Official Title: **Blood Lipopolysaccharide (LPS) Rifaximin Study**

Internal title: “Dietary fat, lipoprotein and lipopolysaccharide: role in insulin resistance”

ClinicalTrials.gov: NCT02124512

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Approach

Overall Hypothesis and rationale. Humans with obesity/IR have higher levels of postprandial LPS and this will be reduced by treatment with rifaximin. These changes may not be evident in a fasting blood sample because the LPS is carried on gut derived lipoproteins. The elevation of LPS will coincide with chylomicron TG elevation, and the AUC of LPS will depend on the height of the TG elevation, and the rapidity of clearance of TG-rich particles. A consequence of reduced plasma LPS will be improved insulin sensitivity and reduced adipose inflammation.

LPS has been measured in human plasma, but no study has examined postprandial LPS in depth. The IV infusion of LPS induces adipose inflammation and insulin resistance (25), but no studies have examined the consequence of gut-derived LPS. The LPS-LBP-TG-rich lipoprotein complex synergistically increases adipose SAA, and would be expected to result in an inflammatory stimulus to adipose tissue. We hypothesize that the chronic reduction in LPS-lipoproteins will result in less chronic inflammatory insult to adipose tissue in obese/IR subjects. This will be reflected in changes in tissue gene expression (decreased inflammation, possible changes in extracellular matrix (ECM)), and improved insulin sensitivity. Although there are several possible methods to alter the gut microbiota, the antibiotic rifaximin has been shown to reduce plasma LPS (2;3;28).

Recruitment of study subjects. We intend to recruit 40 non-diabetic subjects (sample size below) with obesity (BMI 30-40), ages 45-60, and evidence of MetS or IR. Each subject will undergo a fat tolerance test with both a low fat and high fat meal. We wish to avoid very obese subjects (BMI > 40) because of the large amount of lipid that will be consumed (see below). All subjects will initially have a standard 75g OGTT with measurement of glucose and insulin to characterize glucose tolerance and to obtain measures of insulin sensitivity and secretion using the Matsuda index (64;65). We will include subjects with IGT, IFG, as well as NGT subjects if they have at least 3 features of MetS (Table 1). Based on our considerable experience, essentially all of these obese subjects will be IR when the euglycemic clamp is performed. Exclusion criteria will include GI conditions (IBS, lactose intolerance) and the use of lipid lowering or antiinflammatory drugs. These subjects will have the following procedures performed at baseline and after drug/placebo treatment: OGTT, lipid tolerance tests, body composition (DEX), plasma inflammatory markers, adipose biopsy, insulin sensitivity, and gut microbiota assessment, as described below. Diet information will be recorded using a web-based software (9) at baseline, 4 and 8 weeks, and subjects will be asked to stay on their usual diet.

Table 1. Inclusion criteria	
Males & females, all ethnic groups, A1C<6.5	
BMI	30-40
Age	45-60
OGTT; features of MetS*	IGT, IFG, or NGT +3

*IGT: FBS< 126 mg/dl, 2 hr: 140-199; IFG: FBS 101-125, 2 hr ≤199; MetS: TG>150 mg/dl, HDL<40 (M) or <50 (F), BP>130/85 or on treatment, Waist >40" (M) or >35" (F) ; NGT: normal glu tolerance

Specific Aim 1. We will determine whether an alteration in the gut microbiota achieved with rifaximin will decrease circulating LPS. We will recruit obese human subjects with MetS or impaired glucose tolerance. An oral fat tolerance test will be performed at two different fat doses with measurement of plasma chylomicron-associated LPS and LBP, and measurement of LPS and LBP in the other lipoprotein fractions (using Westerns for LBP). Subjects will then be treated with the antibiotic rifaximin for 8 weeks to alter the gut microbiota. The lipid tolerance tests will be repeated to determine whether the antibiotic treatment reduces post-prandial LPS and alters the distribution of LPS in lipoproteins.

All subjects will undergo a fat tolerance test on two different days, in random order, with two different amounts of lipid in the meal, as described above in preliminary data. From the blood samples, we will measure the level of LPS and LBP in plasma, as well as the LPS and LBP in each lipoprotein fraction to determine whether more dietary lipid leads to a higher level of LPS in plasma. We will also determine whether LPS-lipoprotein levels change in a dynamic fashion following the meal. For example, if LPS remains associated with TG-rich lipoproteins, then the LPS will likely be cleared through lipolysis in adipose, and then the remaining remnant-LPS cleared by the liver, and liver insulin resistance may be an important effect of lipoprotein-LPS. However, if LPS is transferred to HDL, this could be due to the LBP content of HDL, and could represent another important function for HDL (i.e. clearance of LPS).

Subjects will then begin taking rifaximin 550 mg BID or placebo for 8 weeks. Rifaximin is poorly absorbed from the gut, has been shown to decrease plasma LPS in several studies (2;3;28), and is used acutely for traveler's diarrhea, and chronically for treatment of diverticulitis, hepatic encephalopathy, bacterial overgrowth, and irritable bowel syndrome (66;67). In clinical trials, the side effects of rifaximin are generally indistinguishable from placebo, and there is no evidence for weight loss or nutrient malabsorption (68) (see human subjects). After 8 weeks of rifaximin, all subjects will repeat the lipid tolerance tests (low- and high fat).

To verify the change in gut microbiota, the major classes of gut bacteria will be assayed from a stool sample before and after rifaximin treatment using nexgen targeted sequencing of 16S RNA, as described (69).

Specific Aim 2. We will determine whether a change in the gut microbiota from rifaximin treatment will decrease adipose inflammation and improve insulin resistance. In this aim we will determine whether the changes in gut microbiota and plasma LPS induced by rifaximin will affect adipose tissue inflammation and insulin sensitivity. After treatment with the antibiotic rifaximin for 8 weeks, the insulin sensitivity testing and adipose biopsies will be repeated to determine whether the antibiotic treatment changes inflammation and insulin sensitivity.

The recruitment of insulin resistant, non-diabetic subjects is described above. In addition to the lipid tolerance tests and OGTTs, these subjects will have an adipose biopsy (SQ, abdominal) and a euglycemic clamp. We routinely perform adipose biopsies, and we obtain enough fat for immunohistochemistry, RNA and protein assays. Fasting plasma will be collected pre- and post rifaximin treatment for measurement of plasma inflammatory markers (TNFα, IL-6, MCP-1, adiponectin, PAI-1, CRP, etc).

Peripheral and hepatic insulin sensitivity will be measured with a euglycemic clamp. For this study, we will use a two-step clamp, with an initial low-dose insulin infusion to measure hepatic insulin sensitivity, followed by a higher dose insulin infusion to assess near maximal peripheral glucose disposal, as described previously (70;71). Subjects will report fasting to the CRC, IV's started (including a retrograde IV in a warming box) and a priming dose of 3 mg/kg D-[6,6-$^2H_2$]glucose will be injected 120 min prior to the start of the insulin infusion followed by a continuous infusion at 0.05mg/kg/min for the duration of the study. A primed insulin infusion (0.25 mU/kg/min) is then begun and continued for 120 min. The blood glucose is allowed to fall during this period until it reaches 90 mg/dl, and blood glucose is then maintained by infusion of 20% dextrose. Following the low dose infusion, a re-primed insulin infusion at 1 mU/kg/min begins and is continued for 2 hr. Blood glucose is measured every 5 min, and blood for insulin and [$^2H_2$]glucose taken every 10 min. Glucose disposal is determined during steady state glucose during the final 30 min of the insulin infusions. This procedure will measure basal hepatic glucose production, suppression at the two glucose concentrations, as well as glucose disposal rate at the two different insulin infusion rates. To assess adipose insulin sensitivity, we will assess lipolysis *in vivo* by measuring plasma FFA levels in relation to fasting insulin, as described recently (17), and also by examining the ability of insulin to suppress catecholamine mediated lipolysis in adipocytes from the fat biopsies, as described previously (18;19).

Anticipated Outcomes. We hypothesize that a change in the microbial flora with rifaximin will reduce chylomicron-associated LPS, and this in turn will reduce adipose tissue inflammation, which in may lead to improved insulin sensitivity. Therefore, we will examine, before and after rifaximin/placebo treatment: 1. LPS associated with lipoproteins, especially TG-rich lipoproteins, during the HF and LF meals, 2. insulin sensitivity and hepatic glucose production, 3. plasma inflammatory markers (TNFα, IL-6, MCP-1, adiponectin, PAI-1, CRP, etc), 4. adipose inflammatory markers (CD68, MCP1, TNFα, PAI1, SAA, IL12, IL10, TLR4 and others), 5. adipose tissue ECM/collagen markers (TSP1, CTGF, collagen VI, collagen V, elastin, and others), 6. macrophage number and polarization (markers of M1 vs M2) by immunohistochemistry; 7. number of capillaries and large vessels by immunohistochemistry.

Changes in plasma and adipose inflammatory markers, ECM, macrophage polarization, and vascularity are all features of IR adipose tissue, and we have published many papers that describe these methods (21;30;31;35;72;73). The reduction in adipose inflammation may be reflected in fewer macrophages, a shift in macrophage polarization (eg. M1 to M2), a decrease in crown-like structures, a decrease in gene expression of inflammatory cytokines (eg. TNFα, IL-6, SAA, etc) and a decreased expression of collagen VI. Other immunohistochemical findings may include increases in elastin, decreased collagen V, and increases in capillary number, as suggested by our previous studies (30). From our adipose biopsies, we obtain enough tissue for Western blots, which is important since many genes of interest are not regulated at the mRNA level. Based on a previous study (37) showing alteration of fasting-induced adipose factor (Fiaf), a LpL inhibitor by germ free conditions, we will measure LpL activity and mRNA in adipose of these subjects. The clamp will include an assessment of hepatic insulin sensitivity, which is important because the liver is important to the clearance of gut-derived remnant lipoproteins and may be an important target of LPS.

Potential experimental problems. Although previous studies in rodents observed decreases in adipose inflammation following antibiotic treatment (44), it is possible that rifaximin treatment may have no effect on any of these metabolic features, or perhaps an effect may require longer treatment. We will perform an interim analysis of our data and change the time period if warranted. Although previous studies with rifaximin are encouraging (1-3;28), this drug will not eliminate all bacteria, and LPS may still be present in the intestine or may come from sources that are not impacted by the antibiotic, such as from food. Finally, this protocol is

focused on LPS, but this is an emerging field and secondary changes may occur. Changes in the gut microbiota are associated with other hormonal and metabolic effects (74;75); e.g. alterations in the ratio of Bifidobacteria/firmicutes could impact insulin sensitivity through mechanisms other than LPS. In humans, diet can alter the gut microbiota and this can be associated with small increases in nutrient loss (40), in part dependent on obesity and excess calories. Rifaximin will alter the gut microbiota, but no weight loss or malabsorption symptoms are reported in clinical trials (actually there is weight gain and less diarrhea when the drug is used for colitis or bacterial overgrowth). There could be changes in incretins. This can be assessed by examining the insulin response to the OGTT, which is dependent on GLP1 and GIP. If we observe changes in the insulin response or glucose tolerance, we will measure GLP1 levels in the OGTT samples.

We believe that this trial will significantly impact the field of "metabolic endotoxemia" (76). The literature is full of studies suggesting that the gut flora are important in exacerbating inflammatory pathways. The rodent data are exciting, but there are no intervention or mechanistic studies in humans to indicate whether this is relevant or significant. Therefore, this study will help investigators translate important rodent data into humans.

Sample size. An important outcome will be the measurement of LPS in plasma and lipoprotein fractions from the low-fat and the high-fat meals, and we will examine the peak LPS levels, as well as area under the curve. In a previous study in lean subjects, baseline LPS was 0.39± 0.07 EU/ml and increased to 0.58 EU/ml with a fatty meal (53). Assuming that SD of the group and SD of change is 0.1 and an effect size of 1.0, we will need 10 subjects to detect a change of 0.1 EU/ml between a low-fat and high fat meal (paired T-test) and 17 subjects to detect the same difference between groups (drug vs placebo), with α of 0.05 and 80% power. Our preliminary data support the effect size described. To estimate the sample size needed to observe changes in adipose tissue inflammation, we will target adipose tissue macrophages. In previous studies of obese/IR subjects, macrophage number was 34±5.8/mm^2 (31). Assuming the SD of change is equal to the SD 5.8 in these obese subjects, 20 subjects in each group will allow us to detect a change in macrophage number of 4/mm^2 with 80% power using a paired t-test. For comparisons between groups, if we assume an effect size of 1.0, we will need to recruit 17 subjects in each group. In previous interventional studies, we detected changes in macrophage number in adipose in response to fish oil and pioglitazone treatment (35;77). These sample size calculations project adequate power with a total of 40 subjects (20 in each group) who complete the whole protocol. We recognize that we are asking a lot of these subjects, however we have considerable experience performing similar in-depth studies. We anticipate a higher drop-out rate than usual, and we estimate that we will need to recruit 60 subjects to obtain 40 that complete the protocol.

Protection of Human Subjects.

1. Risks to the Subjects

a. Human Subjects Involvement and Characteristics

The subjects recruited for this study will be between the ages of 45 and 60. All subjects will be obese and have evidence of insulin resistance or metabolic syndrome (see inclusion criteria, above) and with a BMI between 30-40. Women will be post-menopausal. Exclusion criteria will include any unstable medical condition (recent or unstable cardiovascular disease), cancer, renal insufficiency GFR<30), use of steroids, chronic inflammatory conditions, use of anticoagulants, and lipodystrophy. Subject who may not tolerate the lipid tolerance tests because of GI conditions will be excluded, along with subjects who take medications that could interfere with the studies, such as lipid lowering drugs, or antiinflammatory drugs. Other medical conditions will be evaluated by the study physicians.

This study involves a standard oral glucose tolerance test, a euglycemic clamp to measure insulin sensitivity, and a lipid tolerance test. Subjects will consume a low fat meal (Subway sandwich or equivalent) and unlimited vegetables and fruit the evening before the study, fast for 12 hr, and refrain from alcohol. The next morning, the subjects will consume either a high-fat or low-fat breakfast shake, consisting of Boost with added cream and corn oil in a fixed proportion to provide a mix of fatty acids. Daily energy requirement will be calculated using the Harris-Benedict equation, and the high fat shake will encompass 40% of their daily energy requirements, and will be 50% fat. The low fat shake will use the same formula, but will be 25% fat, through the addition of less cream and corn oil, so the amount of CHO and protein in each shake will be very similar. Blood samples for lipoprotein isolation will be obtained prior to the meal, and hourly for 8 hr after the meal. These studies will take place on the CRC. Subjects will be able to walk around, watch TV, and drink water during this time.

This study involves fat biopsies from subjects under local anesthesia. Because it is difficult to obtain enough adipose tissue from a needle biopsy, incisional fat biopsies will be necessary. Dr. Kern has performed hundreds of such biopsies on outpatients undergoing studies on the effects of feeding, obesity, and diabetes, and insulin sensitizer drugs, and the complication rate has been very low, and patient acceptance of these procedures has been excellent. The biopsies will be performed under local anesthesia by the principal investigator or his designate (eg, a well-trained physician assistant). No patient will have a biopsy if it appears to represent an unacceptable risk, such as platelets <75,000, evidence of moderate platelet dysfunction, chronic aspirin use, or hematocrit < 30. In addition, we will measure body composition by DEXA.

We will recruit subjects without regard to ethnicity, and will study no vulnerable populations.

Intervention. After the above procedures, subjects will then begin taking rifaximin in a dose of 550 mg BID. This antibiotic is poorly absorbed from the gut, and is used routinely for traveler's diarrhea, and for prolonged periods for treatment of diverticulitis, hepatic encephalopathy, bacterial overgrowth, and irritable bowel syndrome (66;67). After 8 weeks of treatment with rifaximin, all subjects will repeat all the procedures above, including clamp, OGTT, lipid tolerance tests (low fat and high fat) and fat biopsies.

Compliance. It will be critical to retain patients in this program, and we will only select patients that are highly motivated. We have projected the need to recruit 60 subjects to reach 40 complete studies, for a potential dropout rate of 33%. To improve retention, the PIs will interact individually with the patients, and we will make maximal use of one-on-one instruction and education, and frequent contact with participants, in addition to small financial incentives. In the past, our drop out rate has been about 10%, once the subject passes the initial screening procedures. This is low, but it reflects the fact that we are experienced at recruiting and motivating subjects. In addition, subjects must be willing to go through a number of initial procedures, so casual study subjects do not usually enroll.

b. Sources of Materials

Adipose tissue, and blood samples will be obtained from subjects, and these tissues will be used exclusively for research purposes. From a blood sample, DNA will be prepared. Under no circumstances will the patients' clinical care be compromised.

c. Potential Risks

Risks of the biopsies include bleeding, infection, and pain and patients are told of this. Dr. Kern is very experienced in this procedure and has an excellent track record. Rarely (<1%) have oral antibiotics been needed following a biopsy.

The lipid tolerance test involves the consumption of a liquid meal containing corn oil and cream, followed by hourly blood draws. Some subjects may have mild GI symptoms, but this will likely be minimal. Of the subjects studied to date, none have had GI symptoms, and all consumed their beverage within 10 min.

The clamp involves frequent blood draws and indwelling catheters. There is some needlestick discomfort, and there is a risk of hypoglycemia, although blood glucose is monitored carefully.

Antibiotic treatment with rifaximin. In clinical studies of rifaximin, the side effects are very low, usually similar to that of the placebo treated group. In studies involving the use of rifaximin for travelers diarrhea, the only side effect of rifaximin that was higher than placebo was flatulence (20% vs 11% in placebo) (Xifaxan® package insert: http://www.salix.com/assets/pdf/prescribe_info/xifaxanpi.pdf). In the package insert, post-marketing experience states that *C. difficile* associated diarrhea has been reported in patients taking rifaximin, and the "Warnings and precautions" statement states that "*C. difficile* diarrhea has been reported with rifaximin as it has been reported with all other antibiotics". Notwithstanding these isolated reports of *C. difficile*, this complication has not been a problem with the use of rifaximin in clinical trials. For example, in the TARGET study, 625 subjects with IBS were randomized to rifaximin, and there were no cases of *C. difficile* (67). Indeed rifaximin is used to treat *C. difficile* diarrhea, including metronidazole-resistant *C. difficile*, and to prevent recurrence (78;79).

To minimize the risk of *C. difficile* associated disease, we will warn subjects of the risk, and we will educate them on the symptoms and monitor their symptoms. Although anyone can get occasional diarrhea, subjects will be told to call us immediately if they have persistent watery stools of >4x/day for 3 days, or any bloody stools, especially if this is associated with systemic symptoms of fever and abdominal pain. Drs. Paul Angulo and Terrance Barrett, who are gastroenterologists at UK, are consultants on this proposal, and will be involved with the care of these subjects.

The alternatives to this study are to not participate. The care of the patient will never be compromised by non-participation.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Patients will be compensated $700 for completion of the study (including both sets of biopsies and the FSIVGTT and lipid tolerance tests), and we will explain the potential risks and benefits, and the patient will be asked to sign a consent form. This protocol and a consent form will undergo thorough review by our institutional IRB.

Consent for this study will be obtained either by Dr. Kern or his designate (coordinator) on the CRC, where the witness for the consent is usually a CRC nurse. Patients will be informed of the voluntary nature of the study, and that their clinical care will not be compromised. A consent form will be approved by the institutional IRB.

b. Protection Against Risk

All procedures will be performed on the CRC of the Center for Clinical and Translational Sciences, a funded CTSA, using skilled nursing, a hospital environment, and sterile procedures. Standard surgical practices ensure safety to the patients. No provision is made to compensate patients for any research related injury, and this is stated in the consent form.

To protect confidentiality, all subjects are assigned a unique number, and this number is used for labeling of all samples and the identification of all laboratory material obtained from subjects, and there is no public release of the name of the subject from whom the material was derived. All data are kept in a password protected computer file (eg. Redcap) or in locked filing cabinets.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Subjects will have an oral glucose tolerance test, which will determine whether the subject has diabetes or impaired glucose tolerance. Routine blood tests of blood count, liver enzymes, cholesterol will be shared with

the patient. All subjects will be informed that there will be no likely benefit to their health from this study. The knowledge gained from these studies may have importance to the subject. However there is no direct benefit from the biopsies and the subject is so informed. The subject is compensated $700 for his/her participation.

4. Importance of the Knowledge to Be Gained

Diabetes, obesity, insulin resistance, and metabolic syndrome are diseases that involve over 1/3 of the American population, resulting in considerable risk of severe medical problems, including hypertension, dyslipidemia, coronary artery disease, and premature death. Subjects with IGT have a very high likelihood of progression to type 2 diabetes. .

Data & Safety Monitoring Plan

All research personnel who work with subjects or subject data or subjects' research samples in this project will have completed training in the protection of human research participants per guidelines issued by the OHRP. This protocol will receive final approval by the institutional IRB.

This protocol will be continuously monitored in real-time by the principal investigators and study coordinator for adverse events (AEs). The research coordinator will contact subjects within 48 hours of each procedure to assess for pain, infection, and other symptoms indicating possible post-procedure complications. Subjects are discharged from the CRC with specific self-monitoring guidelines and instructed to call immediately for any concerning signs or symptoms.

AEs will be graded according to intensity. Mild: Discomfort noticed but no disruption of normal daily activity. Moderate: Discomfort sufficient to reduce or affect normal daily activity. Severe: Incapacitating with inability to work or perform normal daily activity.

The attribution scale for AE reporting will be as follows. Probable: AE is related to the procedure (e.g. infection from a fat biopsy). Possible: AE follows the procedure within a reasonable period (within 7 days), but may have been produced by the subject's clinical state or other factors (e.g. rash 3 days following the clamp). Remote: AE does not follow the procedure or drug within a reasonable period and could readily have been produced by the subject's clinical state or other factors. Unrelated: AE is judged to be clearly due to extraneous causes and does not meet the above criteria.

Plan for unexpected Adverse event (AE) reporting. Serious AEs will be reported to Human Subjects/IRB within 48 hr, and will also be reported to the CR-DOC. Unanticipated events will be reported to the CR-DOC real time, and to the IRB no later than 15 days after the event. Annual reporting of adverse events will be conducted with the Human Subjects annual review/renewal according to their guidelines.

Monitoring of adverse events. Adverse events will be monitored via exams, vital signs, lab tests, review of subject's medical chart, review of subject diaries, etc. and documented. Signs of infection from the biopsies will be monitored by exams. Each visit will be documented with a progress note in the CR-DOC chart. A data safety and monitoring board (DSMB) will oversee the safety of this project. The University of Kentucky CTSA has a Research Participant Advocate who chairs a DSMB, and this DSMB will perform a periodic review of this protocol. No provision is made to compensate patients for any research related injury, and this is stated in the consent form.

Inclusion of Women and Minorities

Subjects and patients will be recruited without regard to sex, race, or ethnic status. All of the patients who enter the study will either be normal subjects, or will be obese or have metabolic syndrome, which is commonly found in obese subjects. Obesity and metabolic syndrome are a common findings in both genders and all ethnic groups, and therefore it is likely that the final make up of subjects in this study will be generally reflective of the population of Northern Kentucky. It is likely that approximately 10% of the subjects recruited will be African-American, based on the local population. The Hispanic, Asian, and Native American population of Kentucky is <1% for each group, although the Hispanic population is increasing rapidly, and the high rate of diabetes and metabolic syndrome in this population may result in a higher representation in this study.

Targeted/Planned Enrollment Table

Total Planned Enrollment: 60

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	29	29	58
Ethnic Category Total of All Subjects*	30	30	60
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	4	4	8
White	26	26	52
Racial Categories: Total of All Subjects*	30	30	60

*The "Ethnic Category Total of All Subjects" must be equal to the "Racial Categories Total of All Subjects."

As described in the research plan, we will screen subjects for IGT and other features of metabolic syndrome. There are exclusion criteria, enumerated above, so some subjects will sign the consent form but then will be excluded. We anticipate that approximately half of the obese subjects will be excluded, usually because they do not have IGT, or do not have features of metabolic syndrome, or have other exclusion criteria. Therefore, we estimate that we will need to recruit about 120 subjects to recruit the 60 subjects needed to start the study. However, the demographics on the 120 subjects will likely be the same as the 60 subjects described above.

Inclusion of Children

Childhood obesity and the resulting insulin resistance are also epidemic and a serious health concern. However, based on recent ruling from the Office of Human Research Protection, the studies proposed herein would be considered too invasive to be appropriate for young children. In addition, we could not properly match the BMIs of children with adults because of the different scales. Although obese children may have IGT, it is much less common than in adults, unless one involves very obese children. Finally, we do not wish to recruit young adults or older adolescents because of the importance of minimizing age-related differences in our subject groups. Because IGT is much less common in young people, this study will concentrated on subjects between the ages of 30 and 60 of both genders and all ethnic groups.

STATISTICAL ANALYSIS PLAN

An important outcome will be the measurement of LPS in plasma and lipoprotein fractions from the low-fat and the high-fat meals, and we will examine the peak LPS levels, as well as area under the curve. In a previous study in lean subjects, baseline LPS was 0.39± 0.07 EU/ml and increased to 0.58 EU/ml with a fatty meal (53). Assuming that SD of the group and SD of change is 0.1 and an effect size of 1.0, we will need 10 subjects to detect a change of 0.1 EU/ml between a low-fat and high fat meal (paired T-test) and 17 subjects to detect the same difference between groups (drug vs placebo), with α of 0.05 and 80% power. Our preliminary data support the effect size described. To estimate the sample size needed to observe changes in adipose tissue inflammation, we will target adipose tissue macrophages. In previous studies of obese/IR subjects, macrophage number was 34±5.8/mm^2 (31). Assuming the SD of change is equal to the SD 5.8 in these obese subjects, 20 subjects in each group will allow us to detect a change in macrophage number of 4/mm^2 with 80% power using a paired t-test. For comparisons between groups, if we assume an effect size of 1.0, we will need to recruit 17 subjects in each group. In previous interventional studies, we detected changes in macrophage number in adipose in response to fish oil and pioglitazone treatment (35;77). These sample size calculations project adequate power with a total of 40 subjects (20 in each group) who complete the whole protocol. We recognize that we are asking a lot of these subjects, however we have considerable experience performing similar in-depth studies. We anticipate a higher drop-out rate than usual, and we estimate that we will need to recruit 60 subjects to obtain 40 that complete the protocol.