Study Title: Study of Acarbose in Longevity
NCT02953093
IRB Number: 2016-6933
IRB Approval Date: 10/05/2018

Study of Acarbose in Longevity (SAIL)

A. Introduction and Specific Aims:

Acarbose, an FDA approved drug for the treatment of type 2 diabetes, has known effects on glucose metabolism. Evidence from mice indicates that acarbose may prolong lifespan. In humans, acarbose improves inflammatory markers and reduces cardiovascular events. Consequently, acarbose is of interest in clinical translational aging research since it may influence fundamental processes that contribute to age-related diseases. We herein propose an exploratory study to examine the effect of acarbose treatment on the biology of aging in humans. Specifically, we plan to investigate whether treatment with acarbose will restore the gene expression profile of older adults to that of young healthy subjects.

Specific Aims: We propose a 5 month randomized, double blind, placebo-controlled, cross-over study of acarbose treatment in older men (age > 60, n=30), with the following aims:

Specific Aim 1: To assess the effect of acarbose on gene expression profile, with a focus on molecular pathways known or suspected to play a role in aging.

Sub-aim 1: As a secondary analysis, we plan to assess the relationship between changes in gene expression with selected phenotypic variables and biomarkers, including changes in insulin sensitivity and glucose tolerance, vascular function, oxidative stress, inflammation, gut bacteria and/or serum microRNA profiles.

Specific Aim 2: To determine if treatment with acarbose will restore the gene expression profile of older, glucose intolerant men to that of young, healthy subjects.

B. Background and Significance:

Aging is a well-established risk factor for many disabling diseases including diabetes, cardiovascular disease, Alzheimer's disease and cancer. In fact, the risk of death from these causes is dramatically accelerated (100-1000 fold) between the ages of 35 and 85 years. There has been growing interest in understanding common pathways that accelerate age-related diseases, with the goal of therapeutically targeting such pathways to maintain health in older age. Several agents increase lifespan and prevent diseases of aging in animals, but studies in humans are needed.

Acarbose, an FDA-approved diabetes medication, is one of the most promising anti-aging agents tested by the National Institute of Aging's Interventions Testing Program. Acarbose is a pseudotetrasaccharide of microbial origin, which lowers blood glucose primarily by inhibiting alpha-glucosidases, a group of enzymes that break down starch in the small intestine.[1] This results in preferential absorption of carbohydrates at the lower small intestine, slowing of digestion time, and flattening of postprandial glucose and insulin excursions. Acarbose is primarily excreted in the feces, and approximately 1 % of acarbose is absorbed systemically, after undergoing fermentation.[1, 2]

In a recent study of genetically heterogeneous, non-diabetic mice, acarbose increased median lifespan by 22% in males and 5% in females.[3] Maximum lifespan was increased by 11% and 9% in males and females, respectively, despite unchanged hemoglobin A1c

levels. Other notable effects include an increase in FGF-21, and decreases in weight, fasting IGF-1 and fasting insulin in acarbose-treated mice.[3] In a follow-up study, acarbose started later in life (18 months) extended lifespan by 5% in male mice. Acarbose also improved age–related memory impairment in a separate mouse study.[4, 5] A study in diabetic rats showed that treatment with acarbose significantly altered the expression of miRNAs that target genes in the inflammatory pathways including interleukin 6 (IL-6), mitogen activated protein kinase 1 (MAPK-1) and tumor necrosis factor (TNF), primarily by acting through the MAPK pathway.[41]

In humans, treatment with acarbose has been shown to prevent the development of type 2 diabetes in high-risk individuals.[6] In the STOP-NIDDM trial, acarbose also significantly decreased incident cardiovascular disease, including a decrease in myocardial infarctions.[7] Subsequent studies, including a meta-analysis, have supported these findings,[8, 9] and a large clinical trial of acarbose for CVD prevention is currently underway in Asia. [10]Treatment with acarbose is also associated with a decreased risk of colorectal cancer.[11] Taken together, these findings suggest that acarbose treatment may have pleotropic effects and support the need to study acarbose in human aging.

The mechanism by which acarbose delays aging in mice is not established. Some authors have hypothesized that acarbose acts as a "caloric restriction mimetic." Caloric restriction (CR) is known to extend lifespan in some model organisms and healthspan in human and non-human primates. Although acarbose does not substantially lower net caloric exposure, like CR, it decreases fasting insulin and weight, improves glucose metabolism, and in mice, mimics some of the metabolomic changes seen with CR.[2, 3, 6 12] In humans, acarbose has postprandial effects that include a decrease in lipids[13, 14] and inflammatory mediators,[15-17] an increase in postprandial nitric oxide and nitric oxide synthase[18], and an improvement in endothelial function, suggesting that acarbose protects from adverse consequences of a prandial nutrient load.[19] However, it is not known whether such changes may contribute to the anti-aging effects of acarbose.

Acarbose also alters gut microbiota[20] and increases serum butyrate,[21] a fermentation byproduct that is associated with improved glucose metabolism. Gut microbes are increasingly recognized as important mediators of inflammatory and metabolic processes in humans. With age, there is a change in gut microbial species, and certain profiles have been associated with frailty.[22, 23] So the question naturally arises as to whether human gut microbiota directly influence aging, and if acarbose might alter this process. Acarbose also increases circulating postprandial GLP-1 levels by up to 50% and alters the secretion of other gut hormones, which might also contribute to its beneficial effects.[24, 25] Finally, it is possible that the absorbed metabolites of acarbose have direct effects on tissues.

In summary, acarbose improves lifespan and age-related memory loss in mice and represents one of the most promising anti-ageing compounds tested by the Interventions Testing Program. In humans, it decreases the risk of certain age-related diseases, including diabetes, cardiovascular disease and colorectal cancer. It is an inexpensive, FDA-approved medication that has been used safely for many years.[26] We hypothesize that acarbose might extend healthy lifespan in humans. As an initial step to test this hypothesis, we plan to study changes in gene transcription and a variety of biomarkers in older adults at risk for cardiometabolic disease.

Muscle and adipose are key metabolic tissues that undergo significant age-related changes, and play an active role in the pathogenesis of aging.[27-29] We are characterizing transcriptional changes with aging in these tissues, and studying the effects of therapeutic interventions. RNA-Seq analysis is being used to identify pathways targeted by candidate anti-aging drugs that have been tested in animals. In addition, we are accumulating a repository of muscle and adipose biopsy samples obtained from young healthy controls to establish a unique biological "fingerprint" for aging in these tissues by comparing changes in gene expression in older adults, post-drug therapy to the profiles of young healthy subjects. This overall approach is supported by a grant from the Glenn Foundation for the Study of the Biology of Human Aging and we are currently analyzing data from two prior studies using metformin and resveratrol. We have gene transcription profiles from RNA seq performed on fat and muscle biopsies from 4 young individuals and plan to recruit up to 30 more young participants under a separate protocol.

As part of our secondary analyses, we also plan to assess changes in microRNA levels in the serum. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at a post-transcriptional level. Due to their stability in blood, changes in expression of circulating miRNA might serve as markers for age-related pathologies. Investigators at Einstein have profiled B-lymphocyte miRNA levels in the plasma and have identified microRNA profiles associated with exceptional longevity.[30] We hypothesize that acarbose treatment will alter the expression of circulating miRNAs in a way that may offer insight into anti-aging mechanisms of acarbose. Given the known effects of acarbose on colonic fermentation and composition of gut microbiota we plan to explore changes in gut microbiome using 16s rDNA seq. We plan to correlate changes in transcription (muscle and fat RNAseq), post-translational regulation (miRNA) and gut microbiome with changes in clinical metabolic outcomes and inflammatory biomarkers.

C. Research Methods:

C.1. Study Design & Protocol Summary: We propose a 22-week randomized, double-blind, placebo controlled cross-over study of acarbose in older adults (n=30) with impaired glucose tolerance.

		Treat-ment A			Wash-out	Treat-ment b		
	Visit 1	Visit 2 0	Visit 3 4 wks	Visit 4 10 wks	2 wks	Phone call: 12wks	Visit 5 16 wks	Visit 6 22 wks
Informed consent	x							
OGTT & eligibility labs ($)	x	Dispense study meds (treat-ment A)		Dispense study meds (treat-ment B)		Start treatme nt B		
Safety monitoring (*)			x				X	
serum for miRNA				x				X
Stored samples for biomarkers,				x				X

Vital signs, height, weight	x	x	x	x			x	X
History	x	Interim history	Interim history	Interim history			Interim history	Interim history
Physical exam (**)	X	X						
Standard meal test (#)				x				X
Muscle & adipose tissue biopsy				x				X
Fecal collection				x				X

($) eligibility labs include OGTT, Cr, AST/ALT, CBC, urinanalysis
(*) safety monitoring includes recording of AEs and labs (Cr, AST/ALT)
(#) standard meal test includes: fasting hsCRP, IGF-1, HbA1c and adiponectin, lipid profile, serum microRNA, Fasting and every 30 minute glucose, insulin levels.
Fasting and 2 hour samples stored for possible future use.
(**) Physical exam can be performed during visit 1 or visit 2.

Consent Process:
For this clinical investigation we will be consenting the research participants in compliance with our local IRB policy, Department of Health and Human Services (HHS) regulations for the Protection of Human Research Subjects (45 CFR 46) and FDA regulations (21 CFR 50 & 56).

For an initial phone screening we will be using a phone consent form that contains also HIPAA language. We will ask for permission to collect personal information and if they agree to be contacted for other future studies.
During the screening visits in the Clinical Research Center (CRC), subjects will sign the current IRB-approved and stamped consent document.
Prior to the adipose and muscle biopsies (visit 4 and visit 6), the physician performing the procedures will obtain consent using a standard Montefiore procedural consent, again detailing the procedures and expected risks.

Study visits:
Screening visit (Visit 1): Following informed consent, a history,vitals, anthropometrics , and screening labs, including OGTT, will be obtained.
Visit 2: Subjects for the study intervention who meet eligibility criteria (see below) will be invited to enroll in the study. Study medication (Treatment A: acarbose or placebo, double-blind) will be dispensed for the following 10 weeks. Since flatulence and abdominal discomfort are common with acarbose, slow titration is often needed. Subjects will be instructed to begin with 1 capsule with the first bite of one meal each day, and will be given instructions on titrating study medication as tolerated to 2 capsules with each meal (breakfast, lunch, and dinner) after 2 weeks. Subjects unable to tolerate any study med, will be withdrawn from the study. Subjects will be contacted every other week by phone to assess adherence and to adjust dosing if necessary. Physical exam will be performed in visit 1 or visit 2 by a licensed study physician to confirm no major physical limitations or illness prior to randomization.

Visit 3: Monitoring visit. This visit will be a visit to assess compliance and dose escalation. Subects who able to tolerate 2 capsules three times daily, will be provided with a higher dose capsule, and instructions to titrate to 2 (higher dose) capsules three times daily. If they are only able to tolerate 1 capsule three times daily of the lower dose, they will be given more of the lower dose capsule to take for the remainder of the treatment period. Interim history will be performed. Liver function tests will be checked. A fecal microbiome stool collection kit and instructions will be provided

Visit 4: At the completion of the 10-week treatment period (up to 4 week titration, 6 week maximum dose) a standard mixed meal test (SMT) will be conducted and muscle and adipose tissue biopsies will be performed, after procedural risks are again explained by the physician performing the muscle and fat biopsies and a Montefiore procedural consent is signed by the participant. Fasting and 2hour blood samples will be collected for analyses noted above. Stool sample tubes will be collected. Vital signs and interval history will be obtained. Subjects will bring unused medication for assessment of adherence. Additional interim safety and/or adherence visits will be conducted if indicated. Instructions to maintain usual diet and activity patterns will be reinforced at each visit. Participants will be given treatment B, and will be instructed to start them after a 2-week washout. The same titration instructions detailed above will be provided to participants.

Phone call- After a 2 week washout participants will be reminded to start treatment B with a phone call

Visits 5-6: The procedures in visits 3-4 will be repeated.

C.1.2 Inclusion and Exclusion Criteria:

Subjects with impaired glucose tolerance or with mild, untreated diabetes (defined below) based on a screening OGTT will be enrolled. Patients with a prior diagnosis of diabetes may enroll if they are not taking anti-diabetic medications and they meet OGTT criteria. If a participant has had an OGTT within the past year, results of this test may be used to determine eligibility. Given rodent studies demonstrating pronounced effects on males, we plan to recruit males for this small study, with a plan to study females in a subsequent, larger study if effects are seen. Although this approach decreases the generalizability of our initial findings, by increasing the homogeneity of the study population and targeting the gender most likely to benefit, we hope to improve the power to detect a treatment effect in this small, preliminary, study.

Inclusion:

Men; age >60 years with IFG or IGT based on 75g OGTT (fasting plasma glucose 100 -125 mg/dl and/or 2-hr glucose between 140 – 199 mg/dl,), or mild untreated diabetes based on OGTT (fasting plasma glucose 126-139mg/dL and/ or 2 hr glucose between 200-249, with a hemoglobin A1c <6.5). We chose these inclusion criteria in order to study subjects who have evidence of impaired glucose regulation, but do not meet criteria for pharmacologic treatment of diabetes.

Exclusion:

1. Serious chronic or acute illness: cancer, clinically significant congestive heart failure, COPD, inflammatory conditions, eGFR<45, active liver disease, poorly controlled hypertension, epilepsy, recent (within 3 months) CVD event (MI, PTCA, CABG, stroke), inflammatory bowel disease; history of bariatric or other gastric surgery, cigarette smoking, serious substance abuse.

2. Treatment with drugs known to influence glucose metabolism (other diabetes medications, systemic glucocorticoids, pharmacologic doses of niacin)
3. Hypersensitivity to acarbose or any component of the formulation
4. Patients taking anti-coagulant medications (e.g., warfarin) or anti-platelet drugs are excluded because of the increased risk of bleeding from muscle and fat biopsies. People taking aspirin for primary prevention who can safely stop the medication for 7 days may be included.

C.1.4. Acarbose dose and formulation: Studies have demonstrated the ability of acarbose to delay or prevent the onset of diabetes in people with impaired glucose intolerance or pre-diabetes. The usual therapeutic dose ranges from 150 mg to 300 mg a day. For this study, we plan to use the maximum tolerated dose of up to 300 mg daily of acarbose. The drug will be introduced at 25mg daily, to be taken with the first bite of a meal. This will be increased to 25mg three times daily, then doubled in 1-2 weeks to 50mg 3 times daily as tolerated, and further doubled in another 1-2 weeks to 100mg 3 times daily as tolerated. Participants may continue to actively participate in the study with a reduced, but tolerable, dose of acarbose (minimum 75 mg daily). Capsules that contain 25mg acarbose or a similar appearing placebo containing lactose will be prepared by the Weiler Hospital research pharmacy.

C.1.5. Duration of treatment: We propose 2x 10-week duration of treatment (4 weeks titration, 6 weeks maximal dose) with a 2-week washout between treatment periods. We have reasoned that this time frame should be sufficient to see effects on gene expression.

C.1.6 Assays: The following assays will be performed in the core laboratories of the Einstein Institute for Clinical and Translational Research: chemistry and liver profiles, HbA1c and urinalysis, glucose, lipids, IGF-1, insulin, hs-CRP, adiponectin. 1mL of serum will be used for microRNA sequencing to be performed in the laboratory of Yousin Suh, PhD. Additional samples will be stored for future analysis of inflammatory markers, endothelial function, glycation end products, urinary F2 isoprostanes, FGF-21 and butyrate, or other markers of interest that may emerge.

C.2

Specific Aim 1: To assess the effect of acarbose on gene expression profile, with a focus on molecular pathways known or suspected to play a role in aging.

We hypothesize that treatment with acarbose will result in changes in the transcriptome in older adults. We will test this by identifying changes in gene expression in muscle and adipose tissue with RNA Sequencing (RNA-Seq). We may also study expression of specific target genes (using RT-PCR).

C.2.1 Detailed Methods

Muscle and adipose samples will be collected at baseline in all participants and at the end of each of the treatment period in older participants, in the fasting state following a 60-minute rest period. These procedures have been performed by our study group in IRB approved studies 2010-398 and 2014-3444.

Muscle Biopsy: A muscle sample of up to 200 mg will be obtained with a spring-loaded biopsy needle (Bard Instruments) in the mid-thigh region (vastus lateralis) following local anesthesia extending into the muscle area. The remainder of the ~100 mg muscle samples will be immediately homogenized in Trizol at the bedside, to inhibit any RNAase activity, frozen in liquid nitrogen and kept at -80°C, for subsequent rt-PCR, mRNA extraction and analysis of gene expression (RNA Seq) [31]

The following assays will be performed on the samples: Skeletal muscle total RNA will be extracted using RNAeasy kit (Qiagen). cDNA will be made using SuperScript III First Strand (Invitrogen) and real time RT-PCR will be run using the LightCycler instrument (Roche). Five housekeeping genes will be used as controls. RNA-Seq assay will be performed at Einstein's Sequencing Core Facility, specifically using multiplexed 100bp single-end sequencing on an Illumina HiSeq2500. (http://www.illumina.com/technology/mrna_seq.ilmn).

Adipose Biopsy: An adipose sample will be obtained from the periumbilical region. A small 0.5 cm cutaneous incision will be performed under local anesthesia and 2-6 grams of adipose tissue (which will vary depending on participant's adiposity) will be obtained by aspiration technique. Whole adipose tissue for analysis by rt-PCR will be immediately homogenized in Trizol (Life Technologies, Bethesda, MD) with a Tissumizer homogenizer. Subsequent mRNA extraction and analysis of gene expression (RNA seq) will be performed at Einstein's Sequencing Core Facility using multiplexed 100bp single-end sequencing on an Illumina HiSeq2500.

Remaining fresh adipose biopsy specimens will be immediately digested with collagenase type 1.

C.2.2 Sub-Aim 1: To assess the relationship between changes in gene expression with selected phenotypic variables and biomarkers, including changes in insulin sensitivity and glucose tolerance, vascular function, oxidative stress, inflammation, gut bacteria and/or serum microRNA profiles.

Detailed Methods

Screening OGTT: A 75-gram OGTT will be conducted in the morning following an overnight fast according to standard methods.

Standard Meal Test Protocol: A standard mixed meal challenge, rather than oral glucose challenge, represents a more physiologically relevant stimulus, in that it may more closely mimic metabolic changes experienced by individuals in daily life. Standard meal tests (SMT) with frequent blood sampling and application of mathematical modeling have been used to evaluate insulin sensitivity and insulin secretion, and have been reported to produce results comparable to more invasive and less physiologic tests.[32, 33] Furthermore, the contribution of the incretin hormones to stimulated insulin secretion is captured, unlike intravenous tests. In our protocol, subjects are studied following an overnight fast and after a test meal of 110g CHO, 20g protein, 20g fat. Blood sampling is performed fasting and 30, 60, 120, and 180 minutes following the meal through an indwelling intravenous catheter and will be used to calculate glucose and insulin AUC. Subjects are

provided with a standard meal to consume at home on the night prior to the next SMT, in order to minimize metabolic variability between tests.

Assessment of insulin sensitivity: Insulin sensitivity will be estimated from insulin and glucose levels obtained following the standard meal challenge, using a modification of the Matsuda index, which has been widely used for non-invasive assessment of insulin sensitivity and shows good correlation (r=0.73) with results obtained from euglycemic hyperinsulinemic clamp studies.[34]

Measurement of markers of inflammation and oxidative stress: Measurement of circulating cytokines and acute phase reactants will be performed both fasting and at 2 hours postprandial. Plasma samples will be aliquoted into Eppendorff tubes and stored at –80°C. Subsequent measurements might include hormones, and inflammatory markers, including plasma leptin, TNF-α, PAI-1, IL-1β, IL-6, VCAM, FGF-21, butyrate, alpha dicarbonyls, adiponectin and other aging biomarkers. Urine samples will be stored (in the presence of an antioxidant, such as BHA) and may be assayed for measurement of urine F2 isoprostanes.

Serum microRNA:
miRNA will be extracted from the serum samples using miRNeasy kit (Qiagen, CA) using the manufacturer's protocol. miRNA libraries will be prepared using NEBNext Multiplex Small RNA Library Prep for Illumina (New England Biolabs). Cel-miR-39 mimic will be added to each sample before extraction, for normalization and sent for miRNA sequencing at Einstein's Sequencing Core Facility using multiplexed single-end sequencing on an Illumina HiSeq2500

Fecal analysis for gut microbiome:
Stool collection and DNA extraction:
Stool samples will be collected at visits 4 and 6. 16s ribosomal DNA sequencing will be performed to assess bacterial species clustering. Participants will be provided with a DNA OMNIgene.Gut OMR200 kit (DNA Genotek, London, Canada) along with a stool collection hat, for self-collection of fecal samples, to be brought in for further treatment and analysis, at baseline visit 2of the study and after 12 weeks of study treatment on visit 4. The kits will be stored at room temperature until fecal microbial DNA extraction (within 30 days). Fecal microbial DNA will be extracted from the samples using PowerFecal DNA Isolation kit (MoBio Labs Inc) following manufacturer's protocol and eluted DNA will be divided into 1cc aliquots and frozen immediately at -80^{0}C for further analysis. The purified DNA samples will be sent for 16s rDNA sequencing to Integrated Microbiome Resource, CGEB, Canada or at the Massachussets Institute of Technology (MIT).

C.2.3 Specific Aim 2: To determine if treatment with acarbose will restore the gene expression profile of older, glucose intolerant men to that of young, healthy subjects. We hypothesize that transcriptional changes detected above may reflect some aspects of the transcription profiles of younger adults and provide insight into specific pathways affected by acarbose.
Under a separate protocol (2016-7110), young individuals aged 20-40 will be recruited and will undergo fat and muscle biopsies for RNA sequencing as detailed above. Changes

seen in the acarbose group in muscle and fat transcriptome will be compared to the young transcriptome, to identify aging-specific pathways affected by acarbose

C.3. Data analysis, rationale, limitations of experimental design:

C.3.1 Study design: We plan to conduct a double-blind placebo controlled trial with cross-over design. The previous studies performed by our group (resveratrol and metformin) were cross-over studies with gene transcription data on 16 and 14 individuals, respectively. We identified clear differences in gene transcription profiles between resveratrol and placebo, using the same criteria outlined above. We expect that acarbose would have a similar if not larger effect size, thus 30 individuals in a crossover design is sufficient to detect differences. Data are still being analyzed for the metformin study. Cross-over study design is advantageous in terms of increased power for the study size. However, there is a risk for carryover effects since metabolic, transcriptional, and gut microbial changes may persist after the medication is cleared from circulation during the 2-week washout. Therefore, we plan to also separately analyze those who received treatment first in a sub-analysis to detect any long lasting changes that may result in a carry-over effect. Changes in physical activity and diet could confound interpretation of our metabolic studies, although the double-blind, placebo controlled study design will help to mitigate any potential impact. Changes in weight and body composition will also be carefully monitored. If there are significant changes in physical activity or weight, we will adjust for these in our data analysis.

C.3.2 Statistical analysis and sample size: We plan to enroll up to 30 older male subjects in a cross-over design (30 data points per treatment). Since this is an exploratory study, data obtained will be used to design future interventions that are larger in size and include women, and/or that more specifically investigate pathways that may be affected by acarbose. Data from this study will also be used to estimate effect size and to allow formal power calculations for future, larger studies. Previous studies of gene transcription have demonstrated that effect sizes on gene transcription can be substantial,[35] and differential expression can be detected in the same tissues, before and after an intervention in as few as 10-15 individuals.[36] Our study of resveratrol confirms the ability to detect differential expression in a sample size of 16, using a crossover design even when clinical effects are modest or negligible. Increasing the sample size to up to 30 will increase the ability to detect transcripts with a lower fold change.

RNA-Seq: Transcriptional profiles will be obtained by the use of the RNA-Seq assay at Einstein's Sequencing Core Facility, specifically using multiplexed 100bp single-end sequencing on an Illumina HiSeq2500. Under a separate protocol, #07-534, we have analyzed transcripts of muscle and adipose tissues obtained from unrelated young (20-30 yo) and we plan to submit another protocol to recruit additional young controls whose transcriptional profiles will further expand our "repository". These analyses will help us further define the parameters of biological transcript changes with aging. These results will provide "proof of concept" and help to set the stage for testing more interventions.

We anticipate ~ 30M reads passing quality control to be aligned to the human genome. The alignment process will be implemented by use of the GSNAP and HTSeq packages –

these specifically implement a splice-junction aware alignment protocol. Pair-wise correlation and principal component analyses on each sample's gene expression profile will be used to determine the quality of the experiment, identify batch effects and other systematics. Differential expression between samples (young and old, and after acarbose and after placebo) will be determined, using a negative binomial model approach such as that implemented in the DESeq package. Genes that show an adjusted p-value of less than 0.05 and a fold change greater than 2 will be selected for further scrutiny between placebo and acarbose conditions. Furthermore, our RNA-seq data will permit us to identify splice variants between the experimental conditions. The differential gene list will be processed with Ingenuity Pathway Analysis and GoStat, allowing us to identify dysregulated pathways and common gene ontology terms differentiating the acarbose transcriptome for the tissue types under scrutiny. Finally, the entire differential expression profile will be processed using the Broad's Gene Set Enrichment Analysis platform against over 10,000 curated gene lists, to provide additional regulatory perspectives on the data. These analyses are considered exploratory in nature and will be used to inform future tests that may target specific gene expression pathways. It will also allow us to make collaborative associations with phenotypes, although these associations will be complex considering the numerous potential age-delaying effects that may occur, some of which may be independent of the measured physiologic parameters.

Insulin sensitivity, biomarkers, etc.: Differences in mean acarbose vs. placebo treatment outcomes (insulin sensitivity, inflammatory biomarkers) will be tested for significance using paired t-test or Wilcoxon test (for non-parametric data). A two-tailed alpha level of 0.05 will denote statistical significance.

miRNA analysis:

The circulating miRNA-seq raw files in the FASTQ format will be subjected to adapted trimming, alignment, normalization, removal of low abundance miRNAs and analysis using the SMiRK pipeline.[37] The trimmed files will be aligned with mature miRNA sequences in the latest version of mirBase database with read counts as the output, which will be normalized by rpm (reads per million) method, between libraries. With auto-elimination of miRNAs with <10 rpm, SMiRK will perform analysis on normalized data by arranging miRNAs as unsupervised hierarchical clusters. A Wilcoxon test will be used to compare miRNA expression levels. Differentially expressed miRNAs ($p<0.05$) will be validated with RT-PCR and Megaplex Pools protocol (Applied Biosystems). miRWalk database will be used to validate target gene expression.

Fecal microbiome 16s analysis:

The raw 16s sequence data will be converted to taxonomic profiles by grouping 16s sequences into Operational Taxonomic Units (OTUs) based on sequence similarity using an previously described protocol[38, 39] as well as by generating de-novo OTU generation using *usearch*, a de novo OTU-picking tool (v6.0.307; http://www.drive5.com/).[40, 41] We will use Basic Local Alignment Search Tool (BLAST) to analyze each OTU's representative sequence against the most recent release of the Greengenes 16S database.[42] The microbial diversity among the treatment and placebo groups will be quantified and compressed into a single scalar statistic as previously described.[43] Relative abundance plots will be

generated in QIIME for visual comparison of microbial abundances at each taxonomic level.

D: Potential risks of study participation

D.1.1. Potential adverse effects of Acarbose: Acarbose has been used extensively to treat diabetes internationally and has an excellent safety profile. The most common side effects are gastrointestinal, including diarrhea, flatulence and abdominal pain that result from the fermentation of unabsorbed carbohydrates and resultant gas production. In general, GI side effects develop within the first few days of therapy, are mild to moderate in severity, and decrease over time. There have been rare postmarketing reports of ileus and pneumatosis cystoides intestinalis associated with alpha-glucosidase inihibitors and use is contraindicated in the setting of colonic ulceration, colonic obstruction and in individuals at high risk for colonic obstruction.

Mild serum transaminase elevations can occur in up to 14% of patients, but elevations >3 x ULN occur in only 3% of patients. These elevations are typically dose related and reversible. There have been rare (62) postmarketing reports of transaminase elevations >500IU/L.(http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/020482s023lbl.pdf) However almost all had hepatic improvement with discontinuation and only 5 cases of fatal fulminant hepatitis have been reported in Japan. Prescribing information advises against use in the setting of hepatic disease, and since quarterly monitoring of liver function tests is recommended, liver function tests will be obtained in a safety visit.

D.1.2: Potential risks of study procedures:

Blood withdrawal: The total amount of blood sampled per 10 week-study will not exceed 450 ml per subject, less than that donated by a blood donor. There is a small risk of hematoma formation or infection due to the intravenous line used for blood sampling at visits 4 and 6. Experienced nursing staff at the CRC will perform the phlebotomy and aseptic technique will be used.

Muscle and adipose tissue biopsy: Although generally well-tolerated, these procedures can be associated with discomfort, bleeding, hematoma formation and, rarely, local infection. A common side effect of the muscle biopsy is muscular cramping in the days following the procedure. A common side effect of the adipose biopsy is ecchymosis in the distribution of the biopsy. Other side effects include scar formation (including keloids) and loss of sensation in the skin at the site of the biosy.Strict aseptic technique will be used and local pressure will be applied to minimize bleeding. Participants will be advised on local wound care and to limit vigorous activities for 48 hours following the biopsies. People who are using aspirin for primary prevention of cardiovascular disease will be asked to not take the medication for 7 days prior to biopsy.

D.1.3 Data and Safety Monitoring Plan: The PI, Dr Brutsaert, co-investigators and study staff will review study progress and any adverse events at bi-monthly staff meetings. All abnormal findings from history and physical examination will be documented in the research chart and reviewed by the PIs or co-investigator. Clinical and laboratory data will be reviewed within 24 hours of receipt and adverse events will be reported according to IRB policy. The investigators will periodically assess and review data collection and storage procedures to maintain confidentiality. The progress of study recruitment will be assessed at monthly intervals in order to assure the feasibility of meeting recruitment projections.

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