set	
7			
8			
9	Protocol version	#3 Date and version identifier	2, 5, 17
10			
11			
12	Funding	#4 Sources and types of financial, material, and other	18-19
13			
14		support	
15			
16			
17	Roles and	#5a Names, affiliations, and roles of protocol contributors	1, 18
18			
19	responsibilities:		
20			
21	contributorship		
22			
23			
24			
25	Roles and	#5b Name and contact information for the trial sponsor	18
26			
27	responsibilities:		
28			
29	sponsor contact		
30			
31	information		
32			
33			
34			
35	Roles and	#5c Role of study sponsor and funders, if any, in study	18
36			
37	responsibilities:	design; collection, management, analysis, and	
38			
39	sponsor and funder	interpretation of data; writing of the report; and the	
40			
41		decision to submit the report for publication, including	
42			
43		whether they will have ultimate authority over any of	
44			
45		these activities	
46			
47			
48			
49	Roles and	#5d Composition, roles, and responsibilities of the	18
50			
51	responsibilities:	coordinating centre, steering committee, endpoint	
52			
53	committees	adjudication committee, data management team, and	
54			
55		other individuals or groups overseeing the trial, if	
56			
57			
58			
59			
60			

1 applicable (see Item 21a for data monitoring

2
3 committee)

4
5
6 **Introduction**

7
8
9 Background and [#6a](#) Description of research question and justification for 4-5
10
11 rationale undertaking the trial, including summary of relevant
12
13 studies (published and unpublished) examining
14
15 benefits and harms for each intervention

16
17
18
19 Background and [#6b](#) Explanation for choice of comparators 5
20
21 rationale: choice of
22
23 comparators

24
25
26 Objectives [#7](#) Specific objectives or hypotheses 5

27
28
29 Trial design [#8](#) Description of trial design including type of trial (eg, 5-6
30
31 parallel group, crossover, factorial, single group),
32
33 allocation ratio, and framework (eg, superiority,
34
35 equivalence, non-inferiority, exploratory)

36
37
38
39 **Methods:**

40
41 **Participants,**
42
43 **interventions, and**
44
45 **outcomes**

46
47
48
49 Study setting [#9](#) Description of study settings (eg, community clinic, 5
50
51 academic hospital) and list of countries where data
52
53 will be collected. Reference to where list of study
54
55 sites can be obtained
56
57

1	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	7
2				
3			applicable, eligibility criteria for study centres and	
4			individuals who will perform the interventions (eg,	
5			surgeons, psychotherapists)	
6				
7				
8				
9				
10				
11	Interventions:	#11a	Interventions for each group with sufficient detail to	8
12				
13	description		allow replication, including how and when they will be	
14			administered	
15				
16				
17				
18				
19	Interventions:	#11b	Criteria for discontinuing or modifying allocated	15
20			interventions for a given trial participant (eg, drug	
21	modifications		dose change in response to harms, participant	
22			request, or improving / worsening disease)	
23				
24				
25				
26				
27				
28				
29	Interventions:	#11c	Strategies to improve adherence to intervention	15
30				
31	adherence		protocols, and any procedures for monitoring	
32			adherence (eg, drug tablet return; laboratory tests)	
33				
34				
35				
36	Interventions:	#11d	Relevant concomitant care and interventions that are	15
37				
38	concomitant care		permitted or prohibited during the trial	
39				
40				
41				
42	Outcomes	#12	Primary, secondary, and other outcomes, including	6-7
43				
44			the specific measurement variable (eg, systolic blood	
45			pressure), analysis metric (eg, change from baseline,	
46			final value, time to event), method of aggregation	
47			(eg, median, proportion), and time point for each	
48			outcome. Explanation of the clinical relevance of	
49			chosen efficacy and harm outcomes is strongly	
50			recommended	
51				
52				
53				
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1	Participant timeline	#13	Time schedule of enrolment, interventions (including	8-9
2			any run-ins and washouts), assessments, and visits	
3			for participants. A schematic diagram is highly	
4			recommended (see Figure)	
5				
6				
7				
8				
9				
10				
11	Sample size	#14	Estimated number of participants needed to achieve	15
12			study objectives and how it was determined,	
13			including clinical and statistical assumptions	
14			supporting any sample size calculations	
15				
16				
17				
18				
19				
20				
21	Recruitment	#15	Strategies for achieving adequate participant	8
22			enrolment to reach target sample size	
23				
24				
25				
26	Methods:			
27				
28	Assignment of			
29	interventions (for			
30	controlled trials)			
31				
32				
33				
34				
35				
36	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	8
37	generation		computer-generated random numbers), and list of	
38			any factors for stratification. To reduce predictability	
39			of a random sequence, details of any planned	
40			restriction (eg, blocking) should be provided in a	
41			separate document that is unavailable to those who	
42			enrol participants or assign interventions	
43				
44				
45				
46				
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49				
50				
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52				
53	Allocation	#16b	Mechanism of implementing the allocation sequence	8
54	concealment		(eg, central telephone; sequentially numbered,	
55			opaque, sealed envelopes), describing any steps to	
56				
57				
58				
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1		conceal the sequence until interventions are	
2			
3		assigned	
4			
5			
6	Allocation:	#16c Who will generate the allocation sequence, who will	17-18
7			
8	implementation	enrol participants, and who will assign participants to	
9			
10		interventions	
11			
12			
13	Blinding (masking)	#17a Who will be blinded after assignment to interventions	3
14			
15		(eg, trial participants, care providers, outcome	
16			
17		assessors, data analysts), and how	
18			
19			
20			
21	Blinding (masking):	#17b If blinded, circumstances under which unblinding is	n/a
22			
23	emergency	permissible, and procedure for revealing a	
24			
25	unblinding	participant's allocated intervention during the trial	
26			
27			
28			
29	Methods: Data		
30			
31	collection,		
32			
33	management, and		
34			
35	analysis		
36			
37			
38			
39	Data collection plan	#18a Plans for assessment and collection of outcome,	8-15
40			
41		baseline, and other trial data, including any related	
42			
43		processes to promote data quality (eg, duplicate	
44			
45		measurements, training of assessors) and a	
46			
47		description of study instruments (eg, questionnaires,	
48			
49		laboratory tests) along with their reliability and	
50			
51		validity, if known. Reference to where data collection	
52			
53		forms can be found, if not in the protocol	
54			
55			
56			
57			
58	Data collection plan:	#18b Plans to promote participant retention and complete	9/ 15
59			
60			

1	retention		follow-up, including list of any outcome data to be	
2			collected for participants who discontinue or deviate	
3			from intervention protocols	
4				
5				
6				
7				
8	Data management	#19	Plans for data entry, coding, security, and storage,	17-18
9			including any related processes to promote data	
10			quality (eg, double data entry; range checks for data	
11			values). Reference to where details of data	
12			management procedures can be found, if not in the	
13			protocol	
14				
15				
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21				
22	Statistics: outcomes	#20a	Statistical methods for analysing primary and	15-17
23			secondary outcomes. Reference to where other	
24			details of the statistical analysis plan can be found, if	
25			not in the protocol	
26				
27				
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29				
30				
31				
32	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup	n/a
33	analyses		and adjusted analyses)	
34				
35				
36				
37				
38	Statistics: analysis	#20c	Definition of analysis population relating to protocol	16-17
39	population and		non-adherence (eg, as randomised analysis), and	
40	missing data		any statistical methods to handle missing data (eg,	
41			multiple imputation)	
42				
43				
44				
45				
46				
47	Methods: Monitoring			
48				
49				
50				
51	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	18
52	formal committee		summary of its role and reporting structure;	
53			statement of whether it is independent from the	
54			sponsor and competing interests; and reference to	
55				
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1 where further details about its charter can be found,
 2
 3 if not in the protocol. Alternatively, an explanation of
 4
 5 why a DMC is not needed
 6
 7

8	Data monitoring:	#21b	Description of any interim analyses and stopping	17-18
9				
10	interim analysis		guidelines, including who will have access to these	
11				
12			interim results and make the final decision to	
13				
14			terminate the trial	
15				
16				
17				
18	Harms	#22	Plans for collecting, assessing, reporting, and	9
19				
20			managing solicited and spontaneously reported	
21				
22			adverse events and other unintended effects of trial	
23				
24			interventions or trial conduct	
25				
26				
27				
28	Auditing	#23	Frequency and procedures for auditing trial conduct,	18
29				
30			if any, and whether the process will be independent	
31				
32			from investigators and the sponsor	
33				
34				
35	Ethics and			
36				
37	dissemination			
38				
39				
40				
41	Research ethics	#24	Plans for seeking research ethics committee /	17-18
42				
43	approval		institutional review board (REC / IRB) approval	
44				
45				
46	Protocol	#25	Plans for communicating important protocol	17
47				
48	amendments		modifications (eg, changes to eligibility criteria,	
49				
50			outcomes, analyses) to relevant parties (eg,	
51				
52			investigators, REC / IRBs, trial participants, trial	
53				
54			registries, journals, regulators)	
55				
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1	Consent or assent	#26a	Who will obtain informed consent or assent from	5-6
2				
3				
4			potential trial participants or authorised surrogates,	
5				
6			and how (see Item 32)	
7				
8				
9	Consent or assent:	#26b	Additional consent provisions for collection and use	n/a
10				
11	ancillary studies		of participant data and biological specimens in	
12				
13			ancillary studies, if applicable	
14				
15				
16	Confidentiality	#27	How personal information about potential and	17-18
17				
18			enrolled participants will be collected, shared, and	
19				
20			maintained in order to protect confidentiality before,	
21				
22			during, and after the trial	
23				
24				
25				
26	Declaration of	#28	Financial and other competing interests for principal	19
27				
28	interests		investigators for the overall trial and each study site	
29				
30				
31				
32	Data access	#29	Statement of who will have access to the final trial	17-19
33				
34			dataset, and disclosure of contractual agreements	
35				
36			that limit such access for investigators	
37				
38				
39	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care,	n/a
40				
41	trial care		and for compensation to those who suffer harm from	
42				
43			trial participation	
44				
45				
46				
47	Dissemination	#31a	Plans for investigators and sponsor to communicate	17-18
48				
49	policy: trial results		trial results to participants, healthcare professionals,	
50				
51			the public, and other relevant groups (eg, via	
52				
53			publication, reporting in results databases, or other	
54				
55			data sharing arrangements), including any	
56				
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publication restrictions

1
2
3
4 Dissemination [#31b](#) Authorship eligibility guidelines and any intended use 18
5
6 policy: authorship of professional writers
7

8
9 Dissemination [#31c](#) Plans, if any, for granting public access to the full n/a
10
11 policy: reproducible protocol, participant-level dataset, and statistical
12
13 research code
14
15

16 Appendices

17
18
19 Informed consent [#32](#) Model consent form and other related documentation Supplementary
20
21 materials given to participants and authorised surrogates material
22
23
24

25 Biological [#33](#) Plans for collection, laboratory evaluation, and 11-13
26
27 specimens storage of biological specimens for genetic or
28
29 molecular analysis in the current trial and for future
30
31 use in ancillary studies, if applicable
32
33
34

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36
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38
39 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
40
41 [Penelope.ai](#)
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BMJ Open

The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota (PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a Randomized Controlled Trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-070027.R2
Article Type:	Protocol
Date Submitted by the Author:	15-Aug-2023
Complete List of Authors:	Montenegro, Julia; University of Alberta, Department of Agricultural, Food, and Nutritional Science L. P. Oliveira, Camila ; University of Alberta, Department of Agricultural, Food and Nutritional Science Armet, Anissa M.; University of Alberta, Department of Agricultural, Food and Nutritional Science Berg, Aloys; University of Freiburg, Faculty of Medicine Sharma, Arya; University of Alberta, Department of Medicine Mereu, Laurie; University of Alberta, Department of Medicine Cominetti, Cristiane; Universidade Federal de Goias, Faculdade de Nutrição Ghosh, Sunita; University of Alberta, Department of Oncology Richard, Caroline; University of Alberta, Department of Agricultural, Food and Nutritional Science Nguyen, Nguyen ; Universite catholique de Louvain, Metabolism and Nutrition research group (MNUT); Walloon Excellence in Lifesciences and Biotechnology, WELBIO department Cani, Patrice; Universite catholique de Louvain, Metabolism and Nutrition research group (MNUT); Walloon Excellence in Lifesciences and Biotechnology, WELBIO department Walter, Jens; University of Alberta, Department of Agricultural, Food and Nutritional Science; University College Cork, APC Microbiome Ireland, School of Microbiology, and Department of Medicine Prado, Carla; University of Alberta, Agricultural Food and Nutritional Sciences
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Genetics and genomics, Immunology (including allergy), Research methods
Keywords:	NUTRITION & DIETETICS, MICROBIOLOGY, IMMUNOLOGY, Obesity

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1
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3 1 **The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota**
4
5 2 **(PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a**
6
7 3 **Randomized Controlled Trial**
8
9

10 4 Julia Montenegro^{1*}, Camila L. P. Oliveira^{1*}, Anissa M. Armet¹, Aloys Berg², Arya M.
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18 7

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46 21 * Co-corresponding authors: carla.prado@ualberta.ca and jenswalter@ucc.ie.
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22 ABSTRACT

23 *Introduction:* Excess body weight is associated with a state of low-grade chronic inflammation
24 and alterations of the gut microbiome. Powdered meal replacements (PMR) have been shown
25 to be an effective strategy for weight management; however, their effect on inflammation and
26 the gut microbiome remains unclear. The aim of this 12-week randomized control clinical trial
27 is to investigate the effects of PMR consumption, here given as a soy-yogurt-honey formula,
28 on inflammation, gut microbiome, and overall metabolism in individuals with excessive body
29 weight.

30 *Methods and analysis:* Healthy adults with excess body weight (n=88) are being recruited and
31 randomly assigned to one of the following groups: a) Control group (CON): maintaining usual
32 diet for 12 weeks, or b) PMR group: replacing morning and afternoon snacks daily with a PMR
33 for 12 weeks. Participants are asked to maintain body weight throughout the study and fill out
34 a journal with information about PMR consumption, body weight, food intake, appetite
35 sensations, and medications. Three study visits are required: baseline, week 6, and week 12.
36 Outcome measures include systemic inflammatory biomarkers, gut microbiome composition,
37 metabolic blood markers, host energy metabolism, body composition, appetite sensations, and
38 host gene expression profile.

39 *Ethics and dissemination:* This research protocol was approved by the University of Alberta
40 Ethics Board (Pro00070712) and adheres to the Canadian Tri-Council Policy statement on the
41 use of human participants in research. Procedures and potential risks are fully discussed with
42 participants. Study findings will be disseminated in peer-reviewed journals, conference
43 presentations, and social media.

44 *Registration details:* ClinicalTrials.gov identifier: NCT03235804.

46 **Keywords:** Powdered meal replacement; obesity; inflammation; gut microbiome.

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6 48 **ARTICLE SUMMARY**7
8 49 **Strengths and Limitations of this study**

- 9
- 10 50 • The randomized controlled clinical trial design, coupled with regular assessments and
- 11 follow-up sessions, as well as a comprehensive range of evaluated outcomes,
- 12 51 effectively reduces biases and confounding factors.
- 13 52
- 14 53 • Cutting edge technology, such as the metabolic chamber and dual-energy X-ray
- 15 54 absorptiometry, enables precise outcome measures.
- 16 55
- 17 56 • The exploratory multi-omics approach, incorporating gut microbiome, gene expression,
- 18 57 and genetic polymorphisms, supports the progress of precision nutrition by generating
- 19 58 hypothesis.
- 20 59
- 21 60 • A primary limitation of the study is the absence of a placebo group and the fact it is not
- 22 61 not double-blinded.
- 23 62
- 24 63 • Since the gut microbiome analysis depends on fecal samples, it might not fully
- 25 64 represent changes in the gut microbiome composition occurring in more proximal parts
- 26 65 of the gastrointestinal tract.
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64 **Word-count:** 5115 words.

65 INTRODUCTION

66 Excess body weight can be defined as a body mass index (BMI) ≥ 25.0 kg/m² (1), which
67 encompasses both the overweight and obesity categories (2). This condition has been
68 associated with a state of systemic low-grade chronic inflammation, which is characterized by
69 a persistent activation of immune and non-immune cells and production of cytokines,
70 chemokines, and acute phase proteins (3, 4). Those inflammatory biomarkers include
71 interleukins (IL), such as IL-6 and IL-8, tumor necrosis factor- α (TNF- α), and C-reactive
72 protein (CRP) (3). Systemic low-grade chronic inflammation causes tissue and organ damage,
73 which can, in turn, lead to the onset and progression of chronic diseases, such as diabetes
74 mellitus, cancer, metabolic syndrome, and cardiovascular diseases (3).

75 In individuals with excessive body weight, the state of systemic low-grade chronic
76 inflammation can be mediated by increased adiposity, as well as by mechanisms through the
77 gut microbiota (3). Increased adipocyte size (i.e., hypertrophy) is associated with cellular
78 dysfunction and distress (5, 6). Hypertrophic adipocytes secrete an increased number of pro-
79 inflammatory chemokines, such as TNF- α , IL-6, IL-8, and monocyte chemoattractant protein
80 1 (MCP-1) (4-6). The increased size of adipocytes and cytokine production lead to adipose
81 tissue hypoxia and death, as well as local and systemic inflammation (4-6).

82 Excess body weight is associated with altered gut microbiome composition and reduced
83 microbiome diversity, which might cause metabolic aberrations and enrich for opportunistic
84 pathogens (e.g. at the epithelial interface) that contribute to inflammation (7, 8). Individuals
85 with excessive body weight usually present with altered gut permeability, which elevates
86 systemic levels of endotoxins (i.e., lipopolysaccharides) (3). When in the bloodstream,
87 lipopolysaccharides binds to toll-like receptor 4 leading to activation of nuclear factor kappa B
88 and consequently production of pro-inflammatory cytokines, including IL-6 and TNF- α (8).

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3 89 Considering the numerous negative health outcomes associated with excess body
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5 90 weight, much effort has been made to develop effective weight management strategies. Among
6
7 91 those are meal replacements, which are food products fortified with vitamins and minerals used
8
9 92 to replace one or more meals per day. Meal replacements are commonly used in association
10
11 93 with calorie restriction. Research has shown that the consumption of meal replacements leads
12
13 94 to greater weight loss when compared to reduced-calorie diets alone (9, 10). Improvement in
14
15 95 metabolic parameters is generally observed with weight loss, including improvement in
16
17 96 glucose metabolism, reduction of triacylglycerol, low-density lipoprotein cholesterol (LDL-C),
18
19 97 systolic blood pressure (9, 11, 12), and the inflammatory markers CRP and IL-6 (13).
20
21 98 Considering the positive health effects of weight loss in individuals with excessive body weight
22
23 99 (14-16) and the beneficial health effects of meal replacements (9, 11), it is important to
24
25 100 differentiate the effects of meal replacements from that of weight loss on overall health, which
26
27 101 have not been investigated so far. Therefore, the aim of this study is to compare the effects of
28
29 102 a 12-week consumption of a powdered meal replacement (PMR group), given as a soy-yogurt-
30
31 103 honey formula (17), versus usual diet (control group, CON) on inflammation, gut microbiome,
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33 104 overall metabolic health, gene expression profile, and genetic background in individuals with
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35 105 excessive body weight who are in weight maintenance.
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107 **METHODS**

108 **Study design and ethical procedures**

109 This study is a randomized, controlled, parallel group, clinical trial conducted at the
110 Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada). The
111 study is an Investigator Initialized Trial sponsored by the Almased Wellness Comp.,
112 Bienenbüttel, Germany. The corresponding research protocol fulfils the requirements of the
113 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist (18).

1
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3 114 This research protocol was approved by the University of Alberta Ethics Board (HREB,
4
5 115 identifier Pro00070712) and complies with the standards established by the Canadian Tri-
6
7
8 116 Council Policy statement on the use of human participants in research. Procedures and potential
9
10 117 risks involved in the study are discussed with participants prior to obtaining informed consent
11
12 118 (supplementary material). This protocol is registered on ClinicalTrials.gov (NCT03235804),
13
14
15 119 and recruitment started on April 2019 and is expected to finish in November 2023 (**Table 1**).

16
17 **Table 1.** World Health Organization trial registration dataset

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03235804
Date of registration in primary registry	August 1, 2017
Secondary identifying numbers	University of Alberta Research Ethics Board # Pro00070712
Source(s) of monetary or material support	Almased Wellness-GmbH (Bienenbüttel, Germany)
Primary sponsor	Almased Wellness-GmbH (Bienenbüttel, Germany)
Secondary sponsor(s)	N/A
Contact for public queries	Dr Carla Prado +1 (780) 492-9555 carla.prado@ualberta.ca and Jens Walter +353 (0)21 490-1773 jenswalter@ucc.ie
Contact for scientific queries	Dr Carla Prado +1 (780) 492-9555 carla.prado@ualberta.ca and Jens Walter +353 (0)21 490-1773 jenswalter@ucc.ie
Public title	The impact of a powdered meal replacement on metabolism and gut microbiota (Premium Study)
Scientific title	The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight (The PREMIUM Study)
Countries of recruitment	Canada
Health condition(s) or problem(s) studied	Overweight and obesity
Intervention(s)	Powdered meal replacement

Key inclusion and exclusion criteria	Inclusion Criteria: (a) female/male aged 18 to 50 years; (b) non-smoker; (c) body mass index (BMI) between 25 and 37 kg/m ² ; (d) weight stable; (e) fat mass \geq 20% for men and \geq 25% for women; (f) stable physical activity level. Exclusion Criteria: (a) diagnosis of chronic diseases or acute infections; (b) taking any medication that may alter study outcomes; (c) taking pre- and probiotics; (d) use of antibiotics in the past two months; (e) females that are pregnant or lactating.
Study type	Randomized controlled trial
Date of first enrolment	April 1, 2019
Sample size	88
Recruitment status	Actively recruiting
Primary outcome(s)	Interleukin-6
Key secondary outcomes	Gut microbiota
Ethics review	University of Alberta Research Ethics Board # Pro00070712
Completion date	N/A
Summary results	N/A
Individual Participant Data (IPD) sharing statement	De-identified data will be shared with the participant upon completion of the study (publication)

Outcome measures

The primary study outcome is to compare changes in IL-6 concentration over time (within groups) between the PMR and CON groups. Secondary outcome is to examine shifts in in gut microbiome composition, assessed by relative abundances of amplicon sequence variant (ASV) over time (within groups) between the PMR and CON groups. Exploratory outcomes include:

- Remaining gut microbiome diversity indices and relative abundances of bacteria at different taxonomic levels (i.e., phylum, family, and genus) over time (within groups) between the PMR and CON groups.

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3 131 • Change in markers of systemic inflammation (high-sensitivity CRP [hs-CRP], IL-8, and
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5 132 TNF- α) and immune modulation (IL-10) over time (within groups) between the PMR
6
7 133 and CON groups.
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9
10 134 • Change in concentrations of metabolic blood markers (glucose, insulin, total
11
12 135 cholesterol, LDL-C, high-density lipoprotein cholesterol [HDL-C], triglycerides,
13
14 136 peptide tyrosine-tyrosine [PYY], glucagon-like peptide-1 [GLP-1], ghrelin,
15
16 137 adiponectin, leptin, free glycerol, free fatty acids, and thyroid stimulating hormone
17
18 138 [TSH]) over time (within groups) between the PMR and CON groups.
19
20
21 139 • Change in resting energy expenditure (REE) and respiratory exchange ratio (RER) over
22
23 140 time (within groups) between the PMR and CON groups.
24
25
26 141 • Change in body composition (fat mass [FM] and lean soft tissue [LST]) over time
27
28 142 (within groups) between the PMR and CON groups.
29
30
31 143 • Change in appetite sensations (hunger, satiety, fullness, and prospective food
32
33 144 consumption) over time within the PMR group.
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35
36 145 • Differences in the responses to the intervention according to genetic polymorphisms
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38 146 over time.
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41 147 • Changes in inflammation and excess body weight-related gene expression profile over
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43 148 time (within groups) between the PMR and CON groups.
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150 **Research participants**

151 Inclusion criteria are as follows: male or female; non-smoker; between 18 and 50 years
152 of age; BMI between 25.0 and 37.0 kg/m²; with a stable body weight 6 months prior to study
153 initiation (i.e., variation <5 kg); fat mass $\geq 20\%$ for males and $\geq 25\%$ for females; willingness
154 to maintain stable physical activity level throughout the study; and females must use effective
155 birth control methods.
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3 156 Exclusion criteria includes participation in >3 hours per week of vigorous physical
4
5 157 activity; pregnancy or lactation; diagnosis of any chronic or acute diseases (except for excess
6
7 158 body weight); use of any medication that impacts study outcomes, except for antidepressants,
8
9 159 anxiolytic, and/or thyroid replacement therapy in a stable dose 3 months prior to study initiation
10
11 160 and throughout the study period; use of antibiotics 2 months prior to study initiation; use of
12
13 161 protein supplements 1 month prior to study initiation; allergy to PMR ingredients (soy, honey,
14
15 162 and yogurt); allergy or intolerance to soy, gluten, and/or lactose; following a vegetarian, vegan,
16
17 163 or any other restrictive dietary pattern; claustrophobia; or being unable to comprehend and
18
19 164 complete the required questionnaires. Participants consuming supplements or food items that
20
21 165 contain pre- or probiotics (e.g., kefir or kombucha) before being enrolled in the study will be
22
23 166 asked to discontinue the use of these products and wait 1 month before starting the study. The
24
25 167 use of other nutritional supplements, such as multivitamins and vitamin D₃ will be allowed if
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27 168 on a stable dose.
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33 169

35 170 **Recruitment, randomization, and intervention**

37 171 Study advertisement is done using flyers displayed at the University of Alberta
38
39 172 campuses, surrounding communities, other post-secondary education institutions in Edmonton
40
41 173 (AB, Canada), and health care centres in the city. The study is also advertised in University of
42
43 174 Alberta email lists, newspapers, classrooms presentations, and on social media (e.g., Kijiji,
44
45 175 Facebook, and Twitter). Additionally, a personalized website (premium.ualberta.ca) was
46
47 176 created.
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51 177 Individuals interested in being part of the study will be invited to attend a screening
52
53 178 visit at the HNRU. This visit will include anthropometric measurements (i.e., height, weight,
54
55 179 and waist circumference), body composition assessment (bioelectrical impedance analysis
56
57 [BIA]), blood tests (i.e., creatinine, estimated glomerular filtration rate [eGFR], albumin,
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59 180
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2
3 181 aspartate transaminase [AST], alanine transaminase [ALT], sodium, potassium, chloride, and
4
5 182 TSH), review of medical history, and completion of a physical activity questionnaire. Although
6
7 183 glucose, insulin, or lipid panel tests are not conducted during the screening visit, individuals
8
9 184 who exhibit symptoms of or are taking medications for chronic diseases (e.g., diabetes,
10
11 185 hypertension, and dyslipidemia) are deemed ineligible for participation.
12
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14
15 186 If deemed eligible, participants are randomly assigned into either the CON or PMR
16
17 187 group. Randomization is stratified by sex using a Microsoft Office Excel® spreadsheet. To
18
19 188 guarantee impartial allocation of participants to the groups, a study team member created a list
20
21 189 of random numbers and assigned them to each group using the website Randomization.com
22
23 190 (<http://www.jerrydallal.com/random/randomize.htm>) with the method of randomly permuted
24
25 191 blocks. The list of random numbers is concealed, and a second investigator subsequently
26
27 192 follows the predetermined order of numbers and assigns participants to their respective groups
28
29 193 based on the order of their screening. Although the investigator has access to the randomization
30
31 194 list, they do not refer to it when assigning participants to their respective groups.
32
33

34
35 195 Participants assigned to the CON group are asked to maintain their usual diet for 12
36
37 196 weeks. The ones in the PMR group are asked to replace their morning and afternoon snacks
38
39 197 using a powdered meal replacement (Almased Wellness Comp., Bienenbüttel, Germany) and
40
41 198 otherwise maintain their usual diet for 12 weeks. Each snack is replaced by 50 grams of powder
42
43 199 mixed with 250 mL of water. The nutritional information of the meal replacement is displayed
44
45 200 in **Table 2**.
46
47

48
49 201 **Table 2.** Nutritional information of the tested soy-honey-yogurt formula, a powdered meal
50
51 202 replacement (PMR)
52

Nutrient	50 g of Product (PMR)
Calories (kcal)	180
Total fat (g)	1.0

<i>Saturated fat (g)</i>	0.5
<i>Trans fat (g)</i>	0
<i>Polyunsaturated fat (g)</i>	0.1
<i>Monounsaturated fat (g)</i>	0.4
Cholesterol (mg)	3
Total carbohydrates (g)	15
<i>Dietary fiber (g)</i>	0.5
<i>Sugars (g)</i>	15
Protein (g)	27
Sodium (mg)	340
Potassium (mg)	500
Vitamin A (IU)	794
Vitamin C (mg)	16
Vitamin E (IU)	6
Thiamin (Vitamin B1) (mg)	5
Riboflavin (Vitamin B2) (mg)	6
Vitamin B6 (mg)	7
Calcium (mg)	215
Iron (mg)	4.9

203

204

205 **Experimental protocol**

206 The study design is illustrated in **Figure 1**. The schedule of enrollment, interventions,
 207 and assessments are shown in **Figure 2**. Following the screening visit and randomization
 208 process, enrolled participants are invited to attend 3 study visits: baseline, week 6, and week

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3 209 12. Assessments during each of these visits include: 1-hour resting metabolic rate (RMR),
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5 210 blood draw, body composition, and physical activity questionnaire. They additionally receive
6
7 211 stool collection kits and instructions for fecal sample collection. During the baseline visit,
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9 212 participants receive a scale and a journal to record the following information daily: body
10
11 213 weight, date and time of meal replacement intake (PMR group only), and medication intake (if
12
13 214 any). They are also asked to record on a weekly basis a 24h dietary recall (both groups) and fill
14
15 215 out appetite sensation questionnaires (PMR group only). Instructions on how to fill out the
16
17 216 journal and dietary records are given. Additionally, participants assigned to the PMR group
18
19 217 receive 84 packages of the PMR during visits at baseline and week 6, as well as instructions on
20
21 218 how to prepare it. Those assigned to the PMR group start consuming the supplement the day
22
23 219 after the first stool sample collection. Study materials (study journal and scale) are returned on
24
25 220 week 12.

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28
29
30 221 A member of the study team contacts participants weekly to verify adherence to the
31
32 222 dietary intervention and potential adverse events. Their body weight is also discussed at that
33
34 223 time. If a body weight change greater than $\pm 2\%$ of their initial body weight is noticed, a
35
36 224 nutrition consult with a Registered Dietitian is scheduled to provide instructions on how to
37
38 225 increase or decrease food intake and physical activity levels to return to baseline body weight.
39
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44 227 *Anthropometry and body composition*

45
46 228 At the screening visit, anthropometric measurements are taken twice, and the average
47
48 229 is used for data analysis. Height is measured using a digital stadiometer (235 Heightronic™,
49
50 230 Concepts, Quick Medical, Snoqualmie, WA, USA) to the nearest 0.1 cm. Body weight is
51
52 231 measured to the nearest 0.1 kg using a calibrated digital scale (Health-o-meter® Professional
53
54 232 Remote Display, Sunbeam Products Inc., FL, USA). Waist circumference is measured using a
55
56 233 measuring tape at the level of participant's belly button, as per standard procedure (19).
57
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3 234 A digital scale (HD-314 TANITA Corporation, Tokyo, Japan) is provided to
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6 235 participants during the baseline visit, which is returned at the study completion. Body weight
7
8 236 is recorded daily in the morning in a fasting state, and with an empty bladder.
9

10 237 Body composition is assessed using dual energy X-ray absorptiometry (DXA, GE
11
12 238 Lunar iDXA, General Electric Company, Madison, USA), air displacement plethysmography
13
14 239 (ADP, Bod Pod 1SB-060M, Life Measurement Instruments, Concord, CA, USA), and BIA
15
16 240 (Seca mBCA525, Seca GmbH & Co, Hamburg, Germany). A number of techniques is being
17
18 241 used to explore potential changes in body composition using multicompartiment modeling:
19
20 242 DXA for bone mineral content, BIA for total body water, ADP for body density, which is used
21
22 243 to calculate the remaining compartment: adipose tissue and residues (i.e., dry LST) (20, 21).
23
24
25
26 244

28 245 *Resting energy expenditure*

30 246 Resting energy expenditure is assessed by indirect calorimetry using an open-circuit
31
32 247 metabolic chamber, which measures the volume of oxygen (O₂) and carbon dioxide (CO₂) from
33
34 248 participant's respiration. Participants lie down in a relaxed position without falling asleep and
35
36 249 breathe normally for 60 minutes. Mixed air with the expired CO₂ is drawn from the chamber
37
38 250 at a constant flow rate (60 ± 2 L/min) while fresh air with constant O₂ is passively drawn into
39
40 251 the chamber. The first 30 minutes of the test are considered time for acclimatization and hence
41
42 252 removed from analysis. Gas exchange (volume of CO₂ and O₂) is analysed minute-by-minute
43
44 253 by the Advance Optima AO2000 Series CO₂ analyser (ABB Automation GmbH, Frankfurt,
45
46 254 Germany) and the Oxymat 6 O₂ analyser (Siemens AG, Munich, Germany). Data is transferred
47
48 255 from those analysers to a computer (Acer Aspire AM3910-E3122, Acer Inc., New Taipei City,
49
50 256 Taiwan) via the National Instruments NI USB-6221 device (National Instruments Corporation,
51
52 257 Austin, Tex., USA) using the PMCSS Software version 1.8 (Pennington Metabolic Chamber
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258 Software Suite, Pennington Biomedical Research Center, La., USA). Resting energy
 259 expenditure (kcal/day) is calculated using the average kcal/min multiplied by 1440.

260

261 *Blood analysis*

262 Blood is sampled from participants by venipuncture after an overnight fast during the
 263 screening visit and at baseline, week 6, and week 12. Evaluated biomarkers are listed in **Table**
 264 **3**.

265 **Table 3.** Blood parameters, sample, and laboratory responsible for blood analysis

Parameter	Screening	Baseline	Week 6	Week 12	Sample	Laboratory
Albumin	x				Serum	External
Creatinine/eGFR	x				Serum	External
ALT	x				Serum	External
AST	x				Serum	External
Electrolytes ^a	x				Serum	External
TSH	x	x	x	x	Serum	External
hs-CRP		x	x	x	Serum	External
Glucose		x	x	x	Serum	External
Lipid panel ^b		x	x	x	Serum	External
Free glycerol		x	x	x	Serum	On site
Free fatty acids		x	x	x	Serum	On site
Interleukins ^c		x	x	x	Plasma	On site
TNF- α		x	x	x	Plasma	On site
Insulin		x	x	x	Plasma	On site
Leptin		x	x	x	Plasma	On site
Adiponectin		x	x	x	Plasma	On site

PYY	x	x	x	Plasma	On site
GLP-1	x	x	x	Plasma	On site
Ghrelin	x	x	x	Plasma	On site
Polymorphisms	x			Whole blood	On site
Gene expression	x		x	Whole blood	On site

266 ^aElectrolytes include chloride, sodium, and potassium. ^bLipid panel include triglycerides, total
 267 cholesterol, LDL-C, and HDL-C. ^c Interleukins include IL-6, IL-8, and IL-10. Abbreviations:
 268 ALT alanine aminotransferase; AST aspartate aminotransferase; eGFR estimated glomerular
 269 filtration rate; GLP-1 glucagon like peptide; hs-CRP high-sensitivity C-reactive protein; PYY
 270 peptide tyrosine-tyrosine; TNF- α tumor necrosis factor α ; TSH thyroid stimulating hormone.

271
 272 Blood samples are collected using BD Vacutainer[®] tubes (Becton, Dickinson and
 273 Company, Franklin Lakes, NJ, USA). Tubes containing silica and a polymer are used for serum
 274 separation, tubes containing K2-ethylenediaminetetraacetic acid (EDTA) are used for plasma
 275 separation, and tubes containing K2EDTA and protease inhibitors (dipotassium and tacrine,
 276 BD P800) are used for GLP-1 and ghrelin analysis.

277 Creatinine, eGFR, albumin, AST, ALT, sodium, potassium, chloride, and TSH are
 278 analysed by an external lab (DynaLIFE Medical Labs, Edmonton, AB, Canada) at the screening
 279 visit prior to enrollment. Glucose, lipid panel (triglycerides, total cholesterol, LDL-C, and
 280 HDL-C), TSH, and hs-CRP will be analysed by DynaLIFE Medical Labs (Edmonton, AB,
 281 Canada). Interleukin 6, IL-8, IL-10, TNF- α , insulin, PYY, GLP-1, ghrelin, adiponectin, leptin,
 282 free glycerol, and free fatty acids will be analysed in our laboratory (University of Alberta, AB,
 283 Canada). Interleukin 6, IL-8, IL-10, TNF- α , insulin, PYY, GLP-1, ghrelin, adiponectin, and
 284 leptin will be analysed by electrochemiluminescence immunoassay (MesoScale Discovery[®],
 285 Maryland, USA).

1
2
3 286 An additional blood draw is requested the day following each study visit for hs-CRP
4
5 287 analysis due to this being a sensitive marker which can vary substantially within hours of
6
7 288 collection for several reasons (22). Therefore, the average of CRP measured on two consecutive
8
9 289 days will be taken in case they are similar. If a participant is in an infectious state (i.e., CRP
10
11 290 >10 mg/L or significant changes between the two days measurement) the highest value will be
12
13 291 excluded from analysis.
14
15

16
17 292 For gene expression profile, ribonucleic acids (RNAs) will be sequenced at baseline
18
19 293 and week 12. Whole blood (500 μ L) is aliquoted into an RNase-free microfuge tube and added
20
21 294 1.3 mL of RNeasy lysis solution (Qiagen, Crawley, UK).
22
23 295 Total RNA will be extracted from whole blood using the RiboPure™ Blood Kit (Thermo Fisher
24
25 296 Scientific, Waltham, USA). The RNA purity will be determined by measuring the 260/280 nm
26
27 297 ratio (ideal ratio ~2.0) and the 260/230 nm ratio (ideal ratio 2.0-2.2) using a spectrophotometer.
28
29 298 The quality of RNA samples will be evaluated prior to library preparation for RNA-Seq, using
30
31 299 a bioanalyzer and an RNA Integrity Number (RIN) ≥ 7 will be accepted. Samples of high purity
32
33 300 and quality RNA will be prepared with the TruSeq RNA Sample Prep kit (Illumina, San Diego,
34
35 301 USA). The sequencing will be performed by an external company using the platform Illumina
36
37 302 HiSeq 4000 (Illumina, San Diego, USA), in the paired-end mode, in which the 2 ends will be
38
39 303 sequenced with a length of 100 base pairs (bp) (2 x 100 bp). At the end, 2 files in the 'fastq'
40
41 304 format will be generated for each of the evaluated samples.
42
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46
47 305 We will select a set of candidate genes that are known to be involved in the regulation
48
49 306 of inflammation and/or excess body weight and are differentially expressed after the
50
51 307 intervention. From these genes, we will analyze the most extensively studied polymorphisms.
52
53 308 The reasoning behind this approach is that genetic variations in these candidate genes could
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55 309 potentially impact the expression and/or function of the proteins they encode, ultimately
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57 310 influencing the response to the intervention. Genetic polymorphisms will be analysed at
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59
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1
2
3 311 baseline. Genomic deoxyribonucleic acid (gDNA) will be extracted with the QIAamp DNA
4
5 312 Micro Kit (Qiagen, Hilden, Germany) from leukocytes in peripheral blood. The gDNA purity
6
7 313 will be verified in a spectrophotometer at 260 and 280 nm. The samples will be considered of
8
9 314 good quality if the ratio between absorbances is between 1.7 and 2.0. The gDNA concentration
10
11 315 will be measured on a fluorimeter. For genotyping, a customized Infinium Global Screening
12
13 316 Array-24 + v3.0 Kit (Illumina, San Diego, USA) will be used.
14
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16
17 317

18 19 318 *Fecal sample collection and gut microbiome sequencing*

20
21 319 A total of three fecal samples are collected at baseline, week 6, and week 12. Fecal
22
23 320 samples are either collected at the HNRU the day of the study visits, or at home, kept at room
24
25 321 temperature, and delivered to the HNRU as soon as possible. During the baseline visit,
26
27 322 participants are instructed on how to collect fecal samples using the provided collection kits.
28
29 323 The fecal collection tubes (DNA/RNA Shield, Zymo Research, Irvine, CA, USA) preserve
30
31 324 nucleic acids in the sample and maintain stability at room temperature. Once delivered to the
32
33 325 lab, the fecal sample tubes are frozen at -80°C until processing and analysis.
34
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37

38 326 The microbial DNA will be extracted from all samples including positive and negative
39
40 327 controls, using QIAamp Fast DNA Stool Mini Kit as previously described (23), packed with
41
42 328 dried-ice, and shipped to University of Minnesota Genomic Center (Minnesota, US) for
43
44 329 sequencing. Shipping will adhere the regulation of Environment, Health and Safety
45
46 330 Department, University of Alberta. MiSeq Illumina technology (300 bp pair-end) will be used
47
48 331 to sequence 16S ribosomal ribonucleic acid (rRNA) targeting V5-V6 region to characterize the
49
50 332 fecal microbiome composition using primer pair 784F [5'-RGGATTAGATACCC -3'] and
51
52 333 1064R [5'-CGACRRCCATGCANCACCT-3'].
53
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57 334

58 59 335 *Physical activity questionnaire*

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1
2
3 336 The Godin-Shephard leisure-time physical activity questionnaire will be completed at
4
5 337 baseline, week 6, and week 12 to estimate physical activity levels (24, 25). In this questionnaire,
6
7 338 participants answer how often they perform strenuous, moderate, and light exercise for more
8
9 339 than 15 minutes in one week. A physical activity score is calculated based on intensity = $(9 \times$
10
11 340 strenuous) + $(5 \times$ moderate) + $(3 \times$ light) (25, 26). This will be used to classify participants as
12
13 341 insufficiently active (<14 units), moderately active (≥ 14 and <24 units), or active (≥ 24 units)
14
15 342 (26).
16
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21

22 344 *Dietary intake*

23
24 345 The dietary intake will be assessed using the online Automated Self-Administered 24-
25
26 346 hour Recall (ASA24[®]) Canada (27). A paper-based version is available per individual
27
28 347 participant and is returned to the study team weekly by email or fax. Dietary information is
29
30 348 entered in ASA24[®] to ensure consistency. Three 24h recalls are completed at weeks 1, 6, and
31
32 349 12 (two weekdays and one weekend day) and one 24h recall per week on the remaining weeks
33
34 350 of the study period (one weekday). Energy, macronutrients, and micronutrients intake will be
35
36 351 obtained using ASA24[®] automated coding based on the amount of each food consumed.
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42 353 *Appetite sensations*

43
44 354 To assess how the PMR affects appetite, participants assigned to the PMR group rate
45
46 355 their appetite sensations using the study journals once a week and at five timepoints: 1)
47
48 356 immediately after waking up/fasting, 2) immediately before the morning PMR consumption,
49
50 357 3) 30 minutes after the morning PMR consumption, 4) immediately before the afternoon PMR
51
52 358 consumption, and 5) 30 minutes after the afternoon PMR consumption. Hunger, satiety,
53
54 359 fullness, and prospective food consumption will be assessed using a paper-and-pen 100-mm
55
56 360 visual analogue scale (28). They are instructed to make a single vertical mark between 2
57
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3 361 anchors to indicate the intensity of their subjective states regarding each element, on a scale
4
5 362 from 0 to 100 mm. The following questions are asked: How hungry do you feel? (I am not
6
7
8 363 hungry at all – I have never been more hungry); How satisfied do you feel? (I am completely
9
10 364 empty – I cannot eat another bite); How full do you feel? (not at all full – totally full); How
11
12 365 much do you think you can eat? (nothing at all – a lot).
13
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15 366

16 367 *Adherence and withdraw/discontinuation*

17
18
19 368 Participants are immediately withdrawn from the study if they: 1) have significant
20
21 369 variation in body weight ($>\pm 3\%$ of baseline body weight (29)) that does not return to baseline
22
23 370 2 weeks after the nutrition consult; 2) become pregnant; 3) start or change medications or
24
25 371 supplement intake listed in the eligibility criteria; 4) no longer meet the inclusion criteria.
26
27 372 Participants assigned to the PMR group are asked to return all supplement bags (empty or not)
28
29 373 to the visits on week 6 and 12. These are weighted, and participants are excluded from the
30
31 374 study if the PMR have not been consumed twice daily during the 12 weeks or if there is $>20\%$
32
33 375 of product left inside the bags. In addition, participants can withdraw from the study at any
34
35 376 time.
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41 378 **Statistical analyses**

42 379 *Sample size estimate*

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44
45 380 A total of 74 participants (37 in each group) will be needed to detect a medium effect
46
47 381 size of 0.669. The effect size was calculated based on a previously published study (30), in
48
49 382 which the mean percent change in IL-6 from baseline to 12 months was -6.76 ± 36.95 pg/mL
50
51 383 in a group receiving soy protein versus 17.62 ± 35.92 pg/mL in the control group. Accounting
52
53 384 for a 20% attrition rate, the total sample size of 88 participants (44 in each group) will have a
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3 385 power of 80% with a significance level of 5%. The sample size calculation was done using
4
5 386 G*Power version 3.1.9.2.
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8 387

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10 388 *Data analysis*
11

12 389 Normality of the study variables will be assessed by the Shapiro-Wilk W-test. By
13
14 390 inspecting boxplots, values >1.5 box-lengths from the edge of the box will be considered as
15
16 391 outliers and may be excluded from analysis. Differences between groups of nominal variables
17
18 392 will be analysed by Pearson's χ^2 test or Fisher's exact test. Both group effect and time effect
19
20 393 will be analyzed using a two-way mixed analysis of variance (ANOVA) or analysis of
21
22 394 covariance (ANCOVA) as appropriate. Assumption of homogeneity of variances will be tested
23
24 395 using Levene's test of equality of variances. Correlation between variables will be assessed by
25
26 396 Pearson's correlation. If significant correlations between nutrients and energy intake are
27
28 397 noticed, the residual method will be applied in order to describe the relationship between
29
30 398 aspects of food intake and biochemical characteristics independent of energy intake (31). All
31
32 399 analyses will be performed using IBM® SPSS® Statistics version 24 (International Business
33
34 400 Machines Corporation), considering a critical significance value of 5%, unless otherwise
35
36 401 stated.
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41
42 402 Regarding genetic polymorphisms analysis, adherence to the Hardy-Weinberg
43
44 403 equilibrium will be checked using the χ^2 -square test. The R package 'argyle' will be used to
45
46 404 analyze the genotype data and assess the potential impact on the responses to the intervention
47
48 405 (32). To verify whether the results differ among the genotypes, the dominant model (major
49
50 406 allele x heterozygous + minor allele) will be applied. Effect size (ES) will be assessed by
51
52 407 Cohen's d-test and multivariate analysis (MANOVA) will be applied, including time and
53
54 408 genotype as the two independent variables. For post-hoc analysis, the Stoll Dwass test ($p < 0.05$)
55
56 409 will be applied. Statistical analysis of the gene expression profile will include the evaluation of
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1
2
3 410 the quality of the sequences with the FastQC tool. The Trimmomatic software (33) will be used
4
5 411 to remove low-quality strings and adapters. Then, the libraries will be evaluated again in the
6
7 412 FastQC software, for proper verification. The RNA sequencing data will be subjected to
8
9 413 analysis by RNA-seq using the protocol described in Trapnell, Roberts (34). The functional
10
11 414 annotation of differentially expressed genes will be carried out through the GeneOntology
12
13 415 platform (<http://geneontology.org>). Analyses to identify differentially expressed metabolic
14
15 416 pathways will be performed using the fgsea package of the R software.

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19 417 For the gut microbiome analysis, raw sequencing data will be undergone multiple
20
21 418 quality control steps including primer removal, trimming, chimera removal as previously
22
23 419 described (35, 36). Sequences will be classified using classify-sklearn algorithm (37) against
24
25 420 Silva database v.138 generated for given primers by RESCRIPT (38). To decontamination, non-
26
27 421 target sequences will be removed such as mitochondria, chloroplast and archaea. Possible
28
29 422 contamination detected by positive and negative controls and sequences with raw count <3 and
30
31 423 present in <10% of samples will be removed. Sample with an extremely low number of reads
32
33 424 (< 2000 after filtering) will not be considered in microbiome analysis. Filtered ASV table in
34
35 425 count data will be converted to relative abundant data for visualisation, then centered log-ratio
36
37 426 (CLR) transformed. The indices of α -diversity (e.g., observed species, Shannon, Phylogenetic
38
39 427 Diversity, Inver Simpson) and β -diversity (e.g., Bray-Curtis and Aitchison distance) will be
40
41 428 calculated using Phyloseq (39) and microbiome (40) R packages. Bray-Curtis distance will be
42
43 429 used to generate non-parametric multidimensional scaling ordination plots for β -diversity
44
45 430 metrics with scaled and centered results. Stability over time will also be assessed based on
46
47 431 Bray-Curtis distances. The adonis2 function in R package vegan will be used for permutational
48
49 432 multivariate analysis of variance (PERMANOVA). Aitchison distance will be used for
50
51 433 Principal component analysis (PCA) in mapping microbiome and metabolic markers data to
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3 434 exploring multidimensional association (23). False discovery rate (FDR) will be used to adjust
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5 435 p values, and $FDR < 0.05$ will be considered as statistically significant.
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10 437 **Patient and public involvement:**

11
12 438 None.
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16
17 440 **ETHICS AND DISSEMINATION**

18
19 441 This study is approved by the University of Alberta's HREB (Pro00070712) and is
20
21 442 registered on ClinicalTrials.gov (NCT03235804). This research adheres to the standards as set
22
23 443 out in the Canadian Tri-Council Policy statement on the use of human participants in research.
24
25 444 This study is regulated by Health Canada. Amendments will be submitted to the HREB and
26
27 445 Health Canada review and approval prior implementations. ClinicalTrials.gov will be updated
28
29 446 accordingly.
30
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32

33 447 All personal information is kept private, and participation is anonymous. Participants
34
35 448 are assigned a study ID, which is kept separated from any personal information collected. A
36
37 449 master list with identifiable information and study IDs is cryptographically protected and stored
38
39 450 at the HNRU. The study information will be kept for 15 years after the completion of the study.
40
41 451 If participants withdraw consent, they are asked for permission to use the data collected until
42
43 452 that point; however, if they deny it, their data is destroyed. Absence of answer is considered as
44
45 453 permission to use the data. The Quality Management in Clinical Research (QMCR) Department
46
47 454 at the University of Alberta is independent of investigators and sponsor. The QMCR is
48
49 455 responsible for monitoring the study data and will conduct yearly auditing.
50
51
52

53 456 Following data collection, analysis, and review of findings, manuscripts will be
54
55 457 prepared for submission to peer-reviewed journals and results presented in national and
56
57 458 international conferences. Study findings will also be disseminated through social media. Data
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1
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3 459 will be published regardless of outcomes and the University of Alberta retains the right to
4
5 460 publish. Authorship eligibility will adhere to the International Committee of Medical Journal
6
7 461 Editors' recommended guidelines (41). As a mandate of completing a registered trial, the
8
9 462 results must be published within 12 months of the completion of the trial. Dataset and statistical
10
11 463 code may be provided upon request.
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16
17 465 **Figure legends:**

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19 466 **Figure 1.** Experimental protocol. Abbreviations: CON control group, PMR powdered meal
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21 467 replacement group.

22
23 468 **Figure 2.** Schedule of enrollment, interventions, and assessments (SPIRIT figure).
24
25 469 Abbreviations: CON control group, PMR powdered meal replacement group.
26
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28

29 470

30
31 471 **Author contributions:**

32
33 472 JM, CLPO, AMA, AB, AMS, LM, CC, SG, CR, NKN, PDC, JW, and CMP were involved in
34
35 473 the design of the study. JM, CLPO, AMS, JW, and CMP wrote the study protocol. JM, CLPO,
36
37 474 AMA, AB, AMS, LM, CC, SG, CR, NKN, PDC, JW, and CMP participated in drafting and
38
39 475 revising the manuscript. JM, CLPO, AMA, AB, AMS, LM, CC, SG, CR, NKN, PDC, JW, and
40
41 476 CMP read and approved the final manuscript.
42
43
44

45 477

46
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52
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56
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58
59
60

1
2
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4
5 485 USA Inc.
6
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8 486

9
10 487 **Competing interests' statement:** In addition to what is noted under "Funding", CLPO reports
11
12 488 receiving honoraria and/or paid consultancy from Abbott and AMRA Medical Inc. outside the
13
14 489 scope of this work. AB received research support for their departments and consultant or
15
16 490 speakers' honoraria from the Almased-Wellness-GmbH. AMS reports receiving honoraria
17
18 491 and/or paid consultancy from Novo Nordisk, Johnson & Johnson, Boehringer Ingelheim, and
19
20 492 Xeno Biosciences outside the scope of this work. PDC is inventor on patent applications
21
22 493 dealing with the use of specific bacteria and components in the treatment of different diseases.
23
24 494 PDC was co-founder of The Akkermansia Company SA and of Enterosys S.A. JW has received
25
26 495 research funding and consulting fees from industry sources involved in the manufacture and
27
28 496 marketing of dietary fibers, prebiotics, and probiotics. JW is further a co-owner of Synbiotics
29
30 497 Health, a developer of synbiotic products. CMP reports receiving honoraria and/or paid
31
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33
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35
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40 500

41 42 501 **References**

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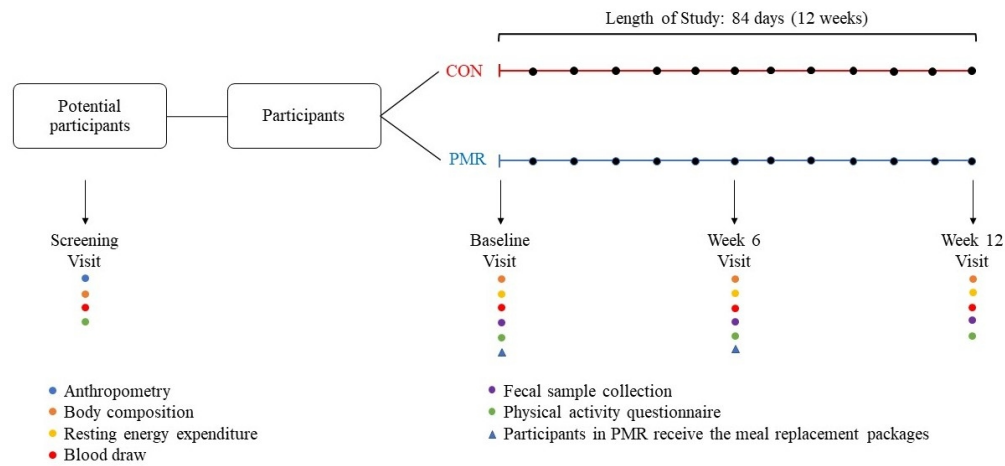


Figure 1. Experimental protocol. Abbreviations: CON control group, PMR powdered meal replacement group.

315x148mm (96 x 96 DPI)

TIMEPOINT		STUDY PERIOD					
		Enrollment	Allocation	Post-Allocation			
		Screening visit	Post-screening	Visit 1 (week 1)	Visit 2 (week 6)	Visit 3 (week 12)	Weekly
ENROLLMENT	Eligibility screen	X					
	Informed consent	X					
	Anthropometry	X					
	Body composition	X					
	Blood tests	X					
	Questionnaires	X					
	Allocation		X				
INTERVENTIONS	CON			●		●	
	PMR			●		●	
ASSESSMENTS	Gut microbiome			X	X	X	
	Systemic inflammatory biomarkers			X	X	X	
	Metabolic blood markers			X	X	X	
	Gene expression			X		X	
	Gene polymorphisms			X			
	Energy metabolism			X	X	X	
	Body composition			X	X	X	
	Appetite sensation						X
	Physical activity questionnaire			X	X	X	
Dietary intake						X	

Figure 2. Schedule of enrollment, interventions, and assessments (SPIRIT figure). Abbreviations: CON control group, PMR powdered meal replacement group.

205x139mm (150 x 150 DPI)



INFORMED CONSENT FORM

Title of Study: The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight (The PREMIUM Study).

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Study Coordinator:

Julia Montenegro	Phone: (780) 492-9010	E-mail: premium@ualberta.ca
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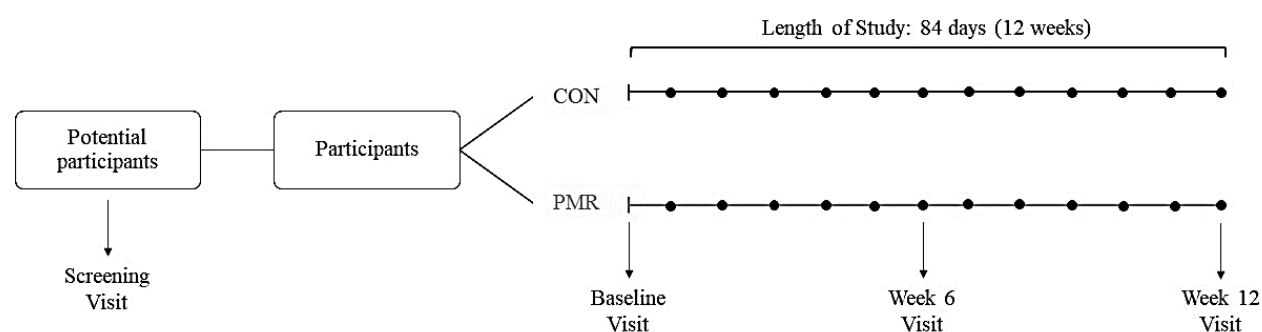
Background

Meal replacements are nutritionally complete formula foods used to substitute a meal. They can be a drink, bar, or soup. These products have been gaining popularity because they can help individuals lose weight. In addition, depending on its ingredients, meal replacements may affect our health. For this reason, meal replacements have been studied for health benefits. However, how meal replacements affect the microbes living in our gut, inflammation, and our genes is not known. Therefore, our study will investigate how a meal replacement affects these factors, as well as metabolism, the amount of fat and muscle of our bodies, and appetite. The powdered meal replacement used in this study is not investigational and is available for purchase by the public. However, because we are using it in this study to see the effect on gut microbes, this meal replacement is considered investigational by Health Canada, which has approved this study.

What will happen in the study?

Participants will attend at least 4 clinic visits over a 14-week period. The day and time of your visits will be decided by you and the study coordinator. We will first collect a blood sample to determine if you are eligible for the study. If eligible, you will be randomly assigned to a powdered meal replacement group or control group. Participants in the powdered meal replacement group will add the meal replacement to their diets twice daily for 12 weeks. Participants in the control group will maintain their usual food intake. The meal replacement is neutral tasting powder which you will add to water and drink. The study coordinator will show you how to do this. Besides from taking the meal replacement, no other lifestyle changes are needed and maintain your normal medication regime and physical activity level is required. You must inform study staff if you make any changes to your current medication or nutritional supplement use. You cannot participate in the study if you take any natural health products which may

alter inflammation, gut microbiome, energy metabolism, body weight and composition, or hormone levels. You should also continue to eat your normal diet. You will weigh yourself daily during the study using a scale we will give you. It is important that you do not lose or gain weight during the study. If this happens you will meet with a registered dietitian to adjust your food intake. Over 12 weeks we will collect three blood samples to measure the level of inflammation, different hormones, genes related to nutrient metabolism and gene expression, fat, and sugar in your blood. We will also collect three stool samples to study the microbes living in your gut and three urine samples to study the substances you consumed with the meal replacement (only if you are in the powdered meal replacement group). You will also complete different questionnaires that ask you about your food intake and level of physical activity. Moreover, for the powdered meal replacement group, you will answer questionnaires about how hungry or full you feel.



Screening visit: Sign “Informed Consent Form”, fasting blood draw, complete questionnaires (personal information, health status, and physical activity), anthropometric and body composition assessments.

Visits at baseline and week 6: Participants in PMR group collect nutritional supplement packages.

Intervention visits (baseline, week 6, and week 12): 1-hour WBCU test, fasting blood draw, urine (PMR group) and stool sample collection, body composition assessment, physical activity questionnaire.

• **Weekly:** Phone call to verify compliance, participants send 1-day dietary record, body weight and appetite sensation (PMR group). On the weeks 1, 6 and 12 there will be 3-day dietary record instead of 1-day dietary record.

Figure: Study design.

Visit 1 – Screening (about 1 hour):

This visit will happen in the morning. For this visit you should not consume alcohol for 3 days and not eat or drink for 12 hours before the visit. You may drink water. First, the study coordinator will explain the study to you. If you are interested in participating, you will be asked to sign this consent form. Then trained personnel will collect 10 mL of your blood (2 teaspoons). This blood sample will help us determine if you are eligible to participate based on your liver, kidney and thyroid function, and hydration status. If one or more of these tests is outside the reference values for a healthy person, you will be notified and considered not eligible to participate in our study. You may not participate in this study if you are pregnant, the blood test will tell us if you are currently pregnant. During the study, those who are of childbearing potential, must practice adequate methods of birth control (e.g., total abstinence, hormonal birth control methods (oral, injectable, transdermal, or intra-vaginal), intrauterine devices, confirmed successful vasectomy of partner etc.). If you become pregnant over the duration of the study, you must stop taking



the product, and immediately inform the study investigators. You will also answer questions related to personal characteristics, health status and physical activity. We will measure your height, weight, and waist circumference. We will also measure the fat in your body using bioelectrical impedance analysis (BIA). You will lie down on a bed and eight self-adhesive electrodes will be connected to both of your hands and feet. You will not feel anything, and the procedure will take 1 to 2 minutes. Or you will be asked to stand on a scale with the ball and the heel of each foot in contact with four metal electrodes. This measurement will take no more than 15 seconds. At the end of this visit we will offer to you a snack and a beverage. Once we test your blood, we will contact you to let you know if you are eligible. If so, you will be randomly assigned to a powdered meal replacement group or control group and we will schedule your second visit.

Visit 2 (about 3 hours):

This visit will also happen in the morning. For this visit, you should not consume alcohol for 3 days and not eat or drink for 12 hours before the visit. You may drink water. You should not exercise 24 hours before this visit and do as little activity as possible before the visit. For example, taking the elevator instead of the stairs.

At the beginning of this visit we will estimate how many calories you burn during a day using the whole-body calorimetry unit (WBCU). The WBCU is similar to a small hotel room. It has a bed, armchair, table, sink, toilet, television, computer/internet and treadmill. You will lie in a relaxed position on a bed. You will breathe regularly and relax without falling asleep. The test will take about 1 hour.

Then, we will measure the fat and muscle in your body using 3 safe and routine techniques. The first technique is a dual energy X-ray absorptiometry [DXA] scan, which may happen in a different day than the visit but in the same week or as close as possible. Before the scan we will make sure it is safe for you by asking some questions. If you are a woman, we will test your blood sample to see if you are pregnant. Pregnant women will not be allowed to participate in this study. During the scan you will lie down on a bed and the technician will position you correctly. The equipment “arm” will pass over your body and it will not cause any discomfort to you. It will take about 20 minutes to finish this test. The second technique is air displacement plethysmography (ADP). During this test you will sit comfortably inside a chamber and stay relaxed and quiet for 1 minute. This will happen twice. The entire test will take about 5 minutes. For this test, you will need to wear minimal, form-fitting clothing and a swim-cap. The last technique is the BIA, you will lie down on a bed and eight electrodes will be connected to both of your hands and feet. You will not feel anything, and the procedure will take 1 to 2 minutes.

After these assessments, certified personnel will collect 24 mL of your blood (5 teaspoons) to assess your level of inflammation, hormones, the interaction between your genes and the diet, fat, and sugar in your blood. If you are in the powdered meal replacement group, you will also collect your urine (~1 cup) to assess the level of substances contained in the meal replacement.

Then, we will give you a snack to eat. You will then answer a physical activity questionnaire. The study coordinator will explain how to monitor your body weight and complete questionnaires for the study. These questionnaires will ask about how hungry and full you feel and the foods you eat.

We will also explain how to collect a stool sample at home using the kit provided. You can bring this stool sample back later the same day or the next day. Once we receive your first stool sample, the research coordinator will give to participants assigned to the powdered meal replacement group the



packages with the product and explain how to use it. The product will be mixed with water and taken twice daily as snacks in the morning and afternoon for 12 weeks (84 days). Lastly, the study coordinator will provide you with another stool collection kit.

Visit 3 – Day 42 (about 3 hours):

This visit will happen in the morning and is similar to Visit 2, except genetic analyses, that will not be assessed. This means that the amount of blood we will collect will be 20 mL (4 teaspoons). For this visit, you should not consume alcohol for 3 days and not eat or drink for 12 hours before the visit. You may drink water. You should not exercise 24 hours before this visit and do as little activity as possible before the visit. During this visit we will complete the same assessments as Visit 2. You will also bring your stool sample to this visit. If you are unable to bring it to this visit, you can bring on the day before or after your scheduled appointment.

The study coordinator will check if your weight has changed from the beginning of the study and review your study journal. If your weight has changed the dietitian will work with you to adjust your diet to make sure your weight remains stable. At the end of the visit, the study coordinator will provide you with another stool collection kit. Participants in the powdered meal replacement group should keep taking the meal replacement mixed with water twice daily as snacks in the morning and afternoon until Visit 4. Participants in the control group will maintain their usual food intake.

Visit 4 – Day 84 (about 3 hours):

For this visit you will prepare as you did for Visit 2. This will be the last visit you will attend. For this visit you should bring back the scale provided during the study period and your final stool sample. As with the other stool sample collections, you are able to bring the sample to the visit, or the day before or after the scheduled visit. The study will have been completed at the end of this visit.

Weekly Assessment / Communication:

At the beginning of the study, we will provide a study journal. This provides reminders of what needs to be done every day throughout the study. Daily and weekly tasks include weighing yourself and completing questionnaires. These questionnaires will be about how hungry and full you feel (only for participants assigned to the powdered meal replacement group) and the foods you eat. Before Visits 2, 3, and 4 you will complete an online questionnaire about the food you ate three days during the weeks of these study visits. This will include one weekend day and one weekday. For the other weeks of the study, you will complete one of these questionnaires each week. The study coordinator will call or email you each week. This is to make sure you are following the recommendations provided. You will also be asked about your daily weight measurements and reminded to complete your study journal.

Results:

You will learn how many calories you burn in a day. You will also receive information about the amount of fat, bone, and lean soft tissue (i.e., everything else, but fat and bone) in your body.



Information about Investigational Product:

Serving Size: 8 tablespoons (50g)

Servings Per Container: 10

Amount Per Serving	% Daily Value*
Calories 180	
Calories from Fat 9	
Total Fat 1.0g*	1.5%
Saturated Fat 0.5g*	2.5%
Trans Fat 0g	**
Polyunsaturated Fat 0.1g	**
Monounsaturated Fat 0.4g	**
Cholesterol 3mg	1%
Sodium 340mg	15%
Potassium 500mg	14%
Total Carbohydrates 15g*	6%
Dietary Fiber 0.5g*	2%
Sugars 15g	**
Protein 27g*	54%

Vitamin A 794 IU	16%
Vitamin C 16mg	27%
Vitamin E 6 IU	20%
Thiamin (Vitamin B1) .5mg	33%
Riboflavin (Vitamin B2) 6mg	350%
Vitamin B6 .7mg	35%
Calcium 215mg	22%
Iron 4.9mg	27%

* Percent Daily Values are based on a 2,000 calorie diet.

** Daily Value not established.

Essential and Potentially Essential

Amino Acid Content of Protein Ingredients

Amino Acid		Per Serving 50g	
L Tyrosine	950mg	L Leucine	2300mg
L Methionine	400mg	L Isoleucine	1400mg
L Cystine	300mg	L Valine	1400mg
L Lysine	1550mg	L Histidine	700mg
L Threonine	950mg	L Arginine	1800mg
L Tryptophan	400mg	L Phenylalanine	1300mg

Ingredients: Soy Protein Isolate, Honey, Skim Milk Yogurt Powder, Potassium Chloride, Magnesium Carbonate, Calcium Citrate, Vitamin C, Niacin, Color Additive: Riboflavin (Vitamin B2), Vitamin E, Zinc Oxide, Ferrous Fumarate, Manganese Sulfate, Calcium Pantothenate, Vitamin B2, Vitamin B6, Vitamin B1, Vitamin A, Folic Acid, Potassium Iodide, Sodium Selenite, Biotin, Vitamin D3, Vitamin B12



What are the risks and discomforts?

There are no known risks of eating the meal replacement, there may be unknown risk with taking this investigational natural health product and potential side effects may include liver related drug adverse events. It is very unlikely to cause you any discomfort. The blood draws are a routine procedure performed by trained personnel. A needle will be inserted into a vein and blood will be withdrawn for lab tests. It is possible you may experience mild pain, fainting, bleeding, and bruising, and or an infection at the insertion site. Bruising is common, but usually goes away after a few days. Infection, dizziness, and fainting are rare during this procedure. There are no risks of having the genetic analysis, only potential general genetic linkages with metabolism of nutrients will be identified. You may also feel uncomfortable being alone in the WBCU. However, the tests will take only 1 hour and there will always be research staff close by and there is an intercom system to talk to them.

The X-ray dose associated with DXA scan is very low and not believed to have any long-term bad effects on your health. Pregnant women are excluded as a precaution. Having a DXA scan does not make it unsafe for you to have other X-rays in the future.

The BIA test is a risk for you only if you have a pacemaker or other internal electrical medical device. This is due to the risk of device malfunction from the weak electrical signal. Individuals with pacemakers or internal medical devices will not be able to participate in this study.

Dr. Laurie Mereu is a member of our research team and medical doctor. She will review your blood tests. If there are abnormal results Dr. Mereu will provide suggestions on how to proceed.

Risk of exposure to COVID-19 with your participation include exposure to others (research personnel and other participants) and increased time within our research unit. Measures undertaken to reduce this risk include ensuring all personnel and participants wear a mask, and frequent hand washing. All hard surfaces and common touched areas are disinfected before and after each visit. One-way traffic and physical distancing of 2 meters are encouraged at all times.

What happens if I am injured because of this research?

If you become ill or injured as a result of being in this study, you will still be able to receive necessary medical treatment. This will occur at no additional cost to you. By signing this consent form, you are not releasing the investigators, institution, or sponsors from their legal and professional duties.

What are the benefits to me?

There are no direct benefits to you for participating in this study. We hope the study will give us more information about how our bodies use the powdered meal replacement.

Do I have to participate?

No. Taking part in this study is your choice. You may stop participating in the study at any time. You can withdraw by contacting a study coordinator. Phone number: (780) 492-9010.

Will I be paid to be in the research?

After you complete the study, we will compensate your time with a \$300 honorarium. We will also give to you a parking pass in case you need to park your car in front of our clinic. There is no cost associated with participating.



Will my information be kept private?

During the study we will collect your health information. This will be kept private. We will not release information containing your name outside of the study investigators office. It will not be listed in the research when published. By law, we may have to release your information with your name so we cannot guarantee absolute privacy. However, we will make every legal effort to make sure that your health information is kept private.

During research studies it is important that the data we get is accurate. For this reason, your health data and name may be looked at by people from the University of Alberta auditors or members of the Research Ethics Board. By signing this consent form, you are giving permission for the study staff to collect your health information and use it for research purposes.

After the study is done, we will securely store your health data that was collected as part of the study. As per Health Canada requirements, your data will be stored and kept confidential for 15 years. If you leave the study, we will ask permission to keep your data. If you do not respond, we will use your data that had been collected so far.

If you leave the study, we will not collect any new information from you. However, we will keep the data that we have already collected, unless you specifically request it to be destroyed.

What if I have questions?

If you have any questions about this research, please contact the principal investigator (Dr. Carla Prado at 780-492-7934) or the study coordinator (Julia Montenegro at 780-492-9010).

If you suffer a research related injury, please contact the study coordinator at this number as well.

If you have any questions about your rights as a research participant, you may contact the Health Research Ethics Board at 780-492-2615. This office is independent of the study investigators.

The study is being sponsored by the ALMASED WELLNESS GMBH, the company that makes the powdered meal replacement. If you need, you can request any details about this product from the Principal Investigator.



CONSENT

Title of Study: The impact of a powdered meal replacement on metabolism and gut microbiota: a 12-week study in individuals with excessive body weight.

Principal Investigators:

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Dr. Arya Sharma		E-mail: amsharm@ualberta.ca

Qualified Investigator:

Dr. Laurie Mereu	Phone: (780) 492-3626	E-mail: laurie.mereu@ualberta.ca
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Study Manager:

Camila Oliveira, Post-doctoral Fellow	Phone: (780) 492-9010	E-mail: premium@ualberta.ca
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Study Coordinator:

Julia Montenegro, PhD Student	Phone: (780) 492-9010	E-mail: premium@ualberta.ca
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By signing below, you understand:

- That you have read the above information and have had anything that you do not understand explained to you to your satisfaction.
- That you will be taking part in a research study.
- That you read and received a copy of the attached Information Sheet.
- The benefits and risks involved in taking part in this research study.
- That you are free to leave the study at any time, without having to give a reason and without affecting your future medical care.
- Who will have access to your records, including personally identifiable health information.

Who explained this study to you? _____

Signature of Research Participant _____

(Printed Name) _____

Date: _____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator or Designee _____ Date _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A SIGNED COPY GIVEN TO THE RESEARCH PARTICIPANT.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

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

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet	2, 6, 22

1		registered, name of intended registry	
2			
3			
4	Trial registration:	#2b All items from the World Health Organization Trial	6-7
5			
6	data set	Registration Data Set	
7			
8			
9	Protocol version	#3 Date and version identifier	2, 6, 22
10			
11			
12	Funding	#4 Sources and types of financial, material, and other	23-24
13			
14		support	
15			
16			
17	Roles and	#5a Names, affiliations, and roles of protocol contributors	1, 23
18			
19	responsibilities:		
20			
21	contributorship		
22			
23			
24			
25	Roles and	#5b Name and contact information for the trial sponsor	23
26			
27	responsibilities:		
28			
29	sponsor contact		
30			
31	information		
32			
33			
34			
35	Roles and	#5c Role of study sponsor and funders, if any, in study	22-23
36			
37	responsibilities:	design; collection, management, analysis, and	
38			
39	sponsor and funder	interpretation of data; writing of the report; and the	
40			
41		decision to submit the report for publication, including	
42			
43		whether they will have ultimate authority over any of	
44			
45		these activities	
46			
47			
48			
49	Roles and	#5d Composition, roles, and responsibilities of the	23
50			
51	responsibilities:	coordinating centre, steering committee, endpoint	
52			
53	committees	adjudication committee, data management team, and	
54			
55		other individuals or groups overseeing the trial, if	
56			
57			
58			
59			
60			

1 applicable (see Item 21a for data monitoring

2
3 committee)

4
5
6 **Introduction**

7
8
9 Background and [#6a](#) Description of research question and justification for 4-5
10
11 rationale undertaking the trial, including summary of relevant
12
13 studies (published and unpublished) examining

14
15 benefits and harms for each intervention

16
17
18 Background and [#6b](#) Explanation for choice of comparators 5
19
20 rationale: choice of
21
22 comparators
23
24

25
26 Objectives [#7](#) Specific objectives or hypotheses 5

27
28
29 Trial design [#8](#) Description of trial design including type of trial (eg, 5-6
30
31 parallel group, crossover, factorial, single group),
32
33 allocation ratio, and framework (eg, superiority,
34
35 equivalence, non-inferiority, exploratory)
36
37

38
39 **Methods:**

40
41 **Participants,**
42
43 **interventions, and**
44
45 **outcomes**
46
47

48
49 Study setting [#9](#) Description of study settings (eg, community clinic, 5
50
51 academic hospital) and list of countries where data
52
53 will be collected. Reference to where list of study
54
55 sites can be obtained
56
57

1	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	8-9
2				
3			applicable, eligibility criteria for study centres and	
4			individuals who will perform the interventions (eg,	
5			surgeons, psychotherapists)	
6				
7				
8				
9				
10				
11	Interventions:	#11a	Interventions for each group with sufficient detail to	10
12				
13	description		allow replication, including how and when they will be	
14			administered	
15				
16				
17				
18				
19	Interventions:	#11b	Criteria for discontinuing or modifying allocated	19
20				
21	modifications		interventions for a given trial participant (eg, drug	
22			dose change in response to harms, participant	
23			request, or improving / worsening disease)	
24				
25				
26				
27				
28				
29	Interventions:	#11c	Strategies to improve adherence to intervention	12, 19
30				
31	adherence		protocols, and any procedures for monitoring	
32			adherence (eg, drug tablet return; laboratory tests)	
33				
34				
35				
36	Interventions:	#11d	Relevant concomitant care and interventions that are	19
37				
38	concomitant care		permitted or prohibited during the trial	
39				
40				
41				
42	Outcomes	#12	Primary, secondary, and other outcomes, including	7-8
43				
44			the specific measurement variable (eg, systolic blood	
45			pressure), analysis metric (eg, change from baseline,	
46			final value, time to event), method of aggregation	
47			(eg, median, proportion), and time point for each	
48			outcome. Explanation of the clinical relevance of	
49			chosen efficacy and harm outcomes is strongly	
50			recommended	
51				
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1	Participant timeline	#13	Time schedule of enrolment, interventions (including	11-12
2			any run-ins and washouts), assessments, and visits	
3			for participants. A schematic diagram is highly	
4			recommended (see Figure)	
5				
6				
7				
8				
9				
10				
11	Sample size	#14	Estimated number of participants needed to achieve	19-20
12			study objectives and how it was determined,	
13			including clinical and statistical assumptions	
14			supporting any sample size calculations	
15				
16				
17				
18				
19				
20				
21	Recruitment	#15	Strategies for achieving adequate participant	9
22			enrolment to reach target sample size	
23				
24				
25				
26	Methods:			
27				
28	Assignment of			
29	interventions (for			
30	controlled trials)			
31				
32				
33				
34				
35				
36	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	10
37	generation		computer-generated random numbers), and list of	
38			any factors for stratification. To reduce predictability	
39			of a random sequence, details of any planned	
40			restriction (eg, blocking) should be provided in a	
41			separate document that is unavailable to those who	
42			enrol participants or assign interventions	
43				
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53	Allocation	#16b	Mechanism of implementing the allocation sequence	10
54	concealment		(eg, central telephone; sequentially numbered,	
55	mechanism		opaque, sealed envelopes), describing any steps to	
56				
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1		conceal the sequence until interventions are	
2			
3		assigned	
4			
5			
6	Allocation:	#16c Who will generate the allocation sequence, who will	10
7			
8	implementation	enrol participants, and who will assign participants to	
9			
10		interventions	
11			
12			
13	Blinding (masking)	#17a Who will be blinded after assignment to interventions	3, 10
14			
15		(eg, trial participants, care providers, outcome	
16			
17		assessors, data analysts), and how	
18			
19			
20			
21	Blinding (masking):	#17b If blinded, circumstances under which unblinding is	n/a
22			
23	emergency	permissible, and procedure for revealing a	
24			
25	unblinding	participant's allocated intervention during the trial	
26			
27			
28			
29	Methods: Data		
30			
31	collection,		
32			
33	management, and		
34			
35	analysis		
36			
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38			
39	Data collection plan	#18a Plans for assessment and collection of outcome,	11-19
40			
41		baseline, and other trial data, including any related	
42			
43		processes to promote data quality (eg, duplicate	
44			
45		measurements, training of assessors) and a	
46			
47		description of study instruments (eg, questionnaires,	
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49		laboratory tests) along with their reliability and	
50			
51		validity, if known. Reference to where data collection	
52			
53		forms can be found, if not in the protocol	
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58	Data collection plan:	#18b Plans to promote participant retention and complete	12, 22
59			
60			

1	retention		follow-up, including list of any outcome data to be	
2			collected for participants who discontinue or deviate	
3			from intervention protocols	
4				
5				
6				
7				
8	Data management	#19	Plans for data entry, coding, security, and storage,	22-23
9			including any related processes to promote data	
10			quality (eg, double data entry; range checks for data	
11			values). Reference to where details of data	
12			management procedures can be found, if not in the	
13			protocol	
14				
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22	Statistics: outcomes	#20a	Statistical methods for analysing primary and	20-22
23			secondary outcomes. Reference to where other	
24			details of the statistical analysis plan can be found, if	
25			not in the protocol	
26				
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32	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup	n/a
33	analyses		and adjusted analyses)	
34				
35				
36				
37				
38	Statistics: analysis	#20c	Definition of analysis population relating to protocol	20-22
39	population and		non-adherence (eg, as randomised analysis), and	
40	missing data		any statistical methods to handle missing data (eg,	
41			multiple imputation)	
42				
43				
44				
45				
46				
47	Methods: Monitoring			
48				
49				
50				
51	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	22
52	formal committee		summary of its role and reporting structure;	
53			statement of whether it is independent from the	
54			sponsor and competing interests; and reference to	
55				
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1 where further details about its charter can be found,
 2
 3 if not in the protocol. Alternatively, an explanation of
 4
 5 why a DMC is not needed
 6
 7

8	Data monitoring:	#21b	Description of any interim analyses and stopping	20-22
9				
10	interim analysis		guidelines, including who will have access to these	
11				
12			interim results and make the final decision to	
13				
14			terminate the trial	
15				
16				
17				
18	Harms	#22	Plans for collecting, assessing, reporting, and	12
19				
20			managing solicited and spontaneously reported	
21				
22			adverse events and other unintended effects of trial	
23				
24			interventions or trial conduct	
25				
26				
27				
28	Auditing	#23	Frequency and procedures for auditing trial conduct,	22
29				
30			if any, and whether the process will be independent	
31				
32			from investigators and the sponsor	
33				
34				
35	Ethics and			
36				
37	dissemination			
38				
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40				
41	Research ethics	#24	Plans for seeking research ethics committee /	22
42				
43	approval		institutional review board (REC / IRB) approval	
44				
45				
46	Protocol	#25	Plans for communicating important protocol	22
47				
48	amendments		modifications (eg, changes to eligibility criteria,	
49				
50			outcomes, analyses) to relevant parties (eg,	
51				
52			investigators, REC / IRBs, trial participants, trial	
53				
54			registries, journals, regulators)	
55				
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1	Consent or assent	#26a	Who will obtain informed consent or assent from	6, 22
2				
3				
4			potential trial participants or authorised surrogates,	
5				
6			and how (see Item 32)	
7				
8				
9	Consent or assent:	#26b	Additional consent provisions for collection and use	n/a
10				
11	ancillary studies		of participant data and biological specimens in	
12				
13			ancillary studies, if applicable	
14				
15				
16	Confidentiality	#27	How personal information about potential and	22-23
17				
18			enrolled participants will be collected, shared, and	
19				
20			maintained in order to protect confidentiality before,	
21				
22			during, and after the trial	
23				
24				
25				
26	Declaration of	#28	Financial and other competing interests for principal	24
27				
28	interests		investigators for the overall trial and each study site	
29				
30				
31				
32	Data access	#29	Statement of who will have access to the final trial	22-23
33				
34			dataset, and disclosure of contractual agreements	
35				
36			that limit such access for investigators	
37				
38				
39	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care,	n/a
40				
41	trial care		and for compensation to those who suffer harm from	
42				
43			trial participation	
44				
45				
46				
47	Dissemination	#31a	Plans for investigators and sponsor to communicate	22-23
48				
49	policy: trial results		trial results to participants, healthcare professionals,	
50				
51			the public, and other relevant groups (eg, via	
52				
53			publication, reporting in results databases, or other	
54				
55			data sharing arrangements), including any	
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publication restrictions

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3
4 Dissemination [#31b](#) Authorship eligibility guidelines and any intended use 22-23
5
6 policy: authorship of professional writers
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8
9 Dissemination [#31c](#) Plans, if any, for granting public access to the full n/a
10
11 policy: reproducible protocol, participant-level dataset, and statistical
12
13 research code
14
15

16 Appendices

17
18
19 Informed consent [#32](#) Model consent form and other related documentation Supplementary
20
21 materials given to participants and authorised surrogates material
22
23
24

25 Biological [#33](#) Plans for collection, laboratory evaluation, and 14-17
26
27 specimens storage of biological specimens for genetic or
28
29 molecular analysis in the current trial and for future
30
31 use in ancillary studies, if applicable
32
33
34

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