University of Minnesota

Clinical Research Study Protocol

Use of Entresto (sacubitril/valsartan) for the treatment of peripheral arterial disease

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Abbreviations

AA	Amino Acid
AE	Adverse Event
ABI	Ankle Brachial Index
ABPM	Ambulatory Blood Pressure Monitoring
AHA	American Heart Association
ANP	A-type Natriuretic Peptide
ATP	Adenosine Triphosphate
BNP	B-type Natriuretic Peptide
CARDIA	Coronary Artery Risk Development in Young Adults
cGMP	Cyclic Guanosine Monophosphate
CGM	Continuous Glucose Monitoring
CKD	Chronic Kidney Disease
CNP	C-type Natriuretic Peptide
CRF	Case Report Forms
CT	Computed Tomography
CTSI	Clinical and Translational Science Institute
DNP	Dendroaspis Natriuretic Peptide
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EF	Effect Size
fMRI	Functional Magnetic Resonance Imaging
GRE-EPI	Gradient Recall Echo Planar [MRI]
GXT	Graded Exercise Test
HIPAA	Health Insurance Portability and Accountability Act
HF	Heart Failure
IC	Intermittent Claudication
ICMJE	International Committee Of Medical Journal Editors
IDS	Investigative Drug Pharmacy
IRB	Institutional Review Board
MCRU	Masonic Clinical Research Unit
MEBRL	Molecular Epidemiology and Biomarker Research Laboratory
MG	Medial Gastrocnemius
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MWD	Maximal Walking Distance
NPs	Natriuretic Peptides
NPR	Natriuretic Peptide Receptor

NT-proBNP	N-terminal pro B-type natriuretic peptide
PAD	Peripheral Artery Disease
PAD-QOL	Quality of Life
PCr	Phosphocreatine
PFWD	Pain Free Walking Distance
PHI	Protected Health Information
PI	Principal Investigator
PKG	Protein Kinase G
Pro-BNP	Prohormone Brain Natriuretic Peptide
PTA	Percutaneous Transluminal Angioplasty
ROI	Region of Interest
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SF-36	36-item Short Form
SI	[MR] Signal Intensities
SNR	Signal to Noise Ratio
Sol	Soleus
SQ	Subcutaneous
TE	Echo Time
UPIRTSO	Unanticipated Problems Involving Risk to Subjects or Others
WIQ	Walking Impairment Questionnaire

1 Introduction

1.1 Background and Significance

Peripheral arterial disease (PAD) affects about 12 million individuals in the United States [1,2]. The functional physical limitations observed in patients with PAD progressively deteriorate from a reduction in mobility to a complete loss of independence [3]. Unfortunately, the available therapeutic options are limited in success rate, adherence and long-term benefits [4]. Functional physical limitations observed in patients with PAD are associated with impaired mitochondrial and microvascular function [5-7]. Because natriuretic peptides have been shown to improve oxygen consumption and blood flow in skeletal muscles [8,9], we propose that increasing the levels of natriuretic peptide (NPs) using sacubitril/valsartan [LCZ696 (Entresto), Novartis Pharmaceuticals Corp.] initially designed to treat patients with heart failure [10], could be used as novel therapeutic option. These are the two most important biological mechanisms implicated in the functional limitations observed in PAD and could provide significant functional beneficial effects to this type of patient.

The mitochondrial and renal effects of NP's are exerted predominantly through activation of NP receptors A (NPR-A), while the endothelial, vascular and anti-fibrotic effects are related to activation of NPR-B [11]. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) act through activation of NPR-A and C-type natriuretic peptide (CNP) activates NPR-C [12]. Because sacubitril inhibits the enzyme that breakdown ANP, BNP as well as CNP, then the activity levels of all NPs should be increased. Therefore, the potential effects of sacubitril should include mitochondrial genesis, promotion of vascular growth, improvement of endothelial function and anti-fibrotic effects.

1.1.1 Peripheral Arterial Disease (PAD) pathophysiological mechanisms

PAD is caused by a decrease in oxygen delivery and utilization that is unable to meet the metabolic demands of active skeletal muscles. Obstruction of large arteries by atherosclerotic plaques prevents adequate delivery of oxygen [5] and the resulting dysfunctional microvasculature [7,8] is unable to distribute blood to those active muscle fibers. Mitochondrial dysfunction, independent of oxygen delivery, cannot regenerate ATP and PCr to satisfy the metabolic demands of those active skeletal muscle fibers [5]. In addition, a decrease in arterial elasticity due to atherosclerosis also impairs blood flow in patients with PAD [13].Therefore, treatment for patients with PAD should be designed to target the mechanisms that impair oxygen delivery, utilization and decrease arterial stiffness.

1.1.2 Treatment options for patients with PAD

The low success rate of the drugs that are used in the treatment of PAD is probably related to the fact that none of them directly targets the mechanisms implicated in PAD [14]. Surgical revascularization with percutaneous transluminal angioplasty (PTA) or with stents leads to significant improvements in blood flow, maximal walking distance (MWD) and pain free walking distance (PFWD) [15,16]. However, despite the initial high success rate [15], restenosis occurs in 63% and 37% of cases within the first year following primarily successful PTA or stent, respectively [15]. Another option, therapeutic angiogenesis, is still in the experimental phase and has not had the expected success in humans [17]. Exercise

rehabilitation programs are more successful than PTA in maintaining MWD and PFWD, as long as patients continue to exercise [18]. Unfortunately, patients with pain at rest are unable to participate in exercise rehabilitation programs and 2 years patient are only of 36% and 68% in center and home based programs, respectively [19].

1.1.3 Natriuretic peptides: a potential treatment option

The benefits in functional clinical parameters maximal walking duration (MWD) and pain free walking duration (PFWD) with exercise training are related to improvements in endothelial function, and increases in capillary and mitochondrial density[20]. These changes improve oxygen delivery and utilization and clearance of metabolic waste products. A potential treatment option that would target two of the most important pathophysiological mechanisms in PAD are NPs. ANP and BNP are synthesized in the atrium and ventricle of the heart in response to pressure and volume overload inducing natriuresis and vasodilation and regulating blood pressure [21], CNP is synthesized by endothelial cells and renal epithelial cells [22]. NPs act by binding to particulate membrane bound guanylyl cyclase-coupled receptors called natriuretic peptide receptor A and B (NPR-A and B) [11]. Once activated, they act by increasing the intracellular messenger cyclic guanosine monophosphate (cGMP), which then acts on a cGMP-dependent protein kinase (PKG) mediating specific physiological functions [22]. ANP and BNP, but not CNP, bind specifically to NPR-A and only CNP binds to NPR-B [22] (Figure 1). ANP and BNP acting by activating NPR-A appears to be related to angiogenesis and mitochondrial genesis [11,22] and CNP acting through NPR-B is implicated in vasodilation, angiogenesis and anti-fibrotic activity [10,11]. The activation of NPR-A is most likely responsible for the increased perfusion of tissues, for increasing muscle oxygen consumption and improvements in insulin sensitivity observed in transgenic mice that overexpressed BNP or were infused with Carperitide, a homolog of ANP [8,23]. Activating NPR-B would induce vasodilation, increase angiogenesis and may decrease fibrosis in the peripheral vasculature, which would improve arterial elasticity.

1.1.4 Entresto (sacubitril/valsartan) biochemical characteristics and physiological properties

Sacubitril is a molecule that inactivates neprilysin, a neutral endopeptidase responsible for degrading natriuretic peptides (Figure 1). The combination sacubitril/valsartan given to patients with heart failure raises serum and urinary levels of cGMP, a marker of NPs activity, by 35% and 25%, respectively [24], due potentially to an increase in ANP, BNP and CNP (Figure 1).

Sacubitril was proposed as a treatment option for patients with heart failure due to the diuretic and vasodilatory effects of natriuretic peptides. Sacubitril cannot be given alone as it raises the levels of angiotensin II and for this reason is combined with valsartan, an angiotensin II receptor blocker. This combination, sacubitril/valsartan, has been shown to decrease mortality for patients with heart failure [25] and it is currently approved by the FDA and it is being used for that purpose [26]. In addition, a similar compound, Omapatrilat, which inhibits both neprilysin and angiotensin converting enzyme (ACE) has been shown to prevent endothelial cell permeability and atheroma formation in a rabbit model of atherosclerosis [27]. The effects of natriuretic peptides on microvascular and mitochondrial function

have not been studied in human skeletal muscle. This study would provide us with the supporting evidence to determine if raising natriuretic peptides improves microvascular and mitochondrial function and if this is positively associated with exercise tolerance. Having a therapeutic option to improve microvascular and mitochondrial function would be clinically important because it could lead to effective therapeutic strategies for patients with peripheral arterial disease, diabetes and in elderly subjects who have limited exercise capacity due to low perfusion and oxygen utilization in skeletal muscles.

1.1.5 Clinical trial with carperitide, what do we still need to learn?

The effects on pain free walking duration (PFWD) of 2 weeks of intravenous infusion of carperitide, a homolog of ANP, were tested in PAD patients with different degrees of severity [8]. That study showed a decrease in pain at rest, an increase in ankle/brachial blood pressure index and in PFWD with minimal side effects. However, the study only had 13 patients, PFWD was assessed in 4 patients and did not have a placebo group and the functional and structural changes in mitochondria and the microvasculature were not determined. Assessing those parameters would provide a sound biological basis for the effects of increasing the activity levels of NPs on the functional clinical limitations and for recommending Entresto for the treatment of PAD.

1.1.6 Significance

Although there are an estimated 12 million individuals in the United States with PAD, this is an underestimation because about 50% of patients with PAD are undiagnosed [2]. Furthermore, patients with PAD have an 80% higher risk of disability than those without PAD [28]. Among patients with PAD, there is a higher risk of disability in those with poor functional performance at baseline [29] and with lower ABI [30]. In addition, those with the greatest rate of decline in mobility had a 3 fold higher rate of disability when compared to those with the lowest rate of decline [31] The inability to be mobile and participate in exercise rehabilitation programs leads to further deterioration culminating in limb amputation, loss of independence and greater risk of mortality [31]. Furthermore, total annual costs associated with vascular-related hospitalizations in patients with PAD were estimated at 21 billion in the year 2004 [32], this greatly increases when the cost for rehabilitation are included [33]. Entresto may be beneficial maintaining and improving mobility, which is a very important step in the prevention of disability, in lowering the risk of mortality and decreasing the costs associated with PAD.

1.2 Study Aims/Objectives

Primary Objective:

1. To assess the effects of 12 weeks of Entresto on the functional clinical parameters maximal walking duration, pain free walking duration and 6 minute walk test of patients with PAD.

Secondary Objective:

1. To assess if changes in functional clinical parameters are related to improvements in microvascular and mitochondrial function and to assess the effects of increasing NP activity on arterial elasticity.
2. To assess the time required for functional improvements (6 minute walk test) to return to baseline

values following 12 weeks of Entresto.

1.3 Hypothesis

We hypothesize that the combined activation of both NPR- A and B will lead to enhanced vascular and mitochondrial function, improving oxygen delivery and utilization leading to substantial improvements in the functional clinical parameters of patients with PAD. We also hypothesize that the combined activation of NPR-A and B results in increased delivery, distribution, diffusion and utilization of oxygen by active skeletal muscle fibers, thus improving clinical functional limitations in patients with PAD.

2 Study Design

2.1 Study Design

This is a double blind, randomized, controlled clinical trial with a goal of enrolling 30 subjects. Individuals who meet the eligibility criteria will be admitted into the study and will be randomly assigned to 12 weeks of Entresto or the placebo group. A variable block randomization design will be used to endure a balance in sample size between the two groups [28]. Each patient will be given a series of life style and health questionnaires, ankle brachial index will be measured and they will perform a graded exercise test to determine, pain free and maximal walking duration. In addition they will also perform the 6 minute walking test. They will also have a fMRI and MRS at pre and post 12 weeks of the clinical trial.

Following the 12 weeks of the treatment period, patients will be evaluated at weeks 4 and 8 to assess if any changes occurred in PFWD MWD and the 6 minute walking test, life style and health questionnaires and the presence or absence of adverse events following withdrawal of Entresto or placebo.

3 Outcome Measures

3.1 Ankle brachial index (ABI), exercise testing, questionnaires, anthropometric measurements and blood pressure monitoring

Patients will have their ABI determined following standard procedures described by the American Heart Association [34]. If the patient's ABI meets the inclusion criteria, then an exercise testing procedure to determine, PFWD and MWDt will be performed. In addition, on that same day the Mini Mental State Examination (MMSE), the Walking Impairment Questionnaire (WIQ), the NIH toolbox assessment test for memory and cognitive function will perform, and Quality of Life Questionnaire (PAD-QOL) [35] will be given to each participant. Body height and weight will be measured using standard anthropometric procedures [36].. Ambulatory blood pressure monitors using the Omron BP742N (©Omron Healthcare Inc. Lake Forest, IL) will be provided and each participant will measure and record their own arm blood pressure three times a day for the duration of the study immediately after waking up, at noon before lunch and in the night before going to bed.

3.2 Laboratory Procedures

Participants will come following an 10 hour fast to provide a venous blood sample for measurements

of plasma Na^+, K^+, glucose, creatinine, NT-proBNP , BNP, serum insulin and also urinary cGMP and creatinine. Urine pregnancy testing will be performed at each visit on women of childbearing potential. Before the blood draw, trained personnel will measure BP three times and the average of those measurements will be recorded.

3.3 Diagnostic Procedure for PAD

Diagnosis of PAD is described in detail in inclusion criteria. Patients will be asked for symptoms suggestive of intermittent claudication (IC) and will also have their brachial and ankle systolic blood pressure measured at both arms and legs. Patients will lie supine and will rest quietly for 5 minutes and systolic blood pressure (SBP) will be measured once in each arm following standard procedures [37]. Systolic blood pressure at the brachial artery and at the dorsalis pedis and posterior tibialis artery will be measured using an 8 MHz doppler vascular probe and the highest blood pressure at those sites will be recorded. A ratio of highest SBP measured at the ankle divided by the highest SBP measured at the brachial (ABI) < 0.90 will meet the diagnostic criteria for PAD. If there are symptoms of IC, but ABI is > 0.90 than patients will perform a walking test using the Gardner protocol and if highest systolic BP at the ankle decreases by > 25 mmHg that is also diagnostic of PAD.

3.4 Determination of maximal and pain free walking duration and the 6 minute walk test

Participant will undergo a baseline symptom-limited treadmill test as described below. The treadmill test will be used to determine MWD and PFWD and will be repeated at follow-up. The graded exercise test (GXT) is initiated at 2.0 mph (3.2 km/h) at 0% grade, and every 2 minutes the grade is increased by 2% [38]. The time at which the participant first starts feeling claudication symptoms is defined as the pain free walking duration and the time at which participants has to stop walking is designated as the maximal walking duration. During the GXT, participants will rate their claudication pain severity every 30 seconds, using the following claudication pain scale: 1 = no pain, 2 = initial onset of mild claudication pain, 3 = moderate pain, 4 = submaximal and 5 = maximal claudication pain (point at which the participant will request to stop). Recent evidence suggest that the 6 minute walk test is an accurate predictor of the response to treatment, mortality rate and mobility loss in patients with PAD [39]. Therefore, we will also perform the 6 minute walk test because it offers the possibility of an inexpensive, accurate and valid tool to assess the effects of Entresto. The 6 minute walk test will be performed in a 20 meter corridor [40]. Patients will be asked to walk as quickly as they can along the corridor for 6 minutes to cover as much distance as possible. They will be allowed to stop if they have to, but will be encouraged to continue again as soon as they are able. During the time that the participant stops walking the clock will continue to run. At the end of test the distance covered is measured.

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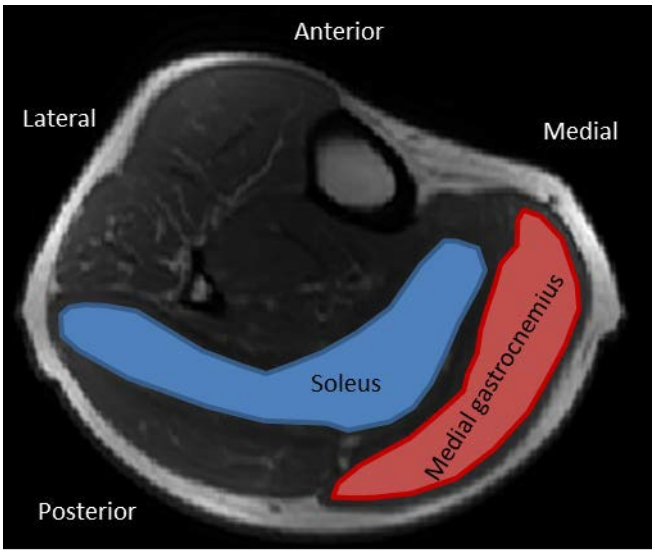


Figure 2. T_1 weighed image of the lower leg showing the distinct location of the soleus and medial gastrocnemius muscles.

3.6 Mitochondrial functional testing with magnetic resonance spectroscopy (MRS)

The bioenergetics of the medial gastrocnemius (MG) and soleus (Sol) muscle (Figure 2) will be assessed using MRS before, during and following a 24 seconds isometric maximal voluntary contraction (MVC). MRS will be performed in a 7 Tesla/90 cm whole-body actively shielded human scanner (Siemens AG, Erlangen, Germany). Optimal positioning of the muscle volume in the isocenter of the magnet will be determined using scout image scans with three orthogonal slices and multislice gradient echo images (matrix size = 108 × 128, 14 slices, 7 mm slice thickness, field of view = 13 × 16 × 20 cm, echo time (TE) = 5 ms, TR = 0.4 s) [37]. These scans will be used to identify the MG and the Sol muscle (Figure 2) and positioned near the center of the ^{31}P coil for acquisitions of a time-resolved ^{31}P MR spectra. Acquisition of MRS will be performed using a pulse-acquire ^{31}P MRS sequence with a small ^{31}P surface coil (6 cm diameter) tuned to the resonance frequency of phosphorus at 7T (120.7 MHz). A nominal 90° square excitation pulse (pulse width of 300 ms) with TR of 1 s, an acquisition bandwidth of 5000 Hz and 2,048 data points will be used to acquire phosphorus data [42,43]. MRS data will be quantified with jMRUI using the AMARES time domain fit routine [44]. The following parameters will be acquired by MRS: PCr content and pH at rest and at the end of a 16 s isometric contraction, the rate constant of PCr recovery (K_{PCr}) and maximal rate of oxidative phosphorylation (V_{max}).

3.6.1 Advantage of high field MRS

Figure 3 shows the ^{31}P spectra acquired using the 7T at the Center for Magnetic Resonance Research of the University of Minnesota by Dr. Wei Chen and his collaborators [45]. Collecting ^{31}P-MRS data at 7T offers more than twice as much signal to noise ratio (SNR) per unit time thus reducing measurement time compared to 3T magnet [46]. In addition, the increase in SNR allows the measurement of MRS parameters in muscles of interest and with high temporal resolution (1 s). This is important because the Sol, as a postural muscle, is predominantly a slow twitch muscle (slow fatigue, primary source of ATP is oxidative phosphorylation) and the gastrocnemius muscles, are more active during locomotion, is a 50% fast twitch muscle (fast fatigue and primary source of ATP is anaerobic glycolysis). The effects of PAD on each muscle may be different and the benefits from treatment will depend on the metabolic characteristics of each muscle. Therefore, measuring oxidative phosphorylation in each muscle separately will provide us with a more accurate assessment of the effects of Entresto on the bioenergetics of skeletal muscles in patients with PAD.

3.6.2 MRS Exercise protocol

Individuals will perform a 24 s maximal isometric voluntary plantar flexion, while inside the magnet, to lower PCr resting levels by 50% [47]. MRS will be performed for 1-min before the contraction, during the 16 s isometric contraction and throughout a 3 min recovery period. Maximal voluntary contraction will be measured in each individual while in the magnet using an MRI compatible device that is composed of a foot holder attached to a force transducer [48].

3.6.3 Rationale for measuring oxidative phosphorylation using MRS

Phosphocreatine resynthesis rates have been used as a surrogate measure of mitochondrial function [49] and has been studied non-invasively using MRS [50]. MRS measured PCr resynthesis rate is independent of arterial occlusion [51] and has been used to study the effects of exercise training and age on mitochondrial function [47,52]. Furthermore, the rate of PCr resynthesis in patients with PAD is 2/3 lower than age matched non-PAD individuals, partially explaining their limitations with mobility [51,53,54]. Therefore, measuring PCr resynthesis non-invasively is especially advantageous in patients with PAD in whom performing serial biopsies can cause wound healing complications. We expect that in patients receiving Entresto the rate of resynthesis of PCr will be increased and it will be associated with improvements in mobility.

3.7 Measurement of tissue perfusion and oxygenation using functional MRI (fMRI)

Validations studies using near infrared spectroscopy showed that changes in tissue oxygenation and blood volume can be assessed using differences in the MR signal intensities acquired using a dual echo gradient recall echo-echo planar (GRE-EPI) MRI procedure [55,56]. This technique found differences in microvascular function between type 2 diabetic patients and healthy individuals [48].

3.8 fMRI data acquisition

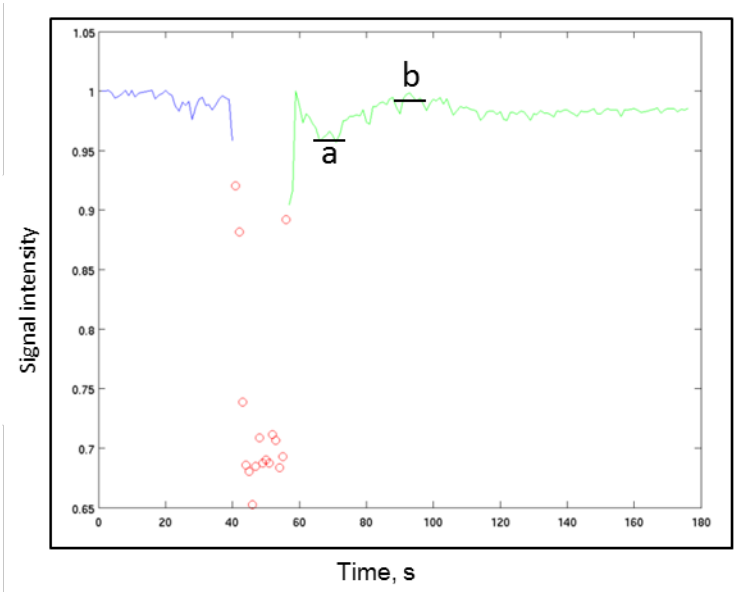


Figure 4. GRE-EPI signal intensity time course before (blue), during (red) and following 16 seconds of maximal voluntary contraction (green) at a 7T magnet. a = post-contraction baseline, b = post-contraction peak. Change SI = b – a.

fMRI and MRS data will be acquired on the same magnet during a procedure that lasts about 90 minutes. MR signal intensities (SI) will be acquired using a custom made 6 cm diameter surface proton/phosphorus coil. The same anatomical site used for the MRS data will be used to acquire the fMRI. Muscle functional MRI (fMRI) data will be acquired using a GRE, echo- planar imaging sequence for 30 s before, during a 3 min cuff-occlusion, and for the following 210 s. . The same procedure will be performed again after a 5 minute rest period using a spin echo (SE) sequence with similar acquisition parameters except with a TE = 21 ms. The functional images will be acquired using an acquired matrix = 128 × 128, slice thickness = 10 mm, repetition time = 1000 ms (defining the time resolution of the measurements), and TE = 15 ms. In addition, MRS data will be acquired using a global signal using a free induction decay and adjusting the parameters to acquired preferentially from the MG muscle using a TR = 6 s Ernst angle = 77º and a a T1 of PCr in muscle for 7T = 4 s.

A couple of blood pressure measurement will be taken following a 5 minute rest and before entering the MRI scanner. The highest blood pressure value will be used to determine the level of cuff inflation during the cuff occlusion procedure. The cuff will be inflated using a Hokanson rapid cuff inflation system, which inflates the cuff around the thigh in a couple of seconds. The cuff will be inflated 75 mmHg above the systolic blood pressure or to a max of 275 mmHg. Before initiating the scanning session, blood pressure cuff will be inflated for about 10 seconds to the target pressure to show participants what they will feel and if they are ok with that level of pressure. Cuff pressure can be lowered by 10 mmHg for patient comfort, if necessary. Images will be acquired from the leg with the more severe claudication symptoms independent of the ABI values. If the patient has a stent or a bypass graft in one leg, images will be acquired from the contralateral leg. If there are stents or bypass graft in both legs then the patient may be excluded from the study. This will be discussed on an individual basis with the Co-PI Dr. Daniel Duprez and the study physician Jiwan Thapa.

3.8.1 Functional MRI data analysis

Images will be analyzed using custom-written Matlab version 7.0.1 (The Mathworks, Natick, MA) routines. The functional images will be co-registered to the anatomical image using a rigid registration procedure [57]. Then the anatomical image will be used to identify the MG and Sol muscles and regions of interest (ROIs) will be specified separately around those two muscles. Resolved connective tissues and blood vessels will be excluded from the ROIs. The mean functional image SI for the ROI will be

calculated as a function of time and divided by the corresponding mean pre contraction SI value. An average SI time course will be obtained by averaging the SI time course from two trials, as previously described [56].

The averaged SI time course will be fitted to a polynomial function, and inflections in the derivative of this polynomial will be used to identify the minimum and maximum post contraction SI values. Figure 4 shows post contraction signal intensity indicating change in oxygenation plotted as a function of time. Data obtained at University of Minnesota CMRR 7T, unpublished observation. The change in SI ($\Delta SI = b - a$) will be determined for the Sol and MG muscle reflecting the average post contraction changes in oxygenation at the muscles microvascular bed.

3.8.2 Rationale for functional MRI measurements

A 12 week supervised exercise training program for patients with PAD showed that increases in peak VO_2 was correlated with increases in capillary density and to a 70% improvement in peak leg blood flow measured using cuff occlusion plethysmography [58]. These results show that the improvements in functional clinical parameters are related to an enhanced tissue perfusion of the leg. The fMRI technique allows for the measurement of post contraction changes in tissue oxygenation with a high temporal and spatial resolution. The assessment of microvascular function in the MG and Sol muscle separately will allow us to determine if the effect of Entresto may be muscle fiber type dependent. Theoretical calculations indicate that at 7T, SE acquired images reflect tissue oxygenation surrounding small vessels, whereas images acquired using GRE are reflective of oxygenation status in tissues surrounding small and large vessels.

4 Subjects

4.1 Study Setting

This study will take place at the University of Minnesota.

4.2 Eligibility Criteria

Inclusion Criteria

Subjects will be eligible to participate in the study if all of the following conditions exist:

1. Age 18-80 years of age
2. Intermittent claudication, such as exercise-induced pain, cramps, fatigue, or other equivalent discomfort, involving large muscle groups of the leg(s) (calf, thigh, buttocks), relieved by rest.
3. Ankle-brachial index ≤ 0.90 acquired according to the American Heart Association guidelines.
4. Highest ankle pressure reduced by at least 25 mm Hg after exercise compared to resting pressure (or loss of previously present Doppler signal for both the posterior tibial and anterior tibial arteries immediately after exercise if arteries were incompressible).
5. Patients on medical treatment for PAD without significant improvement in intermittent claudication within the last 6 months.
6. Absence of more than 60% renal artery stenosis within the last year. If a duplex ultrasound has not been perform within the last year to detect renal artery

stenosis, it will be schedule during the screening visit.

7. For women with reproductive potential, a willingness to use methods of contraception that will prevent the subject from becoming pregnant during the study. Adequate contraception methods include hormonal methods used for two or more menstrual cycles before Screening (eg, oral contraceptive pills, contraceptive patch, or contraceptive vaginal ring), barrier methods (eg, contraceptive sponge, diaphragm in conjunction with contraceptive foam or jelly, or condom in conjunction with contraceptive foam or jelly), intrauterine methods (IUD), sterilization (eg, tubal ligation or a monogamous relationship with a vasectomized partner) and abstinence.

Exclusion Criteria

Subjects will be excluded from participation in the study if any of the following conditions exist:

1. Patients with physician diagnosed heart (HF) stage > II, liver failure or cancer other than skin cancer.
2. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m2.
3. Severe limitations in mobility due to osteomuscular disorders.
4. Dementia or other mental disorders that prevent patients from following a research protocol.
    a. Subjects that score ≤ 23/30 in the Mini Mental State Examination.
5. Patients engaged in an exercise rehabilitation program.
6. Patients schedule to undergo a revascularization procedure during the study.
7. History of angioedema related to use of angiotensin convertase enzyme inhibitor (ACEinh) or angiotensin receptor blocker (ARB).
8. Current use of ACEInh or ARB
9. Concomitant use with aliskiren (renin inhibitors) in patients with diabetes.
10. Renal artery stenosis of more than 60% occlusion in any of the renal arteries by duplex ultrasound.
11. Patients on therapy with lithium compounds.
12. Women who are pregnant or nursing. Women who are not postmenopausal (≥ 12 months of non-therapy- induced amenorrhea) or surgically sterile are defined as being of child-bearing potential, must have a negative result from a urine pregnancy test at Visits 1 and 2. Women with the potential to become pregnant who are not willing to use an acceptable form of birth control, defined as abstinence or use of an intrauterine device (IUD), oral contraceptive, barrier and spermicide, or hormonal implant throughout the study period.
13. Patients with implantable cardiac defibrillators
14. Patients with hyperkalemia (K^+ > 5.2 mEq/L) and patients with conditions that are the most frequent cause of hyperkalemia, such as CKD and ESRD
15. Patients on potassium sparing diuretics

4.3 Recruitment

Recruitment will be performed with the help of the Clinical and Translational Science Institute (CTSI)

of the University of Minnesota recruiting services and by advertisement in clinics and other institutions with a predominantly elderly population. Patients with PAD who have completed a cardiac rehab programs will receive previously IRB approved letters inviting them to participate in our study. In addition, from the Institute of Health and Informatics, a list of patients with PAD, containing information regarding the date of their upcoming appointment, will be made available to the PI at the secure data shelter. The PI will confirm if the patient meets the inclusion and none of the exclusion criteria on Epic and will send an email to the provider through Epic, requesting that the patient be informed about this clinical trial. If the patient is interested, they will contact the PI to request more information. Our research group has ample experience in recruiting patients with PAD, in maintaining high adherence rate and successfully completing clinical trials [38,59]. Although PAD is more frequent in African Americans males, we will make an effort to recruit across all racial and ethnic groups and from both genders.

4.4 Early Withdrawal of Subjects

Any subject may discontinue participation in the study for any reason at any time. Subjects who elect to withdraw may resume other treatments as determined appropriate by their physician. Reasons and circumstances surrounding withdrawal will be documented in the study records.

When a pregnancy has been confirmed in a subject during maternal exposure to treatment, the study drug will be discontinued immediately and the subject will be referred to her primary physician. In the event that any subject discontinues study drug and, subsequently, withdraws from the study before completion, regardless of reason, reasonable efforts will be made to have the subject return for an early termination visit and have the end of treatment procedures completed. If a subject is unable to return to the clinic for an early termination visit, attempts will be made to contact the subject by phone to assess AEs. Subjects will be asked to return to the clinic for further assessments if the PI deems it appropriate.

4.5 Stopping Criteria

Reasons for terminating the study may include but are not necessarily limited to the following:

- The incidence or severity of AEs indicates a potential health hazard to subjects.
- Advice of DSMB
- Subject enrollment is unsatisfactory.

If the study is discontinued, all assessments listed in the end of treatment visit should be completed.

4.6 Data Collection for Withdrawn Subjects

Data of withdrawn subjects will be collected up to the point of withdrawal. This data will still be included in the pooled data of the study for further analysis. For subjects who withdraw from further data collection, there will not be replacement with more subjects. When study drug is discontinued (regardless of the reason), the investigator will encourage that all of the evaluations required at the End of Treatment Visit be performed and that any additional evaluations be completed that may be necessary to ensure that the patient is free of untoward effects. The patient will be encouraged to seek appropriate follow-up for any continuing health problems.

5 Study Drug

5.1 Entresto (sacubitril/valsartan)

Sacubitril is a molecule that inactivates neprilysin, a neutral endopeptidase responsible for degrading natriuretic peptides. Sacubitril cannot be given alone as it raises the levels of angiotensin II 62-64 and for this reason is combined with valsartan, an angiotensin II receptor blocker 65. This combination, sacubitril/valsartan [LCZ696 (Entresto), Novartis Pharmaceuticals Corp.], has been given to patients with heart failure raising serum and urinary levels of cGMP, a marker of NPs activity, by 35% and 25%, respectively 24 and decreases mortality in this type of patients 25. Entresto is currently approved by the FDA for the treatment of patients with heart failure NYHA Class II-IV 26.

5.1.1 Description

ENTRESTO contains a complex comprised of anionic forms of sacubitril and valsartan, sodium cations, and water molecules in the molar ratio of 1:1:3:2.5, respectively. Following oral administration, the complex dissociates into sacubitril (which is further metabolized to LBQ657) and valsartan. The complex is chemically described as Octadecasodiumhexakis(4-{[(1S,3R)- 1-([1,1′-biphenyl]-4-ylmethyl)-4-ethoxy-3-methyl-4-oxobutyl]amino}-4-oxobutanoate)hexakis(N-pentanoyl-N-{[2′-(1H- tetrazol-1-id-5-yl)[1,1′-biphenyl]-4-yl]methyl}-L-valinate)—water (1/15). Its empirical formula (hemipentahydrate) is C48H55N6O8Na3 2.5 H2O. Its molecular mass is 957.99 M.

5.2 Dosage and Administration

Entresto will be given orally at the recommended starting dose of 49/51 mg twice-daily. The dose of Entresto should be doubled after 2 to 4 weeks to the target maintenance dose of 97/103 mg twice daily, as tolerated by the patient [25,60].

12 Weeks of Treatment

We will follow the recommended dosages as reported in the Entresto prescribing information sheet distributed by: Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936. Subjects will initiate with the recommended dose of 49/51 mg (sacubitril/valsartan) twice daily for two weeks. For patients not currently taking an angiotensin-converting enzyme inhibitor (ACEi) or an angiotensin II receptor blocker (ARB) or previously taking a low dose of these agents we will initiate treatment with the lowest dose available (24/26) (sacubitril/valsartan) twice daily. After two weeks of treatment the study physician will progressively increase to the target maintenance dose of 97/103 mg (sacubitril/valsartan). Each participant will receive a package with enough pills containing Entresto/placebo that will last until the following schedule visit. To assure adherence to the treatment, patients will return all the packages with the empty vials at each study visit. Participating patients will be encouraged to follow their standard health care treatment and lifestyle recommendations prescribed

Table 1. Criteria to lower dosage or discontinuation of Entresto
1. Hypotension (symptomatic hypotension or orthostatic hypotension, or hypotension that requires treatment) 2. Renal failure. Increase in creatinine > 50% of baseline value. 3. Hyperkalemia. $K^+ > 5.2$ mEq/L 4. Falls (related to hypotensive episodes) 5. Angioedema
Hypotension = a decrease from baseline in sitting systolic blood pressure (SBP) of ≥ 20 mm Hg and a decrease in sitting SBP to < 90 mm Hg or symptoms.

by their attending physician.

5.2.1 Dose Adjustment

Dose of Entresto is started at the middle level of 49/51 mg twice-daily and if well tolerated by the patient then dosage is increased to the target maintenance dose described above. If manifestations of side effects of Entresto appear (Table 1), dose can be lowered to the lowest dose of 24/26 mg twice- daily. If at the lowest dose level adverse events continue then patient will be withdrawn from the study. Dose of Entresto will be administered following the recommendation as written in the Entresto prescribing information sheet from Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936 as revised by the FDA on August, 2015 (Patient information sheet attached). The study physician Dr. Jiwan Thapa will be responsible for all dose changes in subjects participating in this study. Specific guidelines on dosage change are detailed below.

Dosage modifications guidelines:

Any patient with a serum potassium > 5.2 mEq/L after randomization requires regular, repeated checks of potassium concentration (beyond that prescribed in the protocol) until it is clear that the potassium concentration is stable and not rising into the range of concern (≥ 5.5 and < 6.0 mEq/L) or potential danger (≥ 6.0 mEq/L).

Corrective action for management of hyperkalemia.

Serum potassium > 5.2 and less than or equal to 5.5 mEq/L

- Confirm potassium concentration in a non-hemolyzed sample.
- Reinforce low potassium diet and restriction of food/drinks with high potassium content (e.g. orange juice, melon, bananas, low-salt substitutes etc.).
- Review medical regimen (including dietary supplements and over-the-counter medications) for agents known to cause hyperkalemia. Consider reduction in dose or discontinuation of these agents:
    - Potassium supplements, e.g., potassium chloride.
    - Salt substitutes.
    - Non-steroidal anti-inflammatory drugs (NSAIDs)
    - Herbal Supplements:
        - For example, Noni juice, alfalfa (*Medicago sativa*), dandelion (*Taraxacum officinale*), horsetail (*Equisetum arvense*), nettle (*Urtica dioica*), milkweed, lily of the valley, Siberian ginseng, hawthorn berries.
- Repeat serum potassium measurement within 3 to 5 days.
- If serum potassium remains > 5.2 and ≤ 5.5 mEq/L, regularly monitor serum potassium levels to ensure stability (suggested once monthly).
- Consider down-titration of study medication, according to study physician medical judgment.

Serum potassium > 5.5 and < 6.0 mEq/L

- Confirm potassium concentration in a non-hemolyzed sample.
- Consider down-titration or temporarily discontinue study drug according to study physician medical judgment.

- Apply all measures outlined for serum potassium > 5.2 and ≤ 5.5 mEq/L.
- Repeat serum potassium measurement after 2-3 days.
- If serum potassium < 5.5 mEq/L, consider resumption of study drug at lower dose with repeat potassium within 5 days.

Serum potassium greater than or equal to 6.0 mEq/L

- Immediately discontinue study drug.
- Confirm potassium concentration in a non-hemolyzed sample.
- Urgently evaluate patient and treat hyperkalemia as clinically indicated.
- Apply all measures outlined for serum potassium > 5.2 and < 6.0 mEq/L.
- No resumption of study drug without individualized case discussion with and permission from the DSMB.

Guidelines for the management of renal dysfunction

If, at any time after randomization, eGFR decreases by ≥ 25% from baseline (or if serum creatinine concentration increase to 2.5 mg/dL or by 50% of baseline value, the investigator will check for potentially reversible cases of renal dysfunction such as:

- Non-steroidal anti-inflammatory drug intake, antibiotics, or other treatments known to affect creatininemia
- Volume decrease
- Urinary infection
- Urinary tract obstruction
- Study medication

If a patient eGFR decreases by ≥ 40% from baseline (or if serum creatinine concentration rises above 3 mg/dL, the investigator will check for potentially reversible causes of renal dysfunction (see above). If the study physician judges that the study medications needs to be stopped, the principal investigator needs to be informed and the DSMB contacted.

Guidelines for the management of blood pressure

If symptomatic hypotension occurs:

- Correct any treatable cause, e.g. hypovolemia.
- If hypotension persists, the study drug should be down-titrated or withdrawn at the judgment of the study physician.

5.3 Method for Assigning Subjects to Treatment Groups

A variable block randomization design will be used to endure a balance in sample size between the two groups

5.4 Subject Compliance Monitoring

Compliance to drug administration will be documented at all study visits. Subjects will be asked to bring in their medication at each study visit for monitoring purposes.

5.5 Blinding of Study Drug

Blinding of the drug will be done by the investigational Drug Services (IDS) at the University of Minnesota. Once a subject is eligible to participate in the study, the PI will inform IDS and they will

be in responsible for randomizing the subject into one of the two groups and preparing the first two weeks of treatment. Capsules containing pills with either placebo or Entresto will be placed in a container identified with a code, which will be kept by IDS pharmacy unless it is requested as part of an emergency response or study termination. Each individual pill will be packaged in identical capsules by IDS pharmacy staff. Entresto comes in pills of three dosages: 24/26 mg, 49/51mg and 97/103 mg. Capsules for each of the three dosages (placebo or Entresto) will be prepared by IDS and the study physician (blinded) will provide the capsules following the dosing procedures as described in 5.2.1. The subject will only be unblinded if there is a medical situation where it is necessary to know if the subject is on drug or placebo.

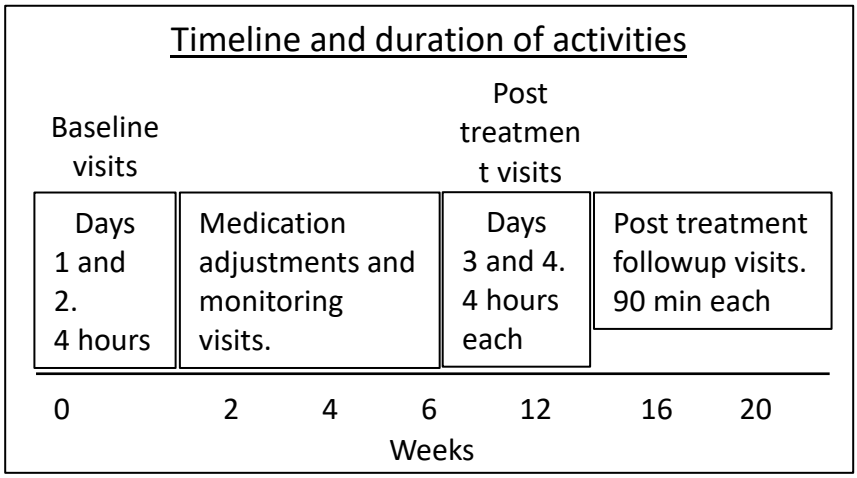
5.6 Receiving, Storing, Dispensing and Return

The Investigational Drug Services (IDS) pharmacy staff will in charge of preparing the pills and the placebo capsules and dispensing the drug for this study.

6 Study Procedures

6.1 Participant Timeline/Procedures

Screening and Baseline



Pre-Screening Phone Interview

An initial phone interview will be used to pre-screen subjects for potential eligibility. Subjects that meet all inclusion criteria and none of the exclusion criteria as described in 4.2, will be invited to participate in the study. If potential participants are interested, they will be asked to come in to discuss the study further and if they wish to participate they will be asked to sign the consent form.

Visit 1 (Screening/Baseline 1)

After consent has been obtained, the following tests and procedures will be done to determine whether the potential subject is eligible to participate.

- Patients will be asked a series of questions about symptoms suggestive of intermittent claudication (IC)
- Brachial and ankle systolic blood pressure will be measured with both arms and legs. Patients will lie supine and will rest quietly for 5 minutes and systolic blood pressure (SBP) will be measured once in each arm following standard procedures. Patients will have their ankle brachial index (ABI) determined following standard procedures described by the American Heart Association [34]. Systolic blood pressure at the ankles will

be measured at the dorsalis pedis and posterior tibialis artery using an 8 MHz doppler vascular probe and the highest blood pressure at those sites will be recorded. A ratio of highest SBP measured at the ankle divided by the SBP measured at the brachial (ABI) < 0.90 will meet the diagnostic criteria for PAD. If there are symptoms of IC, but ABI is > 0.90 than patients will perform a walking test using the Gardner protocol and if highest systolic BP at the ankle decreases by > 25 mmHg that is also diagnostic of PAD.

- 10 hour fasting venous blood sample for measurements of plasma Na^+, K^+, glucose, creatinine, serum insulin and urinary cGMP and creatinine. Measure BP three times and the average of those measurements will be recorded.
- will be collected for measurement of plasma potassium and creatinine/eGFR
- Women of child bearing potential will have a urine pregnancy test to verify they are not pregnant.
- Patients will perform the mini mental state evaluation to test. Patients must have a score of ≥23/30 to be included in the study.

Individuals evaluated by the study physician or the main PI that meet all the inclusion and none of the exclusion criteria will continue with the following tests and procedures.

- Arterial elasticity testing using applanation tonometry in the radial artery. Patients in the supine position will have electrodes placed in their chest to record an EKG, will have a blood pressure taken and arterial pulse wave will be recorded for 30 seconds in the radial artery.
- A graded exercise testing procedure to determine pain free and maximal walking duration will be performed. Participant's blood pressure and EKG will be measured before, during and for 6 minutes following the exercise test. Contraindications outlined by the American College of Sports Medicine to perform and to stop an exercise test will be followed.
- The 6 minute walk test will be performed in a 20 meter corridor. Patients will be asked to walk as quickly as they can along the corridor for 6 minutes to cover as much distance as possible. They will be allowed to stop if they have to, but will be encouraged to continue again as soon as they are able. During the time that the participant stops walking the clock will continue to run. At the end of test the distance covered is measured.
- A Walking Impairment Questionnaire (WIQ), a 36-item short-form health survey (SF-36), and Quality of Life Questionnaire (PAD-QOL) will be given to each participant.
- Body height and weight will be measured using standard anthropometric procedures.
- Total body lean and fat mass will be determined using bioelectrical impedance from Valhalla Scientific body comp scale®.

Visit 2 (Baseline 2/Randomization**)**

The second screening visit will take place within 48 hours of Visit 1. The following tests and procedures will be performed:

- Women of child bearing potential will have a urine pregnancy test to verify they are not pregnant.
- MRS and fMRI will be performed in a 7 Tesla/90 cm whole-body actively shielded human

scanner.

- IDS will randomize and dispense the treatment drug or placebo.
- The subject will be given instructions on how to take the drug and will be provided a diary to keep track of any adverse events they are experiencing.
- Ambulatory blood pressure monitors using the Omron BP742N (©Omron Healthcare Inc. Lake Forest, IL) will be provided and each participant will be asked to measure and record their own arm blood pressure three times a day for the duration of the treatment period. Subjects will be asked to measure their blood pressure immediately after waking up, at noon before lunch and in the night before going to bed. Participants will be instructed on the use of the ambulatory blood pressure monitoring (ABPM) and will have a practice session.

6.2 Treatment Period

Visits 3, 4 and 5 (Treatment visits at Weeks 2, 4 and 6)

Subjects will be required to return to the clinic at weeks 2, 4 and 6 of treatment to perform the following procedures and tests:

- Dose modification to target dose at week 2 and lowering of dose per direction of physician at weeks 4 and 6.
- Blood glucose, creatinine and potassium levels.
- Women of child bearing potential will have a urine pregnancy test to verify they are not pregnant.
- Blood pressure measurement and reading of recording on the blood pressure monitor.
- Review of daily diaries for any side effects.
- Collect and assess study medication; dispense study medication.

6.3 Phone Calls

The PI or Study Coordinator will call subjects every two weeks during the treatment period in order to assess AEs. The phone calls will occur at Week 1, 3, 5 and 7.

6.4 End of Treatment

Visit 6 (End of treatment visit 1)

At the end of the 12 week treatment period, subjects will be asked to return for testing. Subjects will be asked to return within 24 hours of taking their last study pill. The following tests and procedures will be performed:

- Ankle Brachial Index (ABI)
- GXT will be done to determine pain free and maximal walking distance
- A Walking Impairment Questionnaire (WIQ), a 36-item short-form health survey (SF-36), and Quality of Life Questionnaire (PAD-QOL) will be given
- Body height and weight will be measured using standard anthropometric procedures
- Total body lean and fat mass will be determined
- Blood pressure measurement and reading of recording on the blood pressure monitor.
- Review of daily diaries for any side effects.

- Collect and assess study medication.
- Women of child bearing potential will have a urine pregnancy test to verify they are not pregnant.

Visit 7 (End of treatment visit 2)

Visit 7 will occur within 48 hours of visit 6. At that visit the following tests and procedures will be done:

- 10 hour fasting venous blood sample for measurements of plasma Na^+, K^+, glucose, creatinine, NT-proBNP, BNP, serum insulin and also urinary cGMP and creatinine.
- Women of child bearing potential will have a urine pregnancy test to verify they are not pregnant. The 6 minute walk test will be performed in a 20 meter corridor.
- Measure BP three times and the average of those measurements will be recorded.
- fMRI and MRS will be performed in a 7 Tesla/90 cm whole-body actively shielded human scanner.

6.5 Follow-up Visits

Visits 8 (Follow-up, Week 6 after End of Treatment Visit)

Subjects will return 6 weeks after their treatment has ended to assess if any changes have occurred in pain free walking duration, maximal walking duration during the 6 minute walking test. Subjects will have a Walking Impairment Questionnaire (WIQ) and Quality of Life Questionnaire (PAD-QOL) administered. The presence or absence of adverse events following withdrawal of study drug will be assessed.

Schedule of Events

Schedule of Events								
	Visit 1 Screening	Visit 2 Baseline	Study Visits 3-7					Follow-up Visit 8
Time Point		**Day 0**	**Week 2**	**Week 4**	**Week 8**	**Week 12**	**Week 12**	**Week 18**
Time estimates	4 hours	4 hours	1 hour	1 hour	1 hour	4 hours	4 hours	45 min
Obtain Consent	x							
Ankle brachial Index	x					x		
Duplex ultrasound[1]	x							
Arterial elasticity	X					X		
Questionnaires	x					x		x
Memory test	X							
Height, weight, lean and fat mass	X					X		
Treadmill test	x					x		x
6 minute walk	x	x					x	x
MRI		x					x	
Blood draw for labs	X	x	x	x	x	x	x	
Urine test		x					x	
Blood pressure[2]	x	x	x	x	x	x	x	x
Pregnancy test[3]	x	x	X	X	X	X	X	X
Receive drug/placebo		x	x	x	x			
Review blood pressure records and diary			x	x	x	x		

1. Screening visit could be 5 hours long if duplex ultrasound is required, if not it will last 4 hours.
2. Visit should occur within 24 hours of last dose of drug.
3. Blood draw at screening and end of treatment visit 1are following 10 hour fasting to assess serum: creatinine and K^+, sodium, glucose, and serum insulin. Study visits at weeks 2, 4 and 6 are also fasting 10 hours prior to blood draw to assess: serum glucose, creatinine and potassium.
4. Duplex ultrasound will be perform only on those patients without a duplex ultrasound for the diagnosis of renal artery stenosis within the last year.
5. NIH toolbox for cognitive function.

6. The treadmill GXT test will be used to determine pain free walking distance, maximal walking distance.
7. Subjects will be instructed to measure and record their blood pressure at home, 3 times daily during the treatment period.
8. For women of childbearing potential.

7. Data Handling and Record Keeping

6.6 Confidentiality

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

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

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

6.7 Types of Data

Data for this study will include safety, laboratory and outcome measures.

6.8 Record Retention

Per University of Minnesota policy all documents concerning the use of human subjects in research will be maintained for 3 years from completion of IRB-related work and 6 years for HIPAA. No record will be destroyed without the written consent of the sponsor-investigator.

The sponsor-investigator will permit authorized representatives of the University of Minnesota and regulatory agencies to examine (and when required by applicable law, copy) clinical records for the purposes of clinical site monitoring, quality assurance reviews, audits, and evaluation of the study safety and progress.

6.9 Source Documents

A source document is defined as the location where study-related data are initially recorded. Source documents for this study will include hard copy paper and/or electronic forms, laboratory printouts, and medical records onto or into which data will first be recorded.

6.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

When a deviation from the protocol is necessary for an individual subject, the Investigator must complete a description of the deviation from the protocol and justification on the Protocol Deviation Form.

7 Study Auditing, and Inspecting

7.1 Auditing and Inspecting

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

8 Safety and Adverse Events

8.1 Adverse Event Definitions and Reporting Guidelines

9.1.1 Adverse Event

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

9.1.1.1 Serious Adverse Events (SAE)

A serious adverse event (SAE) is any adverse event that is:

- Fatal
- Life-threatening
- Requires or prolongs a hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect

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

Hospitalization

Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse event; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event (e.g., for a preexisting condition not associated with a new adverse event or with a worsening of the preexisting condition; admission for a protocol- specified procedure) is not, in itself, a serious adverse effect.

9.1.1.2 Expected Adverse Events (AE)

Expected adverse events are those that are known to be associated with or have the potential to arise as a consequence of participation in the study.

9.1.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the Protocol at the specificity or severity that has been observed.

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

An adverse event that in the opinion of the Principal Investigator is unexpected and related to the investigational agent.

Assessment of Severity

Categories	**Definition**
Definitely related	This relationship suggests that a definite causal relationship exists between the administration of the investigational agent and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.
Probably related	This relationship suggests that a reasonable temporal sequence of the event with investigational agent administration exists and, based upon the known or previously reported adverse reactions, or judgment based on the investigator's clinical experience, the association of the event with the investigational agent seems likely.
Possibly related	This relationship suggests that treatment with the investigational agent may have caused or contributed to the AE (i.e., the event follows a reasonable temporal sequence from the time of investigational agent administration and/or follows a known response pattern to the investigational agent but could also have been produced by other factors.)
Not related	This relationship suggests that there is no association between the investigational agent and the reported event.

The sponsor-investigator will make an assessment of severity for each AE and SAE reported during the study. The assessment will be based on the sponsor-investigator's clinical judgment.

Assessment of Causality

The sponsor-investigator will estimate the relationship between the investigational agent and the occurrence of

each AE or SAE by using his best clinical judgment. Other elements, such as the history of the underlying disease, concomitant therapy, other risk factors, and the temporal relationship of the event to administration of the investigational agent, will be considered and investigated.

An SAE may be recorded when the sponsor-investigator has minimal information to include in the initial report. The sponsor-investigator may change his opinion of the causality in light of follow-up information, with subsequent amendment of the SAE report.

Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis. A adverse event monitoring form will be use to interview patients during the biweekly phone call to assess presence or absence of adverse events.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events will be collected for the six month period following study product administration. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

9.1.2.1 Data and Safety Monitoring Board (DSMB)

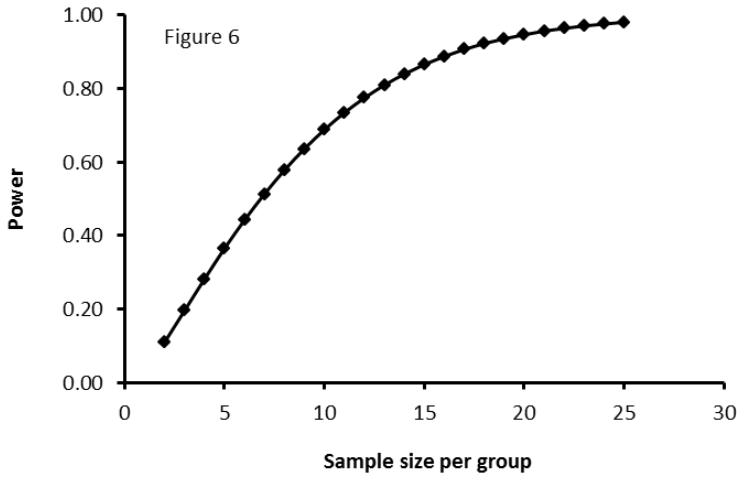
Before recruitment of participants, an independent DSMB will be formed in accord with guidelines published by the U.S. Department of Human Health Services [61] and the National Heart Lung and Blood Institute [62]. This board will be composed by a physician expert in the management of patients with PAD, a biostatistician and at least two investigators with expertise in current clinical trials conduct and methodology. Board members will be independent from the study and at least one will not be affiliated with the University of Minnesota. None of them will have any financial, proprietary, professional, or other interests that may affect impartial, independent decision-making that constitute a potential conflict of interest. This board will be in charge of 1) periodically reviewing and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and 2) make recommendations to the principal investigators (PIs) and the Internal Review Board concerning the continuation, modification, or termination of the trial. A DSMB will be selected and meet before the start of recruitment and will design a plan of action, frequency of meetings and elaborate detail safety guidelines for this study.

9 Data Analysis

9.1 Statistical Methods

General baseline characteristics will be described using mean and proportions. Smirnov-Kolmogorov tests will be used to test normality of distribution of variables. For variables that do not follow the normal distribution, log transformation will be performed. Linear simple and multiple regressions will be performed to assess

association between the effects of Entresto on metabolic and physiological changes with clinical functional variables. Differences between the placebo and the



Entresto group will be assessed using independent sample t-test and Chi-square for quantitative and categorical variables, respectively. Change following the 12 week clinical trial in metabolic and physiological parameters will be assessed using paired sample t-test. Repeated measures analysis of covariance will also be used to evaluate differences between groups as a function of time. A p value < 0.05 will be used to determine statistical significance. Effect size (EF) calculated as EF = (mean Entresto group – mean placebo group)/SD (control group) will be used to assess the clinical significance of the effects of Entresto [63]. The PI will be in charge of performing all statistical calculations using SAS v 9.3 by SAS Institute Inc., Cary, NC. Dr. David R. Jacobs Jr. Professor at the School of Public Health a statistician with more the 800 publications will be a consultant in statistical analysis of the data.

9.2 Power analysis

Sample size for a double blind randomized clinical trial was determined using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Sample size is based on the mean (SD) values obtain in a clinical trial that measured the effects of carperitide on pain free walking distance in patients with PAD 5. The mean (SD) pre post change in pain free walking distance was of 278 (240) m. [We have estimated that for a power 0.85 we will need 15 subjects per group to reject the null hypothesis with Type I error probability of 0.05] (Figure 6).

10 Clinical Management

Dr. Jiwan Thapa Assistant Professor in the Department of Medicine Division of Kidney Diseases and Hypertension will be the study physician for clinical trial. This includes adjusting the dose of Entresto (based on information obtain from the Entresto information sheet, Novartis), development of safety procedures and treatment of medical complications. The main PI (Otto A. Sánchez MD PhD) will be the person directly in charge of admittance of patients into the study, performing and supervising the diagnostic tests and the metabolic, physiological and clinical functional testing.

11 Risks/Benefits

11.1 Potential Risks

As part of the PARADIGM-HF clinical trial adverse reactions occurring at an incidence of ≥ 5% in patients who were treated with ENTRESTO in the double-blind period are shown in Table 2.

Table 2. Adverse effects and percentage of cases in the PARADIGM-HF.

Adverse effects	Entresto	Placebo + Enalapril	Preventive or corrective action
Angioedema, %	0.5	0.2	Discontinue
Hypotension, %	18	12	Dose adjustment of blood pressure lowering medications
Serious hypotension, %	1.5	1.5	Dose adjustment of blood pressure lowering medications
Renal failure, %	5	5	Monitor creat and blood urea
Hyperkalemia	12	14	Monitor K+
Cough, %	9	13	Modify dose
Dizziness, %	6	9	Prevent hypotension
Falls, %	1.90	1.30	Prevent hypotension

11.2 Methods to Minimize Risks

Patients will return to the clinic on weeks 2, 4 and 6 of the treatment period to assess for known adverse events and dosage adjustment if necessary. In addition, the clinical coordinator or the principal investigator will contact the subject by phone every other week to assess presence of adverse events and determine if an additional visit to the clinic is required. Patients will maintain a diary provided by the principal investigator to record blood pressure values and the date and time of the occurrence of known or unexpected adverse reactions. Subjects will be verbally warned and given a handout with a list of medications which are contraindicated or potentially cause an adverse interaction with Entresto. The medications on the list are on the Novartis information sheet on Entresto. The study physician and the PI will highlight these medications and the potential adverse events that can occur.

11.3 Emergency Procedures

Patients will be given detailed instructions of how to recognize any of the known serious or non-serious adverse events that may occur while receiving Entresto and also to report on previously unreported adverse events. If a non-life-threatening serious adverse events occurs, patients are advised to contact their primary care physician and then to contact the principal investigator. If there is a life threatening situation, subjects are advised to call 911 and then to contact the principal investigator. Patients will be given an emergency identification card with the contact information of the principal investigator and the name of the drug, to help inform the health care provider that this patient is in a clinical trial and may have been randomized to either Entresto or a placebo. In case of a serious adverse event, Entresto will be discontinued or dosage may be adjusted depending on the judgement of the study physician. All adverse events will be reported to the DSMB and their recommendations will be followed.

11.4 Unblinding Procedures in Case of Emergency

The Investigational Drug Services (IDS) pharmacy will generate the randomization schedule and will keep the clinical trial code stored in a secure location. The inpatient IDS pharmacy will hold the code, which can be access 24 hours, 7 days a week if necessary. The study physician and the main PI can have access to the code in case of a clinical emergency. The code will be revealed on request by the data safety monitoring board (DSMB) and at the completion of the clinical trial.

12 Ethical Considerations

12.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

12.2 Institutional Review Board (IRB) Review and Informed Consent

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted once IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record. Research staff will ensure that candidates understand all elements of the consent form by addressing questions posed during the consent procedure and by asking for verbal confirmation that the candidate has no additional questions and verbally understands the purpose of the study, study intervention, basic procedures, risks, and that participation is voluntary. Subjects will be given as much time as they need to read and understand the consent form.

12.3 Confidentiality

All biologic specimens and clinical data obtained from this study will be linked to a study specific code and not to personal identifying information (e.g., name, social security number, medical record number). A key which will link the four-digit study code to the personal information will be maintained in a secure pass-word protected file maintained by the site study investigators.

All sites are committed to ensuring that appropriate measures are taken to protect the privacy and confidentiality of all Protected Health Information (PHI) for which it is responsible. This includes compliance with all regulations set forth by the relevant jurisdictions. In the US, this includes the HIPAA Privacy Rule, as well as with existing US State and Federal laws pertaining to PHI. All study volunteers in US will be given a copy of the Notice of Privacy Practices. Good faith efforts will be made to obtain each individual's written acknowledgement of receipt of the Notice(s). The rights mandated by the HIPAA Privacy Rule concerning an individual's ability to access, amend, and disclose certain sections of his or her own PHI will be fully respected, and every effort will be made to assist patients who choose to exercise these rights.

12.4 Declaration of Interests

Novartis is not funding this study and will play no role in the collection, analysis and interpretation of the data or the conduct of the study. None of the researchers have a conflict of interest to disclose regarding Entresto.

12.5 Publication Policy

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a

public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. This protocol will be registered on ClinicalTrials.gov.

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Appendix A: Contraindications from the American College of Sports Medicine

As most potential participants in this study will be a high-risk population for cardiovascular events, every exercise test is monitor using continuous EKG, blood pressure monitoring at rest, at each stage and for 6 minutes following the exercise test. The School of Nursing Exercise Laboratory is equipped with an automated defibrillator and has a standard procedure in place to respond to adverse events that may occur during the performance of an exercise test. Staff and the main PI will rehearse the emergency procedures twice a year and will have up to date CPR certifications. The main PI will supervised all exercise tests and will be responsible for following the guidelines outline below.

The following are absolute and relative contraindications to initiate an exercise test Absolute

1. A recent significant change in the resting EKG suggesting significant ischemia, recent myocardial infarction (within two days), or other acute cardiac event.
2. Unstable angina.
3. Uncontrolled cardiac dysrhythmias causing symptoms or hemodynamic compromise.
4. Symptoms of severe aortic stenosis.
5. Uncontrolled symptomatic heart failure.
6. Acute pulmonary embolus or pericarditis.
7. Suspected or known dissecting aneurism.
8. Acute systemic infection, accompanied by fever, body aches, or swollen lymph nodes.

Relative

1. Left main coronary stenosis
2. Moderate stenotic valvular heart disease
3. Electrolyte abnormalities
4. Severe arterial hypertension (e.g., systolic blood pressure > 200 mmHg and/or diastolic blood pressure > 110 mmHg) at rest.
5. Tachydysrhythmia or bradydysrthymia.
6. Hypertrophic cardiomyopathy or any other form of outflow tract obstruction.
7. Neuromuscular, musculoskeletal, or rheumatoid disorders that are exacerbated by exercise.
8. High degree atrioventricular block.
9. Ventricular aneurism.
10. Uncontrolled metabolic disorders.
11. Chronic infectious disease.
12. Mental of physical impairment leading to inability to exercise adequately.

The following are absolute and relative indications to terminate an exercise test Absolute Indications

1. Suspicion of a myocardial infarction or acute myocardial infarction (heart attack)
2. Onset of moderate-to-severe angina (chest pain)
3. Drop in systolic blood pressure (SBP) below standing resting pressure or drop in SBP with increasing workload accompanied by signs or symptoms
4. Signs of poor perfusion (circulation or blood flow), including pallor (pale appearance to the skin), cyanosis (bluish discoloration), or cold and clammy skin
5. Severe or unusual shortness of breath
6. CNS (central nervous system) symptoms
    1. e.g., ataxia (failure of muscular coordination), vertigo (An illusion of dizzying movement), visual or gait (pattern of walking or running) problems, confusion
7. Serious arrhythmias (abnormal heart rhythms)
    1. e.g.: second / third degree AV block, atrial fibrillation with fast ventricular response, increasing premature ventricular contractions or sustained ventricular tachycardia)
8. Technical inability to monitor the ECG
9. Patient's request (to stop)

Relative Indications

1. Any chest pain that is increasing
2. Physical or verbal manifestations of shortness of breath or severe fatigue
3. Wheezing
4. Leg cramps or intermittent claudication (grade 3 on a 4-point scale)
5. Hypertensive response (SBP >260 mm Hg; DBP>115 mm Hg)
6. Pronounced ECG changes from baseline
    1. >2 mm of horizontal or down sloping ST- segment depression, or >2 mm of ST-segment elevation (except in aVR)
7. Exercise-induced bundle branch block that cannot be distinguished from ventricular tachycardia
8. Less serious arrhythmias (abnormal heart rhythms) such as supraventricular tachycardia