Sympathetic Mechanisms in the Cardiovascular and Metabolic Alterations of Obesity

Principal Investigator:
Italo Biaggioni, M.D., Professor

Co-Investigators:

Amr Wahba, M.D.,
Research Fellow
Division of Clinical Pharmacology
Department of Medicine

Alfredo Gamboa, M.D., M.S.C.I.,
Research Associate Professor
Division of Clinical Pharmacology
Department of Medicine

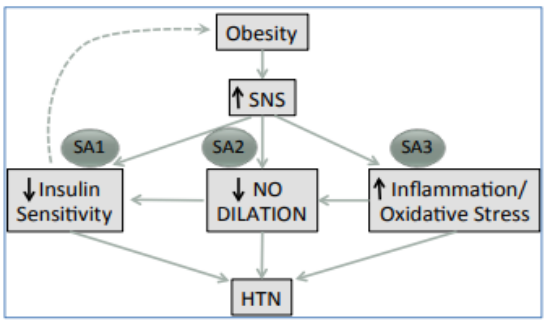
William D. Dupont, PhD.,
Research Professor and Vice-Chair
Department of Biostatistics

Vanderbilt University School of Medicine

Table of Contents:

1.0 Background

As the prevalence of obesity continues to increase, its associated conditions hypertension and diabetes) will contribute to greater health care costs and shortened life expectancy. More than 78 million adults in the U.S. are obese. Medical costs associated with obesity are estimated to be $210 billion annually. Current hypertension treatment guidelines emphasize the need to lose weight, but do not otherwise address the treatment of obesity hypertension. Given the low success rate of long-term weight loss we cannot currently rely on this approach to treat obesity hypertension. Evidence from the literature and our studies indicate that sympathetic activation accompanies obesity and contributes to insulin resistance and endothelial dysfunction. However, first line treatments recommended for hypertension often result in further increases in sympathetic activity and worsening of insulin resistance.



Obesity and insulin resistance. Obesity is associated with decreased responsiveness to insulin (insulin resistance) in the liver, skeletal muscle and the vasculature. The liver is a major source of endogenous glucose production (EGP). Suppression of EGP by insulin reduces blood glucose, but this process is impaired in obesity (hepatic insulin resistance). In the muscle, insulin promotes glucose uptake and this process is also impaired in obesity. In addition, impaired insulin-induced vasodilation, can contribute to insulin resistance by reducing the delivery of substrate (glucose) available for tissue uptake.

Obesity and sympathetic activation. Obesity is widely accepted to be associated with increased sympathetic nerve activity, and this sympathetic activation is preferential to vascular beds that regulate metabolism and blood pressure. For example, muscle sympathetic nerve activity (MSNA), which is tightly coupled to baroreflex regulation of blood pressure, is not only increased in obesity but this increase correlates with body mass index (BMI), body fat, and particularly with visceral fat. Multiple mechanisms explain obesity-associated sympathetic activation, including increased leptin and decreased adiponectin; insulin itself can induce sympathetic activation directly (by acting in CNS centers that increase sympathetic outflow), and reflexively (by inducing peripheral vasodilation with unloading of baroreflexes). (Figure 1)

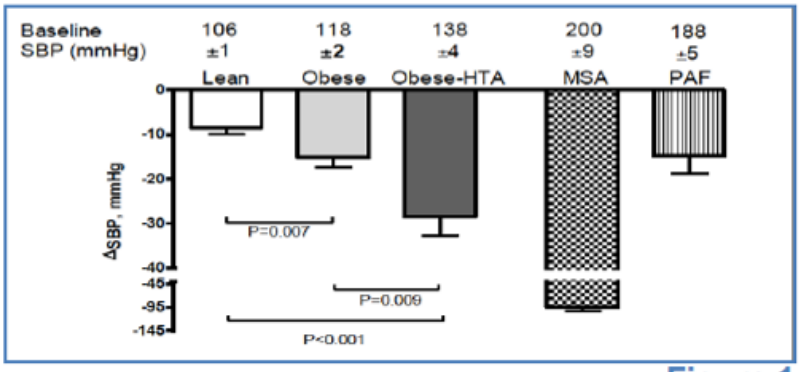


Figure 1

Sympathetic activation and insulin resistance. Evidence from the literature and our laboratory indicates that sympathetic activation contributes to metabolic and vascular insulin resistance. Even physiological sympathetic activation (induced by lower body negative pressure, LBNP) acutely impairs glucose uptake. Conversely, we have shown that autonomic withdrawal improves insulin sensitivity of glucose uptake. Sympathetic activation of hepatic nerves promotes hepatic glucose production and can contribute to resistance to the normal suppression by insulin of hepatic glucose production. We will test this hypothesis in Specific Aim 1.

Sympathetic activation with LBNP also acutely impairs flow-mediated dilation (1) and blunts vasodilation to acetylcholine. Conversely, our preliminary results suggest that autonomic withdrawal reverses "endothelial dysfunction" in obese hypertensives. The relevance of this finding is that this impaired insulin vasodilation can contribute to metabolic resistance (impaired glucose uptake) by reducing glucose availability. Current evidence suggests that the increase in vascular glucose delivery by insulin is mediated mostly by opening new vascular beds (microvascular recruitment). These observations raise the possibility that removal of sympathetic vasoconstriction can result in improvement of insulin-mediated microvascular recruitment and subsequently of sensitivity of insulin-mediated glucose uptake. We will test this hypothesis in Specific Aim 2.

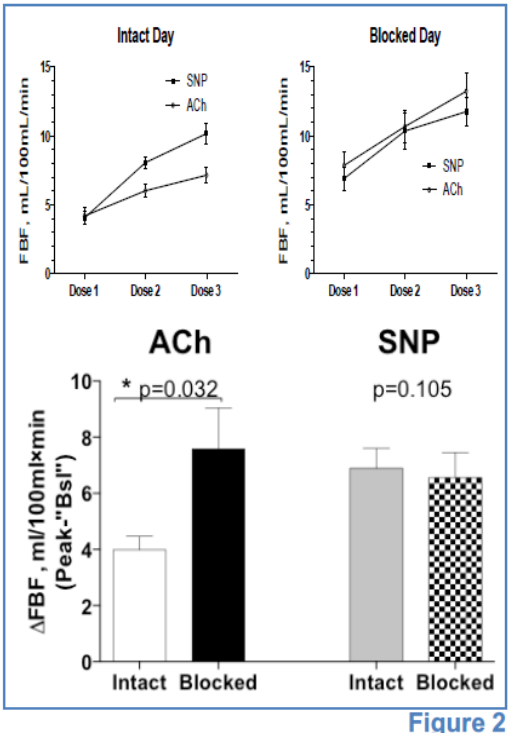


Figure 2

Significance and Scientific Premise. The evidence presented above supports the existence of a negative feedback loop in obesity (insulin resistance, increased levels of insulin, leading to sympathetic activation and promoting further insulin resistance) that can be intervened therapeutically. We propose a mechanistic study that will test the hypothesis that sympathetic inhibition provides a unique benefit compared to calcium channel blockade by improving vascular and metabolic insulin sensitivity and reducing inflammation. We hope our results will challenge current guidelines in the treatment of hypertension.

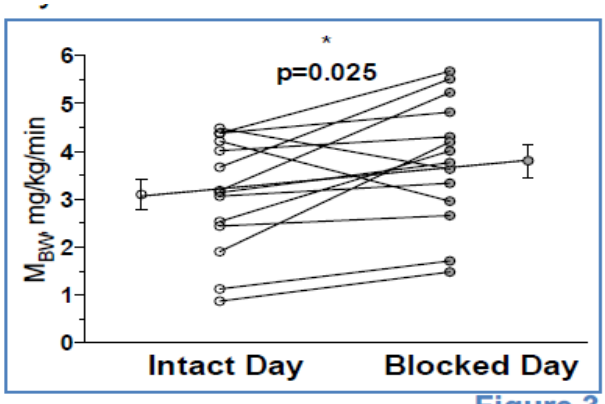


Figure 3

2.0 Rationale and Specific Aims

Current treatment guidelines for hypertension are based on outcome trials that enrolled patients likely to have cardiovascular events during the duration of the trial. They recruited elderly subjects (average age 65 years) who were not obese (average BMI 28). Thus, the data obtained from these studies may not be applicable or reflect the cardiovascular disease evolution in the case of young (15-20 years younger) obese (5 BMI points higher) Our preliminary data suggest that sympathetic activation contributes to obesity hypertension, which is not directly targeted by drugs commonly used to treat hypertension. This is the impetus for this proof-of-concept study comparing the metabolic effects of the central sympatholytic moxonidine and the calcium channel blocker amlodipine, at doses titrated to produce equivalent decreases in blood pressure.

Moxonidine was chosen because it is an imidazoline agonist devoid of the CNS side effects characteristic of older sympatholytic drugs. Amlodipine is one of the most widely used antihypertensives with mostly neutral metabolic effects ("negative" control). A more detailed discussion of drug selection is included below. We hypothesize that, at equipotent antihypertensive doses, moxonidine will be superior to amlodipine in improving insulin resistance (Specific Aim 1) insulin-mediated vasodilation and microvascular recruitment (Specific Aim 2), and obesity associated inflammation and oxidative stress (Specific Aim 3). These three specific aims will be explored within a single clinical study (Figure 6).

Specific Aim 1. To test the hypothesis that sympathetic activation contributes to insulin resistance in obesity hypertension. We propose a two-step hyperinsulinemic euglycemic clamp with stables isotopes to determine the effect of sympathetic activation on insulin-mediated**:**

1a. Suppression of Hepatic Glucose Production
1b. Increase in Glucose Uptake
Our preliminary studies showed that acute autonomic blockade improved insulin resistance (Figure 3). In those studies, we used an acute dose of the ganglionic blocker trimethaphan, and a dose of insulin that suppresses endogenous glucose production (EGP) so we could selectively measure insulin resistance to glucose uptake. Animal studies suggest that sympathetic tone plays an even a greater role in regulating EGP by the liver (2). However, to our knowledge the magnitude of this effect in humans, and in particular in obesity hypertension, has not been assessed. Therefore, we now propose to determine the role of the autonomic nervous system in insulin resistance at baseline, and after central sympathetic inhibition with moxonidine vs. treatment with amlodipine (Figure 6). Subjects will be studied at baseline and during treatment using a two-step hyperinsulinemic euglycemic clamp (Figure 7): first using a low dose insulin infusion, coupled with stable isotopes (glucose tracer) to assess insulin sensitivity of suppression of endogenous glucose production (EGP, Specific Aim 1a), followed by a higher dose of insulin that suppresses EGP, to electively assess glucose uptake (Specific Aim 1b).

Specific aim 2. To test the hypothesis that sympathetic activation contributes to impaired vasodilation in obesity hypertension.
There is significant evidence that obese hypertensive subjects have impaired NO-mediated dilation, and our group confirmed this abnormality in resistance vessels (brachial artery dilation to acetylcholine). In addition, we showed that autonomic

blockade reverses this impairment, indicating that a significant component of impaired NO-dilation is secondary to sympathetic activation, at least in initial stages of obesity hypertension (3).Considering that insulin vasodilation is NO-mediated, sympathetic blockade may improve this mechanism blunted in insulin resistant states. Current evidence suggests that the ability of insulin to increase the number of perfused capillaries (microvascular recruitment) is important for stimulating glucose uptake, by enhancing its delivery to muscle cells (4-6). Therefore, insulin ability to stimulate microvascular recruitment, via NO-mediated dilation, is an important component of insulin sensitivity of glucose uptake (7).

Our previous findings suggest that sympathetic activation contributes to impairments in both NO-mediated dilation (Figure 2) and insulin mediated glucose uptake (Figure 3). Therefore, we propose to test the hypothesis that sympathetic inhibition with moxonidine improves insulin-stimulated microvascular recruitment. To assess this objective, we will use the gold-standard hyperinsulinemic euglycemic clamp combined with contrast-enhanced ultrasonography to address insulin sensitivity and insulin-stimulated microvascular recruitment. To compare the effect of this trial with "selective" blunting of sympathetic activity, we propose to use the vasodilator amlodipine as a control due to its neutral effect on insulin sensitivity.

Specific Aim 3. To test the hypothesis that sympathetic activation contributes to inflammation and oxidative stress in obesity hypertension

Inflammation is increasingly recognized as an important pathophysiological mechanism in hypertension (8). Of relevance to this project, these inflammatory processes are driven by an increase in sympathetic outflow (8, 9). In animal models CNS lesions that interrupt sympathetic pathways prevent angiotensin II-induced inflammation (10), and conversely, activation of sympathetic outflow by a stress paradigm promotes vascular inflammation (11). Furthermore, bilateral renal denervation significantly reduces inflammation and blunts angiotensin II-induced hypertension in animal models (12). Unilateral renal denervation selectively reduces inflammation in the denervated kidney, suggesting a link between sympathetic nerves and organ inflammation even independent of blood pressure (12). Several studies suggest a role for inflammation and immunity in human hypertension. Work from our group showed an increase in the percentage of circulating inflammatory CD4+ and CD8+/ CD45RO+ T cells (Figure 9) in hypertensive subjects (13). These cells have upregulated production of inflammatory cytokines. However, the link between sympathetic activation and inflammatory/immune pathways has not been extensively explored in humans; uncontrolled studies in hypertensive patients found a correlation between sympathetic activity and inflammation. Available data from human studies is in agreement with this postulate, and this provides the rationale for the premise that sympathetic activity contributes to inflammation and oxidative stress in obesity hypertension.

3.0 Previous Human Studies

Moxonidine is a newer generation central sympatholytic that activates imidazoline receptors in the brain, producing less sedation than classic α_2 partial agonists. It also has a longer half-life and has equivalent antihypertensive effect as amlodipine at the doses proposed in this application (14). Furthermore, uncontrolled studies suggest that moxonidine has a positive metabolic profile (15-17). The obvious limitation is that it is

not available in the US (it is approved in Europe and Australia). We should emphasize that this is not a drug treatment clinical trial, but a proof of concept study to test the hypothesis that sympathetic activation contributes to the cardiovascular and metabolic alterations of obesity, and we thought important to use the best pharmacologic probe available. We have an IND for the use of moxonidine and have obtained it through the Hannover Medical Center Pharmacy (Hannover, Germany).

We selected amlodipine because it is one of the most extensively used antihypertensives. Most studies have found amlodipine to be metabolically neutral (18) although a few have shown improvement of insulin sensitivity (19, 20). On the other hand, it may produce a modest sympathetic activation (21). An alternative comparator would have been a thiazide because they are widely used and recommended as first line therapy. However, thiazides are known to worsen insulin sensitivity and it would be seen as stacking the deck in our favor. Thus, we thought important to include a head-to-head comparison between a treatment that specifically targets sympathetic activation (moxonidine) with one that is largely metabolically neutral (amlodipine, "negative" control). We believe this design will help determine if specifically targeting sympathetic activity has a role in the treatment of obesity hypertension compared to medications currently considered standards of care.

4.0 Inclusion/Exclusion Criteria

- Inclusion criteria
    - Adults aged between 18 and 65 years old
    - Able to give informed consent
    - Non-smokers
    - Obese (defined as BMI ≥30 kg/m^2 and 35% of body fat)
    - Hypertensive (at least 2 measurements of 130<SBP<160mm Hg OR 85<DBP<100mmHg while off medications or currently being treated with anti-hypertensives)

- Exclusion criteria
    - Past medical history of long-term diabetes or treatment with insulin, pulmonary, renal or hematopoietic disease , cardiovascular disease other than hypertension (i.e., stroke, myocardial infarction), or other conditions that the study team judges could affect the volunteer's participation in the study (e.g., chronic back pain)
    - Morbid obesity (BMI > 43 kg/m^2)
    - Unable to withdraw from medications that the study team judge to have effects on energy metabolism or autonomic function
    - Highly trained athletes
    - Pregnancy or breastfeeding
    - Patients with Stage 2 hypertension ≥ 160/100 mmHg

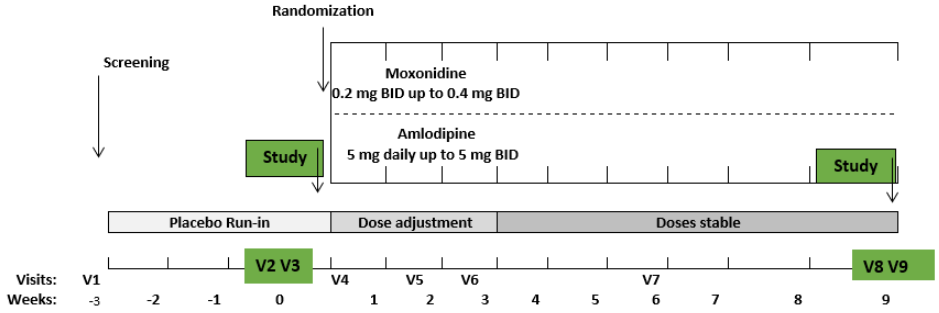
*Of note: chronic medications that can interfere with the autonomic nervous system (i.e., antihypertensives, metformin) will be withheld for at least five half-lives before the first study visit.

5.0 Enrollment/Randomization

For all Specific Aims, subjects will be recruited from the community. A study investigator or trained research assistant will seek eligible subjects and provide them with information regarding the proposed research, including procedures involved, and potential risks and benefits. A written informed consent form will be obtained from all subjects prior to any study procedures. We have extensive experience in conducting acute and longitudinal studies in these patient populations. Nevertheless, several strategies will be used to reduce the dropout rate and increase adherence to treatment. Compliance with the study will be discussed with potential participants before enrollment. Participants will get a schedule of the visits at the beginning of the study, and a research nurse will make regular contact with them by phone calls, text messages and/or emails to send instructions and reminders of upcoming visits and to maximize compliance.

6.0 Study Procedures / Study timeline

As explained before, we propose a double-blind, randomized, 9-week, parallel group study. The clinical trial will consist of 9 visits.



Protocol Activities	V1	V2	V3	V4	V5	V6	V7	V8	V9
Informed consent	X								
H&P, labs	X								
ECGs	X		X						X
Blood drawing	X								
Dexa Scan		X							
BP assessments	X	X	X	X	X	X	X	X	X
Dispense study drugs			X						
Dose adjustments				X	X	X			
MSNA		X						X	
Arterial Stiffness		X						X	
24 Hrs BP monitoring		X							X
Endothelial Cells		X						X	
SA1:Glucose Clamp			X						X
SA2:CEU			X						X
CE3:Circ T Cells, IsoP		X						X	

CEU, contrast-enhanced ultrasonography; Circ T cells, circulating T cells; IsoP, isprostanes

Visit 1 (Screening and clinical characterization): We will enroll obese hypertensive subjects off medications. Antihypertensives and medications that can affect autonomic function will be stopped at least for 5 half-lives (~2-3 weeks). They will be asked to monitor their blood pressure at home and will be provided with blood pressure monitors if necessary. Patients with pre-

hypertension are included if they have insulin resistance and sympathetic activation. Stage II hypertension is excluded because of the likelihood that more than one antihypertensive is needed to control BP.

Visit 2 and 3 (Baseline measurements): After 3 weeks off medications (or at least 5 half-lives), if the patients still meet the inclusion/exclusion criteria, we will perform the following baseline measurements the day before performing the first hyperinsulinemic-euglycemic clamp:

- Sympathetic activity (MSNA) (visit 2 only)
  - We may perform some autonomic nervous system function tests during MSNA such as Valsalva maneuvers
- Heart rate
- Blood pressure (supine and 1, 3, 5, 15, 30 minutes standing)
- Blood samples collected in supine position and after 30 minutes of standing for catecholamines, renin activity, angiotensin II and aldosterone levels) (visit 2 only)
- Blood for FAC analysis of immune cells (visit 2 only)
- Flow mediated dilation and 24-hr blood pressure (visit 2 only)
- FAC analysis of harvested endothelial cells (visit 2 only)
- Non-invasive determination of arterial stiffness using tonometric (Sphygmocor) method (visit 2 only)
- Hyperinsulinemic clamp (visit 3 only)
- Body impedance (visit 2 or 3)
- Blood volume measurement (visit 2 only)
- DEXA scan (visit 2 only)

Visit 4-7 (Randomization): Randomization, blood pressure adjustments and monitoring will be held from visit 4 to visit 7. Patients will be randomized with a permuted block double-blinded scheme. Moxonidine and amlodipine will be started at 0.2mg bid and 5mg, respectively. Blood pressure will be measured using the Sprint protocol with home monitors provided by us, and at weekly visits. A blinded study physician will increase medication dose once if needed. The dose titration period can be extended if necessary, to reach a stable BP.

Visit 8-9: We will repeat the baseline measurements mentioned above after 9 weeks of treatment during these visits. Hyperinsulinemic-euglycemic clamp will be performed in visit 9 (treatment). No DEXA scan will be performed at visit 8.

Study procedures during the hyperinsulinemic-euglycemic clamps:

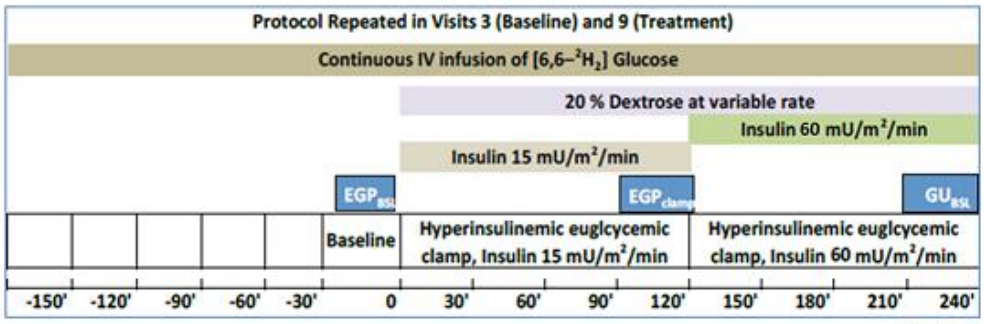


Figure 7

1. We will draw a blood sample to measure baseline levels of [6,6-2H2]-dideuterated glucose. We will then start the continuous infusion of [6,6-2H2]-dideuterated glucose. After at least 90 minutes, we will do baseline measurements of HP and BP. We will take blood samples every 15 minutes for 30 minutes (3 total) to measure plasma glucose, insulin, glucagon, C-peptide, and [6,6-2H2]-dideuterated glucose.

2. We will then perform a two-hour glucose clamp. In ancillary studies (Luther M, unpublished) new found that an autonomic blockade enhances insulin suppression of EGP, we decided to use the lower dose of 15 mU/m^2/min insulin produced a >50% suppression of EGP (Bock et al. 2007). Plasma glucose will be monitored every 5 minutes and euglycemia (90-100 mg/dl) will be maintained with variable infusion of 20% glucose. Potassium chloride 10 mmol/hr will be infused to prevent hypokalemia.

3. Once the glucose clamp is started, the glucose tracer infusion will be gradually reduced to 1.0 mg/m2 (half the starting dose), to account for the expected decrease in hepatic glucose output. To minimize changes in glucose isotopic enrichment, tracer will be added to the 20% Dextrose solution. We will alter tracer delivery commensurate with changes in glucose flux to maintain a constant plasma specific activity of glucose, as done previously (28-31).

4. Within the last 30 minutes after achieving stable glucose levels, samples will be drawn for every 15 minutes for 30 minutes (3 total) to measure glucose and [6,6- 2 H2]-glucose levels. At the third blood draw, we will also obtain samples for insulin, glucagon, C-peptide, catecholamines, and free fatty acids. In particular, glucagon and free fatty acids regulate EGP, but we have previously shown that their suppression during hyperinsulinemia is not altered by sympathetic withdrawal (32).

5. We will then increase the dose of insulin to 60 mU/m2 /min for an additional two hours. At this dose, insulin produces complete suppression of hepatic glucose production even in insulin resistant patients. Glucose utilization under these conditions, therefore, will reflect glucose uptake. The glucose infusion rate during the last 30 minutes of the clamp will be used as a measure of insulin sensitivity of glucose uptake. Within the last

30 minutes after achieving stable glucose levels, samples will be drawn every 15 minutes for 30 minutes (3 total) to measure glucose and [6,6-2H2]-dideuterated glucose levels. At the third blood draw, we will also obtain samples for insulin glucagon, C-peptide, catecholamines, and free fatty acids.

7.0 Potential risks

Hyperinsulinemic-euglycemic clamp: Of note, we have performed more than 40 hyperinsulinemic euglycemic clamps and at least 20 of them during autonomic blockade. We have been able to both maintain glycemia at 5% of fasting levels and clamp systolic blood pressure to ~140 mm Hg. We therefore do not foresee either hypoglycemia or hypotension.

Stopping medications may increase blood pressure: The symptoms of having elevated blood pressure vary among individuals but may include headache, dizziness, blurred vision, nausea and edema. Blood pressure and symptoms will be followed closely and regularly, and a physician will supervise all subjects with medications restarted if necessary.

Moxonidine: Treatment with moxonidine should result in changes in blood pressure. This medication is considered an investigational drug because it is not approved by the FDA in the US. It is approved for use in humans in Europe, Asia, and Australia. Side effects of moxonidine are those expected of sympathetic withdrawal, and include dry mouth, headache, fatigue, dizziness, intermittent facial edema, nausea, sleep disturbances (rarely sedation), vasodilatation, and rarely, skin reactions. Moxonidine will be used under IND 106975, sponsored by Dr. Biaggioni, and approved by the FDA.

Amlodipine : treatment with amlodipine should also result in changes in blood pressure. Common side effects of amlodipine include peripheral edema, fatigue, dizziness, palpitations, headache, somnolence and nausea. For the measurement of glucose turn-over we will use the stable isotope [6,6- 2 H2]-glucose as the tracer.

Peripheral venous catheterization: risks associated with this procedure are mostly related to pain and bruising on insertion site and will be explained in the consent form.

Infusion of the contrast agent (Definity) to measure microvascular blood flow can result in dizziness, chest pain, shortness of breath, or back pain may occur within minutes (1-15 minutes) of being given the drug. A very serious allergic reaction to this drug is extremely rare, but could include rash, itching/swelling (especially of the face/tongue/throat.

Cardiac output: The inert gas rebreathing technique for the assessment of cardiac output carries minimal risk.

Microneurography (MSNA): During this procedure, the nerve is first localized by external electrical stimulation (between 10-15 volts, 1-5 mA through an electrical isolated unit specifically designed for this purpose so the amount of current reaching the volunteer is limited. After the nerve is localized, an ultrafine tungsten electrode is introduced into the nerve to directly record MSNA. A second electrode is placed subcutaneously to serve as a reference. Due to the small size of the electrode, no anesthesia is required for the procedure. There should be no pain or discomfort during MSNA recordings while the electrode is in place. However, during placement of the electrode, paresthesias and/or pain running to the foot can occur if pain fibers are encountered. The electrode is then relocated to avoid this uncomfortable side effect. We have employed this technique at Vanderbilt for over twenty years. The method has very few, if any, side effects. Pain at the site of placement of the electrode may occur some hours following the procedure. Paresthesias distal to the recording site may persist for up to a week after the procedure, and in very rare instances, for longer. Subjects are advised against strenuous leg exercise for at least 24 hours after the procedure to avoid this side effect. Bruising and infection are other possible but very unlikely side effects. All intravenous infusions will be performed in a hospital inpatient setting by trained nurses who are experts in this task. Sterile technique will be used to minimize the risk of a catheter infection.

DEXA scan: The DEXA scan may provide up to the equivalent of 9 days of natural radiation.

Harvesting of endothelial cells for FAC analysis has the potential to increase the risk of bruising. For this procedure a J-tipped wire is advanced 10 cm through the vein catheter. Volunteers may find this sensation unpleasant but not painful.

Non-invasive determination of arterial stiffness will be done using a Sphygmocor device. There are no known risks associated with this measurement. With this method a blood pressure cuff is placed on the arm as with regular blood pressure determinations. This may cause transient discomfort. The measurement takes only a few minutes, as with a regular blood pressure determination.

Body impedance: Measurements of body impedance (electrical resistance) are made by recording the changes in the transmission of a very weak electrical current at the patches placed on the skin. It delivers a very small electrical current (0.6 mA). There are no known risks associated with this procedure.

Blood volume measurement (CO rebreathing): Subjects may experience mild headache and reduction of exercise capacity. It should clear from the body within 12-14 hours.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

The PI will be responsible for data integrity, the safety of study participants, and ensuring that all adverse events are documented, followed and reported to the

IRB and the Data Safety Monitor (DSM) as appropriate. The PI will review the records of the study subjects following the participation of each subject to ensure patient safety. The adverse event will be described with the following information: description of the event, outcome of the event, duration of the event, relationship to study procedure, requirement if any for treatment or intervention and overall outcome. Adverse events will be graded according the following scale:

0 no adverse event or within normal limits
1 mild adverse event (transient and mild in nature with no treatment necessary)
2 moderate adverse event (some intervention and treatment necessary, but patient completely recovers)
3 severe adverse event (the event results in hospitalization, disability, death, or is life-threatening).

Any adverse events (AE) will be rated on a 0-5 scale (0-no AE; 1-mild AE, no treatment required; 2- moderate AE that responds to treatment; 3-severe AE that would limit normal activities and require a prolongation of hospitalization; 4-life-threatening or disabling AE; 5-Fatal AE).

Any AE rated 3 or greater will be immediately reported to the IRB and the DSM. All AEs will be reported to the IRB on an annual basis. In the case of serious AE, the IRB makes an immediate determination about the necessity to modify the protocol, including additional information in the consent form, informing previous participants, temporarily holding enrollment of patients, or terminating the study.

All study procedures and cumulative adverse events are subject to full committee review at least yearly. The DSM will provide objective review of treatment results as they relate to human safety and data quality. Dr. Katherine T. Murray, Professor of Medicine, and Pharmacology, Division of Clinical Pharmacology, with extensive experience in human research and clinical trials, has agreed to take on this role. The PI will provide at least yearly reports to the DSM, or more often if needed. The information produced includes recruiting, safety reporting, data quality, and efficacy. Instances of non-compliance with the protocol will be reported annually.

The DSM will assess safety data including common and serious adverse events. The DSM will have the authority to modify the protocol or to terminate the study if it deems such actions to be warranted. The DSM will also receive quarterly reports of enrollment, protocol adherence, data quality and adverse events via e-mail. The DSM will review all serious adverse events. Any serious adverse event will be reported within 24 hours of the PI's notification of the event.. During regularly scheduled meetings, the PI will provide the DSM with a list of non-serious adverse events. The DSM may choose to perform an interim analysis; however, it is expected that this would not occur without reasonable concern related to either patient safety or data validity.

9.0 Study Withdrawal/Discontinuation

The study investigators or Vanderbilt University Medical Center may withdraw subjects from this study at any time after they have provided informed consent, but before study completion. Study withdrawal may occur for several reasons including but not limited to: non-compliance with the protocol, concern for patient safety, the availability of new information that may affect continued participation, or if the study is stopped. Subjects are free to withdraw from this study at any time. We will cease to collect study information at the time of withdrawal of consent, but data already collected will be retained. Withdrawal of consent or refusal to participate will not prejudice subject health care.

10.0 Statistical Considerations

In this proposal, we will be testing the general hypothesis that nine weeks of treatment with the sympathetic blocker, moxonidine, will improve insulin sensitivity (Specific aim 1), microvascular blood flow (Specific aim 2), and inflammation (Specific aim 3), in obese hypertensive human subjects. We will be comparing the changes before and after treatment with the central sympathetic inhibitor moxonidine (MOX), versus the changes produced with treatment with the calcium channel blocker amlodipine (AML), an antihypertensive medication considered to have neutral metabolic and anti-inflammatory effects. Our main scientific goal is to demonstrate the efficacy of MOX in a superiority trial, and for that reason the study is powered to perform the main statistical analysis between the MOX and AML arms. The clinical comparison between the two responses, not previously done, will provide important new information. Nonetheless, as a secondary analysis, the differences in primary outcomes between the treatment and baseline study days will be compared across the two study arms using a linear regression model. Given the fact that the degree of obesity, baseline sympathetic activity and blood pressure may affect EGP these variables as well as gender and age will be included as covariates in the model to take into account potential confounding effects. We make no a priori assumptions about the outcome of these studies. Any result, therefore will provide important new information.

a) **Specific Aim 1a -** The null hypothesis is that there will be no changes in the insulin-mediated suppression of endogenous glucose production in response to sympathetic blockade with MOX. We will evaluate this hypothesis at Visit 9 (post treatment) by measuring EGP at baseline (EGPBsl) and during the last 30 minutes of a hyperinsulinemic euglycemic clamp (EGPClamp) in a double-blind randomized trial (protocol shown in figure 6). For each study arm, the primary endpoint will be DEGP = (EGPBsl – EGPClamp) the difference in the change of insulin-mediated suppression of basal EGP between the pre and post treatment visit. The primary analysis will be performed between the moxonidine and amlodipine arms, at Visit 9 using Welch's unequal-variance an unpaired t-test to test whether the primary end point will be equal in these two treatment groups. Note that although we are collecting multiple measures over time, this endpoint is based on a single visit and a scalar response feature ΔEGP. Hence, a fixed effects analysis is appropriate for this aim. Appropriate non-parametric analysis methods will also be used if data are not normally distributed. Since the degree

of obesity, baseline sympathetic activity, blood pressure, age and gender may affect EGP, these variables could confound the effect of treatment on the change in EGP. For this reason, in a secondary linear regression analysis we will compare DEGP in the moxonidine and amlodipine arms adjusted for these variables. We will also assess the effects of treatment on EGP over time by conducting a repeated-measures GEE regression analysis of EGP against treatment and these confounding variables at baseline and the 9 subsequent visit times. These analyses will use a normal random component and the identity link function. Spaghetti graphs and residual analyses will be used to assess the model fit. Restricted cubic splines will be used if needed to account for non-linear effects of time on treatment response (33).

Sample size calculation. Power calculations are based on previous studies comparing the suppression of EGP between subjects with impaired fasting glucose and normal fasting glucose (34). In this study among obese subjects with impaired fasting glucose (N=30), the mean and the standard error of mean (SEM) for EGP at baseline was 15.2 ± 0.2 μmol×min-1 × kg-1, and during the clamp, EGP was 10.2±0.7 μmol×min-1 × kg1. Hence, the standard deviation (SD) of EGP at baseline and during clamp was $0.2\sqrt{30} = 1.1$ and $0.7\sqrt{30} = 3.8$, respectively. We will power our study to detect a difference in inhibition of 3 μmol×min-1 ×kg-1. Making the conservative assumption that clamped and baseline EGP values are independent, we estimate the SD of this difference to be *(1.1, + 3.8,) = 4.0. With this SD, 29 subjects per group would provide 80% power to detect a mean difference in the inhibitions of 3 μmol×min-1 × kg-1 with the type I error of a = 0.05. Assuming a drop–out rate of 20%, which includes the subjects that will be enrolled based on HOMA but later shown not to have insulin resistance during the clamps (see Section C.2), we will need to enroll 36 subjects in each study arm. This sample size will also be used in Aims 1b, 2 and 3 even though smaller sample sizes would suffice for these aims.

Specific Aim 1b - The null hypothesis is that chronic sympathetic blockade will not improve whole body insulin sensitivity. We will measure insulin sensitivity as the glucose infusion rate, corrected by body weight (MBW) needed to maintain euglycemia during the last 30 minutes of a hyperinsulinemic euglycemic clamp. The same three groups of subjects will be studied twice, at the beginning (BSL) and the end of 9 weeks of treatment with either the sympathetic blocker moxonidine (MOX) or the calcium channel blocker amlodipine (AML). The null hypothesis is that we will not see any difference in the change in body-weight-corrected insulin sensitivity with MOX as compared with AML. The primary analysis will assess changes in MBW (MBW_clamp – MBW_bsl) between patients receiving MOX and will use a t-test similar to that described for Aim 1a.

Sample size calculation. We estimated the sample size based on previous studies (17) using a similar design to the present study, which found a 21% improvement of insulin sensitivity with moxonidine. From our previous studies, we expect obese insulin resistant hypertensive subjects to have a MBW of 3.1±0.99 mg/kg/min (17). An increase of 21% in the moxonidine arm will translate as a MBWMOX of 3.75 mg/kg/min (a difference of 0.65 mg/kg/min), after 9 weeks of treatment. Assuming that a SD of the difference of 0.75

mg/kg/min, a sample size of of 27 subjects per group will provide 88% power to detect this difference with a = 0.05.

Specific Aim 2 - The null hypothesis is that insulin will not produce any different changes in microvascular blood volume (MBV) using contrast enhance ultrasonography (CEU) in response to nine weeks of treatment with moxonidine as compared to amlodipine. We will measure MBV at baseline (MBVbsl), during the last 30 minutes of the lower insulin dose (15 mU/m2 /min) portion of the hyperinsulinemic euglycemic clamp (MBVegp), and during the last 30 minutes of the higher insulin dose (60 mU/m2/min) portion of the clamp (MBVclamp) on two different occasions (double blind, randomized, protocol shown in figure 6). Subjects will be randomized to treatment with moxonidine (MOX) or amlodipine (AML). The primary endpoint would be the average difference in the change in MBV (MBVclamp-MBVbsl) between the MOX and AML arms. The primary analysis will be conducted between the moxonidine and amlodipine arms using a t-test as described in Aim 1a.

Sample size calculation. We estimated the sample size based on our preliminary results (Figure 8) and previous studies in obese human subjects (35). In our study, on the SAL day, we expect to see a blunted increase in MBV only due to insulin among obese subjects (from 20.4±3.6 to 18.8±3.8, as in the paper by Haenni and Lithell (17)). We believe that an increase of 3.5 arbitrary intensity units (AIU) of MVB (half of the what was seen in healthy subjects) during MOX treatment would be a clinically significant difference. Healthy subjects showed an increase from 18.7±3.3 to 25.0±4.1 in the same paper, and we have observed similar results (see figure 8). With a SD of 3.2 AIU, a sample size of 27 subjects per group would provide 97% power to detect a difference of 3.5 AIU with a = 0.05.

Specific Aim 3 - The null hypothesis is that chronic sympathetic blockade will not improve inflammation in obese hypertensive subjects. We will measure the percent of circulating T cells that express both CD4 and CD45RO (CD4+ /CD45RO+), at baseline and after nine weeks of treatment with each of the study drugs as in the previous specific aims. Obese hypertensive subjects will be studied twice, at the beginning (BSL) and the end of 9 weeks of treatment with either the sympathetic blocker moxonidine (MOX) and the calcium channel blocker amlodipine (AML). The primary outcome variable will be the change from baseline in the CD8+ /CD45RO+ percentage of circulating T cells. The null hypothesis will be that there is no difference in this outcome in patients treated with moxonidine compared to patients treated with amlodipine. The primary analysis will be to conduct an unpaired t-test on the primary outcome that is similar to that described for Aim 1.a. Secondary analyses to account for a possible skewed distribution of the primary outcome, to adjust for potential confounding variables will also be performed in a fashion that is similar to the analyses for Aim 1.a.

Sample size calculation. We estimated the sample size based on previous studies ((36), figure 9) that have found an increased percentage of CD4+

/CD45RO+ circulation T cells in hypertensive human subjects. They found that hypertensive subjects have 20% more circulating T cells expressing both CD4 and CD45RO as compared to healthy controls. We expect to see a reduction of at least 15±5 percent after 9 weeks of treatment with moxonidine. A decrease of 15% in the moxonidine arm will translate as 45% of CD4+ /CD45RO+ (from 60% at baseline), after 9 weeks of MOX treatment. Assuming that a SD of the difference is 15%, a sample size of 27 subjects per arm would provide 95% power to detect a difference of this magnitude with a = 0.05.

11.0 Privacy/Confidentiality Issues

All studies will be done by trained personnel in a quiet room, within or adjacent to the Clinical Research Center. Women of child-bearing potential will have a serum pregnancy test to exclude pregnancy immediately before study participation. In order to minimize any potential risk, a physician and a research nurse will be present and monitor all procedures involving the administration of investigational medications. Careful and continuous monitoring of all vital signs during the studies will help minimize potential risks. Blood pressure and heart rate are monitored continuously using non-invasive techniques whenever drugs are infused. Confidentiality will be maintained for the identity of participants in this study except as necessary for oversight by the Secretary of the Department of Health and Human Services or his designated representative. Subjects will be instructed to contact the investigator immediately if they experience any signs of adverse events during withdrawal of medications. Any adverse events will be reported in a timely fashion to the principal investigator. All adverse events will be documented on the Adverse Event form and reported to the IRB. All subjects who have adverse events will be monitored to determine the outcome. The clinical course of the adverse event will be followed according to accepted standards of medical practice, even after the end of the period of observation. To date, no subjects have had adverse events to the procedures described.

12.0 Follow-up and Record Retention

The project will take 5 years, during the first two quarters of the funding period, we will obtain regulatory approvals and develop the database and case report forms. Patient recruitment will start on the third quarter and continue through Year 5. We propose to enroll a total of 72 subjects. We estimate 30% will be screen failures or dropouts. Eligible participants will be studied on the CRC. Since the same patients will participate in aims 1 and 2, the studies on all aims will be conducted simultaneously. Final data analysis and manuscript preparation will be started the fourth year

The research material obtained in this proposal will be recorded data obtained specifically for research purposes. Blood and urine samples will be obtained for screening purposes and for analysis of the various parameters under study. A separate secure database of patient information will be maintained using the REDCap web-based software, a HIPAA compliant secure database. Only investigators listed in this application will have access to research information and will follow IRB and institutional HIPAA guidelines. Both paper and computer files will be saved under lock/password protection.

13.0 Reference List

1. Engelke K, Williams M, Dietz N, Joyner MJ. Does sympathetic activation blunt nitric oxide-mediated hyperemia in the human forearm? Clinical Autonomic Research. 1997;7(2):85-91.
2. Uyama N, Geerts A, Reynaert H. Neural connections between the hypothalamus and the liver. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists. 2004;280(1):808-20.
3. Gamboa A, Figueroa R, Paranjape SY, Farley G, Diedrich A, Biaggioni I. Autonomic blockade reverses endothelial dysfunction in obesity-associated hypertension. Hypertension. 2016;68(4):1004-10.
4. Bonadonna RC, Saccomani MP, Del Prato S, Bonora E, DeFronzo RA, Cobelli C. Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. Circulation. 1998;98(3):234-41.
5. Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S, et al. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. Diabetes. 2001;50(12):2682-90.
6. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. Diabetes. 2006;55(5):1436-42.
7. Barrett EJ, Wang H, Upchurch CT, Liu Z. Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. American Journal of Physiology-Endocrinology and Metabolism. 2011;301(2):E252-E63.
8. Harrison DG. The immune system in hypertension. Transactions of the American Clinical and Climatological Association. 2014;125:130.
9. Marvar PJ, Lob H, Vinh A, Zarreen F, Harrison DG. The central nervous system and inflammation in hypertension. Current opinion in pharmacology. 2011;11(2):156-61.
10. Marvar PJ, Thabet SR, Guzik TJ, Lob HE, McCann LA, Weyand C, et al. Central and Peripheral Mechanisms of T-Lymphocyte Activation and Vascular Inflammation Produced by Angiotensin II–Induced Hypertension. Circulation Research. 2010;107(2):263-70.
11. Marvar PJ, Vinh A, Thabet S, Lob HE, Geem D, Ressler KJ, et al. T lymphocytes and vascular inflammation contribute to stress-dependent hypertension. Biological psychiatry. 2012;71(9):774-82.
12. Xiao L, Kirabo A, Wu J, Saleh MA, Zhu L, Wang F, et al. Renal denervation prevents immune cell activation and renal inflammation in angiotensin II–induced hypertension. Circulation research. 2015;117(6):547-57.
13. Itani HA, McMaster Jr WG, Saleh MA, Nazarewicz RR, Mikolajczyk TP, Kaszuba AM, et al. Activation of human T cells in hypertension: studies of humanized mice and hypertensive humans. Hypertension. 2016;68(1):123-32.
14. Sanjuliani A, De Abreu V, Francischetti E. Selective imidazoline agonist moxonidine in obese hypertensive patients. International journal of clinical practice. 2006;60(5):621-9.
15. Sharma A, Wagner T, Marsalek P. Moxonidine in the treatment of overweight and obese patients with the metabolic syndrome: a postmarketing surveillance study. Journal of human hypertension. 2004;18(9):669-75.
16. Chazova I, Almazov VA, Shlyakhto E. Moxonidine improves glycaemic control in mildly hypertensive, overweight patients: a comparison with metformin. Diabetes, Obesity and Metabolism. 2006;8(4):456-65.

17. Haenni A, Lithell H. Moxonidine improves insulin sensitivity in insulin-resistant hypertensives. Journal of hypertension Supplement: official journal of the International Society of Hypertension. 1999;17(3):S29-35.
18. Tomiyama H, Yambe M, Yamada J, Motobe K, Koji Y, Yoshida M, et al. Discrepancy between improvement of insulin sensitivity and that of arterial endothelial function in patients receiving antihypertensive medication. Journal of hypertension. 2007;25(4):883-9.
19. Fogari R, Preti P, Zoppi A, Mugellini A, Corradi L, Lazzari P, et al. Effect of valsartan addition to amlodipine on insulin sensitivity in overweight-obese hypertensive patients. Internal Medicine. 2008;47(21):1851-7.
20. Lender D, Arauz-Pacheco C, Breen L, Mora-Mora P, Ramirez LC, Raskin P. A double blind comparison of the effects of amlodipine and enalapril on insulin sensitivity in hypertensive patients. American journal of hypertension. 1999;12(3):298-303.
21. Inomata J-i, Murai H, Kaneko S, Hamaoka T, Ikeda T, Kobayashi D, et al. Differential effects of azelnidipine and amlodipine on sympathetic nerve activity in patients with primary hypertension. Journal of hypertension. 2014;32(9):1898-904.
22. Kintscher U, Marx N, Martus P, Stoppelhaar M, Schimkus J, Schneider A, et al. Effect of high-dose valsartan on inflammatory and lipid parameters in patients with Type 2 diabetes and hypertension. Diabetes research and clinical practice. 2010;89(3):209-15.
23. Ridker PM, Danielson E, Rifai N, Glynn RJ. Valsartan, blood pressure reduction, and C-reactive protein: primary report of the Val-MARC trial. Hypertension. 2006;48(1):73-9.
24. Van Der Zijl NJ, Moors CC, Goossens GH, Hermans MM, Blaak EE, Diamant M. Valsartan improves β-cell function and insulin sensitivity in subjects with impaired glucose metabolism: a randomized controlled trial. Diabetes Care. 2011;34(4):845-51.
25. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Pozzi M, Morganti A, et al. Effects of chronic ACE inhibition on sympathetic nerve traffic and baroreflex control of circulation in heart failure. Circulation. 1997;96(4):1173-9.
26. Klein IH, Ligtenberg G, Oey PL, Koomans HA, Blankestijn PJ. Enalapril and losartan reduce sympathetic hyperactivity in patients with chronic renal failure. Journal of the American Society of Nephrology. 2003;14(2):425-30.
27. Struck J, Muck P, Trübger D, Handrock R, Weidinger G, Dendorfer A, et al. Effects of selective angiotensin II receptor blockade on sympathetic nerve activity in primary hypertensive subjects. Journal of hypertension. 2002;20(6):1143-9.
28. Finegood D, Vranic M. Possible isotope effect of tritiated glucose tracer during hyperinsulinemic, euglycemic glucose clamps. Diabetes. 1985;34(Suppl 1):80A.
29. Bell P, Firth R, Rizza R. Assessment of insulin action in insulin-dependent diabetes mellitus using [6 (14) C] glucose,[3 (3) H] glucose, and [2 (3) H] glucose. Differences in the apparent pattern of insulin resistance depending on the isotope used. The Journal of clinical investigation. 1986;78(6):1479-86.
30. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocrine reviews. 1985;6(1):45-86.
31. Finegood DT, Bergman RN, Vranic M. Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: comparison of unlabeled and labeled exogenous glucose infusates. Diabetes. 1987;36(8):914-24.
32. Gamboa A, Okamoto LE, Arnold AC, Figueroa RA, Diedrich A, Raj SR, et al. Autonomic blockade improves insulin sensitivity in obese subjects. Hypertension. 2014;64(4):867-74.
33. Dupont WD, Dupont WD. Statistical modeling for biomedical researchers: a simple introduction to the analysis of complex data: Cambridge University Press; 2009.
34. Bock G, Chittilapilly E, Basu R, Toffolo G, Cobelli C, Chandramouli V, et al. Contribution of hepatic and extrahepatic insulin resistance to the pathogenesis of impaired fasting glucose: role of increased rates of gluconeogenesis. Diabetes. 2007;56(6):1703-11.

35. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. Diabetes. 2006;55(5):1436-42.
36. Itani HA, McMaster WG, Jr., Saleh MA, Nazarewicz RR, Mikolajczyk TP, Kaszuba AM, et al. Activation of Human T Cells in Hypertension: Studies of Humanized Mice and Hypertensive Humans. Hypertension (Dallas, Tex : 1979). 2016;68(1):123-32.