# STUDY PROTOCOL

**Official Title: Targeting ADMA With Pioglitazone to Reduce Sympathetic Overactivity in CKD Patients**

**NCT Number: NCT03471117**

**Date: November 11, 2020**

**Study Rationale:**

Chronic Kidney Disease (CKD) is a major health problem affecting more than 26 million Americans. An overactive sympathetic nervous system is a well known cardiovascular risk factor present in CKD. The increase in sympathetic nerve activity (SNA) may not only contribute to hypertension, but also accelerates the progression of end organ damage that is independent of any rise in blood pressure. Indeed, elevated SNA is associated with poor prognosis and increased risk of cardiovascular morbidity and mortality. Thus, the sympathetic nervous system constitutes a primary novel drug target needed for improving cardiovascular outcomes in CKD patients. However, limited effort has been directed at identifying the mechanisms driving sympathetic overactivity in CKD and importantly, SNA remains high in these patients despite standard drug therapy including angiotensin converting enzyme inhibitors and angiotensin II receptor blockers. Thus, if we could better understand the mechanism(s) of the elevated SNA, we should be able to devise more effective countermeasures and help reduce the subsequent morbidity and mortality among CKD patients. A potential signal driving SNA involves accumulation of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA). ADMA is elevated in CKD and is a strong, independent predictor of future cardiovascular events in these patients. Much of the work with ADMA has been correlational in nature with a focus on the well-known vascular endothelial properties of nitric oxide. However, increasing functional evidence indicates that nitric oxide is also a key signaling molecule involved in the tonic restraint of sympathetic outflow from the brainstem. Indeed, we recently demonstrated that systemic experimental inhibition of nitric oxide synthase causes sympathetic activation in healthy humans. In the current study, we will target the pathophysiological nitric oxide synthase inhibition caused by elevated ADMA concentrations in CKD and its role in mediating sympathetic overactivity. We will capitalize on the recent work indicating that thiazolidinediones reduce ADMA.

Recent work has reported that thiazolidinediones, such as pioglitazone, reduce ADMA likely by upregulating dimethylarginine dimethylamino-hydrolase (DDAH), the enzyme responsible for the breakdown of ADMA. Indeed, analysis of the DDAH gene revealed the presence of a thiazolidinedione binding site, implying that thiazolidinediones can directly regulate DDAH expression and subsequently ADMA levels. Thus, thiazolidinediones may provide a promising therapy in CKD. Indeed, in a recent study, CKD patients treated with pioglitazone were less likely to reach the composite end points of cardiovascular morbidity and mortality. Importantly, this effect was independent of the level of renal impairment suggesting protective effects even in moderate CKD. However, the mechanisms for these improvements remain unclear. In this study, we hypothesize that these favorable cardiovascular effects are through a lowering of ADMA and SNA. Thus, we will test the ability of pioglitazone to reduce ADMA and SNA in CKD patients.

**Study Design:**

We will perform a randomized, placebo-controlled crossover design study in CKD patients. Patients will be studied before and after one month of treatment with either placebo or pioglitazone (15 mg/day) with a 2-week washout period. Subjects will come to the research laboratory before and after the administration of either placebo or pioglitazone (15 mg/day) for one month. 20 CKD patients will be recruited and studied. Each visit will take approximately four hours.

**VISIT 1**: Prior to any screening/testing, all experimental measurements and procedures will be explained in detail and subjects will provide written, informed consent. A medical health history questionnaire will be filled out and women of child-bearing age will provide a urine sample for a pregnancy testing prior to any study procedures.

Subjects will be familiarized with the experimental measures and procedures prior to actual testing. The following measurements and procedures will be performed: Heart rate (ECG), blood pressure (Welch Allyn and finapres), respiratory rate (pneumobelt), brachial blood flow (duplex Doppler ultrasound), flow-mediated vasodilation, blood samples for metabolic and oxidative stress panel, muscle sympathetic nerve activity, handgrip exercise and cold pressor test.

In addition to laboratory BP measures, 24-hour ambulatory BP monitoring will be performed to better characterize BP fluctuations throughout the day and avoid any "white coat" influence on BP. After the baseline visit, subjects will be instrumented with this monitor that periodically measures BP (e.g., every 30 minutes) and asked to wear it for the next 24 hours. They will return the monitor within 2 days of completing the 24 hour

measurements. This device does not display the BP to the participant, it stores the BP data for downloading after returned to the lab. At the end of this visit, subjects will be given Pioglitazone or placebo pills for 1 month.

**VISIT 2**: This visit will be 1 month after visit 1. All experimental measures and procedures performed during visit 1 will be repeated. If the subjects received placebo treatment at the end of visit 1, they will receive pioglitazone pills at the end of visit 2, or vice versa. Subjects will be sent home with a 24-hour ambulatory BP monitor.

**VISIT 3**: This visit will be 2 weeks after visit 2. Same experimental measures and procedures performed in visit 1 and 2 will be repeated. Subjects will be sent home with a 24-hour ambulatory BP monitor.

**Experimental Measurements and Procedures:**

Anthropometrics: We will measure each subject's height, using a standard stadiometer, and weight, using a balance or digital scale. Waist and hip measurements will be taken using a measuring tape.

Blood draw: A venous catheter will be placed in the antecubital or hand vein for blood sampling. A fasting blood sample will be taken for measures of baseline glucose, insulin, HbA1c, blood urea nitrogen, serum creatinine, cystatin C, lipids, liver enzymes, electrolytes, hemoglobin and hematocrit. In addition, blood will also be collected for measuring norepinephrine, Angiotensin II, ADMA, SDMA, and a complete oxidative stress panel (basic markers such as, protein carbonyls and thiobarbituric acid reactive substances). Blood samples will be taken on all 3 visits and the volume of blood drawn will not exceed 150 mL per visit. Blood samples will be analyzed by Labcorp Inc., the same facility that processes blood samples from Student Health Center at UTA. Each blood sample container is labeled with two identifiers, the subject initials+study number, and the subject's date of birth, as required by Labcorp (e.g., Subject: ABC 01234, DOB 10/14/65) and placed in a locked drop-box. Labcorp is contacted and a courier retrieves the sample from the drop-box on the 5th floor of University Hall the same day. No genetic analysis will be performed. To allow samples for certain assays to be run all at one time and avoid variability, some blood will also be stored in a -80°C freezer in University Hall until analysis. After successful analysis, blood will be discarded in liquid waste containers as per EH&S guidelines. The empty plastic tubes will be collected in biowaste boxes lined with red biohazard bag (Stericycle boxes). Blood containers and Stericycle boxes will be disposed via EH&S making request in CEMS. All stored data will be deidentified using a coding system and frozen until analysis. The code-key will be kept in the PIs lab in a locked cabinet. Only PI and study personnel on this IRB will have access to the files (via key). No other students or faculty will have access.

Electrocardiogram: Standard limb lead electrodes will be used to obtain heart rate measurements.

Respiration: An elastic band will be placed around the subject’s abdomen to measure rate and depth of breathing.

Blood pressure: A blood pressure cuff will be wrapped around the upper arm to obtain blood pressure via an automated oscillometric device (Welch-Allyn). In addition, beat-by-beat blood pressure will be obtained via finger photoplethysmography (Finapres).

Sympathetic Nerve Activity: A tiny microelectrode will be placed in the peroneal nerve of the leg, located just below the knee on the outer part of the leg. Alternatively, the median nerve located in the upper arm will be used. At these points the nerves are closest to the surface of the skin. The course of the nerve will be determined by electrically stimulating through the skin with a pencil shaped electrode. When the nerve is stimulated, involuntary twitching and/or tingling sensations of the foot or hand will occur. The twitching or tingling will disappear when the stimulation is stopped. Once the nerve is found, two tiny, sterile,

microelectrodes will be inserted through the skin. One is a reference electrode placed just above the nerve site (2 cm) and the other is the recording electrode. The recording electrode is advanced into the nerve. When the tip of the electrode enters the nerve, the subject may briefly notice either pressure or tingling sensations. At this point, minor adjustments in the position of the electrode will be made until an optimal nerve signal is obtained.

Peripheral Blood Flow: Blood flow will be determined by using pulsed Doppler ultrasound to non-invasively measure mean arterial blood velocity and diameter. Peripheral blood flow (e.g., femoral artery, brachial artery) can be obtained by placing a Doppler flow probe on the surface of skin over the respective artery. For Doppler ultrasound measurements, when requested by the participants for comfort purposes, an individual of the same sex will be present to conduct these measurements during the protocol. Ultrasound imaging of artery diameter will be performed at a site matching that at which velocity is measured. The following formula is used to calculate blood flow: π × (artery radius)^2 × velocity.

Baseline measurements (such as heart rate, blood pressure, blood flow) will be recorded during 10-20 minute supine rest. After basline measures, experimental procedures listed below will be performed. All procedures will be explained in detail verbally to the subject during consenting. The FDA approval status for all the devices used in this protocol is provided in an attached document.

Flow mediated dilation (FMD): Endothelial function will be assessed via flow mediated dilation (FMD) which includes inflating a blood pressure cuff above the participant's systolic blood pressure for 5 minutes, and making continuous blood flow measures before and after the release of the blood pressure cuff. This will be performed on a peripheral artery (e.g., brachial artery, popliteal artery).

Handgrip Exercise and Post-Exercise Ischemia: Subjects will perform handgrip exercise using a hand dynamometer with their arm supported on an adjustable bedside table. Handgrip exercise will be performed for either three minutes or until they feel like they cannot maintain the exercise (i.e., fatigue). Five seconds before stopping the exercise, a cuff around the upper arm will be inflated to impede blood flow for up to three minutes. This traps the metabolites that were produced during exercise in the area of the muscle, which maintains stimulation of skeletal muscle metaboreceptor afferents. Since these metaboreceptor afferents contribute to the rise in blood pressure during exercise, continued stimulation of these receptors maintains a blood pressure response.

Cold Pressor Test: The subject will be asked to place their hand in ice water for 2 minutes. This procedure will be used to cause transient increases in heart rate and blood pressure.

24-hour ambulatory blood pressure: After each visit, subjects will be instrumented with a 24-hour ambulatory blood pressure monitor that measures blood pressure every 30 minutes. The device does not display blood pressure to the subject but stores the data for downloading after returned to the lab (2 days after completion of 24 hour measurements).This is performed to better characterize BP fluctuations throughout the day and avoid any "whit coat" influence on BP.

**Study Drugs:**

Pioglitazone is in a class of anti-diabetic drugs called thiazolidinediones that are primarily used in the treatment of type 2 diabetes. We aim to determine if this drug also reduces ADMA and SNA in CKD patients. This drug will be taken orally as a pill or capsule for one month. The dosage is 15 mg/day. This is on the lower dosage side for pioglitazone with the maximum dosage being 45mg/day. The study will utilize a randomized, crossover placebo-controlled study design. The randomization for study drugs will be carried out by research personnel. The research subject is not responsible for the cost of the drug or for drug administration costs.

Placebo pills: Placebo will consist of sugar capsules of similar color and appearance as Pioglitazone pills.

**INCLUSION CRITERIA:** We will study CKD patients classified as Stage 3 and 4 of National Kidney Foundation Classification with estimated GFR between 15 and 59 mL/min/1.73 $m^2$ according to the Modification of Diet in Renal Disease (MDRD) formula based on serum creatinine, age, gender, and race. Men and women 35 to 70 years of age will be studied.

**EXCLUSION CRITERIA:** We will study patients on their existing drug therapy.
Exclusion criteria include: 1) allergy to Glitazones 2) myocardial infraction 3) heart failure 4) angina 5) history of kidney stones 6) liver disease (abnormal liver enzymes) 7) anemia (hemoglobin <8 g/dl) 8) cancer with current treatment 9) previous organ transplantation 10) immunosuppressant therapy 11) human immunodeficiency virus infection 12) pregnancy or lactating 13) current tobacco use 14) Dilantin and oral contraceptive usage due to potential drug interaction with glitazones and 15) self-identified history of hypoglycemia.

**References:**


1) **Hidaka T, Nakagawa K, Goto C, Soga J, Fujji Y, Hata T, Idei N, Fujimura N, Chayama K, Kihara Y, Higashi Y.** Pioglitazone improves endothelium-dependent vasodilation in hypertensive patients with impaired glucose tolerance in part through a decrease in oxidative stress. *Atherosclerosis* 210 (2): 521-4, 2010.

Patient population: Hypertensive patients
Drug and dose used: Pioglitazone, 30 mg/day
Period of use: 12 weeks
Adverse effects reported: none
Change in glucose: significantly decreased glucose (from 6.4 to 5.9 mmol/dL)

2) **Horio T, Suzuki M, Takamisawa I, Suzuki K, Hiuge A, Yoshimasa Y, Kawano Y.** Pioglitazone-induced insulin sensitization improves vascular endothelial function in nondiabetic patients with essential hypertension. *Am J Hypertens* 18 (12): 1626-1630, 2005.

Patient population: Hypertensive patients
Drug and dose used: Pioglitazone, 30 mg/day
Period of use: 6 months
Adverse effects reported: Mild leg edema
Change in glucose: no significant decrease in fasting blood glucose

3) **Dana E. King, Marty Player, Charles J. Everett.** The impact of pioglitazone on ADMA and oxidative stress markers in patients with type 2 diabetes. *Primary Care Diabetes* 6: 157-161, 2012.

Patient population: Type 2 Diabetes
Drug and dose used: Pioglitazone, 30 mg/day
Period of use: 12 weeks
Adverse effects reported: None
Change in glucose: did not report anything (only reported HbA1c levels and those did not change with pioglitazone)