

STUDY PROTOCOL

STUDY TITLE: ISCHEMIC CONDITIONING CHRONIC STROKE STUDY

DATE OF DOCUMENT: 7/26/2019

Research Strategy. This is a prospective, randomized, cross-over study.

Subjects and Recruitment. A total of 15 individuals with chronic stroke (>1 year post-stroke) will be enrolled to complete the aim of this study. Males and females will be recruited equally. Inclusion criteria: Study participants must be between 18-85 years of age, able to give informed consent, >1 year post diagnosis of unilateral cortical or sub-cortical stroke, have residual lower limb paresis. Exclusion criteria: History of blood clots in the extremities or any condition in which compression of the thigh or transient ischemia is contraindicated (e.g. wounds in the leg), chronic pain syndrome, history of head trauma, comorbid neurological disorder, any uncontrolled hypertension (>160/100 mmHg), peripheral vascular disease, a myocardial infarction in the previous year, inability to follow 2 step commands, or history of multiple strokes.

Study participants will be recruited from two sources, a HIPPA compliant database of >40 stroke survivors managed by Dr. Hyngstrom and a REDCap database managed by Dr. Durand which contains the names of >200 individuals with stroke consented to be contacted for research studies. The PI's have successfully used these resources to recruit stroke survivors for previous studies. Participants will be paid \$50 per visit.

General Methods: Study participants will be instructed to fast for 8 hours prior to visiting the adult TRU. A member of the TRU staff will measure height, weight and blood pressure and collect a list of current medications. 10 mL of venous blood will be drawn from an indwelling catheter in the antecubital vein of the non-affected arm into a vacutainer tube with lithium heparin. A total of three blood draws will be performed – at rest, immediately following IC or Sham IC, and immediately following the CPT. The tubes will immediately be centrifuged at 2500 RPM for 15 minutes at 4°C to separate the plasma fraction. The plasma will be transferred to storage vials, frozen in liquid nitrogen, and stored at -80°C until analysis.

IC and IC Sham: IC will be performed in accordance with other studies which have used IC as an intervention¹⁻³ and consistent with our previous work.⁴⁻⁶ Briefly, in supine with legs level with the heart, a rapid inflation cuff (Hokanson SC12 thigh cuff) will be placed around the proximal, paretic thigh and inflated to 225 mmHg for 5 minutes, then released for a 5 minute recovery period. Five cycles of inflation/recovery will be performed. For IC Sham, the cuff will only be inflated to 10 mmHg as participants still perceive the cuff tightness, however the inflation pressure is not high enough to occlude arterial or venous blood flow.

Cold Pressor Test: Water will be cooled to 4-6°C in a bucket and circulated with a sous vide precision cooker. Study participants will submerge their non-affected hand, up to the wrist, into the water for two minutes. Blood pressure and subjective pain will be measured before, during (1 minute) and after the test. Blood will be drawn immediately after the test. Skin temperature will be assessed with a skin thermometer.

Plasma Epinephrine and Norepinephrine Measurements. The concentration of epinephrine and norepinephrine in the plasma samples will be measured by the Physiology Biochemical Analytical Laboratory at MCW using high performance liquid chromatography (HPLC) and established protocols.

1 Martin A, Albrecht IS, Mohammad K, Daniel D, Johann B, Richard C, Ewald M, Michael W. Effect of ischemic preconditioning in skeletal muscle measured by functional magnetic resonance imaging and spectroscopy: a randomized crossover trial. *Journal of Cardiovascular Magnetic Resonance* 2011; **13**: 32.

2 Patricia CE, Dick HJT, Manuel S, Reinier E, Maria TEH. Ischemic preconditioning improves maximal performance in humans. *European Journal of Applied Physiology* 2009; **108**: 141-6.

3 Tom G B, Helen J, Warren G, Greg A, Nigel Timothy C, Dick H. J T. Effect of Ischemic Preconditioning on Lactate Accumulation and Running Performance. *Medicine and Science in Sports and Exercise* 2012; **44**: 2084-9.

4 Durand MJ, Boerger TF, Nguyen JN, Alqahtani SZ, Wright MT, Schmit BD, Gutterman DD, Hyngstrom AS. Two Weeks of Ischemic Conditioning Improves Walking Speed and Reduces Neuromuscular Fatigability in Chronic Stroke Survivors. *J Appl Physiol (1985)* 2019.

5 Durand MJ, Nguyen J, Wright MT, Gutterman DD, Schmit BD, Hyngstrom AS. Ischemic Conditioning Improves Brachial Artery Flow Mediated Dilatation in Chronic Stroke Survivors. *Stroke* 2019; **50**: A60-A.

6 Hyngstrom AS, Murphy SA, Nguyen J, Schmit BD, Negro F, Gutterman DD, Durand MJ. Ischemic conditioning increases strength and volitional activation of paretic muscle in chronic stroke: a pilot study. *J Appl Physiol (1985)* 2018; **124**: 1140-7.