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Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota (PREMIUM) in individuals with excessive body weight: a study protocol for a randomised controlled trial

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ABSTRACT

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

Methods and analysis Healthy adults with excess body weight (n=88) are being recruited and randomly assigned to one of the following groups: (1) Control group (CON): maintaining usual diet for 12 weeks, or (2) PMR group: replacing morning and afternoon snacks daily with a PMR for 12 weeks. Participants are asked to maintain body weight throughout the study and fill out a journal with information about PMR consumption, body weight, food intake, appetite sensations and medications. Three study visits are required: baseline, week 6 and week 12. Outcome measures include systemic inflammatory biomarkers, gut microbiome composition, metabolic blood markers, host energy metabolism, body composition, appetite sensations and host gene expression profile.

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

Trial registration number NCT03235804.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The randomised controlled clinical trial design, coupled with regular assessments and follow-up sessions, as well as a comprehensive range of evaluated outcomes, effectively reduces biases and confounding factors.
⇒ Cutting edge technology, such as the metabolic chamber and dual-energy X-ray absorptiometry, enables precise outcome measures.
⇒ The exploratory multiomics approach, incorporating gut microbiome, gene expression and genetic polymorphisms, supports the progress of precision nutrition by generating hypothesis.
⇒ A primary limitation of the study is the absence of a placebo group and the fact it is not not double-blinded.
⇒ Since the gut microbiome analysis depends on faecal samples, it might not fully represent changes in the gut microbiome composition occurring in more proximal parts of the gastrointestinal tract.

INTRODUCTION

Excess body weight can be defined as a body mass index (BMI)≥25.0 kg/m^2,[1] which encompasses both the overweight and obesity categories.[2] This condition has been associated with a state of systemic low-grade chronic inflammation, which is characterised by a persistent activation of immune and non-immune cells and production of cytokines, chemokines and acute phase proteins.[3 4] Those inflammatory biomarkers include interleukins (IL), such as IL-6 and IL-8, tumour necrosis factor-α (TNF-α), and C-reactive protein (CRP).[3] Systemic low-grade chronic inflammation causes tissue and organ damage, which can, in turn, lead to the onset and progression of chronic diseases,

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such as diabetes mellitus, cancer, metabolic syndrome and cardiovascular diseases.[3]

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

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

Considering the numerous negative health outcomes associated with excess body weight, much effort has been made to develop effective weight management strategies. Among those are meal replacements, which are food products fortified with vitamins and minerals used to replace one or more meals per day. Meal replacements are commonly used in association with calorie restriction. Research has shown that the consumption of meal replacements leads to greater weight loss when compared with reduced-calorie diets alone.[9 10] Improvement in metabolic parameters is generally observed with weight loss, including improvement in glucose metabolism, reduction of triacylglycerol, low-density lipoprotein cholesterol (LDL-C), systolic blood pressure[9 11 12] and the inflammatory markers CRP and IL-6.[13] Considering the positive health effects of weight loss in individuals with excessive body weight[14–16] and the beneficial health effects of meal replacements,[9 11] it is important to differentiate the effects of meal replacements from that of weight loss on overall health, which have not been investigated so far. Therefore, the aim of this study is to compare the effects of a 12-week consumption of a powdered meal replacement (PMR group), given as a soy-yoghurt-honey formula,[17] versus usual diet (control group, CON) on inflammation, gut microbiome, overall metabolic health, gene expression profile and genetic background in individuals with excessive body weight who are in weight maintenance.

METHODS

Study design and ethical procedures

This study is a randomised, controlled, parallel group, clinical trial conducted at the Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada). The study is an Investigator Initialized Trial sponsored by the Almased Wellness Comp., Bienenbüttel, Germany. The corresponding research protocol fulfils the requirements of the Standard Protocol Items: Recommendations for Interventional Trials checklist.[18] This research protocol was approved by the University of Alberta Health Research Ethics Board (HREB, identifier Pro00070712) and complies with the standards established by the Canadian Tri-Council Policy statement on the use of human participants in research. Procedures and potential risks involved in the study are discussed with participants prior to obtaining informed consent (online supplemental material). This protocol is registered on ClinicalTrials.gov, and recruitment started in April 2019 and is expected to finish in November 2023 (table 1).

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 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 (ie, phylum, family and genus) over time (within groups) between the PMR and CON groups.
- Change in markers of systemic inflammation (high-sensitivity CRP (hs-CRP), IL-8 and TNF-α) and immune modulation (IL-10) over time (within groups) between the PMR and CON groups.
- Change in concentrations of metabolic blood markers (glucose, insulin, total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides, peptide tyrosine–tyrosine (PYY), glucagon-like peptide-1 (GLP-1), ghrelin, adiponectin, leptin, free glycerol, free fatty acids and thyroid stimulating hormone (TSH)) over time (within groups) between the PMR and CON groups.
- Change in resting energy expenditure (REE) and respiratory exchange ratio over time (within groups) between the PMR and CON groups.
- Change in body composition (fat mass (FM) and lean soft tissue (LST)) over time (within groups) between the PMR and CON groups.
- Change in appetite sensations (hunger, satiety, fullness and prospective food consumption) over time within the PMR group.
- Differences in the responses to the intervention according to genetic polymorphisms over time.
- Changes in inflammation and excess body weight-related gene expression profile over time (within groups) between the PMR and CON groups.

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Table 1 WHO trial registration data set

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03235804
Date of registration in primary registry	1 August 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: (1) female/male aged 18–50 years; (2) non-smoker; (3) body mass index between 25 and 37 kg/m²; (4) weight stable; (5) fat mass ≥20% for males and ≥25% for females; (6) stable physical activity level. Exclusion criteria: (1) diagnosis of chronic diseases or acute infections; (2) taking any medication that may alter study outcomes; (3) taking prebiotics and probiotics; (4) use of antibiotics in the past 2 months; (5) pregnancy or lactation.
Study type	Randomised controlled trial
Date of first enrolment	1 April 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 sharing statement	De-identified data will be shared with the participant on completion of the study (publication)

Research participants

Inclusion criteria are as follows: male or female; non-smoker; between 18 and 50 years of age; BMI between 25.0 and 37.0 kg/m^2; with a stable body weight 6 months prior to study initiation (ie, variation <5 kg); FM≥20% for males and ≥25% for females; willingness to maintain stable physical activity level throughout the study; and females must use effective birth control methods.

Exclusion criteria includes participation in >3 hours per week of vigorous physical activity; pregnancy or lactation; diagnosis of any chronic or acute diseases (except for excess body weight); use of any medication that impacts study outcomes, except for antidepressants, anxiolytic and/or thyroid replacement therapy in a stable dose 3 months prior to study initiation and throughout the study period; use of antibiotics 2 months prior to study initiation; use of protein supplements 1 month prior to study initiation; allergy to PMR ingredients (soy, honey and yoghurt); allergy or intolerance to soy, gluten and/or lactose; following a vegetarian, vegan or any other restrictive dietary pattern; claustrophobia; or being unable to comprehend and complete the required questionnaires. Participants consuming supplements or food items that contain prebiotics or probiotics (eg, kefir or kombucha) before being enrolled in the study will be asked to discontinue the use of these products and wait 1 month before starting the study. The use of other nutritional supplements, such as multivitamins and vitamin D_3 will be allowed if on a stable dose.

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Recruitment, randomisation and intervention

Study advertisement is done using flyers displayed at the University of Alberta campuses, surrounding communities, other post-secondary education institutions in Edmonton (AB, Canada) and healthcare centres in the city. The study is also advertised in University of Alberta email lists, newspapers, classroom presentations and on social media (eg, Kijiji, Facebook and Twitter). Additionally, a personalised website (premium.ualberta.ca) was created.

Individuals interested in being part of the study will be invited to attend a screening visit at the HNRU. This visit will include anthropometric measurements (ie, height, weight and waist circumference), body composition assessment (bioelectrical impedance analysis (BIA)), blood tests (ie, creatinine, estimated glomerular filtration rate (eGFR), albumin, aspartate transaminase (AST), alanine transaminase (ALT), sodium, potassium, chloride and TSH), review of medical history and completion of a Physical Activity Questionnaire. Although glucose, insulin or lipid panel tests are not conducted during the screening visit, individuals who exhibit symptoms of or are taking medications for chronic diseases (eg, diabetes, hypertension and dyslipidaemia) are deemed ineligible for participation.

If deemed eligible, participants are randomly assigned into either the CON or PMR group. Randomisation is stratified by sex using a Microsoft Office Excel spreadsheet. To guarantee impartial allocation of participants to the groups, a study team member created a list of random numbers and assigned them to each group using the website Randomization.com (http://www.jerrydallal.com/random/randomize.htm) with the method of randomly permuted blocks. The list of random numbers is concealed, and a second investigator subsequently follows the predetermined order of numbers and assigns participants to their respective groups based on the order of their screening. Although the investigator has access to the randomisation list, they do not refer to it when assigning participants to their respective groups.

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

Table 2 Nutritional information of the tested soy-honey-yoghurt formula, a powdered meal replacement (PMR)

Nutrient	50 g of product (PMR)
Calories (kcal)	180
Total fat (g)	1.0
Saturated fat (g)	0.5
Trans fat (g)	0
Polyunsaturated fat (g)	0.1
Monounsaturated fat (g)	0.4
Cholesterol (mg)	3
Total carbohydrates (g)	15
Dietary fibre (g)	0.5
Sugars (g)	15
Protein (g)	27
Sodium (mg)	340
Potassium (mg)	500
Vitamin A (IU)	794
Vitamin C (mg)	16
Vitamin E (IU)	6
Thiamin (vitamin B_1) (mg)	5
Riboflavin (vitamin B_2) (mg)	6
Vitamin B_6 (mg)	7
Calcium (mg)	215
Iron (mg)	4.9

Experimental protocol

The study design is illustrated in figure 1. The schedule of enrolment, interventions and assessments are shown in figure 2. Following the screening visit and randomisation process, enrolled participants are invited to attend three study visits: baseline, week 6 and week 12. Assessments during each of these visits include: 1-hour resting metabolic rate, blood draw, body composition and Physical Activity Questionnaire. They additionally receive stool collection kits and instructions for faecal sample collection. During the baseline visit, participants receive a scale and a journal to record the following information daily: body weight, date and time of meal replacement intake (PMR group only) and medication intake (if any). They are also asked to record on a weekly basis a 24 hours dietary recall (both groups) and fill out appetite sensation questionnaires (PMR group only). Instructions on how to fill out the journal and dietary records are given. Additionally, participants assigned to the PMR group receive 84 packages of the PMR during visits at baseline and week 6, as well as instructions on how to prepare it. Those assigned to the PMR group start consuming the supplement the day after the first stool sample collection. Study materials (study journal and scale) are returned on week 12.

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

Anthropometry and body composition

At the screening visit, anthropometric measurements are taken twice and the average is used for data analysis.

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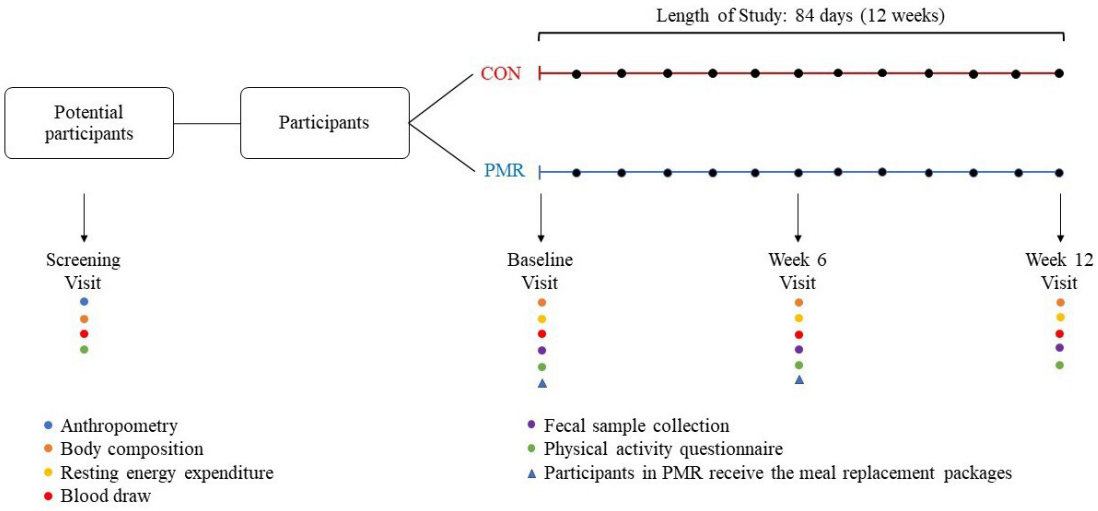


Figure 1 Experimental protocol. CON control group, PMR powdered meal replacement group.

Height is measured using a digital stadiometer (235 Heightronic, Concepts, Quick Medical, Snoqualmie, Washington, USA) to the nearest 0.1 cm. Body weight is measured to the nearest 0.1 kg using a calibrated digital scale (Health-o-meter Professional Remote Display, Sunbeam Products, Florida, USA). Waist circumference is measured using a measuring tape at the level of the participant's belly button, as per standard procedure.[19]

A digital scale (HD-314 Tanita Corporation, Tokyo, Japan) is provided to participants during the baseline visit, which is returned at the study completion. Body weight is recorded daily in the morning in a fasting state, and with an empty bladder.

Body composition is assessed using dual energy X-ray absorptiometry (DXA, GE Lunar iDXA, General Electric Company, Madison, USA), air displacement plethysmography (ADP, Bod Pod 1SB-060M, Life Measurement Instruments, Concord, California, USA) and BIA (Seca mBCA525, Seca GmbH & Co, Hamburg, Germany). A number of techniques is being used to explore potential changes in body composition using multicompartment modelling: DXA for bone mineral content, BIA for total body water, ADP for body density, which is used to calculate the remaining compartment: adipose tissue and residues (ie, dry LST).[20 21]

		STUDY PERIOD					
		Enrollment	Allocation	Post-Allocation			
TIMEPOINT		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 enrolment, interventions and assessments (Standard Protocol Items: Recommendations for Interventional Trials figure). CON control group, PMR powdered meal replacement group.

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Resting energy expenditure

REE is assessed by indirect calorimetry using an open-circuit metabolic chamber, which measures the volume of oxygen (O_2) and carbon dioxide (CO_2) from participant's respiration. Participants lie down in a relaxed position without falling asleep and breathe normally for 60 min. Mixed air with the expired CO_2 is drawn from the chamber at a constant flow rate (60±2 L/min) while fresh air with constant O_2 is passively drawn into the chamber. The first 30 min of the test are considered time for acclimatisation and hence removed from analysis. Gas exchange (volume of CO_2 and O_2) is analysed minute-by-minute by the Advance Optima AO2000 Series CO_2 analyser (ABB Automation GmbH, Frankfurt, Germany) and the OXYMAT 6 O_2 analyser (Siemens AG, Munich, Germany). Data is transferred from those analysers to a computer (Acer Aspire AM3910-E3122, Acer, New Taipei City, Taiwan) via the National Instruments NI USB-6221 device (National Instruments Corporation, Austin, Texas, USA) using the PMCSS Software V.1.8 (Pennington Metabolic Chamber Software Suite, Pennington Biomedical Research Center, Los Angeles, USA). REE (kcal/day) is calculated using the average kcal/min multiplied by 1440.

Blood analysis

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

Blood samples are collected using BD Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Tubes containing silica and a polymer are used for serum separation, tubes containing K2-EDTA are used for plasma separation and tubes containing K2-EDTA 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 laboratory (DynaLIFE Medical Labs, Edmonton, AB, Canada) at the screening visit prior to enrolment. Glucose, lipid panel (triglycerides, total cholesterol, LDL-C and HDL-C), TSH and hs-CRP will be analysed by DynaLIFE Medical Labs (Edmonton, AB, Canada). IL-6, IL-8, IL-10,

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*	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†		x	x	x	Serum	External
Free glycerol		x	x	x	Serum	On-site
Free fatty acids		x	x	x	Serum	On-site
Interleukins		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

*Electrolytes include chloride, sodium and potassium.
†Lipid panel include triglycerides, total cholesterol, LDL-C and HDL-C.
‡Interleukins include IL-6, IL-8 and IL-10.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GLP-1, glucagon like peptide; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C reactive protein; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; PYY, peptide tyrosine–tyrosine; TNF-α, tumour necrosis factor-α; TSH, thyroid stimulating hormone.

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TNF-α, insulin, PYY, GLP-1, ghrelin, adiponectin, leptin, free glycerol and free fatty acids will be analysed in our laboratory (University of Alberta, AB, Canada). IL-6, IL-8, IL-10, TNF-α, insulin, PYY, GLP-1, ghrelin, adiponectin and leptin will be analysed by electrochemiluminescence immunoassay (Meso Scale Discovery, Maryland, USA).

An additional blood draw is requested the day following each study visit for hs-CRP analysis due to this being a sensitive marker which can vary substantially within hours of collection for several reasons.[22] Therefore, the average of CRP measured on two consecutive days will be taken in case they are similar. If a participant is in an infectious state (ie, CRP>10 mg/L or significant changes between the 2 days measurement) the highest value will be excluded from analysis.

For gene expression profile, RNAs will be sequenced at baseline and week 12. Whole blood (500 µL) is aliquoted into an RNase-free microfuge tube and added 1.3 mL of RNAlater stabilisation solution (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Total RNA will be extracted from whole blood using the RiboPure Blood Kit (Thermo Fisher Scientific, Waltham, USA). The RNA purity will be determined by measuring the 260/280 nm ratio (ideal ratio ~2.0) and the 260/230 nm ratio (ideal ratio 2.0–2.2) using a spectrophotometer. The quality of RNA samples will be evaluated prior to library preparation for RNA sequencing (RNA-seq), using a Bioanalyzer and an RNA Integrity Number ≥7 will be accepted. Samples of high purity and quality RNA will be prepared with the TruSeq RNA Sample Prep kit (Illumina, San Diego, USA). The sequencing will be performed by an external company using the platform Illumina HiSeq 4000 (Illumina, San Diego, USA), in the paired-end mode, in which the two ends will be sequenced with a length of 100 base pairs (bp) (2×100 bp). At the end, two files in the 'fastq' format will be generated for each of the evaluated samples.

We will select a set of candidate genes that are known to be involved in the regulation of inflammation and/or excess body weight and are differentially expressed after the intervention. From these genes, we will analyse the most extensively studied polymorphisms. The reasoning behind this approach is that genetic variations in these candidate genes could potentially impact the expression and/or function of the proteins they encode, ultimately influencing the response to the intervention. Genetic polymorphisms will be analysed at baseline. Genomic DNA (gDNA) will be extracted with the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) from leucocytes in peripheral blood. The gDNA purity will be verified in a spectrophotometer at 260 and 280 nm. The samples will be considered of good quality if the ratio between absorbances is between 1.7 and 2.0. The gDNA concentration will be measured on a fluorimeter. For genotyping, a customised Infinium Global Screening Array-24+v3.0 Kit (Illumina, San Diego, USA) will be used.

Faecal sample collection and gut microbiome sequencing

A total of three faecal samples are collected at baseline, week 6 and week 12. Faecal samples are either collected at the HNRU the day of the study visits, or at home, kept at room temperature, and delivered to the HNRU as soon as possible. During the baseline visit, participants are instructed on how to collect faecal samples using the provided collection kits. The faecal collection tubes (DNA/RNA Shield, Zymo Research, Irvine, California, USA) preserve nucleic acids in the sample and maintain stability at room temperature. Once delivered to the laboratory, the faecal sample tubes are frozen at −80°C until processing and analysis.

The microbial DNA will be extracted from all samples including positive and negative controls, using QIAamp Fast DNA Stool Mini Kit as previously described,[23] packed with dried-ice and shipped to University of Minnesota Genomic Center (Minnesota, USA) for sequencing. Shipping will adhere to the regulation of the Environment, Health and Safety Department, University of Alberta. MiSeq Illumina technology (300 bp pair-end) will be used to sequence 16S ribosomal RNA targeting V5–V6 region to characterise the faecal microbiome composition using primer pair 784F [5'-RGGATTAGATACCC −3'] and 1064R [5′-CGACRRCCATGCANCACCT-3′].

Physical Activity Questionnaire

The Godin-Shephard leisure-time Physical Activity Questionnaire will be completed at baseline, week 6 and week 12 to estimate physical activity levels.[24 25] In this questionnaire, participants answer how often they perform strenuous, moderate and light exercise for more than 15 min in 1 week. A physical activity score is calculated based on intensity=(9×strenuous)+(5×moderate)+(3×light).[25 26] This will be used to classify participants as insufficiently active (<14 units), moderately active (≥14 and <24 units) or active (≥24 units).[26]

Dietary intake

The dietary intake will be assessed using the online Automated Self-Administered 24-hour Recall (ASA24) Canada.[27] A paper-based version is available per individual participant and is returned to the study team weekly by email or fax. Dietary information is entered in ASA24 to ensure consistency. Three 24 hours recalls are completed at weeks 1, 6 and 12 (2 weekdays and 1 weekend day) and one 24 hours recall per week on the remaining weeks of the study period (1 weekday). Energy, macronutrients and micronutrients intake will be obtained using ASA24 automated coding based on the amount of each food consumed.

Appetite sensations

To assess how the PMR affects appetite, participants assigned to the PMR group rate their appetite sensations using the study journals once a week and at five time points: (1) immediately after waking up/fasting, (2) immediately before the morning PMR consumption, (3)

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30 min after the morning PMR consumption, (4) immediately before the afternoon PMR consumption and (5) 30 min after the afternoon PMR consumption. Hunger, satiety, fullness and prospective food consumption will be assessed using a paper-and-pen 100 mm Visual Analogue Scale.[28] They are instructed to make a single vertical mark between two anchors to indicate the intensity of their subjective states regarding each element, on a scale from 0 to 100 mm. The following questions are asked: How hungry do you feel? (I am not hungry at all—I have never been more hungry); How satisfied do you feel? (I am completely empty—I cannot eat another bite); How full do you feel? (not at all full—totally full); How much do you think you can eat? (nothing at all—a lot).

Adherence and withdraw/discontinuation

Participants are immediately withdrawn from the study if they: (1) have significant variation in body weight (>±3% of baseline body weight[29]) that does not return to baseline 2 weeks after the nutrition consult; (2) become pregnant; (3) start or change medications or supplement intake listed in the eligibility criteria; (4) no longer meet the inclusion criteria. Participants assigned to the PMR group are asked to return all supplement bags (empty or not) to the visits on week 6 and 12. These are weighted, and participants are excluded from the study if the PMR have not been consumed twice daily during the 12 weeks or if there is >20% of product left inside the bags. In addition, participants can withdraw from the study at any time.

Statistical analyses

Sample size estimate

A total of 74 participants (37 in each group) will be needed to detect a medium effect size (ES) of 0.669. The ES was calculated based on a previously published study,[30] in which the mean per cent change in IL-6 from baseline to 12 months was −6.76±36.95 pg/mL in a group receiving soy protein versus 17.62±35.92 pg/mL in the CON. Accounting for a 20% attrition rate, the total sample size of 88 participants (44 in each group) will have a power of 80% with a significance level of 5%. The sample size calculation was done using G*Power V.3.1.9.2.

Data analysis

Normality of the study variables will be assessed by the Shapiro-Wilk W-test. By inspecting boxplots, values >1.5 box-lengths from the edge of the box will be considered as outliers and may be excluded from analysis. Differences between groups of nominal variables will be analysed by Pearson's χ^2 test or Fisher's exact test. Both group effect and time effect will be analysed using a two-way mixed analysis of variance (ANOVA) or analysis of covariance (ANCOVA) as appropriate. Assumption of homogeneity of variances will be tested using Levene's test of equality of variances. Correlation between variables will be assessed by Pearson's correlation. If significant correlations between nutrients and energy intake are noticed, the residual method will be applied in order to describe the relationship between aspects of food intake and biochemical characteristics independent of energy intake.[31] All analyses will be performed using IBM SPSS Statistics V.24 (International Business Machines Corporation), considering a critical significance value of 5%, unless otherwise stated.

Regarding genetic polymorphisms analysis, adherence to the Hardy-Weinberg equilibrium will be checked using the χ^2 test. The R package 'argyle' will be used to analyse the genotype data and assess the potential impact on the responses to the intervention.[32] To verify whether the results differ among the genotypes, the dominant model (major allele x heterozygous+minor allele) will be applied. ES will be assessed by Cohen's d-test and multivariate analysis (MANOVA) will be applied, including time and genotype as the two independent variables. For post hoc analysis, the Steel Dwass test ($p<0.05$) will be applied. Statistical analysis of the gene expression profile will include the evaluation of the quality of the sequences with the FastQC tool. The Trimmomatic software[33] will be used to remove low-quality strings and adapters. Then, the libraries will be evaluated again in the FastQC software, for proper verification. The RNA-seq data will be subjected to analysis by RNA-seq using the protocol described in Trapnell *et al* .[34] The functional annotation of differentially expressed genes will be carried out through the GeneOntology platform (http://geneontology.org). Analyses to identify differentially expressed metabolic pathways will be performed using the fgsea package of the R software.

For the gut microbiome analysis, raw sequencing data will be undergone multiple quality control steps including primer removal, trimming, chimaera removal as previously described.[35 36] Sequences will be classified using classify-sklearn algorithm[37] against Silva database V.138 generated for given primers by RESCRIPt.[38] To decontamination, non-target sequences will be removed such as mitochondria, chloroplast and archaea. Possible contamination detected by positive and negative controls and sequences with raw count <3 and present in <10% of samples will be removed. Sample with an extremely low number of reads (<2000 after filtering) will not be considered in microbiome analysis. Filtered ASV table in count data will be converted to relative abundant data for visualisation, then centred log-ratio transformed. The indices of α-diversity (eg, observed species, Shannon, phylogenetic diversity, Inver Simpson) and β-diversity (eg, Bray-Curtis and Aitchison distance) will be calculated using phyloseq[39] and microbiome[40] R packages. Bray-Curtis distance will be used to generate non-parametric multidimensional scaling ordination plots for β-diversity metrics with scaled and centred results. Stability over time will also be assessed based on Bray-Curtis distances. The adonis2 function in R package vegan will be used for permutational multivariate analysis of variance (PERMANOVA). Aitchison distance will be used for principal component analysis in mapping microbiome and metabolic markers data to exploring multidimensional association.[23] False discovery rate

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(FDR) will be used to adjust p values, and FDR<0.05 will be considered as statistically significant.

Patient and public involvement

None.

ETHICS AND DISSEMINATION

This study is approved by the University of Alberta's HREB (Pro00070712) and is registered on ClinicalTrials.gov. This research adheres to the standards as set out in the Canadian Tri-Council Policy statement on the use of human participants in research. This study is regulated by Health Canada. Amendments will be submitted to the HREB and Health Canada review and approval prior to implementations. ClinicalTrials.gov will be updated accordingly.

All personal information is kept private, and participation is anonymous. Participants are assigned a study ID, which is kept separated from any personal information collected. A master list with identifiable information and study IDs is cryptographically protected and stored at the HNRU. The study information will be kept for 15 years after the completion of the study. If participants withdraw consent, they are asked for permission to use the data collected until that point; however, if they deny it, their data is destroyed. Absence of answer is considered as permission to use the data. The Quality Management in Clinical Research (QMCR) Department at the University of Alberta is independent of investigators and sponsor. The QMCR is responsible for monitoring the study data and will conduct yearly auditing.

Following data collection, analysis and review of findings, manuscripts will be prepared for submission to peer-reviewed journals and results presented in national and international conferences. Study findings will also be disseminated through social media. Data will be published regardless of outcomes and the University of Alberta retains the right to publish. Authorship eligibility will adhere to the International Committee of Medical Journal Editors' recommended guidelines.[41] As a mandate of completing a registered trial, the results must be published within 12 months of the completion of the trial. Data set and statistical code may be provided on request.

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Competing interests In addition to what is noted under 'Funding', CLPO reports receiving honoraria and/or paid consultancy from Abbott and AMRA Medical Inc. outside the scope of this work. AB received research support for their departments and consultant or speakers' honoraria from the Almased-Wellness-GmbH. AMS reports receiving honoraria and/or paid consultancy from Novo Nordisk, Johnson & Johnson, Boehringer Ingelheim and Xeno Biosciences outside the scope of this work. PDC is inventor on patent applications dealing with the use of specific bacteria and components in the treatment of different diseases. PDC was co-founder of The Akkermansia Company SA and of Enterosys S.A. JW has received research funding and consulting fees from industry sources involved in the manufacture and marketing of dietary fibres, prebiotics and probiotics. JW is further a co-owner of Synbiotics Health, a developer of synbiotic products. CMP reports receiving honoraria and/or paid consultancy from Abbott Nutrition, Nutricia, Nestle Health Science, Fresenius Kabi, Pfizer, and AMRA medical outside the scope of this work. Other authors declare no conflict of interest.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

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UNIVERSITY OF ALBERTA

The PREMIUM Study



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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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

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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.

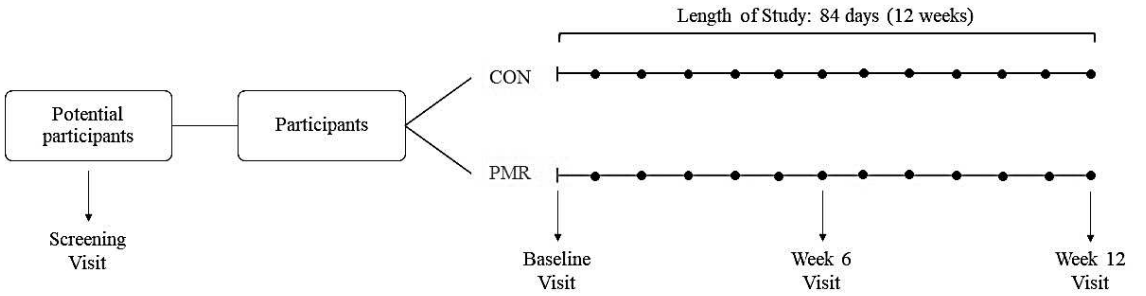


Figure: Study design.

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.

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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.