Effects of Niacin on Intramyocellular Fatty Acid Trafficking in Upper Body Obesity and Type 2 Diabetes Mellitus

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Effects of Niacin on Intramyocellular Fatty Acid Trafficking in Upper Body Obesity and Type 2 Diabetes Mellitus

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	Effects of Niacin on Intramyocellular Fatty Acid Trafficking in Subjects with Upper Body Obesity and Type 2 Diabetes Mellitus
Running Title	Effects of niacin on FFA in UBO and T2DM in insulin resistant states
Protocol Number	17-009977
Phase	Pilot Study
Methodology	Randomized, placebo-controlled; cross-over design
Overall Study Duration	2 years
Subject Participation Duration	6-8 weeks
Single or Multi-Site	Single site
Objectives	Evaluate the effect of niacin or placebo on intramyocellular fatty acid trafficking in subjects with upper body obesity and type 2 diabetes mellitus
Number of Subjects	10 upper body obese and 10 Type 2 diabetic participants will be recruited for this study for a total of 20 participants between the ages of 18-65 years.
Diagnosis and Main Inclusion Criteria	Individuals with upper body obesity and Type 2 diabetics will be included in the study.
Study Product, Dose, Route, Regimen	Niacin (nicotinic acid). Dose: 0.6 mg/minute - 2.8 mg/min for 12 hours. Dose route: intravenous Dose regime: ~ 1.4 mg/min for 12 hours
Duration of Administration	17 hours on one occasion

Reference therapy	The volunteers will undergo a separate study where they will receive intravenous saline without niacin
Statistical Methodology	The incorporation of plasma free fatty acids into intramyocellular lipids will be compared between the saline control day and the niacin infusion day (paired t-test). We will assess whether the incorporation of plasma fatty acids into intramyocellular lipids is related to insulin sensitivity measured by a euglycemic, hyperinsulinemic clamp using linear regression approaches. We will also assess whether the incorporation of plasma fatty acids into intramyocellular lipids is correlated with the activation of the insulin signaling cascade (Western blotting) in muscle using linear regression approaches.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Under normal circumstances adipose tissue is extremely insulin sensitive. Insulin inhibits adipose tissue lipolysis and thereby causes a rapid decrease in plasma free fatty acid concentrations. This insulin-mediated inhibition of lipolysis is impaired in upper body obese compared to lower body obese and normal weight adults (1-3). Humans with type 2 diabetes mellitus (T2DM) have an even more pronounced insulin resistance with regards to lipolysis (4, 5). This insulin resistance results in over-exposure of muscle to free fatty acids (FFA), which can cause insulin resistance in muscle. Thus, it is unsurprising that adults with upper body/visceral obesity (UBO) and Type 2 Diabetes are typically more insulin resistant with respect to glucose metabolism than those with lower body obesity (LBO).

These excess FFA can cause insulin resistance by interfering with insulin signaling. Increased FFA concentration and flux is thought to be a major contributor to insulin resistance in muscle (6) and to stimulate hepatic gluconeogenesis (7). Although insulin resistance is conventionally defined in terms of insulin's effects on glucose metabolism, it is clear that insulin resistance is also present at the level of the adipocyte, with marked resistance to the antilipolytic effects of insulin in obesity (2) and T2DM (8, 9)

Despite a large number of studies of how FFA contribute to health and disease (10-26) ,we and others (27) contend that the mechanisms by which fatty acids affect muscle insulin resistance remains to be fully delineated. The extent to which observed differences in muscle insulin action are the result of altered delivery (elevated adipose tissue lipolysis/FFA concentrations) or altered intramyocellular FFA trafficking into lipotoxic intermediates has not been studied in an integrated fashion. We propose to address these questions by comprehensively assessing muscle fatty acid metabolism in humans with varying degrees of pre-existing and experimentally altered insulin sensitivity. By determining the mechanisms by which elevated plasma FFA

concentrations and differences in intramyocellular trafficking, we will gain a better understanding of insulin resistance and therefore potential therapies for insulin resistance and T2DM.

We therefore propose to address the following questions: 1) Do UBO and T2DM differ with respect to muscle FFA uptake and muscle fatty acid trafficking (i.e. are lipotoxic intermediates from FFA greater in T2DM than UBO relative to muscle FFA exposure) 2) Will overnight, sustained suppression of FFA in UBO and T2DM improve insulin action with respect to glucose metabolism; 3) If so, will this be statistically related to the contribution of FFA to intramyocellular diacylglycerols (DG) and/or ceramides?

Hypothesis: Compared to a saline control experiment, overnight suppression of FFA with intravenous niacin will decrease trafficking of plasma FFA into intramyocellular ceramides and/or DG and will improve insulin signaling and insulin-stimulated glucose disposal.

Rationale: Niacin at high doses suppresses adipose tissue lipolysis via the PUMA-G receptor, independent of insulin. Suppression of FFA with Acipimox (a lipid-lowering niacin derivative) improved muscle insulin sensitivity with regards to glucose metabolism and Akt phosphorylation (66). Surprisingly, this was not accompanied by reductions in whole muscle ceramide or DG concentrations. While it is possible that neither DG nor ceramides were responsible for the abnormal insulin signaling (66), it is also possible that specific DG or ceramide species or the subcellular localization of those species, which were not measured by Liang et al (66), accounted for the improved insulin signaling/action.

1.2 Preclinical and clinical data

Niacin, as a lipid-lowering treatment, is given chronically (for years) and safely at doses of 3g/day. We have given niacin (as Niaspan®) 1 g orally each hour for two hours {Ali, 2015 #107} with no adverse events. Intravenous niacin has been a component of the standard vitamin cocktail for parenteral nutrition for several decades. The daily therapeutic parenteral requirement in adults is 40 mg per day {Bistrian, 2015 #106}. Nelson et al {Nelson, 2012 #18} administered niacin as an

intravenous infusion for two hours starting at 0.6mg/min and increasing to 2.8 mg/min over 30 min; the only observed effect was asymptomatic flushing. In our proposed study we will use a one-time dose that is less than the typical daily dose used for chronic treatment of hyperlipidemia. Given the safety of the route (intravenous) and the moderate dose (~1 gm over 12 hours), we anticipate this is a safe means to temporarily lower plasma FFA concentrations for the purposes of this NIH funded study.

The proposed studies will measure the relative contribution of plasma FFA to intramyocellular signaling molecules (DG and ceramides), as well as the incorporation into imTG and subsequent appearance in long-chain acylcarnitines in adults with a wide range of insulin sensitivity with respect to glucose metabolism. By integrating information regarding the concentrations of lipid signaling molecules, the contribution of plasma FFA to these molecules, and the insulin signaling pathway we will be able to determine the extent to which varying degrees of insulin resistance are due to altered intramyocellular fatty acid trafficking.

Suppression of FFA – an insulin sensitizing approach. Acipimox, a long-acting niacin analog, has been used to suppress FFA through a non-insulin mechanism (72) and thereby study the adverse effects of elevated FFA (66, 73-76). Seven days of Acipimox treatment improved insulin mediated stimulation of glucose disposal in T2DM, but not undifferentiated obese subjects (66). This treatment also increased insulin stimulated Akt phosphorylation in T2DM (66), but did not reduce the elevated whole muscle ceramide content in obese or T2DM and did not change muscle DG concentrations, which were not different between lean, obese and T2DM at baseline (66). Unfortunately, these authors could not document whether FFA were suppressed in a sustained manner, did not have measures of the DG and ceramides species, and measured only whole muscle content of these molecules. Furthermore, they did not characterize their non-diabetic obese volunteers with regards to fat distribution, which may explain why insulin-stimulated glucose disposal per kg fat free mass was equal to or greater in obese than lean controls. Our colleague, Dr. John Miles, has developed a protocol for intravenous niacin administration that suppresses FFA in a sustained manner (77); he has shared with us his protocol to facilitate the application for our own IND. By administering overnight intravenous niacin infusion we propose to test the hypothesis that lowering FFA in truly insulin resistant UBO and T2DM improves: 1) whole body

insulin sensitivity; 2) muscle insulin signaling; 3) subcellular (SS vs. IMF) content of DG and ceramides; 4) the contribution of plasma FFA to these molecules.

In this study we propose to assess the subcellular location of DG and ceramides to better understand their relationship to altered insulin signaling. We will also test the hypothesis that sustained suppression of FFA alone can alter the trafficking of FFA into less "toxic" metabolites and thereby improve insulin action.

1.3 Investigational Agent

Name of product: Nicotinic acid
Brief description: Niacin will be received by Research Pharmacy in powder form. The drug will be administered intravenously by a Baxter infusion pump model Sigma Spectrum, after reconstitution by pharmacy.

1.4 Dose Rationale

Subjects will first receive a titrated dose starting from 0.6 mg/min to a maximum of 2.8 mg/min (likely needed dose = 1.4 mg/min) or 0.9% NaCl. We will collect blood samples overnight during the infusion to determine the lowest possible dose of niacin needed to suppress plasma FFA concentrations to those observed in overnight, postabsorptive, lean, healthy adults. The infusion rate will be titrated to a maximum of 2.8 mg/min only if lower doses do not result in the needed reductions in plasma FFA. The dose of 2.8 mg/min has been shown to result in sustained suppression of plasma FFA concentrations (a reflection of adipose tissue lipolysis) to much lower levels than are needed for the purposes of this study {Nelson, 2012 #18}. Therefore, we anticipate considerably lower niacin doses will be needed. The gradual dose escalation and a pre-study dose of 325 mg of aspirin will prevent or minimize the side effects of flushing due to niacin.

1.5 Risks and Benefits

Radiation: Radiation exposure from the studies supported by this grant include the radiation from the dual energy x-ray absorptiometry, and computed tomography scans of the abdomen.

Blood sampling: Blood samples are collected from an indwelling intravenous catheter for this study. Bruising can occur with venipuncture, as can fainting.

Infusion of substrates: We will infuse minute quantities of glucose and palmitate that are labeled with stable isotopes as part of these studies. The quality assurance plans for these compounds are reviewed and approved by the Mayo Clinic Radioactive Drug Research Committee as the designated representative of the FDA. We will also infuse insulin intravenously and give local anesthetics for the tissue biopsies. The insulin is infused using clinical safeguards as well as research pharmacy safeguards under an IND exemption from the Mayo IRB.

Adipose and muscle biopsies: The risks of these procedures include pain, hematomas, bruising, infection, and scarring of the biopsy site.

Insulin infusion: The risk of insulin infusion is hypoglycemia.

Niacin: the risk of niacin infusion is flushing, pruritus, nausea or vomiting.

2 Study Objectives

Primary Objective

To assess the effect of overnight suppression of FFA with intravenous niacin on trafficking of FFA into intramyocellular ceramides and/or DG

Secondary Objective

To assess the effect of overnight suppression of FFA with intravenous niacin on insulin signaling and insulin-stimulated muscle glucose uptake

3 Study Design

This study is a non-randomized, niacin vs. saline control trial of the effects of niacin on intracellular fatty acid trafficking in insulin resistant states. Subjects will be screened at outpatient clinic visit appointments and interested qualified subjects will be consented and offered participation in this trial. Once consent has been obtained baseline values will be established and subjects will begin treatment and follow-up for up to the next 8 weeks.
A final evaluation and collection of lab samples will be conducted at the end of the study.

3.1 General Description

All volunteers will be weight stable for two months prior to the study and will consume an isoenergetic diet (weight stable) eating all meals (45% carbohydrate, 20% protein and 35% fat) from the Mayo Clinical Research Unit (CRU) for 3 days prior to the study. This will ensure consistency of energy intake and nutrient composition prior to the studies. Body composition (body fat, fat free mass (FFM), leg fat, upper body subcutaneous and visceral fat) will be measured using DEXA. A single slice CT abdomen will be performed to measure visceral fat. A treadmill exercise test will be conducted to assess fitness (peak VO_2) > 1 week prior to any tracer studies in order to allow us to determine whether variation in muscle oxidative capacity (a surrogate for mitochondrial function) contributes to differences in lipotoxicity (78). We will be performing muscle and fat biopsies on two visits separated by a period of at least 4 weeks. Following admission to the CRU the evening prior to the studies a forearm intravenous catheter will be placed for infusion of niacin (or saline), insulin, glucose and isotopic tracers. T2DM volunteers taking insulin will be asked to withhold their long-acting insulin for 3 days prior to the study, manage their blood sugar with short acting insulin, and receive an overnight, monitored infusion of regular insulin to maintain blood glucose values between 4.5-5.5 mmol/L. At 2100 h the infusion of intravenous niacin (or saline) will begin. The initial niacin dose will be 0.6 mg/min and, depending upon symptoms, the rate increased by 0.4 mg/min increments to a goal of 1.4 mg/min. We will obtain blood samples from the intravenous catheters at 2200, 1200, 0200 and 0400 h to measure plasma FFA concentrations. The niacin infusion will be continued until1420 h. The niacin infusion will be gradually weaned off in a pattern designed to avoid a

rebound increase in FFA concentrations. Depending upon the degree of overnight suppression of FFA concentrations, the dose may be adjusted downwards or upwards (maximum rate of 2.8 mg/min) in subsequent studies. At 0400 h an infusion of [U-^{13}C]palmitate (330 nmol/min) will be initiated to trace FFA contribution to intramyocellular lipids in the euinsulinemic state (13,15). At 0600 h an infusion of [6-$^{2}H_2$]glucose will be started to trace glucose metabolism and continued till the end of the study. Resting energy expenditure will be measured by indirect calorimetry to permit interpretation of FFA flux data. A series of blood samples will be collected between 0830 and 0900 h to measure steady state plasma palmitate and glucose enrichment and concentrations. The volunteers will then undergo a vastus lateralis muscle biopsy as well as abdominal subcutaneous adipose biopsy together for measurement fatty acid metabolites and to provide material for the "pre-clamp" measures of insulin signaling; the niacin infusion will then be discontinued.

Beginning at 0915 h the volunteers will receive a primed, constant infusion of insulin (1 mU/kg/min) together with an infusion of 50% dextrose labeled with [6-$^{2}H_2$]glucose in order to maintain euglycemia. At the start of the insulin clamp the [U-^{13}C]palmitate will be stopped and [$^{2}H_9$]palmitate (400 nmol/min) will be started to measure the contribution of FFA to intramyocellular lipids during hyperinsulinemia (35). At the end of the 5 hour insulin clamp a second muscle biopsy will be collected from the contralateral leg from the first biopsy to avoid the effects of local trauma. This biopsy is taken at 5 h to allow us to measure the accumulation of [$^{2}H_9$]palmitate in the intramyocellular lipid metabolites listed above, as well as to detect the loss of [U-^{13}C]palmitate from the metabolites as a function of their turnover. A series of blood samples for glucose and palmitate enrichment will be collected just prior to each of these biopsies.

The change in the ratio of phosphorylated to total insulin signaling effector proteins between the first and second muscle biopsy will be used to assess the insulin-mediated activation of insulin signaling pathways. We will assess insulin signaling in biopsies taken under basal and insulin stimulated conditions at the level of Akt phosphorylation using capillary Western blot approaches to quantify phosphorylation (80). Glucose disappearance rates under euglycemic, hyperinsulinemic conditions are an excellent reflection of muscle glucose uptake, and this will be

used as our index of muscle glucose disposal. Indirect calorimetry will be done prior to the first and second muscle biopsies to provide context for substrate utilization and energy expenditure. We will perform abdominal subcutaneous adipose tissue biopsies in order to measure fat cell size and the inflammatory characteristics of adipose tissue because these factors have been related to insulin resistance in muscle. The biopsies will be done using sterile technique under local anesthesia and will be done on different sides of the abdomen.

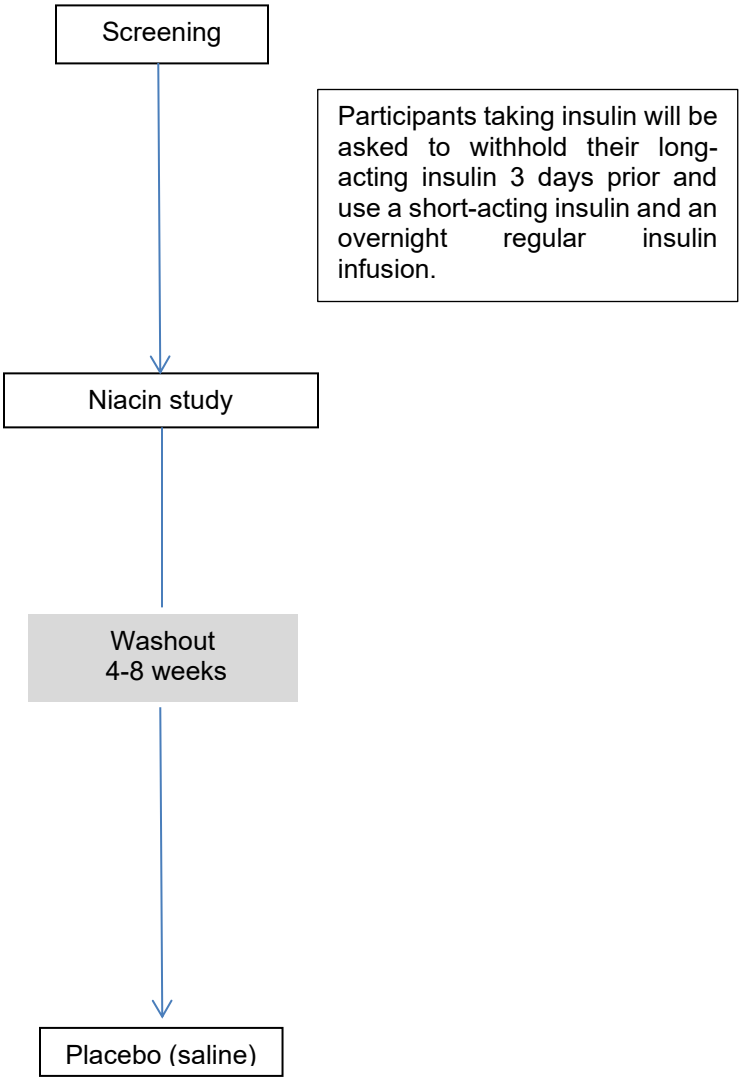
3.2 Number of Subjects

Ten upper body obese and 10 Type 2 diabetics (5 male and 5 female in each group) will be recruited to participate in this study to assure we have truly insulin resistant participants in whom improvements in insulin action can be expected.

3.3 Duration of Participation

The duration of participation for each subject would take anywhere between 6 weeks to 3 months, including the 4-8 week interval period, all the visits and overnight stay at the CRTU. The overall recruitment and completion of the study will take about 4 years due to disruptions in scheduling from the COVID pandemic.

Flowchart of Study Events



3.4 Primary Study Endpoints

To determine the contribution of plasma palmitate ([U-^{13}C] and [$^{2}H_9$]palmitate) to muscle triglycerides, DG and ceramides in saline control and niacin (FFA suppressed) conditions.

3.5 Secondary Study Endpoints

We will also measure the insulin signaling assays and glucose disposal rates, specifically to test the effects of the lower FFA concentrations on insulin signaling and glucose disposal

We will assess whether the incorporation of plasma palmitate into muscle DG is related to changes in the proximal activation by phosphorylation of the insulin signaling pathway during the insulin clamp.

3.6 Primary Safety Endpoints

The only know adverse effects of short term exposure to niacin are skin flushing and pruritus. We will document the duration and severity of any symptoms during the niacin infusion. (ICH E6, section 6.8)

3.7 Identification of Source Data

The following source data will be directly recorded on the Case Report Form (CRF):

- Patient Reported Outcomes – flushing
- Smoking Status

The following source data will not be directly collected in the Case Report Form (CRF), but will be captured in supportive documentation (study source documents, EMR, study staff maintained excel spreadsheets):

- Laboratory results and clinical interpretation of the values.
- Blood pressure, temperature, pulse rate. Will be recorded in the EMR by CRTU nursing staff.
- Height and weight are directly recorded in excel files by study staff.
- Patient Questionnaire (VAS) flushing are recorded in the EMR by CRTU nursing staff.
- CT scan analysis

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Subjects who meet the following criteria may be included in the study:

1. Males and females between 18 and 65 years of age who are able to comprehend instructions, follow study procedures, willing to sign an informed consent form, and consume an isoenergetic diet eating all meals from Mayo GCRC for at least 3 days prior to study.
2. **Overweight/Obese volunteers** will have a BMI 29.0 – 40.0 kg/m^2
    - **Upper body/visceral obesity (UBO)** in women will be those with a waist-hip ratio (WHR) > 0.85 and/or increased visceral fat by single slice CT scan and/or biochemical evidence of metabolic syndrome as determined by the investigators. Upper body obesity in men will be defined as a waist-hip ratio of >0.95 and/or increased visceral fat by single slice CT scan and/or biochemical evidence of metabolic syndrome.
3. Volunteers with Type 2 Diabetes will have HbA1C between **6.5 and 11.5%.**
    Hold metformin and sulfonylurea for 1 week prior study
    Avoid pioglitazone due to long half life

4. Resting vital Signs are within the following range: 95 mmHg < systolic blood pressure < 160 mmHg
   45 mmHg < diastolic blood pressure < 100 mmHg
   40 bpm < heart rate <100 bpm
5. Female subjects are eligible if they meet the following criteria:
   - Are not pregnant or nursing
   - All women of childbearing potential will have a negative serum pregnancy test at screening and a negative urine pregnancy test within 48 hours before administering study drug.
   - All women of childbearing potential will use an appropriate contraceptive method including barrier method, oral contraceptive medication, contraceptive device or abstinence while participating in the study.
6. Recent or Current research participation?

If Yes look at consent form and f/u visits:

- Medications that alter fat metabolism possibly given: if yes, exclude
- Weight possible changing in the study
- Amount of blood drawn during the study (if our study plus this one draw ≥ 450 ml these should be separated by 8 weeks

7. Previous labs:
   - HbA1C < 6.5% for non-diabetic UBO
   - Fasting glucose < 126 mg/dl for non-diabetic UBO
   - Hb ≥ 11.0 for women and ≥ 12 for men
   - platelets > 100 000

4.2 Exclusion Criteria

The following will exclude potential subjects from the study:

1. Individuals with a history of a disease process such as:
   - Ischemic heart disease
   - Atherosclerotic valvular disease
   - Blood pressure greater than 160/95 despite antihypertensive medication

2. Smokers > 20 cigarettes per week
3. Concomitant use of medications that can alter serum lipid profile:
    - High dose fish oil (>3g per day),
    - STATINS (if yes hold for 6 weeks and receive PCP's approval),
    - Niacin
    - Fibrates
    - thiazolidinediones
    - Beta-blockers
    - Atypical antipsychotics

4. Allergy to Lidocaine, niacin and/or Niaspan
5. Subjects with any of the following parameters greater than 1.5 times the upper limit of normal:
    a. Serum creatinine
    b. Alkaline phosphatase
    c. Aspartate aminotransferase (AST) unless participant has fatty liver disease, in which case AST can be < 2 times upper limit normal
    d. Alanine aminotransferase (ALT) unless participant has fatty liver disease, in which case AST can be < 2 times upper limit normal
    e. Total bilirubin (unless the patient has documented Gilbert's syndrome)

4.3 Subject Recruitment, Enrollment and Screening

Subjects will be contacted by letters, email and telephone calls (maximum number of contact attempts with subject will be 3). Copies of the letters, email scripts and telephone scripts will be submitted to IRB.

At the time of enrollment the investigator or personnel involved in recruitment will address the following:

Amount of time needed for reading or reviewing the consent document Amount of time needed for the prospective subject to make a decision

The location or methods of communicating and the related privacy needs for the initial and on-going discussions.

The need for including a medical interpreter, Legally Authorized Representative, family member(s), witness, or advocate who may need to be present and observe the discussions within the informed consent process.

The screening procedure will involve the following:

- Evaluation and documentation of inclusion/exclusion criteria
- Blood draws to measure labs (see table 1)
- Serum pregnancy test unless postmenopausal
- DEXA scan, Data from DEXA scans conducted in the CRTU for other protocols within a 3 month time period may be used in place of repeating the scan in order to reduce radiation risk.
- CT scan.*
- Meeting with the registered dietician to discuss food preferences, food allergies, etc. for the three-day meal plans.
- Maximum physical exertion level will be examined. This will include periodic monitoring of heart rate and blood pressure. An EKG will also be a part of this test.

* Single slice abdominal L2-L3 CT scans conducted in a previous 3 month window, may be used in place of repeating the CT scan.

4.3.1 Early Withdrawal of Subjects

4.3.2 When and How to Withdraw Subjects

Subjects must be withdrawn from the study for any of the following reasons:

- Withdrawal of informed consent
- Pregnancy
- If the investigator concludes that it would be in the subject's best interest for any reason

Subjects may voluntarily withdraw from the study for any reason at any time.

If withdrawal occurs for any reason, the investigator will document the reason for withdrawal in the source notes.

4.3.3 Data Collection and Follow-up for Withdrawn Subjects

Subjects who withdraw after enrollment will be replaced to complete the study.

5 Study Drug

5.1 Description

The investigational product to be studied will be an infusion of nicotinic acid (niacin) or saline. The chemical formula is C6H5NO2. It is a white, crystalline, powder that is reconstituted for intravenous infusion. It is soluble in water.

A. Mechanism of Action

As B complex vitamins, niacin and niacinamide have identical physiological effects. However, these vitamins differ in some of their actions. Niacin produces peripheral vasodilation and flushing, an effect that generally does not occur with niacinamide. Niacinamide is formed in vivo from metabolism of niacin. Niacinamide is an essential precursor of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are the physiologically active forms of niacin. Serving as coenzymes for several dehydrogenases, NAD and NADP are functional groups of electron-transfer agents active in cellular respiration, glycolysis, and lipid synthesis. When taken in amounts considerably greater than needed to meet vitamin requirements, niacin causes a partial suppression of the release of free fatty acids from adipocytes.

Niacin can lower serum triglyceride and LDL-cholesterol concentrations. Rate of hepatic TG synthesis are decreased and thereby decreased VLDL. Subsequent clinically lowered apo B and LDL concentrations are found (81).

B. Pharmacokinetics

Absorption

Nicotinic acid undergoes rapid first-pass hepatic metabolism after oral ingestion, which we will avoid by infusing it intravenously. Niacin concentrations in the systemic circulation are dose-dependent.

A) Bioavailability – intravenous – 100%

B) Effects of Food – none known with intravenous niacin

Distribution

Nicotinic acid is widely distributed and in concentrated in liver, spleen, and adipose tissue.

Metabolism

When administered orally the pharmacokinetic profile of niacin is complicated due to extensive first-pass metabolism. The profile is dose-rate specific and, at the doses used to treat dyslipidemia, saturable. In humans, one pathway of metabolizing niacin is through a simple conjugation step with glycine to form nicotinuric acid (NUA). NUA is excreted in the urine, although there may be a small amount of reversible metabolism back to niacin. The other metabolic pathway results in the formation of nicotinamide adenine dinucleotide (NAD). It is unclear whether nicotinamide is formed as a precursor to, or following the synthesis of, NAD. Nicotinamide is further metabolized to at least N-methylnicotinamide (MNA) and nicotinamide-N-oxide (NNO). MNA is further metabolized to two other compounds, N-methyl2-pyridone-5-carboxamide (2PY) and N-methyl-4-pyridone-5-carboxamide (4PY). The formation of 2PY appears to predominate over 4PY in humans. The activity of these metabolites is unknown.

Excretion

Following single and multiple doses, approximately 60 to 76% of the niacin dose administered orally is recovered in urine as niacin and metabolites; up to 12% was recovered as unchanged niacin after multiple dosing. The ratio of metabolites recovered in the urine was dependent on the dose administered. Niacin is excreted as nicotinuric

acid in urine after conjugation with glycine. Whether the same distribution of metabolites in the urine occurs with intravenous as with oral administration is unknown.

5.2 Treatment Regimen

Subjects will receive a titrated dose starting from 0.6 mg/min to a maximum of 2.8 mg/min (likely needed dose = 1.4 mg/min) or 0.9% NaCl for 12 hours.

5.3 Method for Assigning Subjects to Treatment Groups

Subjects will first receive niacin and for the second study they will receive intravenous saline as the placebo.

Preparation and Administration of Study Drug

Nicotinic acid will be received in powder form and compounded at Research Pharmacy following Compounding Procedures and an approved compounding formula sheet, for intravenous administration which runs in a Baxter infusion pump model Sigma Spectrum. The drug will be aliquoted in single dose amber vials of 10 mL each and refrigerated at a temperature of 2-8°C.

5.4 Subject Compliance Monitoring

We are not expecting non-compliance as the drug administration is a constant monitored intravenous infusion. In cases where a subject requests to stop study drug, it will be recorded and reported as a note to file or a protocol deviation.

5.5 Prior and Concomitant Therapy

The following medications will be withheld 3 days prior to beginning of the study:

- Participants with Type 2 Diabetes on insulin will have their long-acting insulin replaced with a short-acting insulin.

5.6 Packaging

Contents of kits/boxes associated with labelling: The study drug or saline/placebo is labelled with a patient specific label by the RESEARCH pharmacy. This label shall bear the statement "Caution: New Drug--Limited by Federal (or United States) law to investigational use."

5.7 Masking/Blinding of Study

This study is not a blinded study.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Nicotinic acid will be obtained in powder form and be compounded in the Research Pharmacy for intravenous administration, following Compounding Procedures and an approved compounding formula worksheet specific to each patient.
Upon receipt of the drug, an inventory will be performed and logged by research pharmacy technicians accepting the shipment.

5.8.2 Storage

Niacin storage: The investigational drug will be packaged in 10 mL amber vials and be refrigerated away from light in a temperature setting of 2-8°C. After compounding, a sterility date applied is 90 days with refrigerated storage. Niacin preparation is sterilized by a sterile filtration procedure using a sterile 0.2 micron filter.

Placebo storage: 0.9% NaCl will be stored at USP controlled temperature.

Storage location: Study drug and placebo will be stored at SMH Research Pharmacy that is secure and can be only accessed by research pharmacy personnel.

Light conditions: Amber glass vials provide light protection.

Container closure components: 10 mL amber Type 1 glass vials Wheaten item # 223761 are used for the container. The vials are depyrogenated through a washing cycle and are sterilized through a steam sterilization cycle.
The stoppers are West Pharmaceutical Flurotec 20 mm stoppers item #19700021. We purchase them as 'ready to sterilize' and subsequently sterilize them through repackaging and a steam sterilization cycle.

Sterility and bacterial endotoxins test methods: Sterility testing is accomplished through membrane filtration, using Millipore Steritest canisters. In a sterile environment, the contents of an appropriate number of vials (based on USP 71) are filtered through the canister set and are flushed with 0.9% sodium chloride for injection, USP. The canisters are incubated at appropriate temperatures for the media for 14 days.
Endotoxin testing is completed by an outside laboratory (ARL Bio Pharma). Limits are calculating according to USP 85 or if present, the USP monograph for the injection.

5.8.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form and reported by Research Pharmacy.

5.8.4 Return or Destruction of Study Drug

All medications would be given on site. Any patient specific unused medication will be destroyed on site.

6 Study Procedures

Table 1 below lists all of the assessments and indicates with an "x" the visits when they are performed. Subjects should be seen for all visits on the designated day and time.

Subjects who are discontinued before completing the study, and those who withdraw prematurely from the study for any reason, should be scheduled for a visit as soon as possible, at which all of the assessments listed for the "end of Study" visit will be performed. Study visits will occur at Screening, Visit 1 and Visit 2/End of Study.

The maximum duration of the clinical investigation is up to 8 weeks from enrollment (signing of consent, which allows for a 14 day screening period).

Schedule of Events			
	Screening	Inpatient Visit 1	Inpatient Visit 2
Study Day	-14	0	28-56
Written informed consent	X		
History and demographics	X		
Concurrent med reconciliation	X		
Height	X		
Weight	X	X	X
Vitals	X	X	X
Laboratory tests [a]	X*		
Single-slice abdominal CT	X		
DEXA scan	X*		
Adverse event evaluation	X	X	X
Pregnancy test	X[b]	X[c]	
Study Agent (niacin administration)		X	
Insulin and glucose administration		X	X
Isotope administration		X	X
Resting energy expenditure		X	X
Muscle Biopsies		X	X
Adipose biopsies		X	X

a: Complete metabolic panel, CBC w/differential, Lipid Panel, HbgA1C, liver function tests if not part of complete metabolic panel

b:Serum pregnancy test (women of childbearing potential) at screening

c: Urine pregnancy test (women of childbearing potential) ≤ 48 hours prior to study drug administration.

*Research DEXA scan, labs, and CT scan completed in the previous 3 months may be used in place of repeating these.

6.1 Screening Visit

The data that will be collected on each subject at the screening visit are as follows:

- Confirmation of informed consent
- Vital signs (Blood pressure, pulse rate, respiratory rate, and body temperature)
- Body weight and height
- Confirmation of meeting all the Inclusion criteria and none of the exclusion criteria
- Demographics and medical history
- Pregnancy test – serum pregnancy test (for females of childbearing potential)
- Laboratory examinations: Complete Blood Count, Fasting lipid profile, Alkaline Phosphatase, Electrolyte Panel, AST, ALT, HbA1c (for diabetics), Fasting Glucose, Serum Creatinine. Overnight fasting is required and will be documented in the EMR by CRTU nursing staff.
- Body composition as measured by Dual-energy x-ray absorptiometry* (see section 4.3) and abdominal CT scan * (see section 4.3).

6.2 Visit 1 (In-patient visit, 1 night stay)

At visit 1 the subjects will undergo the following:

- Admission to in-patient facility the evening prior to scheduled biopsies
- Negative urine pregnancy test (for females of childbearing potential only) 48 hours prior to dosing.
- Vital signs (blood pressure, pulse rate, respiratory rate, and body temperature)
- Body weight
- Concomitant and non-drug therapies
- Niacin or saline infusion started the evening prior

Volunteers will be asked to stop taking any aspirin or ibuprofen from three days before to three days after their overnight stays.

All participants will consume an isoenergetic diet (weight stable, 45% carbohydrate, 20% protein and 35% fat) from the Mayo Clinical Research and Trials Unit (CRTU) for 1-3 days prior to the niacin infusion study and 3 days before the saline control study. This will assure consistency of energy intake and nutrient composition prior to the study saline control study that is helpful in reducing variability in plasma FFA concentrations.
Following admission to the CRU the evening prior to the studies a forearm intravenous catheter will be placed for infusion of niacin, insulin, glucose and isotopic tracers. T2DM volunteers taking insulin will be asked to withhold their long-acting insulin for 3 days prior to the study, manage their blood sugar with short acting insulin, and receive an overnight, monitored infusion of regular insulin to maintain blood glucose values between 4.5-5.5 mmol/L. At 2100 h the infusion of intravenous niacin (or saline) will begin. The initial niacin dose will be 0.6 mg/min and, depending upon symptoms, the rate increased by 0.4 mg/min increments to a goal of 1.4 mg/min. A retrograde intravenous catheter will be placed to allow for blood sampling at 2200 h, 2400 h, 0200 and 0400 h. The niacin/saline infusion will be continued until 0900 h the following morning. The niacin infusion will be weaned off gradually in order to prevent a rebound increase in plasma FFA. Depending upon the degree of overnight suppression of FFA concentrations, the dose may be adjusted downwards or upwards in subsequent studies to a maximum of 2.8 mg/min in subsequent studies. At 0400 h an infusion of [U-^{13}C]palmitate (330 nmol/min) will be initiated to trace FFA contribution to intramyocellular lipids in the euinsulinemic state (13,15). At 0600 h an infusion of [6-$^{2}H_2$]glucose will be started to trace glucose metabolism and continued till the

end of the study. Resting energy expenditure will be measured by indirect calorimetry to permit interpretation of FFA flux data. A series of blood samples will be collected between 0830 and 0900 h to measure steady state plasma palmitate and glucose enrichment and concentrations.

The volunteers will then undergo a vastus lateralis muscle biopsy for measurement fatty acid metabolites and to provide material for the "pre-clamp" measures of insulin signaling and the niacin infusion will be tapered to 25% of their overnight dose.

Insulin regulation of muscle glucose uptake and fatty acid metabolism will be assessed using a primed, constant infusion of insulin at a dose of 1.0 mU/kg/min. Arterialized venous blood samples will be collected from the same retrograde IV catheter using the hot box with a temperature of 131 degrees Fahrenheit, to allow measurement of hormone, tracers and substrate concentrations over the 30 min prior to the insulin clamp and the last 30 min of the insulin clamp. Blood glucose will be measured every 10 min throughout to allow the glucose infusion rate to be adjusted to maintain euglycemia. Resting energy expenditure will be measured by indirect calorimetry to permit interpretation of FFA flux data. (79).

Adipose tissue biopsies will be collected from the abdominal region under local anesthesia using sterile technique (82) at the same time as the first and last muscle biopsies. The adipose tissue samples (2-3 g from each site) will be immediately rinsed of blood and transported to our laboratory (~ 3 min walk from the CRTU).

There we will divide the specimen into smaller aliquots for collagenase digestion to be used for cell sizing (83), and immunohistochemistry.

The volunteers will be able to order a general meal after the biopsies. After this, the participants will be discharged from the CRTU.

6.3 Visit 2 (In-patient visit, 1 night stay)

After ~4 weeks subjects will return to the CRTU and the same procedure and guidelines as Visit 1 will be repeated with the saline/placebo.

7 Statistical Plan

7.1 Sample Size Determination

Power calculation:
Acipimox increased insulin-stimulated glucose disposal in undifferentiated obese subjects by ~10% (P = 0.07) and in T2DM by ~ 50%, whereas insulin-stimulated Akt phosphorylation increased by ~80% in both groups (66); IRS-1 tyrosine phosphorylation increased only in obese by 300%. In our hands, the reproducibility of insulin-stimulated glucose disposal is within ~5% with no intervention. If we obtain similar responses as Liang et al (66), we would have 90% power to detect a 10% improvement in insulin sensitivity in the obese group at a P of 0.05 with 5 subjects per group. However, because women may respond differently than men to changes in FFA (85), we propose to include more participants in order to allow sex-specific analysis. By selecting UBO volunteers we will likely have a more insulin resistant group than Liang (66), which should improve our statistical power to detect changes in lipid metabolites and insulin signaling. We can also expect some biological heterogeneity in the responses, which should be helpful in that we intend to understand the extent to which changes in insulin sensitivity relate to changes in intramyocellular DG and ceramides (86) and insulin signaling, for example by grouping participants post hoc to groups that have the greatest and least response to FFA suppression.

7.2 Statistical Methods

Descriptive Statistics

Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for the treatment groups. These analyses will help identify potential confounding variables to be used as covariates in sensitivity analyses. Distributions across subgroups used in randomization will be compared to assess whether the randomization was successful in equalizing distributions of these prognostic variables across treatment groups.

Our hypothesis will be tested by measuring the concentration of plasma palmitate (and total FFA) as well as the enrichment ([U-^{13}C] and [$^{2}H_9$]palmitate), and therefor flux of palmitate. We will measure the incorporation of [U-^{13}C] and [$^{2}H_9$]palmitate in DG and ceramides relative to plasma palmitate, as well as the insulin signaling assays and glucose disposal rates, specifically to test the effects of the lower FFA concentrations and lesser contribution of plasma FFA combined with improvements in insulin signaling and glucose disposal (ANCOVA). If glucose disposal and insulin signaling improvements in each group are accompanied by selective reductions in either ceramides or DG (especially the saturated species), this will suggest which of these is most likely responsible for the baseline impairments relative to the lean control state. It is possible that the UBO and T2DM will have different responses in ceramides and DG. For example, the portion of insulin resistance due to lipotoxicity in T2DM could be the combined effect of both DG and ceramides, whereas in UBO may be just one of these compounds. If the insulin signaling measures coincide with the changes in ceramides and DG in such a way as to confirm the pathways that have been described using cell/animal models are operative in humans, in vivo, this will provide an important confirmation that we can translate basic findings to human physiology. To the extent reduced lipolysis reduces FFA during the insulin clamp, we anticipate a lesser contribution of the [9-$_2$H]palmitate to DG and ceramides (repeated measures ANOVA).

A subtext to the DG and ceramide enrichment measures relative to plasma and palmitoyl-CoA is that, to the extent these molecules are synthesized from sources other than FFA, this will provide clues to alternate pathways that may be active in generating lipid signaling molecules. We will be able to understand whether excess ceramides are due to de novo synthesis as opposed to sphingomyelin hydrolysis and whether elevated DG is due to de novo synthesis or other sources, potentially including phospholipid hydrolysis. We believe that our ability to go beyond measures of concentrations by making more mechanistic assessments of the source of lipotoxicity is a strength of the proposed studies.

Handling of Missing Data:

The frequency and pattern of missing data will be evaluated to assess whether the data is missing completely at random, or whether a pattern exists that may suggest a systematic bias

with respect to the presence or absence of data. If the missing data appear to be missing completely at random, and if the amount of missing data is relatively small, the analysis will utilize all available data. Depending on the pattern and amount of missing data, multiple imputation methods may be considered, however given the proposed study has relatively modest sample size, the amount of missing data is anticipated to be small.

Primary Hypothesis:

In order to test Hypothesis 1 we will use the following pieces of data: a) subsarcolemmal (SS - largely equivalent to membrane associated DG (68)) DG concentrations, focusing primarily, but not solely, on 16:0/18:0, 16:0/16:0 and 18:0/18:0, as well as the enrichment in 16:0/18:0, 16:0/16:0 and 16:0/18:1 relative to plasma palmitate. This will provide us with as an index of de novo synthesis of DG from FFA; b) the baseline and insulin-stimulated changes in phosphorylation of Akt (66, 80); c) the increase in glucose disposal as a function of the increase in plasma insulin concentrations.

We will assess whether the SS DG concentrations and the incorporation of plasma FFA into DG (ratio of DG palmitate:plasma palmitate) differs between UBO and T2DM (ANOVA).

We will also test whether the concentrations or incorporation of plasma palmitate into the SS vs. IMF DG is related to changes in the proximal activation by phosphorylation of the insulin signaling pathway during the insulin clamp.

Secondary Hypotheses:

To address hypothesis 2 we will use a similar statistical approach, except using intramyocellular ceramide concentrations and enrichments relative to plasma palmitate in conjunction with Akt phosphorylation. Robust Akt phosphorylation in the face of high ceramide concentrations/synthesis from FFA would argue against the ceramide-PP2A mechanism of insulin resistance.

Interim Analysis

No interim analysis is proposed.

7.3 Subject Population(s) for Analysis

- All-treated population: Any subject entered into the study started the drug infusion and/or biopsy procedure.
- Protocol-compliant population: Any subject who was entered and received the protocol required study drug exposure and required protocol processing
- All-completed population: Only subjects who completed ALL study related procedures and follow-up will be included.

8 Safety and Adverse Events

Safety Review Plan –Study progress and safety will be reviewed quarterly with the research fellow and study coordinator and, because these are relatively small studies, the data summarized at 6 month intervals to allow for a large enough sample to assess outcomes. Progress reports, including patient recruitment and AE's will be provided to the Endocrine Research Committee following each of the 6 month reviews in the event that no participants are accrued in the study a report will not be provided. An annual report will be compiled and will include a list and summarization of adverse events at the time of the non-competing renewal. In addition, the annual report will address (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study. The annual report will be reviewed by the Endocrine Research Committee and the results included in the minutes. These will be forwarded to the IRB and NIDDK and, if applicable, the Mayo CTSA CRU and FDA as indicated.

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the

subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**

- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event

An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

Other important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the follow-up period is up to 6 weeks from the 1st biopsy visit. Any adverse event occurring during the course of the study period will be reported.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-

investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values

Research lab results taken between study drug administration and study completion are expected to be abnormal as a result of adipose tissue and muscle biopsies (catecholamines) done during the study. Screening lab values are reviewed by the physician principal investigator in the EMR and documented as reviewed in the EMR. Clinical lab results obtained by a primary care provider or other physician that is not involved in the study in the time that a participant receives the study drug will not be assessed for abnormality.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results will be recorded in the source document.

Adverse events are considered events that are not already described as routine research risks in the consent form. We will not report pain at time of the biopsy as an AE, nor will we report bruising from biopsy or line placement procedures as these are nearly guaranteed to occur with all participants. All adverse events occurring during the study period will be recorded. The clinical course of each event would be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation will be recorded and reported immediately.

We will use the following standardized system for classification and grading of Adverse Events *http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf*

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The IRB requirements reflect the guidance documents released by the Office of Human Research Protections (OHRP), and the Food and Drug Administration (FDA) in early 2007 and are respectively entitled "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events" and "Guidance for Clinical Investigators, Sponsors, and IRBs: Adverse Event Reporting – Improving Human Subject Protection."

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

According to Mayo IRB Policy any serious adverse event (SAE) which the Principal Investigator has determined to be a UPIRTSO would be reported to the Mayo IRB as soon as possible but no later than 5 working days after the investigator first learns of the problem/event.

The following information to collect when developing any forms for documentation of adverse events. Adverse events are considered events that are not already described as routine research risks in the consent form. We will not report pain at time of the biopsy as an AE, nor will we report bruising from biopsy or line placement procedures as these are nearly guaranteed to occur with all participants.
Information collected on the adverse event worksheet (*and entered in the research database)*:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)

- Date of Resolution:

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

The use of a table of tables to define Relationship of an Event to the Research and to define the severity of each event.

Relationship Index

The relationship of an AE to the Investigational Drug is a clinical decision by the sponsor-investigator (PI) based on all available information at the time of the completion of the CRF and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate that the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject' s clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other

drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfi1 this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

Severity Index

The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient's daily activities.

2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities but are usually ameliorated by simple therapeutic measures.

3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Unmasking/Unblinding Procedures

Not applicable to our study design.

8.5 Stopping Rules

This is unlikely as our study does not involve a treatment or interventional strategy and subjects are dismissed on the day of biopsies. However the entire study may be discontinued at the discretion of the PI based on the occurrence of the following:

- Adverse effects that seriously impact the risk-benefit ratio have been observed
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in recruitment of subjects
- Any new information becomes available during the trial that necessitates stopping the trial
- Other situations that may warrant stopping the trial

Subjects will be informed that they are free to withdraw from the study at any time for any reason. The Principal Investigator (PI) may remove a subject from the study if, in the PI's opinion, it is not in the best interest of the subject to continue the study. Subjects may be discontinued due to a change in compliance with an inclusion/exclusion criterion that is clinically relevant and affects subject safety, occurrence of AEs, occurrence of pregnancy, or administration of non-permitted concomitant medication that might affect subject safety or study assessments/objectives. Notification of discontinuation will be made immediately to the Sponsor's Medical Monitor. In case of premature discontinuation of study participation, efforts will be made to perform end of study/follow up assessments. The date the subject is withdrawn from the study and the reason for discontinuation will be recorded on the subject's Case Report Form (CRF). All withdrawn subjects will be followed until resolution of any AEs or until any unresolved AEs are judged by the PI to have stabilized.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to

all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Safety Review Plan –Study progress and safety will be reviewed monthly with the research fellow and study coordinator and, because these are relatively small studies, the data summarized at 6 month intervals to allow for a large enough sample to assess outcomes. Progress reports, including patient recruitment and AE's, if any occur, will be provided to the Endocrine Research Committee following each of the 6 month reviews if any studies have been done. An annual report will be compiled and will include a list and summarization of adverse events at the time of the non-competing renewal. In addition, the annual report will address (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study. The annual report will be reviewed by the Endocrine Research Committee if any studies have been done and the results included in the minutes. These will be forwarded to the IRB and NIDDK if applicable, the Mayo CTSA CRU and FDA as indicated.

8.6.1 Internal Data and Safety Monitoring Board

Designation of an Independent Monitor – The Endocrine Research Committee, composed of experienced investigators with appointments in the Division of Endocrinology, will serve as the independent monitor for the parts of the study. The PI will present relevant information to the committee at the indicated intervals and answer questions. The findings and recommendations of the committee will be included in the meeting minutes. If the PI presenting the data is the Chair of the Endocrine Research Committee another member of the committee will serve as chair for that meeting. The Endocrine Research Committee meets monthly. The nursing staff in the CRTU will also be involved with the monitoring of the respective protocols.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All missing data will be explained. All data requested on the CRF will be recorded in the study excel files. Clinical plasma data is maintained in the EMR. The Inclusion/exclusion sheet will serve as

the CRF for smoking status, and waist hip measurements and medications taken at time of screen. If the space on the CRF is left blank because the procedure was not done, or question was not answered it will be documented as “N/D”. If the item is not applicable to the individual case, it will be documented as “N/A” Errors will be identified by a strike-through and corrected data.

Data Management

Study data will be managed in a study specific Excel database.

Data Security and Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

As part of the screening for these studies a medical history and physical examination will be performed, blood tests and body composition measurements will be made. None of the research data will be entered into the Mayo Electronic Medical Record. Any paper records that are generated are kept in Dr. Jensen's laboratory, which is locked at all times to prevent hallway entry. Because it often takes several months from the initial volunteer contact until the studies are complete, and because we need to communicate with multiple units (Radiology, Nuclear Medicine, Mayo CTSA CRTU, etc.) over the course scheduling, it is not possible to de-identify paperwork immediately. The paperwork with patient identifiers is kept in a locked room when

not in use. Whenever possible the paper records generated will be such that once the study is complete the identifiers can be removed. A paper matching the subject name and other identifying information with the subject code should be kept in a separate place from the study information. No information will be given to anyone without permission from the subject. If samples are sent outside Mayo for analysis they are de-identified per Mayo Biospecimens Committee rules.

Data Quality Assurance

1) Description of Plan for Data Quality and Management: The PI will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. A statement reflecting the results of the review will be sent to the NIDDK in the annual report (non-competing continuation). Although there are additional reports to be produced by the study coordinator as a result of this DSMP, there are no substantive changes to the study protocol that might require review by the NIDDK.

2) Frequency of Review. The personnel and frequency of data review in these studies varies depending upon the source and type of data. The frequency of data review for this study is summarized in the following table.

Data type	**Frequency of review**	**Reviewer**
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Monthly	Study coordinator, research fellow and Principal Investigator
Adverse event rates (injuries)	Quarterly	Study coordinator, research fellow, Principal Investigator and Endocrine Research Committee as independent monitor
Out of range research laboratory data	As results become available	Research fellow and Principal Investigator*
Overview of data collection as regards statistical power implications of drop outs and missing data	Yearly	Research Fellow and Principal Investigator

* the research data generated by these studies is unique, entirely novel and requires profound expertise/experience to identify analytical, experimental or other research laboratory problems that may have arisen.

B. Subject Accrual and Compliance

1) Measurement and reporting of subject accrual, adherence to inclusion/exclusion criteria
Review of the rate of subject accrual, adherence to inclusion/exclusion criteria will occur during the weekly meetings Dr. Jensen has with the research fellow and/or study coordinator working on each protocol. In addition to these reviews Dr. Jensen will conduct an informal quarterly progress report with the fellow in charge of each protocol where more comprehensive overall progress is discussed and we determine whether changes need to be made to our recruitment strategy. Given that small numbers of volunteers are recruited for each protocol and some are linked to clinical patient care populations we will track whether overall participation in the 2 different projects combined include participants that meet our ethnic diversity goals.

2) Measurement and reporting of participant compliance to treatment protocol- the only compliance issues relate to volunteers returning for the second study.

Data Clarification Process

A report will be run in Excel to identify missing elements in the data capture. A designated study team member will make corrections in the data and the principle investigator will verify.

9.4 Records Retention

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted]

Whichever is longer

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

I. Study Identification Information

A. NIH Study Number – "RO1 DK45343"
B. Study Title – "Regional Fatty Acid Metabolism in Humans"
C. Name of Principal Investigator (PI) – Michael D. Jensen, M.D."

II. Study Overview

A. Brief Description of the Purpose of the Study – This proposal includes the study "Muscle lipid metabolism and insulin resistance following suppression of FFA in individuals with obesity and type 2 diabetes mellitus". This study includes the administration of isotopically labeled fatty acids, muscle biopsies, blood and breath sampling. The Data Safety Monitoring Plan (DSMP) for these studies involves monitoring for potential adverse events by the PI together with independent observers - the Mayo Clinical Research and Trials Unit nursing personnel; adverse events will be reported to the NIH and to the Mayo Clinic IRB.

B. Adherence Statement –The Data Safety Monitoring Plan (DSMP) outlined below for RO1 DK45343 will adhere to the protocols approved by the Mayo Clinic IRB.

III. Confidentiality

A. Protection of Subject Privacy – As part of the screening for these studies a medical history and physical examination will be performed, blood tests and body composition measurements will be made. None of the research data will be entered into the Mayo Electronic Medical Record. Any paper records that are generated are kept in Dr. Jensen's laboratory, which is locked at all times to prevent hallway entry. Because it often takes several months from the initial volunteer contact until the studies are complete, and because we need to communicate with multiple units (Radiology, Nuclear Medicine, Mayo CTSA CRTU, etc.) over the course scheduling, it is not possible to de-identify paperwork immediately. The paperwork with patient identifiers is kept in a locked room when not in use. Whenever possible the paper records generated will be such that once the study is complete the identifiers can be removed. A paper matching the subject name and other identifying information with the subject code should be kept in a separate place from the study information. No information will be given to anyone without permission from the subject. If samples are sent outside Mayo for analysis they are de-identified per Mayo Biospecimens Committee rules.

B. Database Protection – Electronic research data will be kept on password protected computer servers that can be accessed only by those with human studies/privacy training and only with Dr. Jensen's direct approval. Electronic communication with outside collaborators involves only unidentifiable information.

C. Confidentiality during AE Reporting –AE reports and annual summaries will not include participant-identifiable material. Each will include the identification code only.

IV. Adverse Event Information

A. Definition - An adverse event (AE) is any untoward medical occurrence in a subject temporally associated with participation in the clinical study. An adverse finding can

include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these.

A Serious Adverse Event (SAE) is any adverse event that results in one or more of the following outcomes:

- Death
- A life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- Important medical event based upon appropriate medical judgment

B. Classification of AE Severity – AE's will be labeled according to severity which is based on their impact on the volunteer. An AE will be termed 'mild' if it does not have a major impact on the participant, 'moderate' if it causes considerable inconvenience and 'severe' if it causes a substantial disruption to the volunteer's well-being.

C. AE Attribution Scale – AE's will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled either definitely, probably, possibly or unrelated to the study intervention.

D. Expected Risks –

Vascular access. Catheter insertion, intravenous infusion, and blood withdrawal are associated with small risks of phlebitis. This will be minimized by careful attention to sterile technique. As superficial phlebitis occurs, it will be treated conservatively with heat and, when appropriate, antibiotics. Superficial burns to the hand when using the hot hand technique to obtain arterialized venous samples are possible. This has not been a problem with the brief use of the heated box.

Muscle biopsies. The risks of these procedures include pain, hematomas, bruising, infection, and scarring of the biopsy site. In <1% of biopsies, a small area of numbness can develop due to cutting of a superficial sensory nerve. We advise volunteers that this can occur and make them aware that if it does, the re-growth of the nerve will take 6-12 months.

Risk Monitoring/Risk Reduction:

The risk of pain from muscle biopsies is virtually 100%, and the volunteers are given this information. We reduce the risk of infection by performing all biopsies under sterile conditions. We have yet to have an infection related to a muscle biopsy in the last 20 years. Nonetheless, each volunteer is told of the signs symptoms of infection and is told to call the study coordinator immediately should any of they occur. A record is kept of all adverse reactions to biopsies. If an increase in the number of adverse reactions relative to historical controls is observed during the course of a particular study a careful assessment of technique and approaches is made. The protocol might then be modified or additional instruction or supervision of the individual performing the biopsies could be undertaken. The subsequent results are then audited to determine how effective the intervention has been.

Radiation exposure. Dual energy x-ray absorptiometry and the CT scan result in small amounts of radiation exposure. The lowest dose that can be reliably results in good image quality for will be employed. In all instances the amount of radiation that a volunteer will receive is well below the level that results in a significant risk of harmful effects.

Infusion of substances (intravenously or subcutaneously) for studies. A number of substances are administered as part of the studies supported by this grant. These include the administration hormones, niacin, isotopic tracers and local anesthetics. We do expect to see flushing with niacin infusion. This would be managed by reassuring the subject that

it is self-resolving and causes no potential harm in the short or long-term and subjects will be advised to keep themselves hydrated to ease the symptoms.

Risk Monitoring/Risk Reduction:

1. In order to assure the safety of tracers administered to volunteers, a series of safety checkpoints are in place. This begins with the ordering of the tracer from the company. When compound is received the logbook is checked, recorded and initialed to assure that the substance ordered is the substance that is received.

2. If the compound is to be prepared for intravenous infusion, the substance is prepared in a Class 2 sterile hood by individuals who have been certified by the pharmacy at Mayo to be competent with respect to the proper procedures. Periodic competency tests are performed to assure continued sterile technique. If substances are to be made for single unit dosage administration and stored in the investigator's laboratory, the Class 3 pharmaceutical room at Rochester Methodist Hospital is used by a pharmacy technician and pharmacist trained and experienced in the procedures. All lots of tracers are tested for pyrogens and sent for cultures. No tracers are administered to volunteers until the results of the pyrogen testing and culture are available. The tracers are prepared for the final intravenous infusion in the Class 2 sterile hood also by individuals trained to pharmacy specifications in proper technique.

3. All tracers are administered in the Clinical Research and Trials Unit where comprehensive hospital facilities are available should a study participant have an allergic and/or untoward reaction to either tracers, hormones, anesthetics, or other substances.

4. For administration of anesthetics, care is taken to administer the minimum amount of lidocaine necessary in order to achieve adequate anesthesia. We are careful to limit the amount administered to much less than that which could cause cardiac arrhythmias.

The most prominent risks to each individual volunteer are pain and bruising related to vascular access and adipose biopsies. These are addressed in the protocol and consent form.

E. SAE Reporting

- SAEs that are unanticipated, serious, and/or possibly related to the study intervention will be reported to the independent monitors for each separate protocol as indicated above, the IRB, the Mayo CTSA Clinical Study and Trials Unit, NIDDK and other oversight organizations as appropriate.
- Anticipated or unrelated SAEs are reported to the IRB, the Mayo CTSA Clinical Study and Trials Unit and NIDDK in accordance with their requirements. As the incidence of pain and bruising with biopsies is 100% none of the oversight organizations or nursing staff has requested this information.

Because these studies do not involve interventions/treatments or follow up laboratory testing, we do not anticipate that abnormal lab values will need to be addressed as they would only be discovered at screening and would make the volunteer ineligible for the study.

V. Data Quality and Safety Review Plan and Monitoring

A. Data Quality and Management

1) Description of Plan for Data Quality and Management: The PI will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. A statement reflecting the results of the review will be sent to the NIDDK in the annual report (non-competing continuation). Although there are additional reports to be produced by the study coordinator as a result of this DSMP, there are no substantive changes to the study protocol that might require review by the NIDDK.

2) Frequency of Review. The personnel and frequency of data review in these studies varies depending upon the source and type of data. The frequency of data review for this study is summarized in the following table.

Data type	Frequency of review	Reviewer
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Quarterly	Study coordinator, research fellow and Principal Investigator
Adverse event rates (injuries)	Quarterly	Study coordinator, research fellow, Principal Investigator and Endocrine Research Committee as independent monitor
Out of range research laboratory data	As results become available	Research fellow and Principal Investigator*
Overview of data collection as regards statistical power implications of drop outs and missing data	Yearly	Research Fellow and Principal Investigator

* the research data generated by these studies is unique, entirely novel and requires profound expertise/experience to identify analytical, experimental or other research laboratory problems that may have arisen.

B. Subject Accrual and Compliance

1) Measurement and reporting of subject accrual, adherence to inclusion/exclusion criteria Review of the rate of subject accrual, adherence to inclusion/exclusion criteria will occur during the weekly meetings Dr. Jensen has with the research fellow and/or study coordinator working on each protocol. In addition to these reviews Dr. Jensen will conduct an informal quarterly progress report with the fellow in charge of each protocol where more comprehensive overall progress is discussed and we determine whether changes need to be made to our recruitment strategy. Given that small numbers of volunteers are recruited for each protocol and some are linked to clinical patient care populations we will track whether overall participation in the 2 different projects combined include participants that meet our ethnic diversity goals.

2) Measurement and reporting of participant compliance to treatment protocol – The weight loss intervention provided as part of these studies is purely voluntary and no compliance issues are anticipated.

C. Justification of Sample Size – We used published data regarding the improvement in an insulin sensitivity from a study that used Acipimox to determine that 10 subjects/group will be sufficient to detect a clinically meaningful change in insulin action with 90% power at a P of <0.05.

D. Stopping Rules - Because this is not a treatment studies per se, but instead one physiological measurements that take advantage of a clinical treatment protocol with its own monitoring, there is no stopping rule based on interim data analysis clearly demonstrates an effect. This study will be stopped prior to its completion if: (1) adverse effects that significantly impact the risk-benefit ratio have been observed; (2) study recruitment or retention becomes futile; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial.

E. Designation of an Independent Monitor – The Endocrine Research Committee, composed of experienced investigators with appointments in the Division of Endocrinology, will serve as the independent monitor for the parts of the study indicated in the table. The PI will present relevant information to the committee at the indicated intervals and answer questions. The findings and recommendations of the committee will be included in the meeting minutes. If the PI presenting the data is the Chair of the Endocrine Research Committee another member of the committee will serve as chair for that meeting. The Endocrine Research Committee meets monthly. The nursing staff in the Departments of Surgery, Nuclear Medicine and the CRTU will also be involved with the monitoring of the respective protocols.

F. Safety Review Plan –Study progress and safety will be reviewed monthly with the research fellow and study coordinator and, because these are relatively small studies, the data summarized at 6 month intervals to allow for a large enough sample to assess outcomes. Progress reports, including patient recruitment and AE's will be provided to the Endocrine Research Committee following each of the 6 month reviews. An annual report will be compiled and will include a list and summarization of adverse events at the time of the non-competing renewal. In addition, the annual report will address (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study. The annual report will be reviewed by the Endocrine Research Committee and the results included in the minutes. These will be forwarded to the IRB and NIDDK and, if applicable, the Mayo CTSA CRTU and FDA as indicated.

VI. Informed Consent

Written informed consent will be obtained from each subject at entry into the study. Informed consent is obtained by the following process:

- The subject will be asked to review the study consent form;
- The PI or an IRB approved individual authorized to obtain consent will meet with the subject to review the form, to confirm the subject's understanding of the study, and to answer any questions that the subject might have; and
- Once the subject demonstrates understanding of the study and agrees to participate in the study, the consent will be signed in the presence of the PI or an IRB approved individual authorized to obtain consent. The consent is entered into the Mayo electronic medical record.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

The study is financed through a grant from the US National Institute of Health.

12.2 Conflict of Interest

Not applicable

12.3 Subject Stipends or Payments

The remuneration will be $[redacted] per visit. Study compensation will be prorated if needed.

13 Publication Plan

The study team at Mayo Clinic holds the primary responsibility for publication of the results of the study.

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