PROTOCOL COVER PAGE

Protocol Title	The Influence of Physical Activity on the Gut Microbiome of Pre-Diabetic Adults
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Scientific Assessment	Nationally-based, federal funding organizations
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Version Number/Date:	Version 1: 02/28/2020 Version 2: 03/25/2020 Version 3: 04/14/2021 Version 4: 05/25/2021 Version 5: 07/21/2021 Version 6: 09/08/2021 Version 7: 05/11/2022

ANCILLARY REVIEWS

Which ancillary reviews do I need and when do I need them? Refer to HRP-309 for more information about these ancillary reviews.			
Select yes or no	**Does your study…**	*If yes…*	***Impact on IRB Review***
☐ **Yes** ☒ **No**	Include Gillette resources, staff or locations	*Gillette Scientific review and Gillette Research Administration approval is required. Contact: research@gillettechildrens.com*	**Required prior to IRB submission**
☐ **Yes** ☒ **No**	Involve Epic, or Fairview patients, staff, locations, or resources?	*The Fairview ancillary review will be assigned to your study by IRB staff Contact:* ancillaryreview@Fairview.org	**Approval must be received prior to IRB committee/ designated review.** **Consider seeking approval prior to IRB submission.**
☐ **Yes** ☒ **No**	Include evaluation of drugs, devices, biologics, tobacco, or dietary supplements or data subject to FDA inspection?	*The regulatory ancillary review will be assigned to your study by IRB staff Contact: medreg@umn.edu* *See: https://policy.umn.edu/research/indide*	
☐ **Yes** ☒ **No**	Require Scientific Review? Not sure? See guidance on next page.	*Documentation of scientific merit must be provided. Contact: hrpp@umn.edu*	
☐ **Yes** ☒ **No**	Relate to cancer patients, cancer treatments, cancer screening/prevention, or tobacco?	*Complete the CPRC application process. Contact: ccprc@umn.edu*	
☐ **Yes** ☒ **No**	Include the use of radiation? (x-ray imaging, radiopharmaceuticals, external beam or brachytherapy)	*Complete the AURPC Human Use Application and follow instructions on the form for submission to the AURPC committee. Contact: barmstro@umn.edu*	**Approval from these committees must be received prior to IRB approval;** **These groups each have their own application process.**
☐ **Yes** ☒ **No**	Use the Center for Magnetic Resonance Research (CMRR) as a study location?	*Complete the CMRR pre-IRB ancillary review Contact: ande2445@umn.edu*	
☐ **Yes** ☒ **No**	Include the use of recombinant or synthetic nucleic acids, toxins, or infectious agents?	*Complete the IBC application via eprotocol.umn.edu Contact:*	
☐ **Yes** ☒ **No**	Include the use of human fetal tissue, human embryos, or embryonic stem cells?	*Contact OBAO for submission instructions and guidance*	

☒ **Yes** ☐ **No**	Include PHI or are you requesting a HIPAA waiver?	*If yes, HIPCO will conduct a review of this protocol.* *Contact: privacy@umn.edu*	
☐ **Yes** ☒ **No**	Use data from the Information Exchange (IE)?	*The Information Exchange ancillary review will be assigned to your study by IRB staff* *Contact: ics@umn.edu*	**Approval must be received prior to IRB approval.** **These groups do not have a separate application process but additional information from the study team may be required.**
☐ **Yes** ☒ **No**	Use the Biorepository and Laboratory Services to collect tissue for research?	*The BLS ancillary review will be assigned to your study by IRB staff.* *Contact: cdrifka@umn.edu*	
☐ **Yes** ☒ **No**	Have a PI or study team member with a conflict of interest?	*The CoI ancillary review will be assigned to your study by IRB staff* *Contact: becca002@umn.edu*	
☒ **Yes** ☐ **No**	Need to be registered on clinicaltrials.gov?	*If you select "No" in ETHOS, the clinicaltrials.gov ancillary review will be assigned to your study by IRB staff* *Contact: kmmccorm@umn.edu*	
☐ **Yes** ☒ **No**	Require registration in OnCore?	*If you select "No" or "I Don't Know" in ETHOS, the OnCore ancillary review will be assigned to your study by IRB staff* *Contact: oncore@umn.edu*	**Does not affect IRB approval.**

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	3/25/2020	We have made changes to clarify two points. First, we ensured that the compensation listed for all documents is $80. Second, we have clarified how the Physical Activity Readiness Questionnaire will be scored as it pertains to inclusion and exclusion (Sections 8.1 and 13.1). Notably, we have also better clarified scoring in the phone-based screening questionnaire to be used by the research team to screen participants for inclusion in the trial.	No.
2	4/14/2021	We have updated this protocol to reflect: (1) the precautions our research team will be taking to reduce the potential for SARS-CoV-2 transmission; (2) the methodology we will use to deliver this trial in a remote manner if/when in-person exercise sessions are not possible due to the COVID-19 pandemic and/or participant preference (Sections 4.1 and 5.2). Additionally, the protocol was edited for clarity and to avoid redundancies.	No.
3	05/25/2021	We have updated this protocol to reflect: - The removal of waist circumference measures at the in-person visits - the removal of a fasting blood draw measure at week 4 - the removal of a study questionnaire at each assessment visit	Yes. See updates dated 5-25-2021
4	07/21/2021	We have updated this protocol to specifically add ResearchMatch as a recruitment methodology (See Section 11.1). We have also re-considered our compensation amount and have made a nominal increase in amount (See Section 11.4).	Yes. See updates dates 7-21-2021.
5	09/08/2021	We removed the use of Proton-Pump Inhibitors as one of the exclusionary criteria (see section 8.2)	

Revision #	Version Date	Summary of Changes	Consent Change?
6	05/11/2022	We have removed the upper limit for BMI as one of the inclusion criteria(see section 8.1 & 8.2)	

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ABBREVIATIONS/DEFINITIONS

- **CMD: cardiometabolic disease** - envelops numerous disease states like prediabetes, diabetes, metabolic syndrome, and cardiovascular disease.
- **DNA: deoxyribonucleic acid** - to be extracted from the stool samples provided by participants.
- **PreD: prediabetes** - the cardiometabolic disease of interest in the current study, with the formal definition provided within text.
- **PA: physical activity** - any bodily movement that raises energy expenditure above resting levels.
- **PCR amplified: polymerase chain reaction amplification** - widely used process to generate information on the relative abundance of different microbial groups when analyzing the 16S rRNA obtained from stool samples.
- **rRNA: ribosomal ribonucleic acid**
- **SCFA: short chain fatty acids** - fatty acids with fewer than 6 carbon atoms that are generated in the gut and perform select physiological functions important to, among other things, gastrointestinal health.
- **T2D: type 2 diabetes** - highly prevalent cardiometabolic disease that up to 70% of prediabetics will convert to if steps are not taken to impede this conversion.
- **QIIME, DADA2, phyloseq, DESeq2, and SAS:** software packages to be used in the proper analyses of all study data.

1.0 Objectives

1.1 **Purpose:** We propose a 100-participant randomized controlled 2-arm parallel trial which employs a metagenomic approach to examine how 8 weeks of supervised moderate-intensity treadmill walking exercise (MWE)[1,2] for 30-45 min 3 times/week alters the gut microbiome, serum SCFAs, and the cardiometabolic profile, body weight, and body composition of individuals 30-64 years old who are overweight or obese and have PreD.

Aim 1: Compare the effect of 8 weeks of MWE vs. control on shifts in the gut microbiome of overweight or obese individuals with PreD. ***H1*:** Gut microbial diversity and functional capacity (specifically, genes relevant to SCFA production) will increase improve after 8 weeks in the experimental group vs. control.

Aim 2: Compare the effect of 8 weeks of MWE vs. control on changes in serum SCFAs. ***H2*:** Serum Levels of SCFAs butyrate, acetate, and propionate will increase after 8 weeks in the experimental group vs. control.

Aim 3: Use formal mediation methods to estimate the proportion of MWE-induced changes in participants' cardiometabolic profiles, body weight, and body composition that are mediated by changes in the gut microbiome and/or serum SCFAs. ***H3*:** MWE-induced changes in the gut microbiome and serum SCFAs will partly explain changes in participants' cardiometabolic profile (e.g., blood lipids, fasting insulin/glucose, blood pressure, waist circumference), body weight, and body composition.

2.0 Background

2.1 **Significance of Research Question/Purpose:** In the U.S, 91.8 million adults have prediabetes (PreD).[3] The prediabetic state is a prominent public health problem as up to 70% of individuals with PreD convert to type 2 diabetes (T2D).[4] The risk of cardiovascular disease (CVD) is high among those with PreD and T2D, and nearly $330 billion/year is spent treating T2D.[3,5-7] The *American Heart Association (AHA)* and *American Diabetes Association (ADA)* have thus called for continued investigation into mechanisms connecting health behaviors to pre-clinical cardiometabolic disease (CMD; e.g., PreD) to reduce future disease risk.[8,9] Physical activity (PA) is a key modifiable determinant of good health.[10] Literature suggests PA recommendation[10] adherence is related to a 25-35% dose-dependent reduction in all-cause mortality[1,11,12], with as little as 75 min/week of regular moderate-intensity PA beneficial.[1] Data from the Diabetes Prevention Program (DPP) suggested that, among those with PreD, a behavioral intervention emphasizing PA and diet to be at least as successful as metformin in preventing T2D, partially due to weight loss during this

trial.[13] Yet, many individuals do not fully realize the cardiometabolic benefits of PA, and mechanistic pathways linking PA and cardiometabolic improvements are not fully understood.[8,9,14,15] The gut microbiome[16] has been posited as a mechanistic intermediate linking heath behaviors such as PA to CMD development.[14,17-19]

The gut microbiome may be important for immunological,[20,21] metabolic,[16,22] inflammatory,[18,23,24] and neurobehavioral processes[22,24,25], some of which might partially explain how health behaviors influence CMD risk. Yet, limited evidence exists characterizing the effect of PA on the human gut microbiome. To date, most studies of the impact of PA interventions on the gut microbiome have used animal models,[26-41] with these studies supporting regular chronic PA (i.e., exercise) as a modulator of the gut microbiota. Animal studies have most often reported exercise-related changes to the Firmicutes[27-31,38] or Bacteroidetes[26,34,35,40] phyla while also noting beneficial alterations to microbial community diversity[26,29,30,32-35,38-40] and short chain fatty acid (SCFA)-producing taxa[33-36,38,41]. SCFAs are reported to promote gastrointestinal health, increase energy expenditure[42], and to be related to a lean phenotype.[14,27,41] The five existing human gut microbiome trials[43-47] most often implemented non-controlled studies of 5- to 8-weeks of moderate-intensity exercise on 3 days/week for 30-60 min/session. Among overweight or obese adults, prior studies[43-45] note increased □-diversity and altered β-diversity as a result of exercise; similar findings emerged in another study of healthy older adults.[46] Additionally, one study noted exercise to result in increases in SCFAs (in stool), and corresponding changes to SCFA-related bacterial genes and taxa,[45] while two other studies observed improvements in the metabolic potential of the gut microbiome[43,44]. These findings corroborate observational studies in humans wherein more physically active individuals have greater microbiota diversity and/or improved capacity to produce SCFAs than less active individuals.[48-53] **To our knowledge, no randomized controlled parallel design study has been conducted using metagenomic profiling (i.e., assessments of microbial functional capacity) to examine the influence of exercise on the human gut microbiome and serum SCFA levels in a population with a pre-clinical CMD. Moreover, no study has tested if changes in these outcomes mediate changes in cardiometabolic profiles or body weight and composition.**

The proposed study is innovative for the following reasons. First, we know of no other study in individuals who are overweight or obese with PreD that has examined how an exercise program may modify the gut microbiome and serum SCFAs. Second, we will study, for the first time, the interrelationship between exercise and the gut metagenome using a recently validated method for 'shallow shotgun' sequencing.[54] The shallow shotgun approach enables interrogation of the gut metagenome at a cost

that scales to longitudinal repeated measures study designs. As such, we will generate novel data on longitudinal changes in the gut metagenome resulting from MWE in individuals with PreD via thorough examination of the function of various microbial taxa and their effect on health over time.[55] Finally, the use of formal mediation analyses to examine the degree to which MWE-related changes in the preceding outcomes mediate changes in cardiometabolic risk profiles is novel and will inform the relative importance of the gut microbiome in exercise-related cardiometabolic improvements. These mediation analyses will also allow for a deeper interpretation of the physiological mechanisms by which exercise promotes improved health—addressing calls by the *AHA* and *ADA*.[8,9].

2.2 **Preliminary Data:** To date, only five human trials have investigated the effect of exercise on the gut microbiome.[43-47], with none of these studies having been conducted in a population with a subclinical CMD (e.g., PreD). Further, there are notable limitations to these human studies. First, aside from Kern et al.[43], no study employed a true control group. Second, most studies were conducted in small samples of apparently healthy adults, with only two studies[44,47] incorporating a true metagenomic sequencing approach. Incorporating a metagenomic approach (i.e., assessing microbial genes and functional capacity) allows for a deeper understanding of microbial diversity and ecology as well as better elucidation of the function of various microbial taxa and their effect(s) on health.[55] Finally, Allen et al.[45] only assessed SCFAs changes in stool and not in serum.

2.3 **Existing Literature:** The gut microbiome[16] has been posited as a mechanistic intermediate linking heath behaviors such as PA to CMD development.[14,17-19] The gut microbiome may be important for immunological,[20,21] metabolic,[16,22] inflammatory,[18,23,24] and neurobehavioral processes[22,24,25], some of which might partially explain how health behaviors influence CMD risk. Yet, limited evidence exists characterizing the effect of PA on the human gut microbiome. To date, most studies of the impact of PA interventions on the gut microbiome have used animal models,[26-41] with these studies supporting regular chronic PA (i.e., exercise) as a modulator of the gut microbiota. Animal studies have most often reported exercise-related changes to the Firmicutes[27-31,38] or Bacteroidetes[26,34,35,40] phyla while also noting beneficial alterations to microbial community diversity[26,29,30,32-35,38-40] and short chain fatty acid (SCFA)-producing taxa[33-36,38,41]. SCFAs are reported to promote gastrointestinal health, increase energy expenditure[42], and to be related to a lean phenotype.[14,27,41] The five existing human gut microbiome trials[43-47] most often implemented non-controlled studies of 5- to 8-weeks of moderate-intensity exercise on 3 days/week for 30-60 min/session. Among overweight or obese adults, prior studies[43-45] note increased □-diversity and altered β-diversity as a result of exercise; similar findings emerged in

another study of healthy older adults.[46] Additionally, one study noted exercise to result in increases in SCFAs (in stool), and corresponding changes to SCFA-related bacterial genes and taxa,[45] while two other studies observed improvements in the metabolic potential of the gut microbiome[43,44]. These findings corroborate observational studies in humans wherein more physically active individuals have greater microbiota diversity and/or improved capacity to produce SCFAs than less active individuals.[48-53]

3.0 Study Endpoints/Events/Outcomes

3.1 **Primary Endpoint/Event/Outcome: Expanded data collection procedures for our primary and secondary outcomes are available in Section 5.2.** We have two **Primary Outcomes.** The first primary outcome is changes to the gut microbiome, assessed via metagenomic sequencing, from pre- to post-intervention (**Specific Aim 1**). The Shannon Index—a measure of microbial community diversity with good reproducibility (ICCs>0.50)[56]—will be our primary indicator for changes in gut microbiota composition, and will be assessed at baseline, and following the 4th and 8th weeks. The second primary outcome is serum SCFA levels. Participants will undergo fasted blood draws to assess serum SCFA levels (**Specific Aim 2**). This work will be performed using the Metabolon pipeline.[57]

3.2 **Secondary Endpoint(s)/Event(s)/Outcome(s):** We have several **Secondary Outcomes**. For **Specific Aim 3** cardiometabolic profiles will be assessed via assays for high-density lipoprotein cholesterol (HDL-C), triglycerides, fasting insulin/glucose, and c-reactive protein (CRP). All blood assays will be conducted by the *UMN Advanced Research and Diagnostic Laboratory* (see 'Facilities' document). Blood samples collected will be stored at -80°C prior to batch-processing. Resting blood pressure (BP) will be taken using an Omron Automatic BP Cuff (Omron; Lake Forest, IL), with the average of the last two BP measurements reported. These cardiometabolic indices form the foundation of the 'metabolic syndrome',[59-63] and we will form a composite continuous score for cardiometabolic disease risk from HDL-C, fasting glucose, triglycerides, the average of systolic and diastolic BP, and waist circumference. Specifically, z-scores for each biomarker will be calculated and the within-participant average across z-scores computed and referred to as *cardiometabolic disease risk* (*CMDrisk*; the primary dependent variable for Specific Aim 3). This score has been used in epidemiologic, experimental, and clinical studies.[62,64-67] Individual components will be described in secondary analyses as needed. We will also measure HbA1c using whole blood analyzed at the UMN Advanced Research Diagnostic Laboratory; the A1cNOW+® device may also be used in a limited capacity to assess validity.[68] The oral microbiome will be

assessed using a widely employed salivary testing technique. Briefly, we will ask that participants chew a paraffin tablet (Orion Diagnostica) for 5 minutes after which the participant will be asked to spit into a 50mL tube until 5mL of saliva is collected. We have experience with this non-invasive assessment[69], with the assessment taking no longer than 5-10 minutes overall and presenting no greater than minimal risk. Other secondary outcomes are necessary to measure to ensure we can properly control for confounding. Dietary composition will be assessed via 3 phone-based dietary recalls during the run-in phase, and again during the final two weeks of the study. We will use these dietary recalls collected during the run-in period to develop personalized 3-day meal plan to standardize individual dietary behavior prior to stool sample collection. Employing dietary recalls at these time points will also allow us to adequately statistically control for participants' dietary changes—changes which, if not controlled, might confound study results.[70,71] Physical activity will be measured for the duration of the study using the Fitbit Inspire 2 smartwatch that uses accelerometry to measure physical activity metrics such intensity, steps, etc. Finally, anthropometric outcomes will include height, weight, and body composition. These measures, along with the other secondary outcomes aside from PA and Dietary composition, will be measured at the end of the 3-week run-in phase, and at the end of the 4th and 8th study weeks.

4.0 Study Intervention(s)/Investigational Agent(s)

4.1 **Description:** Following baseline data collection, participants will be randomized into one of two study groups using PROC PLAN (SAS 9.4) and a 1:1 allocation ratio.
GROUP 1 (aka, Experimental group): participants will complete 3 walking sessions/week for a total of 8 weeks (24 total sessions). Walking sessions will either take place on new commercial treadmills in the Epidemiology Clinical Research Center or remotely at or around a participant's home (see below). Regardless of the walking location, walking sessions will be 30 min in duration during intervention weeks 1 through 4 and a minimum of 45 min each during intervention weeks 5 through 8.
GROUP 2 (aka, Control group): participants will be asked to maintain their usual PA during the initial 8-week intervention period but will undergo all data collection procedures as outlined for the experimental group (See Section 5.2).

Experimental group participants who want to forego in-person exercise sessions on the University of Minnesota campus due to logistical constraints or the COVID-19 pandemic will modify the exercise intervention in the following manner:

Experimental group participants will engage in the same frequency and duration of walks as described above, but with a change in location to either: (a) in neighborhood or backyard walk (weather permitting, maintaining physical distancing); (b) on a treadmill within their home; and (c) in the hallway of their home if options a. and b. are not possible.

To monitor physical activity levels and ensure remote compliance: we will monitor to ensure that participants are completing their home-based walks at the proper duration and intensity using the Fitabase® platform. (See more information on the data security of Fitabase® in Section 16.7)

4.2 **Drug/Device Handling:** N/A

4.3 **Biosafety:** N/A

4.4 **Stem Cells:** N/A

5.0 Procedures Involved

5.1 **Study Design:** We will employ a 100-participant, 8-wk, randomized 2-arm parallel trial design with a 1:1 allocation ratio.

5.2 **Study Procedures:** All eligible and consented participants will complete a 3-week run-in phase during which baseline outcome assessments will be performed. During run-in weeks 1 and 2, participants will wear a Fitbit Inspire 2 smartwatch and complete three unannounced dietary recalls. During the run-in week 3 (see table below), participants will consume their 3-day standardized meal plan on days three through five, obtain a fecal sample, and complete a study assessment visit. To quantify compliance with the 3-day meal plan, participants will be provided with a paper checklist of all foods to be consumed, and will be asked to indicate which foods were consumed and to document any deviation. The study assessment visit will include a fasting blood draw, blood pressure in triplicate, weight measure and body fat assessment in duplicate, and a saliva sample. Following successful completion of all measures, participants will then be randomized into one of the two study groups using PROC PLAN (SAS 9.4) and a 1:1 allocation ratio.

Group 1 (Experimental group): Participants will complete 3 treadmill walking sessions/week (24 total sessions) over 8 weeks at or around their home or in-person within the Epidemiology Clinical Research Center. Each walking sessions will be 30 min in duration during intervention weeks 1

through 4 and 45 min during intervention weeks 5 through 8. Notably, this exercise dose (i.e., intensity x session duration x weekly frequency x intervention length) is equivalent to, or greater than, that employed in most existing human studies of exercise and the gut microbiome in addition to the exercise dosage observed necessary to yield meaningful cardiometabolic changes.[72] Therefore, while the dose is 'modest', it reflects what is relevant in real-world interventions and appropriate for overweight or obese sedentary individuals.

During week 4, participants will again consume their 3-day standardized meal plan, obtain a fecal sample, and complete a study assessment visit (same measures as described above for the 3-week run-in, except no fasting blood draw will be taken).

During weeks 7 and 8, participants will complete 3 unannounced phone-based dietary recalls. During week 8, participants will again consume their 3-day standardized meal plan, obtain a fecal sample, and complete a study assessment visit (same measures as described above in run-in week 3).

Table 1. Timing of Data Collection events during the 3-Week Run-In and Intervention Weeks 4 & 7/8.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
				Stool (pm)*	Stool (am)*	Stool
		Standard Diet Day1	Standard Diet Day2	Standard Diet Day3		
						Assess. Visit

**Preferred timing stool collection is the evening of Day 5 or morning of Day 6, though anytime in the Day 5 – 7 window is acceptable.*

Group 2 (Control group): Participants will be asked to maintain their usual PA during the initial 8-week intervention period but will undergo all data collection procedures as outlined above for the experimental group.

Expanded Data Collection Procedures: We have two **Primary Outcomes.** The first primary outcome is changes to the gut microbiome, assessed via metagenomic sequencing, from pre- to post-intervention (**Specific Aim 1**). The Shannon Index will be our primary indicator for changes in gut microbiota composition. Participants will use the OMNIgene-Gut stool collection kit to obtain their fecal samples collected at baseline, week 4 and

week 8. The home-collected samples will be returned to our lab within 30 days. DNA will be extracted from the stool samples using the 96 well plate Mo Bio Power soil kit on an Epmotion robot and eluted into 50ul aliquots. Each extraction will contain negative and positive controls representing defined bacterial communities (Zymo; Irvine, CA). DNA will be quantified using Quant-it and stored at -80°C. Metagenomic libraries will be prepared at the *UMN Genome Center* (see 'Facilities' document) using the Nextera XT kit (Illumina; San Diego, CA) and barcoded. DNA libraries will be denatured with NaOH and diluted to 8-12 pM in Illumina's HT1 buffer, spiked with 1% PhiX and a HiSeq 1x100 cycle v3 ki Illumina HiSeq, with an expected output of at least 0.5 million total sequences per sample. This approach has been shown to be valid[54] and represents an approach that can address both gut microbial taxonomy and community functional content at a cost similar to 16S studies.

The second primary outcome is serum SCFA levels. Participants will undergo fasted blood draws to assess serum SCFA levels (**Specific Aim 2**). This work will be performed using the Metabolon pipeline.[57] Briefly, serum samples are spiked with a solution of eight stable labelled internal standards and subjected to protein precipitation. After centrifugation, an aliquot of the supernatant is derivatized. The reaction mixture is analyzed by LC MS/MS on an Agilent 1290/ AB Sciex 5500 system. Peak areas of the respective analyte product ions are measured against the peak area of the corresponding internal standard product ions. Quantitation is performed using a weighted least squares regression analysis generated from fortified calibration standards prepared immediately prior to each run.

We have several **Secondary Outcomes.** For **Specific Aim 3** cardiometabolic profiles will be assessed via assays for high-density lipoprotein cholesterol (HDL-C), triglycerides, fasting insulin/glucose, and c-reactive protein (CRP). All blood assays will be conducted by the *UMN Advanced Research and Diagnostic Laboratory* (see 'Facilities' document). Blood samples collected will be stored at -80°C prior to batch-processing. Resting blood pressure (BP) will be taken using an Omron Automatic BP Cuff (Omron; Lake Forest, IL), with the average of the last two BP measurements reported. These cardiometabolic indices form the foundation of the 'metabolic syndrome',[59-63] and we will form a composite continuous score for cardiometabolic disease risk from HDL-C, fasting glucose, triglycerides, the average of systolic and diastolic BP. Specifically, z-scores for each biomarker will be calculated and the within-participant average across z-scores computed and referred to as *cardiometabolic disease risk* (*CMDrisk*; the primary dependent variable for Specific Aim 3). This score has been used in epidemiologic, experimental, and clinical studies.[62,64-67] Individual components will be described in secondary analyses as needed. We will also measure HbA1c using standardized procedures and the blood

collected. The oral microbiome will be assessed using a widely employed salivary testing technique. Participants will be asked to drool into a 50mL tube until 5mL of saliva is collected. For participants that are having difficulty generating saliva, we will ask those participants chew a small piece of orthodontic wax for 5 minutes and then drool into the 50mL tube. We have experience with this non-invasive assessment[69], with the assessment taking no longer than 5-10 minutes overall and presenting no greater than minimal risk. Other secondary outcomes are necessary to measure to ensure we can properly control for confounding. Dietary composition will be assessed via 3 phone-based dietary recalls. These recalls will be performed on 2 weekdays and 1 weekend day by trained interviewers. Assessment of diet on multiple days allows us to capture the individual variation in participants' diets.[71] We will use these dietary recalls to develop personalized 3-day meal plan to standardize individual dietary behavior prior to stool sample collection. Although randomization should lead to approximately balanced groups with respect to diet, employing dietary recalls at these time points will allow us to adequately statistically control for participants' dietary changes—changes which, if not controlled, might confound study results.[70,71] Physical activity will be measured during the duration of the study using the Fitbit Inspire 2 smartwatch. Finally, anthropometric outcomes will include height measured using a Seca stadiometer (Seca; Hamburg, Germany), with weight and body composition measured using the Tanita TBF-300a Body Composition Analyzer (Tokyo, Japan).

To collect blood samples and saliva samples as well as body weight, body fat percentage, and blood pressure, we are proposing to employ the following procedures in the designated settings while following all necessary COVID-19 precautions:

Setting: Baseline, week 4, and week 8 visits will occur in the Epidemiology Clinical Research Center (ECRC). We will follow all UMN, OVPR and ECRC approved guidelines for conducting research during the COVID-19 pandemic.

COVID-19 Screening: Participants will complete the OVPR-approved ECRC COVID-19 phone screener the day prior to their visit. Prior to entering the ECRC, participants will call the study coordinator and complete the screening questionnaire again. Upon entry into the building, study staff will assess the participant's temperature.

- **Personal Protective Equipment (PPE):** Participants will be asked to wear a mask. All ECRC staff coming within 6 feet of study participants (e.g. for blood or blood pressure collection) will be adhering to UMN guidelines for PPE (e.g. masks, gloves).

ECRC Data Collection:

- Each participant will be directed to a well-ventilated exam room, outfitted with a HEPA air purifier for additional protection where all non-lab measurements will take place.
- Participant will step upon the study scale and state to us their weight and body fat percentage while we stand at least 6 feet away. No other participants will be in the exam room, so confidentiality will be assured.
- Study staff will obtain blood pressure and a trained phlebotomist will collect the blood samples. As always, juice and snacks will be on hand in case needed after the blood draws. We will then have participants collect their saliva samples.
- Participant will be directed out of the building. Blood samples will be processed immediately and placed into a -80 freezer.
- We will thoroughly disinfect all touchpoints and research materials while properly disposing of biohazards.

If ECRC visits cannot occur due to continued COVID-19 outbreaks, we will conduct Home-Based Visits in the following manner:

- Participants will complete the OVPR-approved ECRC COVID-19 phone screener the day prior to their visit.
- Upon staff arrival, study staff will call the participant to again complete the OVPR-approved ECRC COVID-19 phone screener, and a temperature reading of the participant will be taken when they come outside.
- The scale platform will be placed outside the participant's home on a flat surface. The scale reader is connected to the platform by a 6 foot cord, enabling the staff to read the output while maintaining physical distance.
- Blood pressure will be obtained by the study staff member completing the research session.
- The study phlebotomist will collect the blood samples with participant seated in the chair. Juice and snacks will be made available in case the need arises. Blood will be placed in a cooler with dry ice for transport back to the ECRC for processing and placement into a -80 freezer.
    - We will also thoroughly disinfect all touchpoints and research materials while properly disposing of biohazards.

We have outlined the procedures we will take to ensure participant safety during the intervention within section 13.1. This study presents no greater than minimal risk to participants. All data collection forms are available on ETHOS.

5.3 **Study Duration:** The total duration of study participation will be approximately 11 weeks from initial contact with the researchers to the conclusion of all study procedures.

As for the data analyses associated with this study, we anticipate these data analyses to take approximately 4 months—allowing time for the *UMN Genomics Center* and the *UMN Advanced Research and Diagnostic Laboratory* to batch process participants' stool samples and blood work, respectively.

5.4 **Individually Identifiable Health Information:** Each participant will be assigned a unique study ID that will consist of a series of numbers and letters that will have no meaning to any of the individual's personal identifying characteristics. The investigators will use this ID to track each individual participant in the research database. There will be not identifiers in this database. The identifiers will be maintained in separate, secure, encrypted, password-protected database in a different physical location. This separate database for the identifying information will also include the study ID so that the investigators can link research data to individuals when necessary.

5.5 **Use of radiation:** N/A

5.6 **Use of Center for Magnetic Resonance Research:** N/A

6.0 Data and Specimen Banking

6.1 **Storage and Access:** We will initially collect data on paper after which we will transfer these data to computer spreadsheets for export to statistical packages, with this data entry conducted separately by two individuals to ensure data integrity. All PHI will be stored in a folder on the University of Minnesota's Box drive which is HIPAA compliant. All paper-based data will be stored in a locked file cabinet within the West Bank Office Building near the PI's office for a minimum of three years after the last participant has completed data collection. We will also store the de-identified electronic data for a minimum of three years on a server within the Division of Epidemiology and Community Health.

Buffy coat and serum from blood draws and saliva from salivary collection will be stored for up to 20 years in the PI's freezers located at the

Epidemiology Clinical Research Center or MoosT 1-355. All NIH procedures and University policies for the protection of confidentiality will be followed. Identifying data will be kept confidential, and only de-identified data will be shared.

6.2 **Data:** We will collect the following data: Serum, plasma, buffy coat, saliva, stool samples, Fitabase activity app data, food recall data, questionnaire data, and anthropometric data. The following data will be banked for future use if the participant consents: Serum, plasma, buffy coat, stool, and saliva. Otherwise, we will properly dispose of these specimens. All data will only be linked via a unique identification number. No identifying information will be included.

6.3 **Release/Sharing:** Materials generated under the project will be disseminated in accordance with University and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement. Access to databases and associated software tools generated under the project will be available for educational, research, and non-profit purposes. Such access will be provided using web-based applications, as appropriate. Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices. Research data which documents, supports and validates research findings will be made available after the main findings from the final research data set have been accepted for publication. Such research data will be redacted to prevent the disclosure of personal identifiers.

7.0 Sharing of Results with Participants

7.1 **Sharing Results:** Following the conclusion of the study, we will share with each participant their anthropometric (weight, waist circumference) and cardiometabolic data (fasting insulin/glucose, triglycerides, cholesterol levels, and blood pressure) collected during the 2-week run-in visit/baseline fasting visit and following the 4^{th} and 8^{th} weeks. We will share these data in a personalized letter to each participant. Further, we will provide participants with an overall aggregated summary of the study's observations and/or any published manuscripts.

7.2 **Sharing Genetic Results:** N/A

7.2.1 **Disclosure of Results:** N/A

7.2.2 **Returning Results to Participants: N/A**

Aggregate or individual results: N/A

Laboratory results: N/A
Plan for return of results to participants: N/A

Types of results to be returned to participants: N/A

7.2.3 **Future analysis of genotypes:** N/A

8.0 Study Population

8.1 Inclusion Criteria:

(1) 30-64 years old.
(2) Classified as overweight or obese with BMI >25.0kg/m^2, no upper limit
(3) Documentation* of a PreD diagnosis within one year of enrollment by physician or primary care provider based on lab tests showing a fasted blood glucose of 100-125 mg/dL, a 2-hour oral glucose tolerance test of 140-199 mg/dL, or an HbA1c level of 5.7%-6.4%[76]; OR a study screening lab value of HbA1C within the afore mentioned range.
(4) Currently engaged in <100 min/week of PA—confirmed via the Modifiable Activity Questionnaire.[77]
(5) No exercise contraindications as assessed by the Physical Activity Readiness Questionnaire (PAR-Q)[78]—this Questionnaire involves seven "yes" or "no" questions regarding an individual's health status, with answering "yes" to any one of these questions requiring a prospective participant to acquire a written doctor's note stating they can safely participate in the trial's exercise intervention. They would not be enrolled until this doctor's note is received.
(6) No self-reported physical/mental disabilities or gastrointestinal conditions.
(7) No antibiotic usage within the last 45 days.
(8) Stable weight over the last 6 months (<10% change).
(9) Not currently pregnant, planning to become pregnant, or currently breastfeeding.
(10) Willing to maintain current dietary and exercise habits, aside from any changes to be made per the study exercise protocol.

*Note: Documentation can include either a print out or screen shot of the lab value illustrating eligibility, along with the date of the test and the participant's name. If a hard copy is provided, the date and name

will be redacted.

8.2 **Exclusion Criteria:**

(1) Individuals <30 or >64: younger individuals may have not yet reached physiological maturity and in whom PreD prevalence is low; older individuals are more likely to have contraindications to PA and other comorbidities.
(2) BMI< 25kg/m^2. No Upper limit.
(3) Individuals with an HbA1c level <5.7% or >6.4%.
(4) Currently engaged in ≥100 min/wk of PA.
(5) Individuals with contraindications to exercise participation as indicated by the PAR-Q.
(6) A self-reported physical/mental disability that would prevent them from being able to adhere to the intervention.
(7) A self-reported a gastrointestinal illness or condition.
(8) Antibiotic use within the last 45 days.[79-81]
(9) Self-reported use of metformin and/or other medications that could interfere with the primary outcome.
(10) Unstable weight over the last six months (> 10% change).
(11) History of bariatric surgery or a history of other medical interventions that would interfere with the primary outcome.
(12) Currently pregnant or planning to become pregnant during the study period.
(13) Currently breastfeeding.
(14) Unwilling to be randomized to a study group.

8.3 **Screening:** Interested individuals will be asked to complete a red-cap screening questionnaire (sent via email or with study staff over the phone) which will enable staff to pre-review possible participants for eligibility. Everyone who completes a screener will be contacted, and, if needed, screening responses can be clarified.

Those who meet all eligibility criteria can then be scheduled for their consent/first study visit.

Those who *appear* eligible but need to confirm that their HbA1c is within eligibility range will be scheduled to complete a consent/screening visit. At this visit, following completion of the consent/HIPAA form, participants will have their HbA1c tested. To determine their current HbA1c value, we will either use the A1cNOW+® device or collect a 2mL whole blood sample via venipuncture. [68] Of note, one of these methods will only be employed only after the participant has consented to be screened for the study. Briefly,

the A1cNOW+® device will use a small drop of blood obtained from a finger of a prospective participant to determine the individual's HbA1C levels. This device has been validated against clinical laboratory measurements of HbA1C and is certified by the National Glycohemoglobin Standardization Program for this purpose.[85] If a venipuncture sample is collected, this sample will be brought to the Advanced Diagnostic Research Laboratory for analysis.

9.0 Vulnerable Populations

9.1 Vulnerable Populations:

Population / Group	Identify whether any of the following populations will be targeted, included (not necessarily targeted) or excluded from participation in the study.
Children	Excluded from Participation
Pregnant women/fetuses/neonates	Excluded from Participation
Prisoners	Excluded from Participation
Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders	Excluded from Participation
Non-English speakers	Excluded from Participation
Those unable to read (illiterate)	Excluded from Participation
Employees of the researcher	Excluded from Participation
Students of the researcher	Excluded from Participation
Undervalued or disenfranchised social group	Excluded from Participation

Active members of the military (service members), DoD personnel (including civilian employees)	Excluded from Participation
Individual or group that is approached for participation in research during a stressful situation such as emergency room setting, childbirth (labor), etc.	Excluded from Participation
Individual or group that is disadvantaged in the distribution of social goods and services such as income, housing, or healthcare.	Excluded from Participation
Individual or group with a serious health condition for which there are no satisfactory standard treatments.	Excluded from Participation
Individual or group with a fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior).	Excluded from Participation
Any other circumstance/dynamic that could increase vulnerability to coercion or exploitation that might influence consent to research or decision to continue in research.	Excluded from Participation

9.2 **Additional Safeguards:** N/A

10.0 Local Number of Participants

10.1 **Local Number of Participants to be Consented:** We will recruit participants on a rolling basis with the help of our clinical partners within community-based health clinics and community outreach around Minneapolis-St. Paul. We will assume a 20% attrition rate based on documented attrition rates of PA interventions,[82-84] along with a 30% rate of individuals who fail to fall within the HbA1c eligibility range during screening. With these rates in mind, we will recruit 150 individuals total to meet our desired overall *N* of 100 participants (*n* = 50 per group). Given the need for participants to be

able to participant in the exercise intervention at the Epidemiology Clinical Research Center, all participants will be recruited locally.

11.0 Local Recruitment Methods

11.1 **Recruitment Process:** Participants will be recruited on a rolling basis through a combination of the following methods: clinical referrals via partners within community-based health clinics, mass emails via University of Minnesota-Twin Cities campus listservs (all permissions will be obtained prior to this method), emails to past study participants who gave permission for future contact, and fliers posted on physical (e.g. campus buildings, community billboards) and virtual (e.g. Facebook, NextDoor) walls. Our study will also be searchable through clinicaltrials.gov and studyfinder.umn.edu.

Should additional recruitment methods be needed, we may also use established recruitment databases and EMR obtained through partial HIPAA waiver (which we have had approved). For instance, we may use the patient access database overseen by the Clinical and Translational Science Institute.

This study will also be listed at ResearchMatch.org. ResearchMatch is an electronic volunteer recruitment registry that allows people from anywhere in the country to self-register and express an interest in being prospectively considered for participation in research studies. This registry provides information about those volunteers to researchers who are looking for people to participate in studies, while protecting the privacy of the volunteers.

We intend to begin this recruitment process in early 2021, and we believe we can recruit the requisite number of participants over one year.

11.2 **Identification of Potential Participants:** Participants will be referred by one of our partnering care providers or will self-identify in response to fliers, emails, or internet postings.

- Potential participants will make the initial contact with study staff by telephone, mail, text, or via completion of our online screening survey. Only after this initial contact will study staff reach back out to potential participants.

11.3 **Recruitment Materials:** All study materials (fliers, postcards, emails) will contain a brief description of the study, primary inclusion criteria, a link to our study screening survey, and our contact information. Clinical

recruitment partners will receive materials in their preferred method, such as a small study postcard for placement in their lobby, or the same flier that we intend to post within community-based spaces such as local community centers. Emails sent will include the community flier as an attachment, while also containing similar text within the body of the email, along with a direct link to our study screening survey.

11.4 **Payment:** Participants will have the choice of one of two incentives for study completion with greater than 80% adherence: (1) they may request $100 following their completion of the entire study, with this payment provided using the Greenphire ClinCard; OR (2) they may request to keep the Fitbit Inspire 2 along with a $25 ClinCard. This amount is reasonable given the study's protocol and procedures.

12.0 Withdrawal of Participants

12.1 **Withdrawal Circumstances:** We do not foresee any circumstances under which participants will be withdrawn from the research without their consent. Participants who discontinue from the active intervention due to illness or injury will still be invited to complete all study measures at all data collection time points.

12.2 **Withdrawal Procedures:** For participants who ask to withdraw from the study voluntarily, no additional data will be collected and participants would be compensated at the prorated rate as described in Section 11.4. would still be compensated according to the table above. However, data from the participant's time in the study will still be used in data analysis when employing an intent-to-treat analysis approach.

12.3 **Termination Procedures:** We do not foresee any circumstances that would result in the termination of the study for any individual.

13.0 Risks to Participants

13.1 **Foreseeable Risks:** The current study present no greater than minimal risk to participants. However, we will be taking precautions to ensure any potential risk from study participation is minimized. Per standard procedures during exercise intervention studies, we will ensure participants have no contraindications to exercise participation. However, we must note that any type of physical activity participation, even a walking exercise intervention like that of the current study, does carry risk in any population. Risks associated with exercise participation include

dizziness, nausea, muscle soreness, fatigue, fainting, joint pain, shortness of breath, muscular strains/sprains, and cramping. We will minimize these risks by allowing participants to complete a 5-10-minute warm-up of slow walking prior to each exercise session.

Some potential psychological stress or embarrassment may also be associated with filling out the study questionnaire and/or undergoing measurements of weight, waist circumference, and blood pressure in addition to the collection of stool samples and saliva. Risks will be minimized by collecting this data in a private setting, either at their home or at the ECRC, depending upon the measure in question.

As with all blood draws, there may be a modest amount of pain (equivalent to that of a pin prick) and lightheadedness associated with the collection of approximately 3 tablespoons of blood for each of three blood draws. We will minimize this risk by employing an experienced and trained phlebotomist for blood draws. Overall, the anticipated benefits to science outweigh the risks.

Finally, to prevent any threat of a COVID-19 infection, we will follow all UMN/OVPR/ECRC protocols while the participant is at the ECRC and encourage masking and physical distancing if any walks are done outside of the ECRC environment.

13.2 **Reproduction Risks:** N/A

13.3 **Risks to Others:** N/A

14.0 Potential Benefits to Participants

14.1 **Potential Benefits:** Throughout the course of the 8-week intervention, participants may improve cardiometabolic indices (e.g., blood lipids, fasting insulin/glucose, blood pressure, c-reactive protein) while possibly experiencing weight loss too.

15.0 Statistical Considerations

15.1 **Data Analysis Plan:** Chi-square and independent *t*-tests will evaluate baseline group differences as appropriate. Mediation analyses will also be conducted. We outline our analysis plans in Section 15.3. [86-92]

15.2 **Power Analysis:** Power for Aim 1 is based on analyses using alpha-diversity (Shannon Index) as the primary outcome. Taniguchi et al.[46], reported a mean±SD baseline Shannon Index of 5.7±0.6 which is consistent with preliminary data from gut samples collected by Dr. Demmer in other recent projects. Given this variance, and with a balanced design of *n*=50 in the experimental and control arms (*N*=100 total), we will have >99% power to detect a 1 unit increase in Shannon Index. Power for Aim 2 is based on changes in serum SCFAs. Our SCFA assays will be performed at Metabolon and our colleagues at Metabolon provided information on mean±SD for these outcomes in their lab. They are as follows: acetate=1806±2500 ng/mL, butyrate=35±43 ng/mL, and propionate=50±52 ng/mL. With these distributions, we will have >80% power to detect a difference in SCFA levels between treatment and control groups with a magnitude of at least 50% of 1 SD.

The primary outcome for Aim 3 will be a composite score based on the average of the z-scores for the following 5 biomarkers: HDL-C, fasting glucose, triglycerides, average of systolic and diastolic BP, and waist circumference. The standardized mean±SD of this *CMDrisk* z-score in Dr. Pereira's sedentary behavior intervention among 575 participants (Grant #: R01CA198971) is 0±0.7. This provides 80% power to detect a difference in the *CMDrisk* z-score of 0.4, between intervention and control at the conclusion of the study.

15.3 **Statistical Analysis: *Aim 1 analysis*:** Gut microbiome data, derived via metagenomic sequencing, will be operationalized as described above. In a primary analysis, generalized linear regression models will regress Shannon Index on intervention assignment. The model is formalized as: E[Y]= intercept + β1*A + β2*C. E[Y]=expected Shannon value; β1=coefficient for intervention arm; β2=coefficient for a vector of confounders should this be necessary in our randomized design. Secondary analyses will consider metagenomic features (genes and functional profiles). Any metagenomic features not present at >0.1% relative abundance in at least 10% of samples will be removed. Zero-inflated log-linear generalized linear models with a Gaussian error term will be fitted, with treatment status as predictor, as implemented by the metagenomeSeq Bioconductor package for shotgun metagenomic data analyses.[93] The generalized model, per metagenomic outcome, is formalized as: log E[Y]= intercept + β1*A + β2*C. E[Y]=expected normalized sequence count value; β1=coefficient for intervention arm; β2=coefficient corresponding to the vector of baseline confounders. Total read count is incorporated as an offset term. This analysis will generate a "top list" of genes, gene families, and pathways with altered abundance by intervention assignment. Separate models will

be developed for intervention status predicting week 4 and week 8 outcomes. We will also consider time*treatment status models to incorporate stool sampling at all three timepoints. P-values will be corrected for multiple hypothesis testing using the false-discovery rate as we have done previously with high dimensional data.[86-92,94-98]

***Aim 2 analysis*:** As with Aim 1, generalized linear regression models will be used. Each of three SCFAs (acetate, butyrate and propionate) will be regressed on intervention assignment. The model is formalized as: E[Y]= intercept + β1*A + β2*C. E[Y]=expected SCFA value; β1=coefficient for intervention arm; β2=coefficient for a vector of confounders.

***Aim 3 analysis*:** We will use PROC CAUSALMED (SAS 9.4) to explore **mediation** effects on *CMDrisk*. This regression-based model was developed by Valeri and VanderWeele[99] and VanderWeele.[100] The predictor variable will be treatment (experimental vs. control) and the outcome will be *CMDrisk*. We will include age, *sex as a biological variable*, race/ethnicity, and PA duration as covariates. Mediation will be tested by adding parameters, *in separate models*, for gut microbiota diversity (8-week change in -diversity) and serum SCFAs (8-week change in levels of serum SCFAs).

15.4 **Data Integrity:** All data will be double entered separately by two individuals. Blood and stool sample data will be examined for computational value errors associated with out-of-range variables. All data will be examined for outliers and improbable values. More details provided in section 17.1.

16.0 Health Information and Privacy Compliance

16.1 **Select which of the following is applicable to your research:**

☐ My research does not require access to individual health information.

☒ I am requesting that all research participants sign a HIPCO approved HIPAA

Disclosure Authorization to participate in the research (either the standalone form or the combined consent and HIPAA Authorization).

☐ I am requesting the IRB to approve a Waiver or an alteration of research participant authorization to participate in the research.

Appropriate Use for Research:

16.2 **Identify the source of Private Health Information you will be using for your research (Check all that apply)**

☐ I will use the Informatics Consulting Services (ICS) available through CTSI (also referred to as the University's Information Exchange (IE) or data shelter) to pull records for me

☒ I will collect information directly from research participants.

☐ I will use University services to access and retrieve records from the Bone Marrow Transplant (BMPT) database, also known as the HSCT (Hematopoietic Stem Cell Transplant) database.

☐ I will pull records directly from EPIC.

☐ I will retrieve record directly from axiUm / MiPACS

☐ I will receive data from the Center for Medicare/Medicaid Services

☐ I will receive a limited data set from another institution

☐ Other. Describe: N/A

16.3 **Explain how you will ensure that only records of patients who have agreed to have their information used for research will be reviewed.**

This information will come only from the participants themselves, with all review of any health information occurring in a private room within the Epidemiological Clinical Research Center.

16.4 **Approximate number of records required for review:** N/A

16.5 **Please describe how you will communicate with research participants during the course of this research. Check all applicable boxes**

☐ This research involves record review only. There will be no communication with research participants.

☐ Communication with research participants will take place in the course of treatment, through MyChart, or other similar forms of communication used with patients receiving treatment.

☒ Communication with research participants will take place outside of treatment settings. If this box is selected, please describe the type of communication and how it will be received by participants. Participants will be asked their preferred communication method; if text and/or email is selected, the appropriate consents will be obtained. Diet recalls will be conducted by phone, but only at times provided by study participants.

Participants will be contacted only as it pertains to the study (scheduling/confirming visits, assessing compliance, troubleshooting

technical issues, etc).

Importantly, we will not request access to medical records or any other source of private information about the participants.

16.6 **Explain how the research team has legitimate access to patients/potential participants:**

Potential participants will make the initial contact with study staff. Only after this initial contact will study staff reach back out to potential participants.

16.7 **Location(s) of storage, sharing and analysis of research data, including any links to research data (check all that apply).**

☐ In the data shelter of the Information Exchange (IE)

☐ Store ☐ Analyze ☐ Share

☐ In the Bone Marrow Transplant (BMT) database, also known as the HSCT (Hematopoietic Stem Cell Transplant) Database

☐ Store ☐ Analyze ☐ Share

☒ In REDCap (recap.ahc.umn.edu)

☐ Store ☐ Analyze ☐ Share

☐ In Qualtrics (qualtrics.umn.edu)

☐ Store ☐ Analyze ☐ Share

☐ In OnCore (oncore.umn.edu)

☐ Store ☐ Analyze ☐ Share

☒ In the University's Box Secure Storage (box.umn.edu)

☒ Store ☐ Analyze ☒ Share

☐ In an AHC-IS supported server. Provide folder path, location of server and IT Support Contact:

☐ Store ☐ Analyze ☐ Share

☐ In an AHC-IS supported desktop or laptop.

Provide UMN device numbers of all devices:

☐ Store ☐ Analyze ☐ Share

☒ Other:

Indicate if data will be collected, downloaded, accessed, shared or stored using a server, desktop, laptop, external drive or mobile device (including a tablet computer such as an iPad or a smartform (iPhone or Android devices) that you have not already identified in the preceding questions

☒I will use a server not previously listed to collect/download research data

We will monitor participants physical activity using the Fitabase® platform. Fitabase® is a platform provided by Fitbit that allows for research participants to upload their Fitbit smartwatch data each day. We will be able to use this platform and an associated walk log to ensure participants are engaging in their home-based walking. Importantly, Fitabase® houses data in secure, encrypted Microsoft Azure servers and do not sell, release, or otherwise make data available to third parties. Further, we will provide only de-identified participant IDs to Fitabase®, with Fitabase® only having access to de-identified IDs but otherwise unable to access participants' email address or account names.

☐I will use a desktop or laptop not previously listed

☐I will use an external hard drive or USB drive ("flash" or "thumb" drives) not previously listed

☐I will use a mobile device such as an tablet or smartphone not previously listed

16.8 **Consultants. Vendors. Third Parties:** N/A

16.9 **Links to identifiable data:** Each participant will be assigned a unique study ID that will consist of a series of numbers and letters that will have no meaning to any of the individual's personal identifying characteristics. The investigators will use this ID to track each individual participant in the research database. There will be not identifiers in this database. The identifiers will be maintained in separate, secure, encrypted, password-protected database in a different physical location. This separate database for the identifying information will also include the study ID so that the investigators can link research data to individuals when necessary. Electronic data bases, encrypted on University servers and password protected, only accessible to the investigators. Limited paper files may be maintained in locked filing cabinets in locked offices, but these will be minimized and will include consent form copies and a minimal amount of participant information if needed in hard copy. No research information will be placed in any sort of medical, employment, or educational record.

16.10 **Sharing of Data with Research Team Members:** We will share research data on a limited basis between research team members. However, when we do share data, we will do so via our University Box accounts.

16.11 **Storage and Disposal of Paper Documents:** Limited paper files may be maintained in locked filing cabinets in locked offices near the PI, but these materials will be minimized and will include consent form copies and a minimal amount of participant information if needed in hard copy. No research information will be placed in any sort of medical, employment, or educational record. Any paper materials will be kept for a minimum of 3 years after which the materials will be shredded and disposed of via proper means.

17.0 Confidentiality

17.1 **Data Security:** As reviewed previously, we have numerous safeguards in place to protect data integrity—all of which will be under the oversight of the PI. Our research team is qualified to conduct this oversight given past experience conducting randomized trials. First, all data will be double entered separately by two individuals which will allow us to check for data entry errors. Blood, stool, and salivary sample data will be examined for computational value errors associated with out-of-range variables. All data will be examined for outliers and improbable values. These procedures will ensure protection of the study's internal validity. Second, to protect the participants' health-related information, we will deidentify each participant's data. In detail, and as discussed in section 16.1, each participant will be assigned a unique study ID that will consist of a series of numbers and letters that will have no meaning to any of the individual's personal identifying characteristics. The investigators will use this ID to track each individual participant in the research database. There will be not identifiers in this database. The identifiers will be maintained in separate, secure, encrypted, password-protected database in a different physical location. This separate database for the identifying information will also include the study ID so that the investigators can link research data to individuals when necessary. Electronic data bases, encrypted on University servers and password protected, only accessible to the investigators. Limited paper files may be maintained in locked filing cabinets in locked offices, but these will be minimized and will include consent form copies and a minimal amount of participant information if needed in hard copy. No research information will be placed in any sort of medical, employment, or educational record.

18.0 Provisions to Monitor the Data to Ensure the Safety of Participants

18.1 **Data Integrity Monitoring.** Our research team is qualified to conduct this oversight given past experience conducting randomized trials. First, all data will be double entered separately by two individuals which will allow us to check for data entry errors. Blood, stool, and salivary sample data will be examined for computational value errors associated with out-of-range variables. All data will be examined for outliers and improbable values. These procedures will ensure protection of the study's internal validity. Second, to protect the participants' health-related information, we will deidentify each participant's data. In detail, and as discussed in section 16.1, each participant will be assigned a unique study ID that will consist of a series of numbers and letters that will have no meaning to any of the individual's personal identifying characteristics. The investigators will use this ID to track each individual participant in the research database. There will be not identifiers in this database. The identifiers will be maintained in separate, secure, encrypted, password-protected database in a different physical location. This separate database for the identifying information will also include the study ID so that the investigators can link research data to individuals when necessary. Electronic data bases, encrypted on University servers and password protected, only accessible to the investigators. Limited paper files may be maintained in locked filing cabinets in locked offices, but these will be minimized and will include consent form copies and a minimal amount of participant information if needed in hard copy. No research information will be placed in any sort of medical, employment, or educational record.

18.2 **Data Safety Monitoring.** N/A

19.0 Provisions to Protect the Privacy Interests of Participants

19.1 **Protecting Privacy:** We will have participants complete the demographic questionnaire in a private room well-ventilated within the Epidemiology Clinical Research Center. Further, we will conduct all measurements of weight, waist circumference, and blood pressure in this same well-ventilated room within this building as well. These data will not be spoken aloud. Finally, all blood collection will occur at the Epidemiology Clinical Research Center and performed by an experienced phlebotomist in the well-ventilated private room, with the salivary collection occurring within a private room in this facility too.

19.2 **Access to Participants:** We will only request access to each participant's home address, email, and phone numbers to ensure that we can contact participants to set up weekly walking sessions or determine a schedule of home-based walking sessions. We will otherwise not request access to medical records or any other source of private information about the participants.

20.0 Compensation for Research-Related Injury

20.1 **Compensation for Research-Related Injury:** This research does not involve greater than minimal risk to participants.

20.2 **Contract Language:** In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care, as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study know right away.

21.0 Consent Process

21.1 **Consent Process (when consent will be obtained):** During the recruitment process, potential participants will be provided with a copy of the study consent form to review. An additional copy will also be attached to their consent visit confirmation email/letter for their records. The day prior to the first study visit, the study coordinator will call to confirm the visit and complete the COVID-19 screening questionnaire. The study coordinator will confirm that the participant understands the study goals, design, purpose, and risks/benefits and is sure they would like to participate in the study, and answer any study questions at that time (to minimize in-person time at the ECRC). At the consent visit, the study coordinator will answer any additional questions and have the participant sign the official consent form. A signed copy of the consent form will be offered to the participant. The consent process will take place within a conference room within the Epidemiology Clinical Research Center immediately prior to baseline testing. We will employ no formal process to ensure ongoing consent, but we will continually ensure that the participant is aware of the voluntary nature of the study by ensuring the OVPR Participant Information sheet is sent 24-hours prior to any in-person visit and knows that they may withdraw from study participation at any time for any reason.

21.2 **Waiver or Alteration of Consent Process (when consent will not be obtained):** N/A

21.3 **Waiver of Written/Signed Documentation of Consent (when written/signed consent will not be obtained):** N/A

21.4 **Non-English Speaking Participants:** N/A

21.5 **Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age):** N/A

21.6 **Cognitively Impaired Adults, or adults with fluctuating or diminished capacity to consent:** N/A

21.7 **Adults Unable to Consent:** N/A

- **Permission:** N/A
- **Assent:** N/A
- **Dissent:** N/A

22.0 Setting

22.1 **Research Sites:** We will identify and recruit participants primarily from the health clinics of our clinical partners, but we will also recruit individuals from the Minneapolis St-Paul community. We will not be compensating any of our clinical partners for their involvement. Research procedures will be performed at the Epidemiology Clinical Research Center.

22.2 **International Research:** N/A

23.0 Multi-Site Research

N/A

24.0 Coordinating Center Research

N/A

25.0 Resources Available

25.1 Resources Available:

- We have clinical partners in Minneapolis-St. Paul health clinics which should allow us to complete the recruitment of 100 participants within two years in addition to our ability to leverage University listservs for flier distribution and the posting of fliers within local community centers and on social media. We will devote 2 years to conducting this research.
- The West Bank Office Building has ample room for the current study. The Division of Epidemiology and Community Health has 48 faculty, 350 staff, and a combined office space totaling more than 75,000 square feet. Meeting rooms are equipped with state-of-the-art video communication technology for communication with external consultants and staff working off-site. It is anticipated that the current space will be adequate to accommodate the needs of this proposal. Office equipment, such as conference telephones, printers, photocopiers, and postage services are available to the proposed study. Additionally, we already possess the two treadmills that will be used to implement the current study, with more to be purchased. The Epidemiology Clinical Research Center (ECRC) is located one block away from the offices of the Division of Epidemiology and Community Health, about 0.5 miles from the University of Minnesota Hospital on the Minneapolis campus, and is readily accessible from all parts of the Twin Cities metropolitan area (within three blocks from two major interstate highways). **Participants in this trial will visit the ECRC for the blood draws, saliva sample collection, and other clinic measures during the baseline fasting visit and during the 4th and 8th week time points. Assays from blood draws will be analyzed at the University of Minnesota's Advanced Research and Diagnostic Laboratory, with all necessary precautions taken to limit any chance of COVID-19 infections (as outlined earlier in the protocol).** Parking is free. The ECRC occupies one floor (17,758 square feet) of a two-story building and includes reception area, offices for staff, examination rooms, interview rooms, ultrasound, phlebotomy and blood processing rooms, freezer room, lounge, conference rooms, and storage space. The building meets current regulations for handicapped accessibility

and has 100 free adjacent parking spaces. The site was established in February 2000 and is currently providing space and infrastructure for 20+ research projects including multicenter epidemiologic studies and clinical trials

- We do not anticipate any medical or psychological risk associated with participation, but the West Bank Office Building and Epidemiology Clinical Research Center has general first aid equipment and a defibrillator onsite if any medical needs arise. Further, we will ensure that individuals involved in the monitoring of study participation are CPR certified.
- The PI and Co-Is will train any individual assisting with data collection procedures/measurements and will supervise these individuals performing these procedures/measurements for 1 week following training.

26.0 References

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