PARTNERS HUMAN RESEARCH COMMITTEE

DETAILED PROTOCOL

Exploration of potential alterations in bone perfusion after spinal cord injury

Version 7

05/24/22

Clinicaltrials.gov # NCT04083794

I. BACKGROUND AND SIGNIFICANCE

Spinal cord injury (SCI) results in rapid and severe osteoporosis with decreases in bone mass of up to 45% within the first 3 years after injury. Although this osteoporosis is primarily attributed to loss of weight bearing and associated muscle contractions in the paralyzed limbs, there are important reductions in bone blood flow that impact bone metabolism and bone turnover. Bone is a highly vascularized organ with an extensive network of arteries, arterioles, and capillaries that is critical for bone development, maintenance, and repair; [1], [2] without sufficient and well-regulated blood flow, bone cannot maintain its integrity. [3] Thus, reductions in bone perfusion may be a key contributor to bone loss post SCI.

There is extensive arterial remodeling below the lesion that occurs soon after injury, leading to significant structural maladaptations such as decreased femoral artery diameter and increased wall thickness. [4], [5] These changes relate to significant alterations in both vasoconstrictor and vasodilatory response. For example, decreases in flow with leg dependency are greater in those with SCI, indicating enhanced myogenic vasoconstriction. [6], [7] Moreover, increases in leg blood flow with with reactive hyperemia are markedly less in those with SCI, indicating impaired myogenic vasodilatory capacity. [7]–[9] Hence, the extensive structural changes post SCI likely result in diminished flow to the leg due to both greater vasoconstriction and lesser vasodilation. This potentially leads to restricted flow to bone in those with SCI. Furthermore, these hypothesized alterations in vasoconstrictor and vasodilatory capacity may also relate to changes in functional blood flow responses in bone. Compressive loading increases bone metabolism requiring greater blood flow. However, if bone perfusion in response to loading is impaired in SCI due to structural changes, strategies to improve bone health based on loading could be largely ineffective. In addition, the structural changes occurring post injury, relate to alterations in nitric-oxide (NO) mediated vasodilation, another potential key mechanism of maintaining appropriate perfusion to bone. While there is almost no research investigating NO vasodilation in bone in humans, a handful of studies suggest that in skeletal muscle, those with SCI have increased NO-mediated vasodilation compared to controls.[10], [11] This implies that there is either increased NO production or similar peripheral NO that are unopposed by sympathetic vasoconstriction. However, given the lack of information on NO mediated vasodilation in the tibial vasculature, it would be prudent to first establish it potential role in healthy individuals prior to exploration in those with SCI. Afterwards, we will investigate the impact of peripheral vascular remodeling on bone perfusion after SCI. Furthermore, we propose to investigate the effect of mechanical loading, on bone perfusion in those with SCI compared to able-bodied individuals.

A critical limitation to the study of bone blood flow in humans has been the lack of noninvasive assessments. The dense nature of bone makes it difficult to investigate perfusion and the techniques used to quantify circulation in other tissues are either difficult or impossible to apply to bone *in vivo.* This study will use a recently developed custom-made near infrared spectroscopy (NIRS) device that can non-invasively assess blood content in bone. [12] While NIRS has been used to assess perfusion in soft tissue, [13], [14] it has not been extensively used to assess perfusion in bone. However, NIRS has been shown to provide reproducible results in controlled

conditions, [15] and can track dynamic changes in bone perfusion. [16], [17] In our preliminary work, we used NIRS to assess physiological changes in blood content in the tibial cortical bone in humans. Here, we aim to extend our preliminary work by *examining the impact of structural and functional vascular changes on bone perfusion in SCI and determine bone flow response to different loading conditions.*

II. SPECIFIC AIMS

1) Determine if popliteal artery structural changes are reflected in altered myogenic control of tibial bone perfusion post SCI.

Hypothesis: Compared to able-bodied, those with SCI will demonstrate greater popliteal artery wall thickness and smaller diameter that will relate to both greater myogenic vasoconstriction (in response to leg dependency) and lesser myogenic vasodilation (in response to reactive hyperemia) in tibial bone.

2) Determine if popliteal artery structural changes are reflected in lesser increases in tibial bone perfusion during compressive loading post SCI.

Hypothesis: Compared to able-bodied, those with SCI will demonstrate greater popliteal artery wall thickness and smaller diameter that will relate to smaller increases in bone perfusion with increasing loading conditions.

3) Determine if nitric-oxide mediated vasodilation is present in the tibial bone vasculature of able-bodied.

Hypothesis: In able-bodied, the tibial bone vasculature vasodilates in response to a nitric oxide donor.

All able-bodied volunteers will complete both aim1 and aim2. Only a subset of volunteers with SCI – those with time since injury between 3 and 24 months post injury – will be included for aim 2. The proposed study will investigate the control of bone perfusion in the SCI population as it relates to vascular remodeling and loading conditions.

III. SUBJECT SELECTION

Inclusion criteria

We anticipate we will recruit 29 individuals (males and females) aged 18 to 40 years with injuries, American Spinal Injury Association Impairment Scale (AIS) A and B and C. For aim 2, only individuals with SCI with complete injuries between 3 and 24 months post injury will be included. In addition, we will recruit 29 healthy individuals (males and females) age and gender matched to enroll for this study.

Exclusion criteria (for both able-bodied and SCI groups)

Clinical signs or symptoms of heart disease, hypertension, coronary disease, diabetes, other neurological disease, cancer, abnormal resting ECG, pregnant women, underweight and obese individuals (body mass index between 18.5 and 29.9), use of amphetamines (Ritalin, Adderall, Concerta) in the past 48 hours, tibial fracture or tibial stress fracture in the past 2 years.

Healthy individuals should have no recent weight changes $\geq$ 15 pounds, should not be allergic to nitroglycerin patches, capsules, tablets, ointments, or spray, should not use erectile dysfunction drugs, and should not use guanylate cyclase stimulators, such as riociguat. Concomitant use can cause hypotension.

Healthy individuals should not use PDE-5 inhibitors, such avanafil, sisildenafil, tadalafil, vardenafil hydrochloride. Concomitant use can cause severe hypotension, syncope, or myocardial ischemia.

Healthy indivduals should not have severe anemia. Nitroglycerin is contraindicated in patients with severe anemia (large doses of nitroglycerin may cause oxidation of hemoglobin to methemoglobin and could exacerbate anemia).

Those with SCI will have no extreme spasticity to avoid spontaneous contractions and cannot be on baclofen due to its potential autonomic effects.

IV. SUBJECT ENROLLMENT

We will recruit from eligibile ASRT participants study (Partners IRB Protocol # 2013P000604. Additionally, we will reply on word of mouth and referrals from the spinal cord injury program, the outpatient therapy department, and the Exercise for Persons with Disabilities (ExPD) program at Spaulding Rehabilitation Network to enroll participants in this study. Flyers will be posted at SRH outpatient sites and/or given to pontential subjects along with a phone number to call if they are interested in learning more about the study. Our laboratory has been regularly enrolling SCI patients for research from the SRN. For the able-bodied participants, in the past, we have successfully recruited young males and females by posting flyers on college campuses and/or by posting on internet websites. The website ad has a short description of the project and a number to contact if they would like more information. When the potential subject makes contact with the study staff, we use a phone screening of health questions and a short description of the study. This process usually takes 15 to 20 minutes. If individuals meet eligibility requirements from the phone screening and are interested in participating, they are then scheduled for a physical screening. At that time, we mail a consent form to give opportunity to read and understand the specifics of the study.

Research study staff will obtain volunteer consent in accordance with guidelines established by the Institutional Review Board during the study visit to the Cardiovascular Laboratory. Participants are encouraged to ask questions and are reminded that participation is strictly voluntary and will not affect their current or future care at Spaulding Rehabilitation Hospital or any of its affiliates.

V. STUDY PROCEDURES

Study Visit

Once recruited for the study, the volunteers will visit the laboratory for a health screening session to further determine eligibility. This 30-minute screening session includes obtaining informed consent, health history, and obtainingt weight and tibial length measurements.

Once volunteers complete the screening process and meet the study inclusion criteria they will continue the visit to investigate tibial perfusion in response to leg dependency, reactive hyperemia, and increasing loading conditions . The study visit will take approximately 3 hours for participants that complete both aims and approximately 1.5 hours for those with SCI that complete only aim 1.

Detailed procedures

At the beginning of the study visit, we will obtain measurements of the popliteal artery thickness and lumen diameter using Doppler ultrasound (SonoSite Edge II, Fujifilm SonoSite). Throughout the study for the first aim, we will measure whole leg blood velocity in the popliteal artery using Doppler ultrasound (Multidop T2, DWL). Throughout the protocol, volunteers will be instrumented for measurement of tibial blood flow using a NIRS system (see Measurements below). The measurements will be taken in the same leg.

Assessing tibial perfusion in response to leg dependency and reactive hyperemia (able-bodied and volunteers with SCI)

We will use leg dependency and reactive hyperemia to assess myogenic vasoconstriction and myogenic vasodilation, respectively. This approach will provide insight to the relation between popliteal artery structure and myogenic vasoconstrictor response (leg dependency) and myogenic vasodilator response (reactive hyperemia), respectively.

Leg dependency (i.e. the limb is lowered below heart level such that the knee is flexed at 90deg) results in an increased arterial transmural pressure, thereby engaging myogenic vasoconstriction. [6] Past work showed that those with SCI have more pronounced decreases in blood flow during leg dependency compared to able-bodied. [7] Thus, myogenic vasoconstrictor responses might be enhanced in the paralyzed legs. In addition, reactive hyperemia (i.e. increased calf blood flow in response to sustained ischemia via proximal thigh cuff inflation) is significantly lesser in SCI compared to able-bodied. [7]–[9] Thus, the myogenic vasodilatory capacity of the arterieres in the legs of those with SCI might be impaired.

Throughout this aim, we will monitor continuously calf blood flow and tibial bone perfusion in the same leg. With the subject in supine position, we will obtain a 5 minutes resting baseline, followed by 5 minutes with the limb lowered below heart level (knee flexed at 90 deg). After 10 minutes of recovery, we will us reactive hyperemia to increase blood flow to the leg. With the subject supine, a pressure cuff placed above the knee will be inflated to supersystolic values (>200 mmHg) for 10 minutes. Data will be obtained for a 5 minutes resting baseline, 10 minutes during cuff inflation, and 5 minutes after cuff release.

Assessing tibial perfusion in response to exogenous nitric-oxide (NO) (able-bodied)

We will use an exogenous NO donor to assess NO-mediated vasodilation in the tibial bone vasculature of able-bodied. We will use one tablet of sublingual nitroglycerine (Nitroglycerine 0.4mg Sublingual table) as the NO donor. With the subject in supine position, we will obtain a 5 minutes resting baseline, followed by one tablet dissolved under the tongue, for 15 minutes. Throughout, we will continuously monitor heart rate and blood pressure, and calf blood flow and tibial bone perfusion in the same leg.

Assessing tibial perfusion in response to increasing loading conditions (able-bodied and individuals with SCI with time since injury between 3 and 24 months)

This approach will define the relationship between popliteal artery structure and magnitude of bone perfusion during loading of different magnitudes.

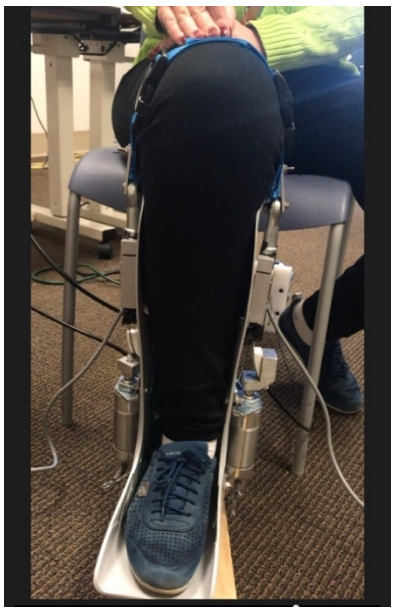
We will use a custom tibial loading device (see Measurements below) to provide three periods of static bone loading, in random order: 1) 10% of body weight, 2) 30% of body weight, 3) 50% of body weight.

After 10 minutes of recovery, the volunteers will undergo the three periods of cycling bone loading described below. The subjects will be seated with the leg secured in the boot of the tibial bone loading device. The knee will be maintained at 90 deg flexion. Bone loading will be applied across the tibia at the 3 magnitudes of loading. Tibial bone perfusion of the same leg will be monitored continuously. Each period of static loading will consist of a 5 minutes resting baseline, 10 minutes of loading, and 5 minutes of recovery.

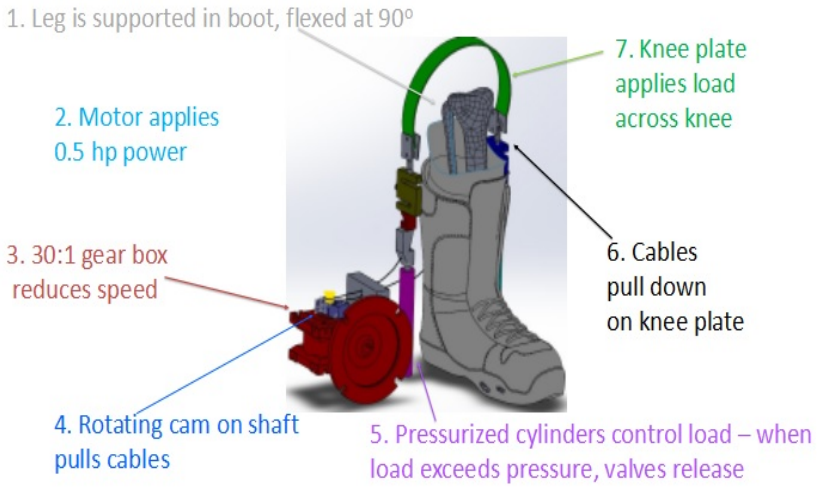
Measurements

Tibial Blood Flow. A custom made bone optical spectropsopy device that uses near-infrared (NIR) light will be used to continuously monitor tibial perfusion. Near infrared spectroscopy (NIRS) provides a safe, non-invasive, and inexpensive alternative to analyze hemodynamic changes in the biological tissues using light in the range of 600 to 1000 nm. The system has been previously used in Protocol #2014P002003 without any side effects. The system has two detectors allowing estimation of hemoglobin changes in a two layer (skin and bone) model and uses a broadband light source and high-sensitivity spectrometers to yield full NIR spectral information. The instrument uses a white-light tungsten halogen lamp (Fostec DCRII, Dallas, TX) to deliver light through a large optical fiber bundle to the skin. Two detector fibers are coupled with spectrometers (Mini-spectrometer TG C9405CB, Hamamatsu Photonics, Japan), placed at 1 and 2 cm distances from the source. Both spectrometers allow measurement of the diffusely reflected light between 650 nm and 800 nm with 5 nm resolution. The light source and the two detectors are attached to a custom made probe head placed on the skin surface directly over the tibia. Light propagates between the source and the detectors in “banana-shaped” sensitivity functions and penetrates shallower for more closely separated source-detector pairs and more deeply for wider separated pairs. Thus, the detector 1 cm from the source probes the relatively thin (2 mm) skin layer, whereas the detector 2 cm from the source probes deeper into tissue and is therefore sensitive to the underlying bone. Placing the probe on the anterior side of the middle third tibia where the bone lies directly under the skin minimizes the effects of soft tissue and local changes in skin

blood flow on the NIRS measurements. The extended modified Beer-Lambert law for a two-layer model will be used to determine hemoglobin content changes in tibial bone from changes in light absorption.



Tibial Loading Device. The custom-made device allows for controlled, compressive loading of the bone. The system consists of a boot with 2 inch strap over the knee. Wires pull vertically down on the brace via a cam-type device on a motor (Lesson, 90V DC). The load is monitored by air pressure cylinders on either side of the boot and the peak load cannot exceed the pressure of the cylinders. The servo-controlled pressure adjusts the load such that it remains at the predetermined level. This allows for full control of the magnitude and pattern of loading on the lower leg. This system can apply static or cycling loading on the tibia. This approach has been modeled after a mouse tibial loading model that has been successfully employed to examine bone adaptations under imposed physical loading conditions. The system is able-to non-invasively apply loading in a physiological direction and axially loads a single skeletal element – the tibia. The system also allows for isometric muscle contractions in the calf (plantar flexion) that can be performed in synchrony with the compressive loading and provides a stimulus that more closely mimics walking. The leg of the volunteer is placed in the boot of the device and secured with the strap over the knee. This ensures that the knee is maintained at 90 deg flexion throughout loading. Bone loading will be applied axially along the tibia and maintained constant at the different loading magnitudes. The loading force will be monitored and recorded via the two load cells place on either sides of the boot



Beat-by-Beat Calf Blood Flow. We will use Doppler derived flow velocities to estimate whole limb blood flow through the popliteal artery. A continuous wave 4 MHZ Doppler probe (Multidop T2, DWL) is placed against the skin under the knee to isolate the popliteal artery proximal to the bifurcation.

Popliteal Artery Structure. A linear 6- to 15- MHz Doppler ultrasound (SonoSite Edge II, Fujifilm SonoSite, Bothell, WA) will be used to measure popliteal artery thickness and lumen diameter. The measurements will be taken at the distal popliteal artery above 2cm above arterial take-off, with the subject in supine position with the knee flexed at 45deg. The arterial diameter will be measured on transverse images, with the ultrasound beam perpendicular to the artery. [18]

Tibial Length. Tibial length will be measured in sitting position with a tape measure.

Body Weight. Weight will be measured on a scale.

VI. STATISTICAL ANALYSIS

Sample Size

Our prior work found a borderline significant increase ($p=0.06$) in tibial perfusion after exercise in able-bodied. Power analysis at 80% of these data showed that 17 subjects would have provided a $p<0.05$ increase in perfusion. Therefore, for this study, we based our sample size on this calculation. We do not expect any sex-related difference; moreover, the SCI population is comprised primarily of males. To ensure a sufficient sample size for NO-mediated vasodilation, we will aim to recruit an additional of 17 individuals in each group. Therefore, we will recruit 29 individuals with SCI (men and women) and 29 healthy individuals (men and women) to participate in the study.

Statistics

Data from NIRS monitoring will be used to determine hemoglobin content changes in tibial bone from changes in light absorption on a moment-by-moment basis. A detailed description of the analysis can be found in our previous work. [12] Briefly, NIRS monitoring provides a change in hemoglobin content from one state to another. This is due to the fact that absolute intensity of the scattered light measured across the tibia cannot be used for an absolute measure of perfusion. However, as perfusion increases, there is a proportional increase in absorption (and vice versa). Thus, percent change in total hemoglobin detected reflects the relative change in perfusion.

The main outcomes assessed will be popliteal artery thickness and lumen diameter, tibial perfusion, and whole leg blood flow. This project will employ a simple and straightforward statistical approach to compare responses within and between groups. Statistical significance will be set at 0.05, and power at 80%. Descriptive statistics will be presented including mean, median, standard deviation, and range. Differences/relations will be considered as statistically significant at alpha=0.05 level. For all parametric statistical tests, conformity of data to required statistical assumptions will be verified using standard methods. If assumptions for parametric tests do not hold, non-parametric alternatives will be employed. The analysis plan is written as though there are no distributional problems; assumptions for all analyses will be checked and analogous methods calling for fewer and/or weaker assumptions will be used, if called for. For the data from aim 1, unpaired t-tests will be used to assess changes in perfusion between groups and reactive

hyperemia/leg dependency. Since the two conditions result in distinct and opposite responses, they should be considered separately. For the data from aim 2, for the three passive loading conditions (10%, 30%, 50% body weight without associated muscle contractions), a 2x3 ANOVA will be used to assess changes in perfusion between groups across loading conditions.

The results of this study will provide invaluable insights into bone perfusion in the paralyzed legs of those with SCI. For both aims, we anticipate that individuals with SCI will have less bone perfusion compared to able-bodied due to the extensive vascular maladaptations. Even if the results are not different between those with SCI and able-bodied, this study will have explored a potentially key contributor to SCI-related bone loss, with major implications in treating osteoporosis.

VII. RISKS AND DISCOMFORTS

The tibial bone loading device should pose minimal risks – the device applies loading at loads less than full body weight – from 10% to 50% body weight. This should pose minimal risk to volunteers with SCI since they will be less than 40 years of age and within 2 years of injury and so should have minimal risk of osteoporosis. Cuff inflation might result in muscle discomfort that will quickly go away after cuff release. In those with SCI, cuff inflation can also cause autonomic dyreflexia. The NIRS system is non-invasive and poses little risk to the patients. Initial trials showed minor reddening of the skin; however, this was temporary and it quickly went away. Nitroglycerin (0.4 mg Nitroglycerin sublingual) at the small doses given is considered low risk, and any higher risk patients are excluded during the eligibility process. Sublingual nitroglycerin may result in headaches, weakness, dizziness, lightheadedness, nausea, mild burning or tingling with the tablet in the mouth, and flushing (warmth, redness, or tingly feeling under the skin) as the body adjusts to the medication. Some serious but unlikely side effects include fainting and fast and irregular heart rate. Blood pressure and heart rate will be monitored continuously, and the nitroglycerin will be removed immediately if large declines in blood pressure (>20 mmHg) occur.

VIII. POTENTIAL BENEFITS

This research may help physicians and scientists explore the potential contribution of vascular maladaptations to SCI related bone loss, with major implications in treating osteoporosis. Subjects will not directly benefit from participation in this protocol.

MONITORING AND QUALITY ASSURANCE

The research coordinator will be responsible for monitoring the completeness of all data and source documents. The Principal Investigator will monitor the informed consent procedures in accordance with the Informed Consent Compliance Checklist of Partners HealthCare Systems HRQIP. The subjects data/protocol adherence will be monitored by the research coordinator at each step in the study including. Checklists and note pages are used to note any deviations or omissions from the protocols. The Cardiovascular Research Laboratory Medical Emergency

Safety Plan will be followed in the case of an adverse medical event. Any clinical/health related issues would be immediately presented to the subject (i.e. abnormal EKG, blood chemistries, etc) to determine appropriate notification (ie. current physician or appropriate specialist) .Based on the seriousness of the situation a physician may be contacted to provide clinical guidance on the appropriate course of action. Event assessment and follow-up will be done by the PI as needed on a case by case basis.

This study will be conducted in compliance with the protocol, International Council for Harmonization/Good Clinical Practice requirements(ICH/GCP) and applicable state, local and federal regulatory requirements.

Reporting to Partners IRB:

Reports of *unanticipated problems involving risks to subjects or others* are to be submitted through Insight/eIRB within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem.

Reporting Unanticipated Problems that are Adverse Events

Any unanticipated untoward or unfavorable medical occurrence, including abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, that indicates that the research places subjects at increased risk of physical or psychological harm than previously known or recognized are to be submitted through Insight/eIRB as an Other Event, Adverse Event. The investigator must provide the following information in the report:
(1) a detailed description of the adverse event;
(2) the basis for determining that the event is unexpected in nature, severity, or frequency;
(3) the basis for determining that the event is related or possibly related to the research procedures;
(4) the basis for determining that the research places subjects at an increased risk of harm (i.e., a serious adverse event); and
(5) whether any changes to the research or other corrective actions are warranted.
NOTE: The PHRC does not accept sponsor IND/IDE safety reports describing adverse events that have occurred at sites other than those subject to this policy unless the report is of an incident that is:
(1) serious; (2) unexpected or unanticipated; (3) related to the investigational drug/device; and
(4) suggests that subjects are at an increased risk of harm and as such warrants changes in the research, consent process, or informing subjects. IND/IDE safety reports that warrant changes are to be submitted through Insight/eIRB as an Amendment.

Reporting Unanticipated Problems that are not Adverse Events

All other unanticipated incidents, experiences, information, outcomes, or other problems that indicate that the research places subjects at an increased risk of physical, psychological, economic, legal, or social harm than was previously known or recognized are to be submitted through Insight/eIRB as an
Other Event. The investigator must provide the following information in the report:
(1) a detailed description of the unanticipated problem;
(2) the basis for determining that the problem is unexpected;
(3) the basis for determining that the problem indicates that the research places subjects at an increased risk of harm; and
(4) whether any changes to the research or other corrective action are warranted.

Reporting to the National Institute of Arthritis and Muscloskeletal and Skin Disease (NIAMS) and Safety Officer (Dr.Ralph Marino).

This protocol has funding from NIAMS. NIAMS has appointed a Saftey Officer, Dr. Ralph Marino, to act in an advisory capacity to the NIAMS to monitor participant safety, evaluate the progress of the study, to review procedures for maintaining the confidentiality of data, the quality of data collection, management and analyses.

The Principal Investigator (PI) will report unanticipated problems (UPs), serious adverse events (SAEs) and protocol violations that impact participant safety to the NIAMS and the Safety Officer, (through the Exective Secretary, KAI-Research) within 48 hours of the PI becoming aware of the event. KAI facilitates the review of the expedited reporting of these events by the Safety Officer. The Safety Officer will review the Safety Reports submitted by the PI to KAI twice a year. KAI facilitates the review of these reports by the Safety Officer and the NIAMS, and provides the final review comments to the PI when the review is complete. During the course of the study, ad hoc meetings may be called by the NIAMS or Safety Officer at any time to discuss potential safety issues.

Definitions:

Adverse event means any untoward or unfavorable medical occurrence in a human subject, includingany abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease,temporally associated with the subject's participation in the research, whether or not considered relatedto the subject's participation in the research (modified from the definition of adverse events in the 1996. International Conference on Harmonization E-6 Guidelines for Good Clinical Practice).

Serious adverse event means any event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Possibly related to the research means there is a *reasonable possibility* that the adverse event, incident,experience or outcome may have been caused by the procedures involved in the research (modified from the definition of *associated with use of the drug* in FDA regulations at 21 CFR 312.32(a)). *Reasonable possibility* means that the event is more likely than not related to participation in the research or,in other words, there is a >50% likelihood that the event is related to the research procedures.

X. REFERENCES

[1] M. L. Brandi and P. Collin-Osdoby, "Vascular biology and the skeleton," *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.*, vol. 21, no. 2, pp. 183–192, Feb. 2006, doi: 10.1359/JBMR.050917.

[2] M. Brookes and R. G. Harrison, "The vascularization of the rabbit femur and tibio-fibula," *J. Anat.*, vol. 91, no. 1, pp. 61–72, Jan. 1957.

[3] J. T. Lacey, "NON-UNION OF FRACTURES: AN EXPERIMENTAL STUDY," *Ann. Surg.*, vol. 89, no. 6, pp. 813–848, Jun. 1929.

[4] D. H. J. Thijssen *et al.*, "Time course of arterial remodelling in diameter and wall thickness above and below the lesion after a spinal cord injury," *Eur. J. Appl. Physiol.*, vol. 112, no. 12, pp. 4103–4109, Dec. 2012, doi: 10.1007/s00421-012-2400-2.

[5] C. R. West, A. Alyahya, I. Laher, and A. Krassioukov, "Peripheral vascular function in spinal cord injury: a systematic review," *Spinal Cord*, vol. 51, no. 1, pp. 10–19, Jan. 2013, doi: 10.1038/sc.2012.136.

[6] K. Okazaki *et al.*, "Vasoconstriction during venous congestion: effects of venoarteriolar response, myogenic reflexes, and hemodynamics of changing perfusion pressure," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 289, no. 5, pp. R1354-1359, Nov. 2005, doi: 10.1152/ajpregu.00804.2004.

[7] M. Kooijman, M. de Hoog, G. A. Rongen, H. J. M. van Kuppevelt, P. Smits, and M. T. E. Hopman, "Local vasoconstriction in spinal cord-injured and able-bodied individuals," *J. Appl. Physiol. Bethesda Md 1985*, vol. 103, no. 3, pp. 1070–1077, Sep. 2007, doi: 10.1152/japplphysiol.00053.2007.

[8] M. Kooijman, G. A. Rongen, P. Smits, and M. T. E. Hopman, "Preserved alpha-adrenergic tone in the leg vascular bed of spinal cord-injured individuals," *Circulation*, vol. 108, no. 19, pp. 2361–2367, Nov. 2003, doi: 10.1161/01.CIR.0000096480.55857.3C.

[9] R. s. Khakha, Z. Bloomer, D. Bain, G. Nicholson, A. Gall, and M. Ferguson-Pell, "Differences in the reactive hyperemia response of bone in able bodied and spinal injured persons using near infrared spectroscopy," *Orthop. Proc.*, vol. 88-B, no. SUPP_II, pp. 305–305, May 2006, doi: 10.1302/0301-620X.88BSUPP_II.0880305c.

[10] L. M. Brewster *et al.*, “Effects of circulating extracellular microvesicles from spinal cord-injured adults on endothelial cell function,” *Clin. Sci.*, vol. 134, no. 7, pp. 777–789, Apr. 2020, doi: 10.1042/CS20200047.

[11] R. Brown, D. Celermajer, V. Macefield, and M. Sander, “The Effect of Nitric Oxide Inhibition in Spinal Cord Injured Humans with and without Preserved Sympathetic Control of the Vasculature,” *Front. Neurosci.*, vol. 10, p. 95, Mar. 2016, doi: 10.3389/fnins.2016.00095.

[12] A. E. Draghici *et al.*, “Near infrared spectroscopy for measuring changes in bone hemoglobin content after exercise in individuals with spinal cord injury,” *J. Orthop. Res. Off. Publ. Orthop. Res. Soc.*, May 2017, doi: 10.1002/jor.23622.

[13] D. A. Boas, C. E. Elwell, M. Ferrari, and G. Taga, “Twenty years of functional near-infrared spectroscopy: introduction for the special issue,” *NeuroImage*, vol. 85 Pt 1, pp. 1–5, Jan. 2014, doi: 10.1016/j.neuroimage.2013.11.033.

[14] M. Ferrari and V. Quaresima, “A brief review on the history of human functional near-infrared spectroscopy (fNIRS) development and fields of application,” *NeuroImage*, vol. 63, no. 2, pp. 921–935, Nov. 2012, doi: 10.1016/j.neuroimage.2012.03.049.

[15] S. M. Aziz, F. Khambatta, T. Vaithianathan, J. C. Thomas, J. M. Clark, and R. Marshall, “A near infrared instrument to monitor relative hemoglobin concentrations of human bone tissue in vitro and in vivo,” *Rev. Sci. Instrum.*, vol. 81, no. 4, p. 043111, Apr. 2010, doi: 10.1063/1.3398450.

[16] P. Farzam, P. Zirak, T. Binzoni, and T. Durduran, “Pulsatile and steady-state hemodynamics of the human patella bone by diffuse optical spectroscopy,” *Physiol. Meas.*, vol. 34, no. 8, pp. 839–857, Aug. 2013, doi: 10.1088/0967-3334/34/8/839.

[17] S. Konugolu Venkata Sekar *et al.*, “In Vivo, Non-Invasive Characterization of Human Bone by Hybrid Broadband (600-1200 nm) Diffuse Optical and Correlation Spectroscopies,” *PloS One*, vol. 11, no. 12, p. e0168426, 2016, doi: 10.1371/journal.pone.0168426.

[18] Y. G. Wolf, Z. Kobzantsev, and L. Zelmanovich, “Size of normal and aneurysmal popliteal arteries: a duplex ultrasound study,” *J. Vasc. Surg.*, vol. 43, no. 3, pp. 488–492, Mar. 2006, doi: 10.1016/j.jvs.2005.11.026.

PARTNERS HUMAN RESEARCH COMMITTEE
DETAILED PROTOCOL

Exploration of blood flow regulation to bone in humans

Version 11

03/24/21

Clinicaltrials.gov # NCT04083794

I. BACKGROUND AND SIGNIFICANCE

All tissues of the human body require adequate perfusion to provide oxygen and nutrients to meet metabolic demands. It has been long recognized that the arterial system in bone is of overwhelming importance, adequate circulation being necessary for nearly all skeletal functions, including development, growth, homeostasis, and repair. [1], [2] There is an extensive network of arteries, arterioles, and capillaries that supplies blood flow to bone and without adequate circulation bone cannot maintain its integrity. [3] For example, inadequate flow has been associated with bone loss,[4] impaired growth,[5] and delayed fracture healing.[6]

Despite the appreciation of the importance of blood circulation to bone health, the regulation of blood flow to bone has been relatively unstudied in humans and the mechanisms that regulate bone blood flow are not well understood. The majority of work in bone perfusion has been in various animal models (mice, rats, rabbits, dogs, cats, etc.) with the preponderance of data suggesting that there are key systemic effectors of acute changes in bone perfusion. One of the potential factors controlling blood flow regulation is the sympathetic nervous system, the bone vasculature being innervated by a rich network of sympathetic nerves. Although no studies in humans have been conducted, most of animal data demonstrates that sympathetic activation reduces blood flow to bone. Application of norepinephrine decreases blood flow to both intact bone [7], [8] and isolated bone. [9] Likewise, stimulation of sympathetic nerves decreases flow to bone [7] via alpha-adrenergic receptor activation.[9] Moreover, the smooth muscle of the arterioles in bone respond as expected to infused vasodilators and vasoconstrictors.[10], [11] Hence, the sympathetic innervation of bone vasculature serves a functional purpose in the control of blood flow. If this were not the case, independent of the link between bone metabolism and bone flow, the arterial network in bone would act as a simple pressure passive system. Here, we propose to investigate the role of vascular sympathetic activity in blood flow regulation in humans.

A critical limitation to the study of bone blood flow in humans has been the lack of noninvasive assessments. The dense nature of bone makes it difficult to investigate perfusion and the techniques used to quantify circulation in other tissues are either difficult or impossible to apply to bone *in vivo.* We recently developed a custom-made near infrared spectroscopy (NIRS) device that can non-invasively assess blood content in bone. [12] While NIRS has been used to assess perfusion in soft tissue,[13], [14] it has not been extensively used to assess perfusion in bone. However, NIRS has been shown to provide reproducible results in controlled conditions,[15] and can track dynamic changes in bone perfusion.[16], [17] In our preliminary work, we used NIRS to assess physiological changes in blood content in the tibial cortical bone in humans. Although our preliminary work is intriguing, it provided insufficient insight to regulation because this study was unable to disentangle the various possible contributors to bone perfusion. Thus, we aim to extend our preliminary work by *examining the response of tibial perfusion to vascular sympathetic activity and systemic perfusion pressure in healty individuals via NIRS monitoring.* In addition, *we will contrast these responses with those seen in individuals with spinal cord injury (SCI).* SCI represents a human 'model' of chronic reduced loading with loss of sympathetic regulation below the level of injury that alters control of bone perfusion. Contrasting the response of tibial perfusion to vascular sympathetic activity and systemic perfusion pressure in able-bodied individuals and individuals with SCI will set the basis for future work that will define whether alterations in perfusion may relate to pathophysiologies of bone.

Hypothesis

We hypothesize that bone blood perfusion decreases proportional to increases in leg vascular sympathetic outflow.

II. SPECIFIC AIMS

1) Determine the effect of increased vascular sympathetic activity with maintained blood pressure on tibial perfusion.

2) Determine the effect of increased vascular sympathetic activity with increased systemic pressure on tibial perfusion.

3) Compare the changes in tibial perfusion in response to systemic factors between able-bodied and those with SCI.

Knowledge of tibial perfusion in response to increased sympathetic outflow, with or without changes in systemic pressure, will allow us to determine the sympathetic contribution to bone blood flow.

III. SUBJECT SELECTION

Inclusion criteria

It is anticipated that 34 healthy males and 34 healthy females aged 18 to 40 years will be recruited and enrolled for this study.

Also, we anticipate we will recruit 17 males with SCI, aged 18 to 40 years, with injuries American Spinal Injury Association Impairment Scale (AIS) A and B and C, with full hand function. We will only enroll males with SCI since the SCI population is predominantly male (about 75%).

Exclusion criteria (for both able-bodied and SCI groups)

Clinical signs or symptoms of heart disease, hypertension, coronary disease, diabetes, other neurological disease, cancer, recent weight change >15 pounds, abnormal resting ECG, pregnant and/or breastfeeding women, underweight and obese individuals (body mass index between 18.5 and 29.9), use of amphetamines (Ritalin, Adderall, Concerta) in the past 48 hours, tibial fracture or tibial stress fracture in the past year.

In addition, those with SCI will have no extreme spasticity to avoid spontaneous contractions and cannot be on baclofen due to its potential autonomic effects.

IV. SUBJECT ENROLLMENT

In the past, we have successfully recruited young males and females by posting flyers on college campuses and/or by posting on internet websites. The website ad has a short description of the project and a number to contact if they would like more information. When the potential subject

makes contact with the study staff, we use a phone screening of health questions and a short description of the study. This process usually takes 15 to 20 minutes. If individuals meet eligibility requirements from the phone screening and are interested in participating, they are then scheduled for a physical screening. At that time, we mail a consent form to give opportunity to read and understand the specifics of the study.

We will recruit SCI participants from the outpatient therapy department and the Exercise for Persons with Disabilities (ExPD) program at Spaulding Rehabilitation Network. Flyers will be posted at SRH outpatient sites and/or given to potential subjects along with a phone number to call if they are interested in learning more about the study. Our laboratory has been regularly enrolling SCI patients for research from the SRN. For the able-bodied participants, in the past, we have successfully recruited young males and females by posting flyers on college campuses and/or by posting on internet websites. The website ad has a short description of the project and a number to contact if they would like more information. When the potential subject makes contact with the study staff, we use a phone screening of health questions and a short description of the study. This process usually takes 15 to 20 minutes. If individuals meet eligibility requirements from the phone screening and are interested in participating, they are then scheduled for a physical screening. At that time, we mail a consent form to give opportunity to read and understand the specifics of the study.

Research study staff will obtain volunteer consent in accordance with guidelines established by the Institutional Review Board during the study visit to the Cardiovascular Laboratory. Participants are encouraged to ask questions and are reminded that participation is strictly voluntary and will not affect their current or future care at Spaulding Rehabilitation Hospital or any of its affiliates.

V. STUDY PROCEDURES

Study Visit

Once recruited for the study, the volunteers will visit the laboratory for a health screening session to further determine eligibility. This 30-minute screening session includes obtaining informed consent, health history, and obtaining height and weight measurements.

Once volunteers complete the screening process and meet the study inclusion criteria they will continue the visit to investigate the effect of sympathetic outflow to tibial perfusion. The study visit will take approximately 3.5 hours to complete. The screening visit and the study visit may occur on the same day or on different days.

Detailed procedures

Throughout the protocol, able-bodied volunteers will be fully instrumented for measurements of tibial blood flow, muscle/bone sympathetic activity, popliteal blood flow velocity, ECG, beat-by-beat arterial pressure, brachial arterial pressure, and respiration (see Measurements below). All those with SCI enrolled in this study will have complete injuries and thus vascular sympathetic control is disrupted below the level of injury. Hence, in individuals with SCI the arterial network in bone might act as a simple pressure passive system. Given the disrupted vascular sympathetic control, in those with SCI we will not record muscle/bone sympathetic activity.

In able-bodied, we will use direct microneurographic measures of nervous activity to the lower leg vasculature in the common peroneal nerve as a measure of sympathetic activity to the bone

vasculature of the tibia throughout the protocol. Our assumption is that peroneal nerve sympathetic activity reflects vasomotor output to both muscle and bone of the lower leg. Though this assumption is not testable prior to having data in hand, we assume that Hilton's law prevails. According to Hilton's law, bone innervation derives from the peripheral nerves of the muscle that overlies the bone. The common peroneal nerve primarily innervates the fibula, but also the deep peroneal branch innervates the tibialis anterior which overlies the tibia. Though we acknowledge this is potentially inexact, our sympathetic nervous activity measure should broadly reflect sympathetic outflow to the lower leg.

We will use three approaches to increase sympathetic outflow to the leg vasculature: head tilt, cold pressor test, and isometric handgrip exercise (IHE) to fatigue. In those with SCI, we expect that bone vasculature will respond as a pressure passive system.

Subjects will lie supine throughout the study protocol. After instrumentation, the subject will perform three maximal voluntary contractions (MVC) on a handgrip dynamometer. This is to determine 30% MVC for sustained isometric exercise. Afterwards, an adequate nerve recording will be obtained. To ensure that a good nerve recording is obtained, the subjects will be asked to perform a Valsalva maneuver, which results in an expected increase in sympathetic activity. Invented by Antonio Maria Valsalva in the 17th century, the Valsalva maneuver, a prolonged expiration against a closed glottis, is widely used because of its safety and ease of quantification. This voluntary act of straining creates transient changes in intra-thoracic and intra-abdominal pressure with concomitant increases in sympathetic activity. To achieve this, subjects will be asked to breathe through a tube with a small vent in the glottis to prevent them from increasing mouth instead of chest pressure. A pressure gauge attached to this tube will allow the subject maintain a target pressure of 40 mmHg for 15 seconds. Data will be obtained for 1 minute prior to the maneuver, 1 minute after, with 2 minutes of recovery.

Assessing tibial perfusion in response to increased sympathetic outflow with maintained blood pressure

This approach will provide insight to the relation between moment-by-moment bone perfusion and beat-by-beat increases in vascular sympathetic activity without alterations in systemic pressure. During head-up tilt, decreases in central blood volume and circulatory adjustments are proportional to the angle of the head-up tilt [20]. Sympathetic activity increases and muscle blood flow decreases significantly by a tilt angle of 30 degrees [21]. In addition, head-down tilt to 6-8 deg results in a decrease in sympathetic activity [22]. Hence, increased levels of head-up tilt provide a strong stimulus for active vasoconstriction in the leg, while maintaining system pressure constant. This provides a stimulus that progressively increases sympathetic activity with a maintained systemic perfusion pressure. Data will be obtained for a 5 minutes resting baseline, followed by 25 minutes of continuous tilt of successive 5 minutes periods at 10 deg head-down tilt, 0 deg tilt, 10 deg head-up tilt, and 20 deg head-up tilt.

Assessing tibial perfusion in response to increased sympathetic outflow and increased systemic pressure.

The purpose of this approach is to provide insight to the relation between bone perfusion and progressive increases in sympathetic outflow accompanied by increases in systemic pressure.

Cold pressor test and sustained isometric handgrip will be used to increase sympathetic nervous activity, with increased systemic pressure.

The cold pressor test provokes a nociceptive response that causes increases in sympathethic activity, blood pressure, and heart rate [23]. Past work showed that these responses are water temperature dependent. Graded increases in sympathetic activity, accompanied by parallel increase in arterial pressure, were seen during immersion at 0 and 7^{o}C, but not at 14^{o}C [24].

A 5 minutes resting baseline will be obtained prior to the subject performing the cold pressor test. Afterwards, participants will undergo a cold pressor task, consisting of immerion of a hand for 3 min in 0^{o}C water. This will be followed by 3 minutes of recovery data collection.

Isometric handgrip to fatigue (IHE) results in time-dependent, progressive increases in peroneal nerve sympathetic outflow and blood pressure [25]. With sustained handgrip forces greater than 25% of maximal voluntary contraction, sympathetic activity increases progressively to end exercise. Hence, IHE provides a strong stimulus for active vasoconstriction in the leg that contributes to the exercise pressor response.

A 5 minutes resting baseline will be obtained prior to the subject performing IHE at 30% MVC sustained to fatigue. An oscilloscope will be calibrated to provide the subject visual feedback on force generation; subjects will be verbally encouraged to maintain force throughout; fatigue will be determined by the investigators as maximal perceived rating of exertion accompanied by inability to maintain target force for more than 3 sec, despite continued effort.

In those with SCI, given the lack of vascular sympathetic control and thus no increase in sympathetic outflow during tilt, cold pressure, and isometric handgrip exercise, we expect that bone blood flow will respond as a pressure passive system and hence will track blood pressure response.

In able-bodied, tibial blood perfusion, an adequate nerve recording, and popliteal flow will be obtained. These time series will allow derivation of relations between nerve activity and tibial perfusion, and between nerve activity and popliteal flow. Nerve activity will serve as an index of vasoconstriction activity to the entire leg, while tibial perfusion and popliteal flow will represent a measure of downstream leg vascular responses. If nerve activity is not obtained, the subjects will still perform the procedures described above. In that case, the relationship between tibial blood perfusion and popliteal flow will be assessed.
In those with SCI, tibial blood perfusion and popliteal flow will be obtained. The relationship between tibial blood perfusion and popliteal flow will be assessed. These approaches will provide insight to the proportionality between sympathetic outflow and bone blood flow, as well as the hypothesized pressure passive bone vasculature in those with SCI.

Subjects may be asked to repeat any of the study assessments if there is difficulty obtaining results that meet our quality standards or there are equipment issues that arise.

Measurements

Tibial Blood Flow. A custom made bone optical spectropsopy device that uses near-infrared (NIR) light will be used to continuously monitor tibial perfusion. Near infrared spectroscopy (NIRS) provides a safe, non-invasive, and inexpensive alternative to analyze hemodynamic changes in the biological tissues using light in the range of 600 to 1000 nm. The system has been previously

used in Protocol #2014P002003 without any side effects. The system has two detectors allowing estimation of hemoglobin changes in a two layer (skin and bone) model and uses a broadband light source and high-sensitivity spectrometers to yield full NIR spectral information. The instrument uses a whitelight tungsten halogen lamp (Fostec DCRII, Dallas, TX) to deliver light through a large optical fiber bundle to the skin. Two detector fibers are coupled with spectrometers (Mini-spectrometer TG C9405CB, Hamamatsu Photonics, Japan), placed at 1 and 2 cm distances from the source. Both spectrometers allow measurement of the diffusely reflected light between 650 nm and 800 nm with 5 nm resolution. The light source and the two detectors are attached to a custom made probe head placed on the skin surface directly over the tibia. Light propagates between the source and the detectors in “banana-shaped” sensitivity functions and penetrates shallower for more closely separated source-detector pairs and more deeply for wider separated pairs. Thus, the detector 1 cm from the source probes the relatively thin (2 mm) skin layer, whereas the detector 2 cm from the source probes deeper into tissue and is therefore sensitive to the underlying bone. Placing the probe on the anterior side of the middle third tibia where the bone lies directly under the skin minimizes the effects of soft tissue and local changes in skin blood flow on the NIRS measurements. The extended modified Beer-Lambert law for a two-layer model will be used to determine hemoglobin content changes in tibial bone from changes in light absorption.

Sympathetic Microneurography. Multiunit, post-ganglionic muscle sympathetic nerve activity will be recorded with a tungsten microelectrode in the peroneal nerve only in the able-bodied population. The peroneal nerve will be mapped at the fibular head via low voltage transcutaneous electrical stimuli. Subsequently, the microelectrode is inserted manually through the unanesthetized skin and initially provides low voltage electrical stimuli (1-4 volts) to help locate the nerve. When the microelectrode enters a nerve fascicle innervating muscle, involuntary twitches are induced. The microelectrode is then used to record sympathetic efferent nerve outflow to calf muscle. This procedure is safe and the principal investigator has performed over 1000 experiments since 1991 without complication. The raw nerve signal will be amplified, rectified and integrated for visual inspection during experiments (Nerve Traffic Analyzer, Model 662c-3, University of Iowa Bioengineering, Iowa City, IA) and the amplified raw signal will be directly sampled at 20 kHz for further signal processing.

Beat-by-Beat Calf Blood Flow. We will use Doppler derived flow velocities to estimate whole limb blood flow through the popliteal artery. A continuous wave 4 MHZ Doppler probe (Multidop T2, DWL) is placed against the skin under the knee to isolate the popliteal artery proximal to the bifurcation.

Electrocardiogram. A standard 4-lead electrocardiogram will be used to obtain continuous heart rate.

Arterial Pressure. The arterial waveform in a finger of the hand will be derived on a beat-to-beat basis by the Finapres Blood Pressure System (Finapres, Ohmeda) with brachial oscillometric blood pressure (Dash 2000, GE) as a standard measure of arterial pressure.

Respiration. A respiratory belt will be placed around the chest for breathing depth and frequency. This signal does not provide exact tidal volumes and will only be used to monitor breathing.

Following the removal of insturments, the able-bodied volunteers will be asked to complete the online MSNA questionnaire administered via REDCap, if the patient had the electrode placed

beneath the surface of their skin. If the subject does not have access to the internet, a paper copy of the MSNA questionnaire will be mailed to his/her address.

VI. STATISTICAL ANALYSIS

Sample Size

Our prior work found a borderline significant increase ($p=0.06$) in tibial perfusion after exercise in able-bodied. Power analysis at 80% of these data showed that 17 subjects would have provided a $p<0.05$ increase in perfusion. Therefore, for this study, we based our sample size on this calculation. However, we will recruit equal numbers of men and women to explore potential sex-related differences. Also, due to individual anatomic differences and day-to-day variability in success, microneurographic recordings can be obtained in approximately two out of three volunteers. To ensure successful sympathetic nerve recordings in 17 individuals, we will need to recruit ~30% more subjects. Thus, we will recruit 34 healthy males, 34 healthy females, and 17 males with SCI.

Statistics

Data from NIRS monitoring will be used to determine hemoglobin content changes in tibial bone from changes in light absorption on a moment-by-moment basis. A detailed description of the analysis can be found in our previous work. [12] Briefly, NIRS monitoring provides a change in hemoglobin content from one state to another. This is due to the fact that absolute intensity of the scattered light measured across the tibia cannot be used for an absolute measure of perfusion. However, as perfusion increases, there is a proportional increase in absorption (and vice versa). Thus, percent change in total hemoglobin detected refects the relative change in perfusion.

The main outcomes assessed will be tibial perfusion, whole leg blood flow, and sympathetic activity. Descriptive statistics of the time series of these outcomes will include mean, median, standard deviation, and range. Differences/relations will be considered statistically significant at alpha = 0.05 level.

For cold pressor test, isometric handgrip exercise, and head tilt, in able-bodied we will assess the relationship between dependent increases in sympathetic outflow and changes in tibial perfusion. Since it is unclear whether absolute or relative changes in sympathethic outflow may best relate to relative changes in tibial flow, both will be explored as within subject predictors. Sex-related differences in response will be assessed simply as group differences in the individual slopes of the relation between tibial flow and sympathetic activity.

For cold pressor test, isometric handgrip exercise, and head tilt, in those with SCI we will assess the relationship between changes in mean arterial pressure and changes in tibial perfusion.

VII. RISKS AND DISCOMFORTS

The proposed study will utilize cardiovascular monitoring techniques with minimal risks. The attachment and removal of ECG electrodes and inflation of the blood pressure cuff may cause mild discomfort. Recordings from the peroneal nerve with a microelectrode may cause the minor discomfort of muscle weakness and/or pins and needles sensation, which resolve spontaneously within a few days. The immersion of the hand in ice-cold water may cause mild to moderate discomfort. The risks associated isometric handgrip exercise are minimal, but may cause mild to moderate discomfort. The NIRS system is non-invasive and poses little risk to the patients. Initial trials showed minor reddening of the skin; however, this was temporary and it quickly went away. The tilt test may cause subjects to feel dizzy, nauseous, sweaty or pale and may increase heart rate or decrease blood pressure; however, this is temporary and it goes away in a few minutes after the subject are brought back to neutral position.

VIII. POTENTIAL BENEFITS

This research may help physicians and scientists better understand how the nervous system controls bone perfusion in humans. Subjects will not directly benefit from participation in this protocol.

MONITORING AND QUALITY ASSURANCE

The research coordinator will be responsible for monitoring the completeness of all data and source documents. The Principal Investigator will monitor the informed consent procedures in accordance with the Informed Consent Compliance Checklist of Partners HealthCare Systems HRQIP. The subjects data/protocol adherence will be monitored by the research coordinator at each step in the study including. Checklists and note pages are used to note any deviations or omissions from the protocols. The Cardiovascular Research Laboratory Medical Emergency Safety Plan will be followed in the case of an adverse medical event. Any clinical/health related issues would be immediately presented to the subject (i.e. abnormal EKG, blood chemistries, etc) to determine appropriate notification (ie. current physician or appropriate specialist) .Based on the seriousness of the situation a physician may be contacted to provide clinical guidance on the appropriate course of action. Event assessment and follow-up will be done by the PI as needed on a case by case basis.

This trial will be conducted in compliance with the protocol, International Council for Harmonization/Good Clinical Practice requirements(ICH/GCP) and applicable state, local and federal regulatory requirements.

Reporting to Partners IRB:
Reports of *unanticipated problems involving risks to subjects or others* are to be submitted through Insight/eIRB within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem.

Reporting Unanticipated Problems that are Adverse Events
Any unanticipated untoward or unfavorable medical occurrence, including abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, that indicates that the research places subjects at increased risk of physical or psychological harm than previously known or recognized are to be submitted through Insight/eIRB as an Other Event, Adverse Event. The investigator must provide the following information in the report:

(1) a detailed description of the adverse event;
(2) the basis for determining that the event is unexpected in nature, severity, or frequency;
(3) the basis for determining that the event is related or possibly related to the research procedures;
(4) the basis for determining that the research places subjects at an increased risk of harm (i.e., a serious adverse event); and
(5) whether any changes to the research or other corrective actions are warranted.
NOTE: The PHRC does not accept sponsor IND/IDE safety reports describing adverse events that have occurred at sites other than those subject to this policy unless the report is of an incident that is:
(1) serious; (2) unexpected or unanticipated; (3) related to the investigational drug/device; and (4) suggests that subjects are at an increased risk of harm and as such warrants changes in the research, consent process, or informing subjects. IND/IDE safety reports that warrant changes are to be submitted through Insight/eIRB as an Amendment.

Reporting Unanticipated Problems that are not Adverse Events
All other unanticipated incidents, experiences, information, outcomes, or other problems that indicate that the research places subjects at an increased risk of physical, psychological, economic, legal, or social harm than was previously known or recognized are to be submitted through Insight/eIRB as an
Other Event. The investigator must provide the following information in the report:
(1) a detailed description of the unanticipated problem;
(2) the basis for determining that the problem is unexpected;
(3) the basis for determining that the problem indicates that the research places subjects at an increased risk of harm; and
(4) whether any changes to the research or other corrective action are warranted.

Reporting to the National Institute of Arthritis and Muscloskeletal and Skin Disease(NIAMS) and Safety Officer (Dr.Ralph Marino)

This protocol has funding from NIAMS. NIAMS has appointed a Saftey Officer, Dr. Ralph Marino, to act in an advisory capacity to the NIAMS to monitor participant safety, evaluate the progress of the study, to review procedures for maintaining the confidentiality of data, the quality of data collection, management and analyses.

The Principal Investigator (PI) will report unanticipated problems (UPs), serious adverse events (SAEs) and protocol violations that impact participant safety to the NIAMS and the Safety Officer, (through the Exective Secretary, KAI-Research) within 48 hours of the PI becoming aware of the event. KAI facilitates the review of the expedited reporting of these events by the Safety Officer. The Safety Officer will review the Safety Reports submitted by the PI to KAI twice a year. KAI facilitates the review of these reports by the Safety Officer and the NIAMS, and provides the final review comments to the PI when the review is complete. During the course of the study, ad hoc meetings may be called by the NIAMS or Safety Officer at any time to discuss potential safety issues.

Definitions:

Adverse event means any untoward or unfavorable medical occurrence in a human subject, includingany abnormal sign (for example, abnormal physical exam or laboratory finding),

symptom, or disease,temporally associated with the subject's participation in the research, whether or not considered relatedto the subject's participation in the research (modified from the definition of adverse events in the 1996. International Conference on Harmonization E-6 Guidelines for Good Clinical Practice).

Serious adverse event means any event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Possibly related to the research means there is a *reasonable possibility* that the adverse event, incident,experience or outcome may have been caused by the procedures involved in the research (modified from the definition of *associated with use of the drug* in FDA regulations at 21 CFR 312.32(a)). *Reasonable possibility* means that the event is more likely than not related to participation in the research or,in other words, there is a >50% likelihood that the event is related to the research procedures.

Deviation means any alteration/modification to the PHRC-approved protocol without prospective PHRC approval. The term *protocol* encompasses all PHRC-approved materials and documents including the detailed protocol, protocol summary, consent form, recruitment materials, questionnaires, and any other information relating to the research study.

Unanticipated problem involving risks to subjects or others means any incident, experience, information, outcome, or other problem that is *unexpected* and related to the research, and that indicates that the research places subjects or others at an increased risk of physical, psychological, economic, legal, or social harm than was previously known or recognized. *Unexpected* means that the incident, experience, or outcome is unexpected (in terms of nature, severity or frequency) given the (a) research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document and (b) the characteristics of the study population being studied.

Unexpected adverse event means any *adverse event* occurring in one or more subjects participating in a research protocol, the nature, severity, or frequency of which is not consistent with either: (1) the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as

product labeling and package inserts; or (2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

X. REFERENCES

[1] M. L. Brandi and P. Collin-Osdoby, "Vascular biology and the skeleton," *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.*, vol. 21, no. 2, pp. 183–192, Feb. 2006.
[2] M. Brookes and R. G. Harrison, "The vascularization of the rabbit femur and tibio-fibula," *J. Anat.*, vol. 91, no. 1, pp. 61–72, Jan. 1957.
[3] J. T. Lacey, "NON-UNION OF FRACTURES: AN EXPERIMENTAL STUDY," *Ann. Surg.*, vol. 89, no. 6, pp. 813–848, Jun. 1929.
[4] M. T. Vogt, J. A. Cauley, L. H. Kuller, and M. C. Nevitt, "Bone mineral density and blood flow to the lower extremities: the study of osteoporotic fractures," *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.*, vol. 12, no. 2, pp. 283–289, Feb. 1997.
[5] J. Trueta and J. D. Morgan, "The vascular contribution to osteogenesis. I. Studies by the injection method," *J. Bone Joint Surg. Br.*, vol. 42-B, pp. 97–109, Feb. 1960.
[6] J. M. Kanczler and R. O. C. Oreffo, "Osteogenesis and angiogenesis: the potential for engineering bone," *Eur. Cell. Mater.*, vol. 15, pp. 100–114, May 2008.
[7] H. Azuma, "INTRAOSSEOUS PRESSURE AS A MEASURE OF HEMODYNAMIC CHANGES IN BONE MARROW," *Angiology*, vol. 15, pp. 396–406, Sep. 1964.
[8] N. E. Shaw, "Observations on the intramedullary blood-flow and marrow-pressure in bone," *Clin. Sci.*, vol. 24, pp. 311–318, Jun. 1963.
[9] M. T. Dean, M. B. Wood, and P. M. Vanhoutte, "Antagonist drugs and bone vascular smooth muscle," *J. Orthop. Res. Off. Publ. Orthop. Res. Soc.*, vol. 10, no. 1, pp. 104–111, Jan. 1992.
[10] P. J. Briggs, C. G. Moran, and M. B. Wood, "Actions of endothelin-1, 2, and 3 in the microvasculature of bone," *J. Orthop. Res. Off. Publ. Orthop. Res. Soc.*, vol. 16, no. 3, pp. 340–347, May 1998.
[11] A. H. Stein, H. C. Morgan, and R. F. Porras, "The effect of pressor and depressor drugs on intramedullary bone-marrow pressure," *J. Bone Joint Surg. Am.*, vol. 40-A, no. 5, pp. 1103–1110, Oct. 1958.
[12] A. E. Draghici *et al.*, "Near infrared spectroscopy for measuring changes in bone hemoglobin content after exercise in individuals with spinal cord injury," *J. Orthop. Res. Off. Publ. Orthop. Res. Soc.*, May 2017.
[13] D. A. Boas, C. E. Elwell, M. Ferrari, and G. Taga, "Twenty years of functional near-infrared spectroscopy: introduction for the special issue," *NeuroImage*, vol. 85 Pt 1, pp. 1–5, Jan. 2014.
[14] M. Ferrari and V. Quaresima, "A brief review on the history of human functional near-infrared spectroscopy (fNIRS) development and fields of application," *NeuroImage*, vol. 63, no. 2, pp. 921–935, Nov. 2012.
[15] S. M. Aziz, F. Khambatta, T. Vaithianathan, J. C. Thomas, J. M. Clark, and R. Marshall, "A near infrared instrument to monitor relative hemoglobin concentrations of human bone tissue in vitro and in vivo," *Rev. Sci. Instrum.*, vol. 81, no. 4, p. 043111, Apr. 2010.

[16] P. Farzam, P. Zirak, T. Binzoni, and T. Durduran, “Pulsatile and steady-state hemodynamics of the human patella bone by diffuse optical spectroscopy,” *Physiol. Meas.*, vol. 34, no. 8, pp. 839–857, Aug. 2013.
[17] S. Konugolu Venkata Sekar *et al.*, “In Vivo, Non-Invasive Characterization of Human Bone by Hybrid Broadband (600-1200 nm) Diffuse Optical and Correlation Spectroscopies,” *PloS One*, vol. 11, no. 12, p. e0168426, 2016.
[18] L. B. Rowell and D. R. Seals, “Sympathetic activity during graded central hypovolemia in hypoxemic humans,” *Am. J. Physiol.*, vol. 259, no. 4 Pt 2, pp. H1197-1206, Oct. 1990.
[19] D. S. Kimmerly and J. K. Shoemaker, “Hypovolemia and neurovascular control during orthostatic stress,” *Am. J. Physiol. Heart Circ. Physiol.*, vol. 282, no. 2, pp. H645-655, Feb. 2002.
[20] S. V. Matalon and L. E. Farhi, “Cardiopulmonary readjustments in passive tilt,” *J. Appl. Physiol.*, vol. 47, no. 3, pp. 503–507, Sep. 1979.
[21] M. Saito, N. Foldager, T. Mano, S. Iwase, Y. Sugiyama, and M. Oshima, “Sympathetic control of hemodynamics during moderate head-up tilt in human subjects,” *Environ. Med. Annu. Rep. Res. Inst. Environ. Med. Nagoya Univ.*, vol. 41, no. 2, pp. 151–155, Dec. 1997.
[22] J. Kawanokuchi *et al.*, “Influence of vestibulo-sympathetic reflex on muscle sympathetic outflow during head-down tilt,” *Environ. Med. Annu. Rep. Res. Inst. Environ. Med. Nagoya Univ.*, vol. 45, no. 2, pp. 66–68, Dec. 2001.
[23] J. Fagius, S. Karhuvaara, and G. Sundlöf, “The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles,” *Acta Physiol. Scand.*, vol. 137, no. 3, pp. 325–334, Nov. 1989.
[24] K. C. Kregel, D. R. Seals, and R. Callister, “Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation,” *J. Physiol.*, vol. 454, pp. 359–371, Aug. 1992.
[25] D. R. Seals, P. B. Chase, and J. A. Taylor, “Autonomic mediation of the pressor responses to isometric exercise in humans,” *J. Appl. Physiol. Bethesda Md 1985*, vol. 64, no. 5, pp. 2190–2196, May 1988.