IRB #	HSC20130458H

COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: Mechanism of microbiome-induced insulin resistance in humans (Aim 2)

NCT number: NCT 02127125

IRB Approval Date: 03/12/2019

Unique Protocol ID: HSC20130458

FORM C

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RESEARCH DESCRIPTION

If an item does not apply to your research project, indicate that the question is "**not applicable**" – do not leave sections blank

For Sections: **1**. "Purpose and Objectives"; **3**. "Study Design"; and **4**. "Study Population," and **5 – 12**, you may copy and paste the relevant passages from the sponsor's full protocol or grant application (citing the page number and section is **unacceptable**). Section 2, "Background" is the only part of this form where you may cite the relevant passages (page number and section) from the sponsor's full protocol or grant application. This section may be used to also describe local standards of practice or add information pertinent to the local IRB review of a multicenter study.

Click once on the highlighted entry in each box to provide your response. Click the item number/letter or word, if hyperlinked, for detailed instructions for that question. If your response requires inserting a table, picture, etc, you may need to first delete the box that surrounds the answer and then insert your table or other special document.

Title of Project:

Mechanism of microbiome-induced insulin resistance in humans (Aim 2)

1. Purpose and objectives. *List the purpose and objectives:*

Insulin resistance in peripheral tissues (i.e. skeletal muscle) is one of the earliest and most significant abnormalities in the pathogenesis of type 2 diabetes (T2DM). However, the molecular basis for the insulin resistance of T2DM is not fully understood. The human gut hosts an enormous number and variety of microorganisms, including at least 1014 bacteria. An increasing number of animal studies suggest that this microbial "organ", also known as the microbiome, is a causative factor in the insulin resistance of obesity and T2DM, and may represent an entirely new therapeutic target against these conditions. These studies suggest that high fat ingestion, obesity, and T2DM, favor a microbiome profile which enhances production and gastrointestinal permeability of lipopolysaccharide (i.e. metabolic endotoxemia). Lipopolysaccharide (LPS or endotoxin) is a component of the outer membrane of gram negative bacteria cell walls which induces an inflammatory response by activating the cell surface receptor toll-like receptor-4 (TLR4). Despite accumulating evidence from animal studies suggesting that intestinal microbiota and metabolic endotoxemia could play a key role in the pathogenesis of insulin resistance, obesity, and T2DM, the relevance of the intestinal microbiome and metabolic endotoxemia on human metabolic disease remains unknown. Thus, the goal of this study is to determine the role that intestinal microbiota and metabolic endotoxemia play in the pathogenesis of insulin resistance and T2DM in humans.
Aim: To determine whether microbiome modulation and an experimental reduction in plasma LPS concentration improve inflammation and insulin action in insulin resistant (obese and T2DM) subjects. We will test the hypothesis that protecting the intestinal barrier with a synbiotic will restore normal gut permeability, reduce LPS, and ameliorate inflammation (TLR4 signaling) and insulin resistance. Also we will assess the role of LPS independently by reducing plasma LPS concentration with sevelamer.

2. Background.

Describe past experimental and/or clinical findings leading to the formulation of your study.

For research involving investigational drugs, describe the previously conducted animal and human studies.

For research that involves FDA approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.

Attach a copy of the approved labeling as a product package insert or from the Physician's Desk Reference.

You may reference sponsor's full protocol or grant application (page number and section) or if none, ensure background includes references.

a. Background

Refer to pages 1-3 of grant application.

b. Current practice

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Three groups of subjects will be enrolled into this study: lean, obese nondiabetic and obese type 2 diabetic.

The current practice for type 2 diabetic subjects is the following:

- Lifestyle modification with dietary counseling.
- If glycemic control is poor after 6-10 weeks, oral agents are initiated. Metformin is considered the first line agent. If control is poor with metformin, a second line agent (sulfonylurea, glitazone, DPP IV inhibitor, or GLP-1 agonist is indicated).

3. Study Design.
Describe the study design (e.g., single/double blind, parallel, crossover, etc.) Consider inserting a scheme to visually present the study design.

Visit 1: Subjects will undergo a medical history, physical examination, and screening blood tests.

Visit 2: Within 3-14 days eligible subjects will return for OGTT.

Visit 3: Within 3-21 days subjects undergo blood (LPS) and stool collection and an intestinal permeability test.

Visit 4: Within 7-21 days from Visit 3, subjects will return to undergo a whole body DEXA, indirect calorimetry and an insulin clamp. Muscle biopsies will be performed before (-30 min) and at the end (+180 min) of the clamp. Following completion of these studies, subjects will be randomized to receive in a double-blind fashion, placebo (maltodextrin, 6 g three times a day), the synbiotic [5 g of oligofrucose + 1 g Bifidobacterium longum (4x10^10 CFU/g) three times a day], or sevelamer (1.6 g sevelamer + 4.4 g maltodextrin three times a day), for 4 weeks, and discharged to home. While at home, subjects will maintain their habitual (isocaloric) diet and physical activity level.

Visit 5: Approximately on day 24of treatment blood (LPS) and stool are collected and an intestinal permeability test is done. Note: Scheduling window varies – ideally to occur within 1 week prior to Visit 6 clamp and total days of study medication.

Visit 6: Approximately on day 28 (between days 26 to 32), subjects undergo a second DEXA, indirect calorimetry, and insulin clamp with muscle biopsies.

Analyses. Plasma LPS, LBP, soluble CD14, IL-6, TNFα and phosphate will be measured before, during, and after treatment (Visits 3, 4, 5 and 6). Assays of TLR4 and insulin signaling in muscle are performed as described for Aim 1. Stool samples will be collected before and during treatments. This will allow us to evaluate differences in microbiome composition between lean, obese, and T2DM subjects at baseline, and establish the relationship between treatment-induced changes in specific bacteria composition/distribution with changes in inflammation and insulin action.

4. Study Population(s).

You will be drawing subjects from one or more populations. In medical research, for example, a population can be individuals with type 2 diabetes controlled with diet, or a population of healthy individuals. In social behavioral research, a population can be individuals attending an education program, etc.

4.a. How many <u>different populations</u> are you enrolling in this study?	3
4.b. <u>For each different population</u>, provide a short descriptive **label**: *(e.g., normal-healthy, diabetics, parents, children, etc.)* *Copy and paste additional labels as needed* →	1. Lean nondiabetic 2. Obese nondiabetic 3. Obese type 2 diabetic

4.c. <u>For each specific population</u> identified in **4b**, provide the following information in the table provided below.
*(For studies with **more than one** population, copy all of table 4.c. and paste to insert additional tables.)*

Population # 1	**Population Descriptive Label:** Lean nondiabetic
(1)	Identify the criteria for **inclusion**:

1) Both genders between ages 18-65 years, without family history of diabetes.
2) All races and ethnic groups.
3) Premenopausal women in the follicular phase, non-lactating, and with a negative pregnancy test.
4) Postmenopausal women on stable dose of or not exposed to hormone replacement for ≥6 months.
5) HCT≥ 34%, serum creatinine ≤ 1.4 mg/dl, electrolytes, urinalysis, and coagulation tests. LFTs up to 2X.
6) Stable body weight (±2%) for ≥ 3 months.
7) Two or less sessions of strenuous exercise/wk for last 6 months
8) BMI <26 kg/m2
9) Normal glucose tolerance based on ADA criteria

(2) Identify the criteria for **exclusion**:

1) Presence of impaired glucose tolerance or type 2 diabetes based on ADA criteria
2) Family history of diabetes (parents, siblings, children)
3) Current treatment with drugs known to affect glucose and lipid homeostasis. If the subject has been on a stable dose for the past 3 months, the following agents will be permitted: calcium channel blockers, beta-blockers, ACE inhibitors, angiotensin receptor blockers, and statins.
4) History of allergy to sevelamer.
5) Non-steroidal anti-inflammatory drugs or systemic steroid use for more than a week within 3 months.
6) Current treatment with anticoagulants (warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsy in accordance with the primary physician.
7) Use of agents that affect gut flora (e.g. antibiotics, colestyramine, lactulose, PEG) within 3 months.
8) History of heart disease (New York Heart Classification greater than grade II; more than non-specific ST-T wave changes on the ECG), peripheral vascular disease, pulmonary disease, smokers.
9) Poorly controlled blood pressure (systolic BP>170, diastolic BP>95 mmHg).
10) Active inflammatory, autoimmune, hepatic, gastrointestinal, malignant, and psychiatric disease.
11) History of gastrointestinal surgery or gastrointestinal obstruction within two years.

(3) **Recruitment Process – identifying potential subjects**

Describe plans about how the population will be **identified** for the purpose of recruiting. *(e.g., database search, personal contacts, referrals, patients under the care of the research team, etc.)*

(Consider Form J, HIPAA Waiver to access PHI for identification of potential subjects)

All subjects will be recruited through advertisement in local newspapers, radio and the web.

Subjects will be recruited by word of mouth and by ads placed in newspapers, digital ads on newspaper websites and affiliated mobile apps**, radio/TV promo spots, flyers posted on bulletin boards in the Medical School at UTHealth, at STVHCS GEM clinic, UHS-TDI research clinic, private practice offices, and displayed at community events to bring attention to the study. Facebook ads on UTHealth Facebook may also be used with approved Form L for Facebook. **When viewers click on a digital ad, the viewer is taken to a landing page that is an image of the approved Form L Newspaper ad.

Study is listed on FindaStudy website. VA subjects will not be emailed for recruitment purposes first, however, FindAStudy respondents who are veterans providing their email and phone number to request contact may receive a phone call as a result

If recruiting from more than one institution and the identification process differ - clearly describe differences here.

New participants will first contact the researchers. The research team will then make an appointment to explain the study to the participant

(4) **Recruitment Process – first contact**

Describe how **initial contact** will be made with potential subjects

(e.g., researchers will contact potential subjects or subjects will contact the researchers or make appoints to see researchers after learning of the study).

Describe how those making initial contact have access to the subjects' identity and the subjects' information. *(Consider whether a Form J, HIPAA Waiver is needed to disclose PHI.)*

New participants will first contact the researchers. The research team will then make an appointment to explain the study to the participant

Study team may receive queried information from Pepper Center Call Center and study team will reach out to potential candidates (if subject answered YES to future contact question in call center REDCap)

If recruiting from more than one institution and the process of making initial contact differs - clearly describe differences here.

Describe differences or insert "N/A"

Potential subjects will contact us. Respondents will be interviewed by telephone or in person by the PI/study staff after verbal consent is obtained. (attached Form M - phone screen form)

Study staff may opt to mail (USPS) a copy of ICF to potential candidates for review prior to screening appointment. If potential subject is not a veteran, study staff may opt to email a copy of the ICF to the potential candidate for review prior to the initial consenting appointment.

Subjects may be referred by another physician after screen failure to another study and study staff calls subject to schedule screening or may accept previous screening tests obtained within past several months per protocol.

For those who contact the Pepper Center Call Center: Subjects are provided contact information for researchers who are recruiting, potential subject may contact the research team or vice versa.

CITRUS LABS: study may request to purchase records from Citrus Labs for potential participants meeting some of the study pre-screening health criteria such as those found in the phone screen form. Citrus Labs brochure attached. This method will not be used to recruit VA patients.

(5)

Recruitment process – setting

Describe the **setting** in which an individual will be initially approached.

(e.g., private room, inpatient unit, waiting area, group setting, over internet, over phone, in public). Also, describe all interaction between the research staff and the potential subject between the time they contact the research team or vice versa and the time they sign a consent form (including pre-screening activities-see instructions for detailed guidance)

Subjects will receive some general information about the study over the telephone. Interested subjects will come to the Bartter Research Unit at the Audie Murphy VA where the study will be described in detail in a private room. The subjects also will be given the consent form. Ample time will be given to read the consent form and to formulate any questions they might have. Subjects can also take the consent at home for their review.

If recruiting from more than one institution and the setting differs - clearly describe differences here.

Describe differences or insert "N/A"

(6)

Recruitment process - advertisements

Will any advertising be used? ☑ Yes (attach) ☐ No ☐ Pending (will submit an amendment after approval)

If yes, please see Section 4, Form L for instructions on attaching copies of the information to be used in flyers or advertisements. Advertisements must be reviewed and approved by the IRB prior to use.

(7)

Consent Process

Describe the consent/assent **procedures** that will be used by the research team.

- Include how: information is provided; the consent interview is conducted; the consent is signed.
- Identify the study staff who will conduct the consent interview by their roles (e.g., investigator, research nurse).

***** If the consent process of a single subject will involve more than one member of the research team, describe how this process will be coordinated from start to finish.

****** If you expect this population will have individuals likely to have diminished decision-making capacity (not including incompetent or impaired decision making capacity), describe the assessment process for determining whether the individual is capable of giving informed consent (i.e., evaluation criteria, time intervals)

All subjects will be interviewed by Dr. Nicolas Musi or one of the co-investigators during the pre-recruitment interview, who will describe in detail the purpose, nature and potential risks of the study. Each subject is then asked to read the consent form. Subjects will then be asked if they have any questions concerning any aspect of the study. All questions will be answered thoroughly. Subjects may also take the consent to home, and sign the consent during a follow-up interview or on Visit 1 (before initiating the procedures). Immediately prior to the study, subjects are asked to sign the consent form and are given a photocopy to keep for themselves. Consent only will be obtained once the investigator is convinced that the subject verbally demonstrates understanding and agrees to the process. The consent forms will be kept at the Bartter Research Unit of the VAMC under restricted access

(8)

Consent Process – time between initial contact and obtaining consent

Describe the **timing** of obtaining informed consent, whether there is any waiting period between informing the prospective subject and obtaining consent. *(e.g., take consent home, waiting period of X hours, after consulting with family members, etc.)*

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Subjects will consent after the purpose, nature, and potential of the risks of the study are explained during a pre-recruitment interview or immediately before initiating Visit 1. Subjects may also take the consent to home, and sign the consent during a follow-up interview or on Visit 1 (before initiating the procedures).

(9) Describe measures taken to **minimize** the possibility of coercion or undue influence during consent.

After explaining the study, subjects will be allowed to read the consent freely and with sufficient time. Subjects will not be pressured in any way to enter the study. We will explain that the study is voluntary and that if they decide not to participate (or exit the study) they (and their family members) will not be punished in any way, and this will not affect their eligibility to enter future studies or to seek medical treatment at the Audie L. Murphy VAMC or UTHSCSA.

(10) Will subjects from this population be assigned to different research groups? *(e.g., treatment and control group)*

☑ Yes ☐ No

If yes, list the groups by inserting a short descriptive title for each group.

E.g., experimental group A, B, etc., control group, etc. - these labels are needed for the Risk: Benefit Analysis section

Control: This group will receive placebo

Experimental/Synbiotic: This group will receive a symbiotic (Bifidobacterium longum plus oligofructose)

Experimental/Sevelamer: This group will receive sevelamer

4.c. For each specific population identified in **4b**, provide the following information in the table provided below.

*(For studies with **more than one** population, copy all of table 4.c. and paste to insert additional tables.)*

Population # 2	**Population Descriptive Label:** Obese nondiabetic

(1) Identify the criteria for **inclusion**:

1) Both genders between ages 18-65 years old, with or without family history of diabetes..
2) All races and ethnic groups.
3) BMI 30-37 kg/m2. Stable body weight (±2%) for ≥ 3 months.
4) Normal glucose tolerance based on ADA criteria.
5) Two or less sessions of strenuous exercise/wk for last 6 months
6) Premenopausal women in the follicular phase, non-lactating, and with a negative pregnancy test. Postmenopausal women on stable dose of or not exposed to hormone replacement for ≥6 months.
7) HCT≥ 34%, serum creatinine ≤ 1.4 mg/dl, electrolytes, urinalysis, and coagulation tests. LFTs up to 2X.

(2) Identify the criteria for **exclusion**:

1) Presence of diabetes or impaired glucose tolerance based on ADA criteria and OGTT.
2) BMI <30 or >37 kg/m2
3) Current treatment with drugs known to affect glucose and lipid homeostasis. If the subject has been on a stable dose for the past 3 months, the following agents will be permitted: calcium channel blockers, beta-blockers, ACE inhibitors, angiotensin receptor blockers, and statins.
4) History of allergy to sevelamer.
5) Non-steroidal anti-inflammatory drugs or systemic steroid use for more than a week within 3 months.
6) Current treatment with anticoagulants (warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsy in accordance with the primary physician.
7) Use of agents that affect gut flora (e.g. antibiotics, colestyramine, lactulose, PEG) within 3 months.
8) History of heart disease (New York Heart Classification greater than grade II; more than non-specific ST-T wave changes on the ECG), peripheral vascular disease, pulmonary disease, smokers.
9) Poorly controlled blood pressure (systolic BP>170, diastolic BP>95 mmHg).
10) Active inflammatory, autoimmune, hepatic, gastrointestinal, malignant, and psychiatric disease.
11) History of gastrointestinal surgery or gastrointestinal obstruction within two years.

(3) **Recruitment Process – identifying potential subjects**

Describe plans about how the population will be **identified** for the purpose of recruiting. *(e.g., database search, personal contacts, referrals, patients under the care of the research team, etc.)*

(Consider Form J, HIPAA Waiver to access PHI for identification of potential subjects)

Subjects will be recruited through advertisement in local newspapers and the web.

If recruiting from more than one institution and the identification process differ - clearly describe differences here.
New participants will first contact the researchers. The research team will then make an appointment to explain the study to the participant

(4)

Recruitment Process – first contact
Describe how **initial contact** will be made with potential subjects
(e.g., researchers will contact potential subjects or subjects will contact the researchers or make appoints to see researchers after learning of the study).
Describe how those making initial contact have access to the subjects' identity and the subjects' information. *(Consider whether a Form J, HIPAA Waiver is needed to disclose PHI.)*

New participants will first contact the researchers. The research team will then make an appointment to explain the study to the participant

If recruiting from more than one institution and the process of making initial contact differs - clearly describe differences here.
Describe differences or insert "N/A"

(5)

Recruitment process – setting
Describe the **setting** in which an individual will be initially approached.
(e.g., private room, inpatient unit, waiting area, group setting, over internet, over phone, in public). Also, describe all interaction between the research staff and the potential subject between the time they contact the research team or vice versa and the time they sign a consent form (including pre-screening activities-see instructions for detailed guidance)

Subjects will receive some general information about the study over the telephone. Interested subjects will come to the Bartter Research Unit at the Audie Murphy VA where the study will be described in detail in a private room. The subjects also will be given the consent form. Ample time will be given to read the consent form and to formulate any questions they might have. Subjects can also take the consent at home for their review.

If recruiting from more than one institution and the setting differs - clearly describe differences here.
Describe differences or insert "N/A"

(6)

Recruitment process - advertisements
Will any advertising be used? ☐ Yes (attach) ☐ No ☑ Pending (will submit an amendment after approval)

If yes, please see Section 4, Form L for instructions on attaching copies of the information to be used in flyers or advertisements. Advertisements must be reviewed and approved by the IRB prior to use.

(7)

Consent Process
Describe the consent/assent **procedures** that will be used by the research team.

- Include how: information is provided; the consent interview is conducted; the consent is signed.
- Identify the study staff who will conduct the consent interview by their roles (e.g., investigator, research nurse).

* If the consent process of a single subject will involve more than one member of the research team, describe how this process will be coordinated from start to finish.

** If you expect this population will have individuals likely to have diminished decision-making capacity (not including incompetent or impaired decision making capacity), describe the assessment process for determining whether the individual is capable of giving informed consent (i.e., evaluation criteria, time intervals)

All subjects will be interviewed by Dr. Nicolas Musi or one of the co-investigators during the pre-recruitment interview, who will describe in detail the purpose, nature and potential risks of the study. Each subject is then asked to read the consent form. Subjects will then be asked if they have any questions concerning any aspect of the study. All questions will be answered thoroughly. Subjects may also take the consent to home, and sign the consent during a follow-up interview or on Visit 1 (before initiating the procedures). Immediately prior to the study, subjects are asked to sign the consent form and are given a photocopy to keep for themselves. Consent only will be obtained once the investigator is convinced that the subject verbally demonstrates understanding and agrees to the process. The consent forms will be kept at the Bartter Research Unit of the VAMC under restricted access

(8)

Consent Process – time between initial contact and obtaining consent
Describe the **timing** of obtaining informed consent, whether there is any waiting period between informing the prospective subject and obtaining consent. *(e.g., take consent home, waiting period of X hours, after consulting with family members, etc.)*

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Subjects will consent after the purpose, nature, and potential of the risks of the study are explained during a pre-recruitment interview or immediately before initiating Visit 1. Subjects may also take the consent to home, and sign the consent during a follow-up interview or on Visit 1 (before initiating the procedures).

(9) Describe measures taken to **minimize** the possibility of coercion or undue influence during consent.

After explaining the study, subjects will be allowed to read the consent freely and with sufficient time. Subjects will not be pressured in any way to enter the study. We will explain that the study is voluntary and that if they decide not to participate (or exit the study) they (and their family members) will not be punished in any way, and this will not affect their eligibility to enter future studies or to seek medical treatment at the Audie L. Murphy VAMC or UTHSCSA.

(10) Will subjects from this population be assigned to different research groups? *(e.g., treatment and control group)*

☑ Yes ☐ No

If yes, list the groups by inserting a short descriptive title for each group.

E.g., experimental group A, B, etc., control group, etc. - these labels are needed for the Risk: Benefit Analysis section

Control: This group will receive placebo

Experimental/Synbiotic: This group will receive a synbiotic (Bifidobacterium longum plus oligofructose)

Experimental/Sevelamer: This group will receive sevelamer

4.c. For each specific population identified in **4b**, provide the following information in the table provided below.

*(For studies with **more than one** population, copy all of table 4.c. and paste to insert additional tables.)*

Population # 3	**Population Descriptive Label:** Obese – type 2 diabetic

(1) Identify the criteria for **inclusion**:

1) Both genders between ages 18-65 years old, with or without family history of diabetes.
2) All races and ethnic groups.
3) BMI 30-37 kg/m2. Stable body weight (±2%) for ≥ 3 months.
4) T2DM with HbA1C ≤ 8.5%
5) Two or less sessions of strenuous exercise/wk for last 6 months
6) Premenopausal women in the follicular phase, non-lactating, and with a negative pregnancy test. Postmenopausal women on stable dose of or not exposed to hormone replacement for ≥6 months.
7) HCT≥ 34%, serum creatinine ≤ 1.4 mg/dl, with normal electrolytes, urinalysis, and coagulation tests. LFTs up to 2X.
8) Based on OGTT (Visit 2), ADA criteria of Type 2 diabetes

(2) Identify the criteria for **exclusion**:

1) Current treatment with drugs known to affect glucose and lipid homeostasis (sulfonylureas only are permitted). If the subject has been on a stable dose for the past 3 months, the following agents will be permitted: calcium channel blockers, beta-blockers, ACE inhibitors, angiotensin receptor blockers, and statins.
2) History of allergy to sevelamer.
3) BMI <30 or >37 kg/m2
4) Non-steroidal anti-inflammatory drugs or systemic steroid use for more than a week within 3 months.
5) Current treatment with anticoagulants (warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsy in accordance with the primary physician.
6) Use of agents that affect gut flora (e.g. antibiotics, colestyramine, lactulose, PEG) within 3 months.
7) History of heart disease (New York Heart Classification greater than grade II; more than non-specific ST-T wave changes on the ECG), peripheral vascular disease, pulmonary disease, smokers.
8) Poorly controlled blood pressure (systolic BP>170, diastolic BP>95 mmHg).
9) Active inflammatory, autoimmune, hepatic, gastrointestinal, malignant, and psychiatric disease.
10) History of gastrointestinal surgery or gastrointestinal obstruction within two years.

(3) **Recruitment Process – identifying potential subjects**

Describe plans about how the population will be **identified** for the purpose of recruiting. *(e.g., database search, personal contacts, referrals, patients under the care of the research team, etc.)*

(Consider Form J, HIPAA Waiver to access PHI for identification of potential subjects)

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Subjects will be recruited through advertisement in local in local newspapers and the web, and through the General Medicine Clinics of the University Center for Community Health and the Audie L. Murphy VA Hospital. Patients who present to the clinic or the VA will have their chart reviewed to determine if the hey meet inclusion criteria. A HIPAA Waiver and a Waiver of Consent will be requested for this activity.

If recruiting from more than one institution and the identification process differ - clearly describe differences here.

New participants will first contact the researchers. The research team will then make an appointment to explain the study to the participant

(4)

Recruitment Process – first contact

Describe how **initial contact** will be made with potential subjects

(e.g., researchers will contact potential subjects or subjects will contact the researchers or make appoints to see researchers after learning of the study).

Describe how those making initial contact have access to the subjects' identity and the subjects' information. *(Consider whether a Form J. HIPAA Waiver is needed to disclose PHI.)*

New participants will first contact the researchers. The research team will then make an appointment to explain the study to the participant. For those who are identified to meet eligibility through records review will be approached during their normal clinic visit. They will be informed of the research and asked if they would be interested in learning more.

If recruiting from more than one institution and the process of making initial contact differs - clearly describe differences here.

Describe differences or insert "N/A"

(5)

Recruitment process – setting

Describe the **setting** in which an individual will be initially approached.

(e.g., private room, inpatient unit, waiting area, group setting, over internet, over phone, in public). Also, describe all interaction between the research staff and the potential subject between the time they contact the research team or vice versa and the time they sign a consent form (including pre-screening activities-see instructions for detailed guidance)

Subjects will receive some general information about the study over the telephone. Interested subjects will come to the Bartter Research Unit at the Audie Murphy VA where the study will be described in detail in a private room. The subjects also will be given the consent form. Ample time will be given to read the consent form and to formulate any questions they might have. Subjects can also take the consent at home for their review.

If recruiting from more than one institution and the setting differs - clearly describe differences here.

Describe differences or insert "N/A"

(6)

Recruitment process - advertisements

Will any advertising be used? ☐ Yes (attach) ☐ No ☑ Pending (will submit an amendment after approval)

If yes, please see Section 4, Form L for instructions on attaching copies of the information to be used in flyers or advertisements. Advertisements must be reviewed and approved by the IRB prior to use.

(7)

Consent Process

Describe the consent/assent **procedures** that will be used by the research team.

- Include how: information is provided; the consent interview is conducted; the consent is signed.
- Identify the study staff who will conduct the consent interview by their roles (e.g., investigator, research nurse).

***** If the consent process of a single subject will involve more than one member of the research team, describe how this process will be coordinated from start to finish.

****** If you expect this population will have individuals likely to have diminished decision-making capacity (not including incompetent or impaired decision making capacity), describe the assessment process for determining whether the individual is capable of giving informed consent (i.e., evaluation criteria, time intervals)

All subjects will be interviewed by Dr. Nicolas Musi or one of the co-investigators during the pre-recruitment interview, who will describe in detail the purpose, nature and potential risks of the study. Each subject is then asked to read the consent form. Subjects will then be asked if they have any questions concerning any aspect of the study. All questions will be answered thoroughly. Subjects may also take the consent to home, and sign the consent during a follow-up interview or on Visit 1 (before initiating the procedures). Immediately prior to the study, subjects are asked to sign the consent form and are given a photocopy to keep for themselves. Consent only will be obtained once the investigator is convinced that the subject verbally demonstrates understanding and agrees to the process. The consent forms will be kept at the Bartter Research Unit of the VAMC under restricted access

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(8)	**Consent Process – time between initial contact and obtaining consent** Describe the **timing** of obtaining informed consent, whether there is any waiting period between informing the prospective subject and obtaining consent. *(e.g., take consent home, waiting period of X hours, after consulting with family members, etc.)*
Subjects will consent after the purpose, nature, and potential of the risks of the study are explained during a pre-recruitment interview or immediately before initiating Visit 1. Subjects may also take the consent to home, and sign the consent during a follow-up interview or on Visit 1 (before initiating the procedures).	
(9)	Describe measures taken to **minimize** the possibility of coercion or undue influence during consent.
After explaining the study, subjects will be allowed to read the consent freely and with sufficient time. Subjects will not be pressured in any way to enter the study. We will explain that the study is voluntary and that if they decide not to participate (or exit the study) they (and their family members) will not be punished in any way, and this will not affect their eligibility to enter future studies or to seek medical treatment at the Audie L. Murphy VAMC or UTHSCSA.	
(10)	Will subjects from this population be assigned to different research groups? *(e.g., treatment and control group)*
☑ Yes ☐ No	
If yes, list the groups by inserting a short descriptive title for each group. *E.g., experimental group A, B, etc., control group, etc.* - these labels are needed for the Risk: Benefit Analysis section Control: This group will receive placebo Experimental/Synbiotic: This group will receive a symbiotic (Bifidobacterium longum plus oligofructose) Experimental/Sevelamer: This group will receive sevelamer	

5. Informed Consent for Research Involving Non-English Speaking Subjects – *choose either A, B or C*	
A. ☐	**N/A**. The primary investigator for this study will request a waiver of consent for all subjects in this study. (go to #6)
☐	**N/A** This study does not involve interaction with living individuals; *(limited to use of identifiable information)*. (go to #6)

OR

B.	☑	**Only individuals who speak English will be enrolled**. *(if checked select one of the two statements below)*
	☑	There is **no** expected direct benefit for those participating. (go to #6)
	☐	There is an expected direct benefit for those participating. Excluding non-English speaking individuals is acceptable because: *(insert the rationale for excluding this population below* -- then go to #6)
		[Insert rationale here]

OR

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C. ☐ **Individuals who do not speak English will be enrolled**.
The translated consent will be submitted to the IRB:
Select one Form B, item 12 should be checked

☐ Immediately following approval of the English consent.
(go to c(1) and c(2) below)

☐ Only after a potential non-English speaking participant is identified. Since this plan will delay enrollment pending IRB approval of a translated consent, provide justification that prospective non-English speaking subjects will not be excluded from beneficial research.
Choose one of the choices below:

☐ There is **no** expected direct benefit for those participating. (go to c(1) and c(2) below)

☐ There is an expected direct benefit for those participating.
Provide justification why the delay is acceptable below, then (go to c(1) and c(2)below)

Insert the reason a delay is acceptable here

C (1) If you are recruiting non-English speaking subjects, Describe the process for obtaining informed consent from prospective subjects in their respective language (or the legally authorized representative's respective language).

Describe here

C (2) In order to ensure that individuals are appropriately informed about the study when English is their second-language, describe a plan for evaluating the level of English comprehension, and the threshold for providing a translation, or explain why an evaluation would not be necessary.

Describe here

6. Research Plan / Description of the Research Methods:

6.a. Provide a **comprehensive narrative** describing the **research methods**.
Provide the **order in which tests/procedures will be performed**,
the **setting** for these events and a description of the **methods used to protect privacy** during the study.
Provide the **plan for data analysis** (include as applicable the **sample size calculation)**

Visit 1: Subjects will come to the BRU at 7 AM for a medical history, physical examination (including anthropometric measurements), screening tests (CBC, chemistry, lipid profile, HbA1c, PT, PTT, UA, pregnancy test if applicable), and ECG.

Visit 2: Within 3-14 days from Visit 1, eligible subjects will return at 7 AM after an overnight fast for an oral glucose tolerance test (OGTT).

Visit 3: Within 3-21 days from Visit 2, subjects will come to the BRU at 7 AM in the fasting state to undergo stool sample collection, plasma LPS, LBP measurement, inflammatory markers and phosphate (see Analyses page 11), and an 5h intestinal permeability (lactulose/mannitol) test. Serum phosphorus also will be measured.

Visit 4: Within 7-21 days from Visit 3, subjects will undergo whole body DEXA, a euglycemic hyperinsulinemic clamp and indirect calorimetry as described (44, 45). A pregnancy test is performed if applicable. DEXA is performed to examine the effect of the interventions on body composition. At time -30 min a biopsy of the vastus lateralis muscle will be performed. At time 0 min, insulin will be infused at 60 mU/m2.min for 180 min. A 2nd biopsy is performed on the contralateral leg at 180 min. Labs for analyses as in Visit 3 are repeated.

Pharmacological intervention: Following completion of Visit 4, subjects will be randomized to receive, in a double-blind fashion, placebo (maltodextrin, 6 g three times a day), the synbiotic [5 g of oligofrucose + 1 g Bifidobacterium longum (4x10^10 CFU/g) three times a day], or sevelamer (1.6 g sevelamer + 4.4 g maltodextrin three times a day), for 4 weeks, and discharged home. Sevelamer is obtained from Amgen (Renvela, Cambridge, MA), oligofructose from Orafti (Tienen, Belgium), Bifidobacterium from Danisco (Copenhagen, Denmark) and maltodextrin from American International Foods (Grand Rapids, MI). Randomization and drug preparation/dispensing is performed by the Research Pharmacist of the BRU. For each dose the powder will be included in identical packets. The subjects from the 3 groups (placebo, synbiotic, sevelamer) will ingest the powder contained in one packet with water 3 times /day. Medication willb e given for up to 32 days. Appointment time frame for study medication will be 26-32 days to allow for patient scheduling and weekends.
Before discharge, subjects are given printed instructions and information about potential side effects.

Visit 5: Approximately on day 24 of treatment, subjects will come to the BRU at 7 AM in the fasting state to undergo stool sample collection, and an 5h intestinal permeability (lactulose/mannitol) testing. Labs for analyses as in Visit 3 are repeated. Note: Scheduling window varies – ideally to occur within 1 week prior to Visit 6 clamp and total days of study medication.

Visit 6: Approximately on day 28 (between days 26 and 32) of treatment, subjects will come to the BRU at 7 AM to undergo DEXA, insulin clamp, indirect calorimetry, and biopsies of the muscle (2) as described above for Visit 4. A pregnancy test is performed if applicable. Labs as in Visit 3 are repeated (see Analyses below).

Analyses. Plasma LPS, LBP, soluble CD14, IL-6, TNFα and phosphate will be measured before, during, and afer treatment (Visits 3, 4, 5, and 6). Assays of TLR4 and insulin signaling in muscle are performed as described for Aim 1. Stool samples (Visits 3 and 5) will be collected before and during treatments. This will allow us to evaluate differences in microbiome composition between lean, obese, and T2DM subjects at baseline, and establish the relationship between treatment-induced changes in specific bacteria composition/distribution with changes in inflammation and insulin action.

Statistical analysis. Experimental results will be expressed as means ± SE. Comparisons of means between all the groups will be done by ANOVA for repeated measures (67). Associations between measures of LPS concentration vs.TLR4 signaling and vs. insulin signaling/sensitivity, within a group, will be determined by Pearson's correlation. For tests of correlation coefficients between groups we will use the Fisher's Z transformation (64). We will also determine the relationship between plasma LPS concentration vs. TLR4 signaling and LPS vs. insulin signaling/sensitivity, by using multiple regression analysis. Scatter plots will be done to look for outliers and to verify linearity. Prof. Alex McMahan is an experienced biostatistician who will direct the statistical analysis of this study. Based on our preliminary results and data from the literature (36, 65) regarding the variability of these measurements, we calculated that an n=12 subjects/group is required to obtain power (1-β) of 0.80 with a 2-sided test at α= 0.05 significance level. Assuming a ~20% dropout rate, 15 subjects/group would be sufficient to achieve the required group sizes. The following data and power analysis (2-tailed) were obtained from an ongoing human study (PI: Musi).

Variable	Mean±SD Lean	N	Mean±SD Obese	n	Power
LPS Concentration	0.049±0.055	12	0.119±0.014	12	0.99
Insulin Sensitivity (M)	11.3±2.8	12	8.1±2.4	12	0.91
Ins Stim IRS1-P-Y	4.0±1.1	12	0.9±0.6	12	0.99
Variable	**Mean±SD Lean**	**N**	**Mean±SD T2DM**	**n**	**Power**
LPS Concentration	0.049±0.055	12	0.130±0.071	12	0.99
Insulin Sensitivity (M)	11.3±2.8	12	4.2±1.7	12	0.99
Ins Stim IRS-1 P-Y	4.0±1.1	12	1.5 ±1.0	12	0.99

For the following variables, data were obtained from published human studies (36, 40, 65).

Variable	Mean±SD Pre-Sevel	N	Mean±SD Post-Sevel	n	Power
LPS Concentration	3.6±3.0	12	1.2±2.3	12	0.99
CRP Concentration	4.8±2.4	12	0.44±0.24	12	0.99
Variable	**Mean±SD Placebo**	**N**	**Mean±SD Synbiotic**	**n**	**Power**
LPS Concentration	87±14	12	54±10	12	0.99

6.b. List of the study intervention(s) being tested or evaluated under this protocol

☐ **N/A -** this study does not test or evaluate an intervention. Skip to item 6.d.

#	Study intervention(s) being tested or evaluated under the protocol *Add or delete rows as needed*	VA Place a check if the intervention will be performed at the VA	Local Standard Practice Indicate whether the intervention is considered acceptable practice locally
1	Sevelamer carbonate	☑	☐
2	Synbiotic (Bifidobacterium longum and oligofructose)	☑	☐

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6.c. Risk:Benefit Analysis of study interventions being tested or evaluated under this protocol

For each study intervention identified in section 6b above, complete a risk:benefit analysis table.

(Two tables are provided, copy & paste additional tables as needed or delete both tables if this study does not test an intervention)

6.c.
Study Intervention #1
Sevelamer carbonate

List each group exposed to this intervention on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this intervention. (Benefits can be directly from the intervention or from a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
Experimental Sevelamer	In obese nondiabetic and obese type 2 diabetic subjects, sevelamer could decrease plasma cholesterol level and improve insulin sensitivity. In obese type 2 diabetic subjects, sevelamer also could improve plasma glucose level.

For this intervention, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)
Do not delete frequency. The need to know what to report promptly later = a requirement to estimate frequency (Instructions).

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 out of 100 subjects.	• Nausea • Vomiting	•
	Not serious	**Serious**
Less likely These risks are expected to occur in 5-20 subjects or less out of 100 subjects.	• Diarrhea • Dyspepsia • Abdominal Pain • Flatulence • Constipation	
		Serious
Rare These risks are expected to occur in less than 5 subjects out of 100		-Fecal impaction, bowel obstruction or bowel perforation: Sevelamer is a very safe drug; its main side effects are gastrointestinal. In a large trial, 1053 subjects with chronic kidney disease received sevelamer (Suki et al Kidney Int. 2007 Nov;72(9):1130-7). Three related serious adverse events occurred in three subjects: constipation, vomiting, and osteoporosis. The subject diagnosed with osteoporosis was a 72-year-old woman who had been treated intermittently for asthma with both oral and inhaled steroids. Subjects with active gastrointestinal disease, or history of gastrointestinal obstruction and/or surgery within the last 2 years, will be excluded. -Hypophosphatemia. Sevelamer does not cause hypophosphatemia in subjects with normal kidney function. As an extra precaution, we will only enroll subjects with normal phosphorus plasma concentration, which will be monitored during treatment.

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6.c.
Study Intervention #2
Synbiotic (Bifidobacterium longum and oligofructose)

List each group exposed to this intervention on a separate line. (e.g., experimental, control, Arm A, Arm B, etc **Or** state All Groups/Subjects	For each group, list the **benefits** of this intervention. (Benefits can be directly from the intervention or from a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
Experimental/ Synbiotic	In obese nondiabetic and obese type 2 diabetic subjects, the synbiotic could decrease plasma cholesterol level and improve insulin sensitivity. In obese type 2 diabetic subjects, the synbiotic also could improve plasma glucose level.

For this intervention, **list the reasonably foreseeable risks**
List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)
Do not delete frequency. The need to know what to report promptly later = a requirement to estimate frequency (Instructions).

	Not serious	**Serious**
Likely These risks are expected to occur in more than 20 out of 100 subjects.	•	•
	Not serious	**Serious**
Less likely These risks are expected to occur in 5-20 subjects or less out of 100 subjects.	• Bloating	•
		Serious
Rare These risks are expected to occur in less than 5 subjects out of 100		•

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6.d. List of ALL other research procedures or components not listed in table 6.b.
All of the research procedures for this study should be listed in either table 6.b. or 6.d.

Consider grouping similar procedures under a single component. E.g., blood work, CT = safety assessments)

(Click here for example)

#	Research component • **individual procedures** *example:* **Eligibility Assessments** • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	Column A **Local Standard Practice** Indicate the number of times each procedure will be performed as stipulated in the research plan **that would be done as part of standard practice.**	Column B **Research Only** Indicate the number of times each procedure will be performed solely for research purposes *(any performed outside frequency or timing for acceptable local practice)*	Column C Place a check if the procedure will be performed at the VA	Column D **Risks** List the reasonably expected risks under the following categories as appropriate: • Serious and likely; • Serious and less likely; • Serious and rare; • Not serious and likely; • Not serious and less likely
1	**Eligibility**				
	Consent	0	1	√	None
	Medical History, Inclusion/Exclusion Review	0	1	√	None
	Physical Exam/assessment	1	0	√	None
	Screening blood tests (chemistry, CBC, lipid profile, A1c, PT,PTT)	0	1	√	Not serious and likely: momentary pain and discomfort during venipuncture bleeding, bruising and infection
	Screening urine analysis	0	1	√	None
	75 gm oral glucose tolerance test including venipuncture	0	1	√	Not serious and likely: Pain: momentary pain and discomfort during venipuncture. bleeding, bruising and infection Blood loss: 50 ml
2	**Safety Assessment**				
	ECG	0	1	√	None
3	**Research Assessment**				
	Pharmacological intervention (placebo, symbiotic, or Sevelamer)	0	2	√	See section 6c
	Blood LPS	0	4	√	Not serious and likely: momentary pain and discomfort during venipuncture bleeding, bruising and infection
	Stool sample	0	4	√	None
	Intestinal permeability test	0	2	√	None
	DEXA	0	2	√	Not serious and likely: Subjects will be exposed to a very small amount of radiation during the DEXA exam (0.1 mrem/DEXA). Rare and serious: cancer
	Euglycemic insulin clamp	0	2	√	Not serious and likely: Pain: momentary pain and discomfort during venipuncture. bleeding, bruising and infection Rare and serious: cancer Radiation.2 Clamps: Tritiated glucose is given during the clamp. The radiation exposure is minimal (19.7 mRem/clamp) which is well within guidelines. Blood loss: 100 ml

					Not serious and less likely: Hypoglycemia is possible; however, since the glucose infusion is designed to counterbalance the metabolic effects of insulin, hypoglycemia will not occur. No other side effects of insulin and glucose are known. Plasma glucose concentration will be determined at 5 minute intervals throughout the period of insulin/glucose administration. The 20% dextrose infusion will be adjusted in order to maintain euglycemia (100 mg/dl) and to avoid hypoglycemia.
	Direct calorimetry (ventilated hood) during insulin clamp	0	4	√	Not serious and less likely: Claustrophobia
	Hot box during insulin clamp		2		Serious and rare: burns and blisters
	Muscle biopsy (thigh)	0	4	√	Not serious and likely: Pain: At the time of biopsy, subjects may feel mild pain, discomfort, or pressure (variably described by different subjects) for about 5-10 seconds. Pain or discomfort ceases as soon as the biopsy needle is withdrawn. Local hematomas: Local hematomas occur rarely and they resolve spontaneously within 2 weeks. Less Likely and Serious: Allergy: Allergic reactions to the local anesthetic (lidocaine) are extremely rare, but could include dermatitis, swelling, or hives. None of our patients have experienced any serious allergic reaction to the local anesthetic. Serious and rare: Bleeding from a muscle biopsy may be severe enough to require hospitalization. This has occurred in less than 1 in 500 of these pro Numbness Very rarely some subjects may experience numbness or tingling at the biopsy site. This usually is temporary and goes away in a few days. There is the possibility, less than 1 in 1000, that nerve damage could be permanent. cedures. Infection: There is a small risk, less than 1 in 100, of infection at the site of the muscle biopsy. Symptoms of an infection would include pain, redness, swelling, and yellow-greenish (pus-looking) discharge in the biopsy site, and is usually accompanied by fever. Because 4 biopsies of the muscle will be performed, the risk of infection and pain in the leg may increase. Infections can be usually treated effectively with antibiotics taken by mouth. In very rare occasions, hospitalization is required to give antibiotics through the vein, and an operation could be needed to clean the infected area. Permanent nerve damage: Permanent nerve damage manifested as localized numbness or decreased sensation is possible, but very rare.

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7. Safety Precautions. *(Describe safeguards to address the serious risks listed above.)*

a. Describe the procedures for protecting against or minimizing any potential risks for each of the more than minimal risk research procedures listed above.

Risks will be minimized by a careful screening process including medical history, physical exam, and review of complete blood count, coagulation tests, chemistry and electrocardiogram. The presence of experienced personnel, and of at least one physician, at all times is also critical to reducing risks.

Insulin Clamp: Plasma glucose concentration will be determined at 5 minute intervals throughout the period of insulin/glucose administration. The 20% dextrose infusion will be adjusted in order to maintain euglycemia (100 mg/dl) and to avoid hypoglycemia.

Muscle biopsies: To avoid local hematoma formation, pressure is applied for 30 min after the biopsy. To avoid infection, the procedure is performed under sterile conditions. Our group has experience with over 1000 biopsies.

Blood loss: The total amount of blood drawn will be less than 500 ml. The subjects will be told that they should not donate blood for two months after the study. Any subject with a hematocrit of less than 34% will not be studied.

b. Where appropriate, discuss provisions for ensuring necessary medical or professional intervention in the event of adverse events, or unanticipated problems involving subjects.

Any expected or unexpected adverse events will be assessed by Dr. Musi or a co-investigator. Any necessary immediate medical care will be arranged in conjunction with the subject's wishes.

c. Will the safeguards be different between/among groups?

☐ Yes ☒ No

If yes, describe here

8. Confidentiality of the Research Information

a. Specify **where** the data and/or specimens will be stored and **how** the researcher will **protect the confidentiality** of subject information. *If you will be collecting Social Security Numbers (SSNs), Scrambled Social Security Numbers, or the last four digits of a Social Security Number, provide details regarding the **security measures** to protect the SSNs.*

Data will be coded with a series of numbers unrelated to the SSN. The key to the link will be stored in a separate location. All data will stored in password-protected VA computer at the Bartter Research Unit under the VA server. Electronic files will be stored on STX_Research\PI_MusiN study folders. Paper research records will be stored in locked cabinet in a locked office on BRU until completion then moved to GRECC A300 for data analysis and permanent storage.

The Specimens will be stored in a restricted access area at the Bartter Research Unit laboratory

b. Will all electronic data be stored in accordance with the institution's information security policy and encryption standards?

☒ Yes ☐ No, if no explain below

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9. Payment. (payment of subjects should be included in the consent form)

a. Describe the incentives (e.g., inducements) being offered to subjects for their time during participation in the research study.

The subjects will receive monetary compensation to cover their time and transportation costs

b. If monetary compensation is offered, indicate how much the subjects will be paid and describe the terms and schedule of payment.

IRB policy requires a provision for providing partial payment to subjects who withdraw before the completion of the research. For *VA research, payment to human subjects participating in research is prohibited when the research is integrated with the patient's medical care and when the research makes no special demands on the patient beyond those of standard medical care. Payment may be permitted, with IRB approval, under certain circumstances. Consult with the VA R&D Office to discuss payment of subjects*.

Subjects will receive :

- $50 after Visit 2 (glucose tolerance test).
- $50 after Visit 3 (intestinal permeability test).
- $300 after Visit 4 (insulin clamp and muscle biopsy).
- $50 after Visit 5 (intestinal permeability test).
- $300 after Visit 6 (insulin clamp and muscle biopsy).

On rare occasions we may agree to reimburse you for unexpected or unusual expenses related to travel or other circumstances related to study participation, but this may not exceed $100. This will be done on an individual basis only and will be subject to approval by the Principal Investigator Dr. Musi.

10. Costs to Subjects. (costs to the subject should be included in the consent form)

a. Describe any costs for care associated with research (including a breakdown of standard of care procedures versus research procedures), costs of test drugs or devices, and research procedure costs that are the subject's responsibility as a consequence of participating in the research.

There will be no cost to study volunteers. Some veterans are required to pay co-payments for medical care and services provided by the VA. These co-payment requirements will continue to apply to medical care and services provided by the VA that are *not* part of this study. There will be no cost to veterans or non-veterans for procedures and drugs that are part of this research study.

b. Describe any offer for reimbursement of costs by the sponsor for research related injury care. (Attach a copy of the section of the clinical trial agreement or contract describing research related injury care – the information in this section must match the injury section of the consent form).

In the event that you sustain an injury or illness as a result of your participation in this VA approved research, all medical treatment (emergency as well as medical treatment beyond emergency care) will be provided by the VA. You will be treated for the injury at no cost to you. However, no additional compensation has been set aside. You have not waived any legal rights or released the hospital or its agents from liability for negligence by signing this form.

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11. PI-Sponsored FDA-Regulated Research

If the PI is the IND/IDE holder, or has agreed to perform any of the IND/IDE holder's sponsor obligations, the PI is considered a sponsor (sponsor investigator) and must meet additional requirements. (Form O, O-1 and P provide details)

[see Office of Clinical Research policies]

[X]	N/A. The PI is not the IND or IDE holder, or has not agreed to perform sponsor obligations

a. Has the PI completed the CITI module: Conducting Investigator-Initiated Studies According to FDA Regulations and Good Clinical Practices?

[X]	Yes	[ ]	No. If no, complete the training prior to submitting this protocol

b. Describe the PI's experience/knowledge/training related to serving as a sponsor-investigator.

Describe here

FORM C

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Abstract / Project Summary

Provide a succinct and accurate description of the proposed research. State the purpose/aims. Describe concisely the research design and methods for achieving the stated goals. This section should be understandable to all members of the IRB, scientific and non-scientific. This summary will also be needed in future IRB Progress Reports.

DO NOT EXCEED THE SPACE PROVIDED.

Purpose/Objectives: Insulin resistance in peripheral tissues (i.e. skeletal muscle) is one of the earliest and most significant abnormalities in the pathogenesis of type 2 diabetes (T2DM). However, the molecular basis for the insulin resistance of T2DM is not fully understood. The human gut hosts an enormous number and variety of microorganisms, including at least 1014 bacteria. An increasing number of animal studies suggest that this microbial "organ", also known as the microbiome, is a causative factor in the insulin resistance of obesity and T2DM, and may represent an entirely new therapeutic target against these conditions. These studies suggest that high fat ingestion, obesity, and T2DM, favor a microbiome profile which enhances production and gastrointestinal permeability of lipopolysaccharide (i.e. metabolic endotoxemia). Lipopolysaccharide (LPS or endotoxin) is a component of the outer membrane of gram negative bacteria cell walls which induces an inflammatory response by activating the cell surface receptor toll-like receptor-4 (TLR4). Despite accumulating evidence from animal studies suggesting that intestinal microbiota and metabolic endotoxemia could play a key role in the pathogenesis of insulin resistance, obesity, and T2DM, the relevance of the intestinal microbiome and metabolic endotoxemia on human metabolic disease remains unknown. Thus, the goal of this study is to determine the role that intestinal microbiota and metabolic endotoxemia play in the pathogenesis of insulin resistance and T2DM in humans.

Research Design/Plan: We will study three groups of subjects: 1) 36 healthy, 18-65 yrs old, normal-glucose tolerant subjects, without a family history of T2DM, and body mass index (BMI) of $\leq$26 kg/m^2; 2) 36 obese, 18-65 yrs old, normal-glucose tolerant subjects, with or without a family history of T2DM, and body mass index (BMI) of 30-37 kg/m^2 ; 3) 36 obese, 18-65 yrs old, T2DM with or without a family history of T2DM, HbA1C $\leq$8.5%, and body mass index (BMI) of 30-37 kg/m^2 . All subjects will undergo pharmacological intervention. All subjects will be studied at the Bartter Research Unit (BRU) at the Audie L. Murphy VA Medical Center, as follows;

Visit 1: Screening tests including a complete history and physical examination/assessment, ECG, blood chemistry, blood cell count, HbA1c, PT, PTT, lipid profile, and UA.

Visit 2: OGTT.

Visit 3: Stool sample collection, plasma LPS measurement, and an 8 h intestinal permeability (lactulose/mannitol) testing.

Visit 4: DEXA, euglycemic hyperinsulinemic clamp, and indirect calorimetry. At time -30 min a biopsy of the vastus lateralis muscle will be performed. At time 0 min, insulin will be infused at 60 mU/m2.min for 180 min. A 2nd biopsy is performed on the contralateral leg at 180 min.

Pharmacological/nutraceutical intervention: Subjects will be randomized to receive, in a double-blind fashion, placebo (maltodextrin, 6 g three times a day), the synbiotic [5 g of oligofrucose + 1 g Bifidobacterium longum (4x10^10 CFU/g) three times a day], or sevelamer (1.6 g sevelamer + 4.4 g maltodextrin three times a day), for 4 weeks. For each dose the powder will be included in identical packets. The subjects from the 3 groups (placebo, synbiotic, sevelamer) will ingest the powder contained in one packet with water 3 times /day for 28 days.

Visit 5: Stool collection, plasma LPS measurement, and intestinal permeability testing as described above for visit 3.

Visit 6: DEXA, insulin clamp, indirect calorimetry, and biopsies of the muscle (2) as described above for Visit 4.

Clinical Relevance: Our findings will improve our understanding of the key role that the microbiome and endotoxemia play in the pathogenesis of insulin resistance. This new knowledge could lead to the development of novel therapies for T2DM.