Protocol version 2
Date: 28 Mar 2022

TITLE: Effects of tDCS on Post-stroke Fatigue and Inflammation

IRB Protocol Number: 118687

PI: Dr. John H. Kindred
Research Associate
Division of Physical Therapy
Department of Rehabilitation Sciences
College of Health Professions
Medical University of South Carolina

BACKGROUND / SIGNIFICANCE

Stroke is the most common cause of long-term disability in the United States. Fatigue is a common sequela of stroke and many other neurological conditions. There is no universally accepted definition of fatigue or post-stroke fatigue. However, it is generally accepted that fatigue can be dichotomized into fatigability, an objectively measured decline in physical or cognitive performance, and the perception of fatigue (Kluger et al. 2014, Enoka et al. 2021). The perception of fatigue is defined as a feeling of weariness/tiredness or mismatch between an individual's perceived and actual effort that negatively affects their ability to perform physical, cognitive, and social activities that is not alleviated with rest. A model of post-stroke fatigue (PSF) is presented Figure 1 (adapted from Enoka et al. 2021). Even though the negative impacts of fatigue on quality of life and social/physical activity participation post-stroke are well known, there are currently no evidence-based treatments for post-stroke fatigue (PSF, Hinkle et al. 2017). The development of effective treatments is lacking in part due to the complex and poorly understood pathophysiology of PSF. The recent NIH-sponsored workshop "Beyond the Symptom: The Biology of Fatigue" highlighted this fact and called for more extensive research into the mechanisms and treatments of fatigue. While specific mechanisms of individual patients are unknown, altered immune function, i.e., inflammation (Wen et al. 2018), changes in tissue metabolism (Roelcke et al. 1997), disruptions in interoception (Gonzalez Campo et al. 2020), and altered neural network function (Høgestøl et al. 2019) have been implicated across neurological conditions.

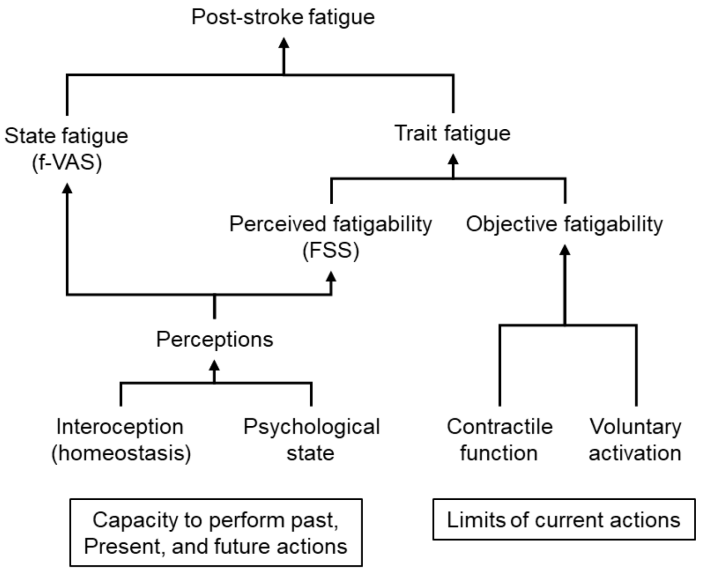

Figure 1: A model defining post-stroke fatigue, adapted from a schema of fatigue in multiple sclerosis by Enoka et al. (2021). An individuals current State fatigue is measured on a 0-10 Visual Analogue Scale (f-VAS) representing their perception of fatigue at the moment of assessment. The most common method to assess and diagnos clinical fatigue is the Fatigue Severity Scale (FSS), which is thought to measure the effect of fatigue on an individual's perceived ability to perform tasks.

Specific to stroke, a meta-analysis reported the prevalence of PSF is 50% (Cumming et al. 2016). A recent survey of stroke survivors listed PSF treatments as the second most prioritized area of need behind walking/mobility (Rudberg et al. 2020). When dichotomizing the survey's respondents by age, younger stroke survivors reported PSF treatment as their number one priority area. The latest scientific statement from the American Heart Association spotlights the inflammatory and altered cortical excitability components of PSF (Hinkle et al. 2017). Transcranial direct current stimulation (tDCS) has been reported to affect inflammation and cortical excitability. A recent small tDCS study reported beneficial effects on PSF but did not investigate conclusive mechanisms of action or response (De Doncker et al. 2021). Our contribution to this line of inquiry will be to further test the clinical effects of tDCS on PSF and measure markers of inflammation within the central nervous system and peripheral circulation. We expect to identify a link between inflammation and PSF which will significantly contribute to a better understanding of the pathophysiology of fatigue and establish neuromodulatory therapies, such as tDCS, as effective treatments for PSF.

INNOVATION

The effect of tDCS on non-neuronal cell populations has received little attention to date. Basic and pre-clinical research shows small electrical currents, such as the ones used by tDCS, can induce cell differentiation and migration (reviewed by Pelletier and Cicchetti 2015) and alter immune cell function leading to reduced inflammatory markers (Rabenstein et al. 2019, Zhang et al. 2021). Studies in humans have shown reduced circulating inflammatory cytokines after five days of tDCS (Osteoarthritis, Suchting et al. 2020). Stroke is known to induce neuroinflammatory/immune responses and certain inflammatory/immune markers remain elevated one year post incident (Beamer et al. 1998). Inflammation has also been shown to interfere with rehabilitation and could possibly contribute to post-stroke depression (reviewed by Fang et al. 2019). There are reports of anodal-tDCS (a-tDCS) leading to reduced fatigue severity post-stroke and in MS. However, these reports have relatively

small samples and did not identify/investigate possible mechanisms of effects/response. This pilot project is innovative because it uses a multimodal approach to investigate the effects of tDCS on measures of cortical excitability **and** effects on inflammation, which likely play roles in the development and severity of PSF. Future investigations derived from this line of inquiry have the opportunity to greatly enhance our understanding of the pathophysiological mechanisms of PSF, identify effective neuromodulatory therapies, and pinpoint a phenotypic response criterion for future clinical trials, i.e. individuals with PSF due to inflammation. An additional innovation is using MR spectroscopy to measure markers of nervous system inflammation. MR spectroscopy is a well-established tool. However, its use in assessing metabolite changes with neuromodulatory therapies is not common due to most tDCS research focusing on cortical excitability and/or neural network function. Using MR spectroscopy will allow us to quantify in-vivo changes in metabolites and inflammatory markers in individuals with PSF in response to tDCS.

APPROACH

Recruitment and Consent

Prospective participants will be recruited from our bioinformatics enabled post-stroke recruitment database which is maintained by the NIH Center of Biomedical Research Excellence in Stroke Recovery at the Medical University of South Carolina. This database, RESTORE, contains over 1200 individuals post-stroke who have consented to be contacted for research purposes. Veterans from our VA-approved recruitment data have agreed to have their contact information placed in RESTORE and their veteran-status is highlighted. Prospective participants more than six months post-stroke will be identified by their response to question 4 of the Personal Health Questionnaire Depression Scale (PHQ-8) stored in their database record. A response of 2 or 3 indicates "Feeling tired or having little energy" on at least half the days over the last 2 weeks. Veterans who participated in the recently completed VA RR&D sponsored study "Fatigue and Mobilility in Stroke: a Biomechanical and Neurophysiological Investigation" (IK1 RX0003126, CDA-1, PI: Kindred JH) will be preferentially contacted and recruited to participate in this project. Initial contact will be made with prospective participants via telephone. The project basics will be explained and interested individuals will be invited to our research laboratory for final eligibility screening and consent. *Full inclusion and exclusion criteria are listed in the "Protection for Human Subjects" section*. Once consented, participants will complete a set of questionnaires to assess levels of state and trait fatigue, depression, anxiety, and pain. State fatigue is defined as a participant's instantaneous perception of fatigue and is measured on a 0-10 visual analogue scale (f-VAS) with 0 signifying no feelings of fatigue and 10 representing the most fatigued an individuals could imagine. Trait fatigue refers to an individual's perception of fatigability, i.e. how fatigue affects their ability to perform activities of daily living and participate in social and physical activities. The most common instrument used to measure trait fatigue is the Fatigue Severity Scale (FSS, Krupp et al. 1997). The FSS is a nine-question, scored on a 1-7 likert scale, self-reported questionnaire assessing the impact of fatigue on a variety of activites over the last seven days. An average score of 4 on the FSS signifies clinically relevant fatigue (total FSS score ≥ 36). Additional clinical tests and questionnaires will be performed to measure physical disability (gait speed), balance, and quality of life. Women of childbearing potential will perform a urinalysis to determine pregnancy status. Pregnant women will be excluded due to unknown effects of tDCS on fetal development and mother's milk. The

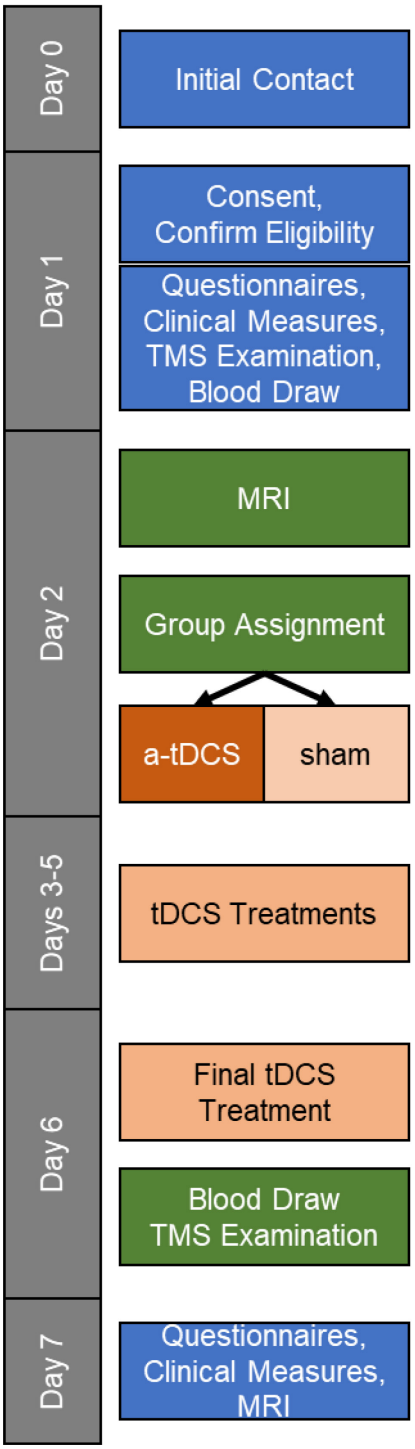

Figure 2: Research protocol timeline. Three additional followup phone-calls to assess fatigue will be performed 1,2, and 4 weeks post the participants' last tDCS treatment.

full list of questionnaires and clinical measures is presented in Table 1. *The primary outcome for this study is trait fatigue measured by the FSS*.

Study Design and a-tDCS Treatment

This pilot proposal is a randomized longitudinal single-blind sham-controlled intervention. Individuals with PSF will be randomly assigned to the sham or a-tDCS treatment group. Before and after five consecutive days of a-tDCS applied bilaterally over the left and right lower extremity motor cortex, PSF and nervous system and peripheral inflammation will be measured, similar to De Doncker et al. (2021). The tDCS treatment will consist of twenty minutes of 4 mA a-tDCS delivered via two carbon rubber electrodes placed inside sponge pads (1x1 tDCS, Soterix, USA). The current will be ramped up 4 mA over 30 seconds. Each electrode has a surface area of 70 cm^2, resulting in a current density of 0.06 mA/cm^2 and a charge density of 1.1 C/cm^2, which are within current safety guidelines (Bikson et al. 2016). The anode electrode will be centered on the interhemispheric fissure and the central sulcus. This area was chosen due to its previous use in fatigue studies using neuromodulation (multiple sclerosis, Ferrucci et al. 2014; fibromyalgia, Altas et al. 2019; Stroke, De Doncker et al. 2021). The motor cortex is being used more often in neuromodulation studies due to its structural and functional connectivity to cortical and subcortical networks/nodes, e.g. basal ganglia, cingulate cortex. The cathodal electrode will be placed on the nonparetic shoulder. Modeling of cephalic and extracephalic tDCS montages indicates that extracephalic placement of the cathode leads to deeper current flow in the somatosensory cortex (Noetscher et al. 2014). The sham tDCS procedure will be performed in the same manner as active a-tDCS, however, the current will be ramped up and down over 30 seconds (Fridricksson et al. 2018). Figure 2 displays the protocol timeline. Days 2-6 will be consecutive, no more than 36 hours will be allowed between treatment visits. If more than 36 hours elapses the participant will be withdrawn from the study. The mearsurement of state fatigue, via f-VAS, will be performed before and after each tDCS treatment visit. Follow-up assessments of state and trait fatigue will be performed via phone at one (Follow-up 1), two (Follow-up 2), and four weeks post-treatment (Follow-up 3). The primary study outcome will be the comparison of the FSS scores obtain at baseline (Visit 1) and one week post-tDCS (Follow-up 1).

Neuroimaging

Anatomical and spectroscopic images (T1, T2, ^{1}H-MRS) will be acquired using the Center for Biomedical Imaging at MUSC's standard imaging protocols. The T1 and T2 images will be used for the computation of stroke lesion locations and volumes. The T1 images will also be used to guide the corticomotor response (CMR) testing listed below. We will use Single-voxel MEshcher-GArwood Point RESolved Spectroscopy (MEGA-PRESS) ^{1}H-MRS to assess central nervous system inflammation. Voxels will be placed in the basal ganglia and insular cortex in both hemispheres. Basal ganglia voxels will be placed with the anterior border abutting the anterior portion of the lentiform nucleus and as medial as possible to avoid the ventricles (Pretzsch et al. 2019). The basal ganglia have been previously implicated in fatigue post-stroke (Tang et al. 2010) and in multiple sclerosis (Roelcke et al. 1997, Tellez et al. 2008). The insular cortex is a prominent brain region involved in interoception. Interoception is the sensing, integrating, interpreting and regulating self-signals and has been theorized to contribute to fatigue (Stephan et al. 2016, Enoka et al. 2021) and implicated in fatigue in multiple sclerosis (Gonzalez Campo et al. 2020). Levels of Myo-inositol (MI), total creatine (tCr), and choline (CHO) and their ratios to N-acetylaspartate (NAA) will be quantified as markers of inflammation (reviewed by Chang et al. 2015). Spectroscopy data will be analyzed using the freely available LCModel software as in our previous works (Moss et al. 2018, 2019). Pre and post tDCS intervention spectroscopy values will be compared to determine changes in inflammatory markers associated with the intervention. The mearsurement of state fatigue will be performed before and after each imaging session.

Blood collection and analysis

Before and after five days of tDCS treatment, participants will provide a blood sample for the measurement of pro-inflammatory cytokines. These cytokines include interleukin- (IL) 1, 6, and 10, tumor necrosis factor (TNF) α, and c-reactive protein. Whole blood will be collected in EDTA-treated tubes and separated into plasma, immune cells (buffy coat), and red blood cells. Separated samples will be frozen and stored until the end of the study. Once all samples are collected they will undergo Simple Plex^{TM} microfluidic ELLA platform immunoassay (ProteinSimple, USA) analysis for listed cytokines (Leligdowicz et al. 2017, Burke et al. 2021). State fatigue will

be measured just prior to blood collection. Blood will be collected at the South Carolina Clinical and Translational Research Institute (SCTR), located on the MUSC/VA shared campus, by skilled medical staff.

Corticomotor Response (CMR)

The CMR of the paretic and non-paretic tibialis anterior will be measured immediately before and after the tDCS intervention using neuronavigated single- and paired-pulse TMS (Brainsight, Rogue Research, CAN) to identify is changes in the CMR are associated with changes in PSF fatigue severity. Preliminary, unpublished, data from our lab indentifed a link between asymmetries in intracortical facilitation (ICF) between the lesioned and non-lesioned hemishperes and PSF severity. Single-pulse TMS will be used to measure resting motor threshold (rMT) and motor evoked potential amplitude (MEP_{amp}) and latency (MEP_{lat}). Paired-pulse TMS will be used to assess glutamatergic activity signified by intracortical facilitation (ICF). Prior to the participants' first interventional visit, a 7x5 grid will be placed over the participants' acquired 3D T1 image. The middle grid point will be centered on the intersection between the interhemispheric fissure and central sulcus. Surface electromyography (sEMG) electrodes will be placed over the left and right TA. The CMR assessment will begin with the identification of the stimulator powers required to elicit a consistent response in the paretic and non-paretic TA. Once these power levels have been determined a single TMS pulse at each of the identified power outputs will be delivered over the respective grid points. The gridpoint resulting in the largest MEP_{amp} for each muscle will be determined to be the “hotspot” (Kindred et al. 2021). Resting MT will be assessed using simple adaptive parameter estimation by sequential testing (PEST, Borckhardt et al. 2006) measured at the respective hotspots. Next, 20 single TMS pulses will be delivered at 120% rMT for measurement of MEP_{amp} and MEP_{lat}. Paired-pulse TMS will be applied to measure ICF with the conditioning pulse delivered at 80% rMT followed by the conditioned pulse at 120% rMT, using an interstimulus interval of 10 ms,. The recorded sEMG signals will be analyzed using the standard laboratory operating procedures (Kindred et al. 2019, 2020). Neurophysiological variables before and after the tDCS interventional will be compared to determine if changes in PSF severity are associated with changes in the CMR. State fatigue will be measured pre and post the CMR assessment.

Table 1: Clinical Outcome Measures

Domain	Instrument
Fatigue (State)	Visual Analogue Scale
Fatigue (Trait)	* Fatigue Severity Scale (FSS) (Krupp et al. 1989) Modified Fatigue Impact Scale (mFIS) (Fisk et al. 1994) Fatigue Assessment Scale (FAS) (Michielsen et al. 2003)
Anxiety	Hospital Anxiety and Depression Scale (Zigmond and Snaith 1983)
Depression	Patient Health Questionnaire 9 (PHQ-9) (Spitzer and Williams 1999)
Pain	Defense and Veterans Pain Rating Scale (DVPRS) (Polomano et al. 2016)
Physical Function / Mobility	10 m walk over GAITRite (Kindred et al. 2019)
Community Participation / Quality of Life	Standard Form 36 (Anderson et al. 1996)

* Primary outcome measure for Aim 1

Statistical Analysis

Aim 1 Hypothesis: Five days of a-tDCS applied to the lower extremity motor cortex will result in a reduction of PSF severity in individuals with chronic stroke. A two-factor, between-subjects factor of GROUP (active a-tDCS, sham) and within-subjects factor of TIMEPOINT (pre, post), mixed model ANOVA will be used to test this hypothesis. The primary outcome variable for this analysis is the FSS scores. We will also calculate measures of effect size to power future studies.

Aim 2 Hypothesis: Five days of a-tDCS applied to the lower extremity motor cortex will result in a reduction of inflammatory markers within the central nervous system and the peripheral blood. Pre- and post-intervention markers of inflammation will be compared using a mixed model, two-factor ((between-subjects factor of GROUP (active a-tDCS, sham) and within-subjects factor of TIMEPOINT (pre, post)), ANOVA. Each outcome measure (MI, tCR, IL-1, TNF-α, etc) will be tested independently.

Additional Analyses: Examine the possible relationship between PSF severity and inflammatory markers. This relationship may provide mechanistic insight into the effect of tDCS on PSF and allow us to possibly predict future responders/non-responders to a-tDCS treatments of PSF. Pearson's correlations will be calculated between baseline measures of inflammation and FSS scores to determine a possible role of current state inflammation on PSF severity. Correlations between the change in FSS scores and inflammation, pre- and post-a-tDCS, will be calculated to provide preliminary evidence of a possible mechanism of reduced inflammation and improved PSF.

Potential Problems / Solutions

Some groups have reported a 50% response rate to tDCS. Due to our small sample, non-responders may reduce our statistical power. A post-hoc response analysis, defined as a change greater than the minimally clinically important difference of 0.45 (Nordin et al. 2016), will be performed to power future studies. Placing the ^{1}H-MRS voxel within the basal ganglia is a logical starting place based on the published literature. However, it is possible that inflammatory markers outside this region are as influential or more so than basal ganglia measures. Future work may be required to determine which brain regions are best to assess with ^{1}H-MRS. Another limitation is that only a-tDCS is being tested. Cathodal tDCS (c-tDCS) has been reported to have similar long-term effects on gene expression and protein synthesis. The benefits of tDCS on fatigue, to date, have mostly investigated a-tDCS although future investigations need to determine if c-tDCS may be a viable treatment option. Another area that will need to be addressed in future studies is if our post-intervention measurements of metabolite changes reflect the last episode of treatment or if they are indicative of long-term changes in response to tDCS. Future studies will also need to assess the optimal intervention length and follow-up periods.

Biostatistical Justification

This pilot work is based on a similar study investigating a-tDCS' effects on post-stroke fatigue (De Doncker et al. 2021). The FSS is the primary measure of fatigue severity and the minimally clinically important difference (MCID) was reported to be 0.45 (Nordin et al. 2016), with changes greater than this resulting in reported improvements in QOL. To detect this level of change with 80% power and an alpha = 0.05 between two groups using a within-between-subjects interaction ANOVA a minimum sample of 8 individuals in each group is required. We will enroll 24 participants (12 a-tDCS, 12 sham) to account for any drop-out, loss to follow-up, or complications during data collection. We have projected to perform recruitment and data collection activities over nine months.

REFERENCES

Altas, E. U., Askin, A., Besiroglu, L., & Tosun, A. (2019). Is high-frequency repetitive transcranial magnetic stimulation of the left primary motor cortex superior to the stimulation of the left dorsolateral prefrontal cortex in fibromyalgia syndrome? Somatosens Mot Res, 36(1),56-62.

Anderson, C., Laubscher, S., & Burns, R. (1996). Validation of the Short Form 36 (SF-36) health survey questionnaire among stroke patients. Stroke, 27(10), 1812-1816.

Beamer, N.B., Coull, B.M., Clark, W.M., … & Sexton, G. (1998). Persistent inflammatory response instrokesurvivors. Neurology,50(6),1722-28

Bikson, M., Grossman, P., Thomas, C., ... Woods, A. J. (2016). Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016. Brain Stimul, 9(5), 641-661.

Borckardt, J. J., Nahas, Z., Koola, J., & George, M. S. (2006). Estimating resting motor thresholds in transcranial magnetic stimulation research and practice: a computer simulation evaluation of best methods. J ect, 22(3), 169-175.

Burke, H., Freeman, A., Cellura, D.C., ... Wilkinson,T.M.A. (2020). Inflammatory phenotyping predicts clinical outcome in COVID-19. RespirRes, 21(1), 245.

Chang, L., Munsaka, S. M., Kraft-Terry, S., & Ernst, T. (2013). Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain. J Neuroimmune Pharmacol, 8(3), 576-593.

Cumming, T. B., Packer, M., Kramer, S. F., & English, C. (2016). The prevalence of fatigue after stroke: A systematic review and meta-analysis. Int J Stroke, 11(9), 968-977.

De Doncker, W., Ondobaka, S., & Kuppuswamy,A. (2021). Effect of transcranial direct current stimulation on post-stroke fatigue. J Neurol. 268(8), 2831-2842

Enoka, R. M., Almuklass, A. M., Alenazy, M., Alvarez, E., & Duchateau, J. (2021). Distinguishing between Fatigue and Fatigability in Multiple Sclerosis. Neurorehabil Neural Repair, 35(11), 960-973.

Fang,M., Zhong,L., Jin,X,...Liu,T(2019).Effect of Inflammation on the Process of Stroke Rehabilitation and Poststroke Depression. FrontPsychiatry, 10,184.

Ferrucci, R., Vergari, M., Cogiamanian, F.,. . . Priori, A. (2014). Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis. NeuroRehabilitation, 34(1), 121-127.

Fisk, J. D., Ritvo, P. G., Ross, L., Haase, D. A., Marrie, T. J., & Schlech, W. F. (1994). Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis, 18 Suppl 1, S79-83.

Fridriksson, J., Rorden, C., Elm, J., Sen, S., George, M. S., & Bonilha, L. (2018). Transcranial Direct Current Stimulation vs Sham Stimulation to Treat Aphasia After Stroke: A Randomized Clinical Trial. JAMA Neurol, 75(12), 1470-1476.

Gonzalez Campo, C., Salamone, P. C., Rodríguez-Arriagada, N.,. . . Sedeño, L. (2020). Fatigue in multiple sclerosis is associated with multimodal interoceptive abnormalities. Mult Scler, 26(14), 1845-1853.

Hinkle, J. L., Becker, K. J., Kim, J. S.,. . . Stroke, C. (2017). Poststroke Fatigue: Emerging Evidence and Approaches to Management: A Scientific Statement for Healthcare Professionals From the American Heart Association. Stroke, 48(7), e159-e170.

Høgestøl, E. A., Nygaard, G. O., Alnæs, D., Beyer, M. K., Westlye, L. T., & Harbo, H. F. (2019). Symptoms of fatigue and depression is reflected in altered default mode network connectivity in multiple sclerosis. PLoS One, 14(4), e0210375.

Kindred, J. H., Kautz, S. A., Wonsetler, E. C., & Bowden, M. G. (2019). Single Sessions of High-Definition Transcranial Direct Current Stimulation Do Not Alter Lower Extremity Biomechanical or Corticomotor Response Variables Post-stroke. Front Neurosci, 13, 286.

Kindred, J. H., Wonsetler, E. C., Charalambous, C. C., ... Bowden, M. G. (2020). Individualized Responses to Ipsilesional High-Frequency and Contralesional Low-Frequency rTMS in Chronic Stroke… Frontiers in Human Neuroscience, 14 (494).

Kindred, J. H., Cash, J. J., Ergle, J. B., … & Bowden, M. G. (2021). Comparing cortico-motor hotspot identification methods in the lower extremities post-stroke: MEP amplitude vs. latency. Neuroscience Letters, 754, 135884.

Kluger, B. M., Krupp, L. B., & Enoka,R.M. (2013). Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology, 80(4), 409-416.

Krupp, L. B., LaRocca, N. G., Muir-Nash, J., & Steinberg, A. D. (1989). The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol, 46(10), 1121-1123.

Leligdowicz, A., Conroy, A. L., Hawkes, … & Kain, K. C.(2017). Validation of two multiplex platforms to quantify circulating markers of inflammation and endothelial injury in severe infection. PLoS One, 12(4), e0175130.

Michielsen, H. J., De Vries, J., & Van Heck, G. L. (2003). Psychometric qualities of a brief self-rated fatigue measure: The Fatigue Assessment Scale. J Psychosom Res, 54(4), 345-352.

Moss, H. G., Brown, T. R., Wiest, D. B., & Jenkins, D. D. (2018). N-Acetylcysteine rapidly replenishes central nervous system glutathione measured via magnetic resonance spectroscopy in human neonates with hypoxic-ischemic encephalopathy. J Cereb Blood Flow Metab, 38(6), 950-958.

Moss, H. G., Jenkins, D. D., Yazdani, M., & Brown, T. R. (2019). Identifying the translational complexity of magnetic resonance spectroscopy in neonates and infants. NMR Biomed, 32(6), e4089.

Noetscher, G. M., Yanamadala, J., Makarov, S. N., & Pascual-Leone, A. (2014). Comparison of cephalic and extracephalic montages for transcranial direct current stimulation--a numerical study. IEEE Trans Biomed Eng, 61(9), 2488-2498.

Nordin, Å., Taft, C., Lundgren-Nilsson, Å., & Dencker, A. (2016). Minimal important differences for fatigue patient reported outcome measures-a systematic review. BMC medical research methodology, 16, 62-62.

Pelletier, S. J., & Cicchetti, F. (2015). Cellular and Molecular Mechanisms of Action of Transcranial Direct Current Stimulation: Evidence from In Vitro and In Vivo Models. International Journal of Neuropsychopharmacology, 18(2).

Polomano, R. C., Galloway, K. T., Kent, M. L., Brandon-Edwards, H., Kwon, K. N., Morales, C., & Buckenmaier, C., 3rd. (2016). Psychometric Testing of the Defense and Veterans Pain Rating Scale (DVPRS): A New Pain Scale for Military Population. Pain Med, 17(8), 1505-1519.

Pretzsch, C. M., Freyberg, J., Voinescu, B.,. . . McAlonan, G. M. (2019). Effects of cannabidiol on brain excitation and inhibition systems... Neuropsychopharmacology, 44(8), 1398-1405.

Rabenstein, M., Unverricht-Yeboah, M., Keuters, M. H., . . . Rueger, M. A. (2019). Transcranial Current Stimulation Alters the Expression of Immune-Mediating Genes. Frontiers in Cellular Neuroscience, 13(461).

Roelcke, U., Kappos, L., Lechner-Scott, J.,. . . Leenders, K. L. (1997). Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. Neurology, 48(6), 1566-1571.

Rudberg, A. S., Berge, E., Laska, A. C., … Lundström, E.(2020)Stroke survivors' priorities for research related to life after stroke.TopStrokeRehabil, 1-6.

Spitzer, R. L., Kroenke, K., & Williams, J. B. (1999). Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. JAMA, 282(18), 1737-1744.

Stephan, K. E., Manjaly, Z. M., Mathys, C. D., Weber, L. A. E., Paliwal, S., Gard, T., . . . Petzschner, F. H. (2016). Allostatic Self-efficacy: A Metacognitive Theory of Dyshomeostasis-Induced Fatigue and Depression. Frontiers in Human Neuroscience, 10.

Suchting, R., Colpo, G. D., Rocha, N. P., & Ahn, H. (2020). The Effect of Transcranial Direct Current Stimulation on Inflammation in Older Adults With Knee Osteoarthritis: A Bayesian Residual Change Analysis. Biol Res Nurs, 22(1), 57-63.

Tang, W. K., Chen, Y. K., Mok, V., Chu, W. C., Ungvari, G. S., Ahuja, A. T., & Wong, K. S. (2010). Acute basal ganglia infarcts in poststroke fatigue: an MRI study. J Neurol, 257(2), 178-182.

Tellez, N., Alonso, J., Rio, J., Tintore, M., Nos, C., Montalban, X., & Rovira, A. (2008). The basal ganglia: a substrate for fatigue in multiple sclerosis. Neuroradiology, 50(1), 17-23.

Wen, H., Weymann, K. B., Wood, L., & Wang, Q. M. (2018). Inflammatory Signaling in Post-Stroke Fatigue and Depression. Eur Neurol, 80(3-4), 138-148.

Zhang, S., Cheng, S., Zhang, Z., Wang, C., Wang, A., & Zhu, W. (2021). Related risk factors associated with post-stroke fatigue: a systematic review and meta-analysis. Neurol Sci, 42(4), 1463-1471.

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. Acta Psychiatr Scand, 67(6), 361-370.

INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN

Prospective participants will be aged 35-80 years. This age range excludes young adults which are generally accepted to have a higher level of innate neuroplasticity. This selection range incorporate the age-phenome knowledge base age groups developed by Geifman, Cohen, and Rubin (2013). Aggregate data on age, race, and gender of each participant will be provided to the NICHD at fixed intervals. Although we do not anticipate differential treatment effects based on age, our analyses will explore clinically important differences due to age.

Exclusion of Children

Stroke is a condition most commonly associated with advanced age and the prevalence of stroke in children is very low. Importantly, these cases may differ in their etiology from the subjects we propose to study. As such, we will not enroll any children in this study.

Maximum Age

Aged nervous systems are known to be less-plastic and may not respond to neuromodulatory therapies as well as younger nervous systems. To reduce the possible effects of aging on the effectiveness of this pilot intervention we will limit enrollment to participants between 35 and 80 years of age (adult to aged groups defined by Geifman, Cohen, and Rubin (2013)).

References

Geifman, N., Cohen, R., & Rubin, E. (2013). Redefining meaningful age groups in the context of disease. Age (Dordr), 35(6), 2357-2366. doi:10.1007/s11357-013-9510-6

INCLUSION OF WOMEN AND MINORITIES

All eligible subjects of both sexes and all minorities will be encouraged to participate in the proposed non-invasive brain stimulation treatment for post-stroke fatigue. Study participants will be recruited from the South Carolina Lowcountry tri-county area, which comprises Berkeley, Charleston, and Dorchester counties. The 2020 US Census1 reported a population of approximately 800k individuals in the tri-county area with women making up 51.3% of the population. The racial and ethnic composition of the counties was White (69.2%), Black (25.7%), Hispanic (5.9%), Two or More Races (2.3%), Asian (2.1%), American Indian & Alaska Native (< 1%), and Native Hawaiian & Other Pacific Islander (< 1%). Women are reported to be approximately 53.5% of new and recurrent strokes2. As this number is similar to the population we will use the population statistic for our recruitment, especially as our recruitment is of only 24 individuals.

We will monitor the diversity of the sample on a monthly basis and if recruitment target(s) are not adequate, we will take appropriate measures to improve diversity in recruitment.

Specific Plans for Maximizing Inclusion of Women and Minorities
Inclusion of Women: Inclusion or exclusion of participants will not differ based on sex and we aim to recruit similar numbers of males and females.

Inclusion of Minorities: Inclusion or exclusion of participants will not differ based on race/ethnicity. Based on the available literature on barriers to minority participation in clinical studies, we also will take the following additional steps to ensure representative enrollment of minority groups:

1. Emphasize that the goal is to recruit patients regardless of race or ethnicity. To assuage common suspicions that a specific race or ethnicity is being targeted,3-5 we will make clear to potential participants and their parents/guardians that patients of all races and ethnicities are being recruited.

2. Clearly explain that the purpose of informed consent is to protect, not relinquish, participants' rights. In several studies on the barriers to minority participation in clinical studies, participants have reported that they perceive the process of written, informed consent as relinquishing, rather than protecting, their rights.6-8 We will attempt to mitigate this source of distrust by explaining the intent of the informed consent process and emphasize the rights that the consent document enshrines.

3. Use straightforward and easy-to-understand language in consent forms. Participants' concerns about providing informed consent also relate to "not understanding technical legal and medical terminology"6 and "difficulty in fully understanding the complexities of research"6. We will attempt to mitigate this barrier to minority participation by taking extra care to write consent forms in easy-to-understand, straightforward language.

References
1. US Census Bureau, www.census.gov, Accessed 28 Feb 2022

2. Virani, S. S., Alonso, A., Aparicio, …, and Tsao, C. W. (2021). Heart Disease and Stroke Statistics - 2021 Update: A Report From the American Heart Association. Circulation, 143(8), e254-e743.

Protocol version 2
Date: 28 Mar 2022

RECRUITMENT AND RETENTION PLAN

Recruitment of Participants
Prospective participants will be recruited from multiple sources including RESTORE, ClinicalTrials.gov, and advertisements placed throughout the community (e.g. flyers posted in community centers and electronic flyers posted on social media sites). RESTORE is a bioinformatics-enabled database that contains over 1100 stroke survivors that have consented to be contacted for participation in research studies. This database is maintained by the NIH Center Of Biomedical Research Excellence (COBRE) for Stroke Recovery at the Medical University of South Carolina.

Research staff will search RESTORE for potential research participants and make initial contact via phone. Research staff will also respond to inquiries received from the community via phone. The research activities will be explained to prospective participants by the research staff. If the prospective participants express interest in partaking in the study they will be invited to the laboratory to confirm eligibility and provide written consent.

Retention
Enrolled participants will receive $100 USD as remuneration for their participation in the proposed study. Remuneration will be delivered by pre-paid debit-type card that will be loaded at pre-determined milestones (after initial MRI, after each (5) treatment visit, after follow-up phone calls.

RISKS TO HUMAN PARTICIPANTS

Dr. Kindred (PI) will be performing the proposed intervention at the College of Health Professions Research building on the Medical University of South Carolina campus. The research building is staffed with licensed clinical professions, physical and occupational therapists, and all research staff are trained in emergency procedures. Research staff also have basic life support (BLS) training from the American Red Cross, or equivalent. All investigators and research staff have completed computer-based CITI Human Subjects Research ethical and safety training, as required by the Medical University of South Carolina and the associated IRB. All research activity, informed consents, and continuing reviews will be reviewed by MUSC's IRB, before the research is started and continuing reviews will occur annually. The research staff will ensure that all information needed for the continuing reviews are available to the IRB in accordance with the IRB's requirements.

Human Subjects Involvement and Characteristics and Design
A total of 24 adults, between 35 and 80 years of age, with unilateral strokes more than 6 months post event will be recruited and enrolled over a one year period. The proposed randomized, single-blind, sham-controlled, trial will investigate the effects of anodal transcranial direct current stimulation (tDCS) on post-stroke fatigue and markers of inflammation. Participants will be randomly assigned to the active a-tDCS or sham group. Clinical and inflammatory markers will be assessed pre and post five consecutive days of a-tDCS applied to the motor cortex.

The inclusion/exclusion criteria for the study are as follows:

Inclusion Criteria:
1. Men and Women, any race or ethnicity, ages 35-80 years
2. 6+ months post-stroke
3. Average Fatigue Severity Score (FSS) >= 4 (indicates clinically significant fatigue)
4. Self-reports persistent fatigue for at least the past 6 months
5. Able to walk 10m unassisted

Exclusion Criteria:
1. Absolute contraindications to MRI, TMS, tDCS
2. Inability to follow 3-step instructions
3. Multiple strokes on opposite hemispheres
4. Diagnosis of comorbid neurological conditions (e.g. multiple sclerosis, Parkinson's disease, and dementia)
5. Severe hypertension (resting SBP > 200 mmHg or DBP > 120)
6. Cerebellar or brainstem strokes
7. Hospital Anxiety and Depression Scale (HADS) score > 7, depression or anxiety
8. Pregnancy

Study Procedures, Materials, and Potential Risks

Source of Materials
Data will be in the form of structured interviews, self-reported rating scales and questionnaires, surface electromyography recorded transcranial magnetic (TMS) evoked potentials, and MRIs. No identifiable information is expected to be collected outside of the consent process. Once consented individuals will be assigned a non-identifiable research ID number that will be used to mark/track collected data.

Research Procedures and Potential Risks
Although unlikely, it is possible that some participants may experience distress when asked questions pertaining to their feelings of fatigue, depression, anxiety, or pain. Some participants may also experience

distress during MRI sessions or TMS assessments. However, previous and ongoing research by our research group indicates that these risks are minimal.

Specific risks, which are all low/minimal, associated with the research procedures are as follows:

tDCS: Commonly reported side effects are as follows: local skin irritation with light itching/tingling/ burning, mild headache, or nausea. There have also been rare cases of mild burns at the procedure site.

TMS: There is a very low risk of a seizure after TMS. The risk of seizure induction by this protocol has been thoroughly assessed and the TMS parameters have been chosen to be well within published safety guidelines for the conduct of TMS studies in human subjects.

Blood Draw / Venipuncture: Risks associated with blood draws include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, or fainting is possible, although unlikely.

MRI: There have been no ill effects reported from exposure to the magnetism or radio waves used in this test. A known risk is that the magnet could attract certain kinds of metal. Therefore, we will perform a detailed screening, provided by the Center for Biomedical Imaging (CBI), before any participants are imaged.

Walking Tests: Some of the activities performed during this study will be performed while the participants are walking or standing. During these activities, participants may lose their balance or become unsteady.

Pregnancy: We do not know if the study intervention will affect mother's milk or an unborn fetus. Therefore, breast-feeding and pregnant women are not allowed to take part in the study. If a potential participant is pregnant or become pregnant they will be removed from the study as there may be risks to the embryo or fetus that are unknown at this time.

Randomization: The treatment you receive may prove to be less effective or to have more side effects than the other study treatment(s) or other available treatments.

Loss of Confidentiality: There is a risk of loss of confidentiality or personal health information. We will collect the participants age, date of stroke, sex, and racial/ethnicity to classify our sample and for use as influencing factors on intervention effectiveness.

Unknown Risks: The experimental treatments may have unknown side effects. The researchers will let you know if they learn anything during the course of the study that might make you change your mind about participating in the study.

Adequacy of Protection Against Risks

Informed Consent Procedures

All personnel are trained in the responsible conduct of human research. Informed consent will be collected by the on-site study coordinators (Mr. Brian Cence, Mrs. Alyssa Chesnutt) or the PI (Dr. John Kindred). All study personnel will be familiar with the treatment protocol. This will ensure each person collecting informed consent is familiar with all aspects of the study. Individuals obtaining informed consent will role-play the consent procedures with the PI until they demonstrate competency.

Written/signed informed consent will be collected in a private and interruption-free room inside the study offices inside the College of Health Professions Research building. Potential candidates will not be required to decide to participate at this initial visit, though that possibility will be available. If potential participants wish to discuss participation with their families and/or significant others, or other healthcare providers they will be encouraged to do so. The informed consent document will outline, a) the sponsorship of the study; b) the nature, purpose, and procedures of the research study; c) the voluntary nature of participation (i.e., participation is not required, participation can be discontinued at any time); d) the duration of the study; e) potential risk and discomforts, as

well as benefits of participating; f) that all information will be kept confidential subject to the provisions of state and federal law; and g) compensation. Participants will be informed that they can discontinue their participation in the study at any time and that this decision will not influence future healthcare or research participation at MUSC. Private information collected as part of the informed consent process is the participants full name and signature.

Protections Against Risk
Specific mitigation plans for each of the known risks are provided below:

tDCS: To reduce the chance for an adverse event (AE)/reaction electrode preparation and contact will be constantly checked. Research staff will also engage with the participants during intervention application and monitor the participants' physiological and emotional state. Our research group has performed over 100 tDCS sessions with no adverse or serious adverse events.

TMS: To minimize risk, we will be using single- and paired-pulse TMS, as the majority of adverse events in the literature have occurred after repetitive TMS and we will follow published safety guidelines. Individuals with a history of seizures, you will not be allowed to participate in this portion of the study. Participants will undergo IRB approved screening prior to enrollment.

Blood Draw / Venipuncture: To reduce venipuncture risks, all procedures will be performed by licensed nursing staff at the South Carolina Clinical and Translational Research Institute on the medical campus.

MRI: We will perform a detailed screening, provided by the Center for Biomedical Imaging (CBI), before any participants are imaged. If there is any question about potentially hazardous metal within a participant's body, they will be excluded from participation in this research study. We will also keep the examining room locked so that no one carrying metal objects can enter while participants are in the scanner.

Walking Tests: To ensure that participants do not fall they will wear a safety harness that is attached to the ceiling. The harness will prevent participants from hitting the ground in the event they lose their balance or become unsteady.

Pregnancy: All prospective participants of childbearing potential will be required to undergo urinalysis to determine pregnancy status.

Loss of Confidentiality: To minimize this risk, we will use a non-identifiable research id number to label all collected data. Any data collected on physical media will be stored in locked file cabinets where only authorized research personnel have access. Electronically collected data will be stored on secured password protected IT network resources.

Potential Benefits of the Proposed Research
Post-stroke fatigue is well known but poorly understood phenomenon that significantly contributes to a poorer quality of life. The proposed research has the potential to reduce the impact of post-stroke fatigue leading to improvements in physical, cognitive, and emotional health. The potential benefit of improving function and quality of life in individuals with post-stroke fatigue outweigh the minimal risks associated with the study's procedures.

Importance of the Knowledge to be Gained
Another benefit is this proposal is the possibility of improving our knowledge of the inflammatory contributions to post-stroke fatigue. Increasing our mechanistic knowledge may guide future refinement and development of treatments for post-stroke fatigue. As stated, the risks associated with the proposed research are minimal leading to a favorable cost-benefit analysis.

DATA SAFETY AND MONITORING PLAN

This section is based on the recommendations in NIDA's "Guidelines for Developing a Data and Safety Monitoring Plan" (www.drugabuse.gov/funding/dsmbsop.html).

Summary of the Protocol

The intervention being studied is a five-session anodal transcranial direct current stimulation (tDCS) intervention delivered over five consecutive days. Measures of post-stroke fatigue and inflammation (central inflammation measured by magnetic resonance spectroscopy and peripheral markers measured via blood analysis) will be made before and after the intervention.

Trial Management. The study will be primarily managed from the Functional Neurostimulation Laboratory (Dr. Kindred, PI) within the Division of Physical Therapy within the College of Health Professions at the Medical University of South Carolina. The target population is adults more than six months post-stroke with clinically present fatigue (average Fatigue Severity Scale > 4).

Data Management and Analysis

The primary outcome of the study is the change in post-stroke fatigue severity (fatigue severity scale (FSS), pre- and post-intervention) and markers of central (measured using magnetic resonance spectroscopy (MRS)) and peripheral (measured via blood analysis) inflammatory markers. Analyses will be guided by the specific hypotheses of the study. Post-hoc exploratory analyses will be conducted with two-tailed tests and more conservative statistical procedures which guard against Type I error (e.g., Tukey tests). All primary hypotheses will be tested at level of significance $\alpha=0.05$. We will also estimate the effect sizes of interest and provide 95% confidence intervals for them.

Quality Assurance

Data quality will be monitored by random inspection of the completed forms by research staff/PI.

Regulatory Issues. All unexpected Adverse Events (AEs) will be reported to the MUSC IRB within 10 working days. Serious AEs (SAEs) will be reported within 24-business hours. Follow-up of all unexpected and serious AEs will also be reported to the IRB. All AEs will be reviewed weekly by the PI and yearly by the IRB. Any significant actions taken by the local IRB and protocol changes will be relayed to NIH. AEs and SAEs occurring during the course of the trial will be collected, documented, and reported in accordance with IRB requirements.

Definition of AE and SAE

Adverse events are defined as any untoward medical occurrence that may present itself during treatment or administration of an intervention, and which may or may not have a causal relationship with the treatment. Serious adverse events are defined as any medical occurrence that:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect. OR
- Requires intervention to prevent one of the above outcomes.

Documentation and Reporting

AEs/SAEs will be documented and reported as per IRB requirements. Research staff will identify adverse events and obtain all available information to assess severity, seriousness, study relatedness, expectedness, outcome, and the need for change or discontinuation in the study intervention. Adverse events will generally be documented on AE Logs and AE Case Report Forms (CRFs). Additional relevant AE information, if available, will be documented in a progress note in the research record as appropriate to allow monitoring and evaluating

of the AE. If the AE meets the definition for serious, appropriate SAE protocol specific reporting forms will be completed and disseminated to the appropriate persons and within the designated timeframes as indicated above. For each AE/SAE recorded, the research staff will follow the AE/SAE until resolution, stabilization or until the subject is no longer in the.

When a reportable SAE is identified, the PI and/or Study Coordinator will initiate an SAE form, and the following individuals will be notified by facsimile transmission, email and/or telephone within 24-business hours of the initial notification of the SAE:

i. The PI and laboratory clinical staff will provide oversight, consultation, assessment, and documentation as appropriate of the SAE.
ii. The research staff will notify the MUSC IRB and complete the AE report form in conjunction with the PI. The MUSC IRB meets monthly and is located at 165 Cannon Street, Rm. 501, Charleston, SC 29425. Communication with the IRB is through email, memos, official IRB forms, and online reporting.
iii. The NIH program officer.

If complete information is not available when the initial 24-hour SAE report is disseminated, follow-up information will be gathered to enable a complete assessment and outcome of the event. This information may include hospital discharge records, autopsy reports, clinic records, etc. The research staff will attach copies of source documents to the SAE report for review by the PIs and for forwarding to the NIH program officer as appropriate within 2 weeks of the initial SAE report. In addition, the PIs will provide a signed, dated SAE summary report, which will be sent to the NIH Medical Safety Officer within two weeks of the initial SAE report.

We will report adverse events to the MUSC IRB online as soon as possible, but no later than 10 working days after the investigators first learns of the event. The MUSC IRB AE reporting requirements are as follows: All deaths that occur during the study or 30 days post termination from the study are required to be reported as adverse events even if they are expected or unrelated. Other adverse events are reportable to the MUSC IRB if the AE is unexpected AND related or possibly related AND serious or more prevalent than expected. All three criteria must be met for an AE to be reported to the MUSC IRB. The IRB definition of unexpected is that the AE is not identified in nature, severity or frequency in the current protocol, informed consent, investigator brochure or with other current risk information. The definition of related is that there is a reasonable possibility that the adverse event may have been caused by the device or intervention. Reportable AEs are reviewed by the IRB Chair and reported to the IRB Board at the next meeting.

Trial Safety
The potential risks and benefits and methods to minimize these risks are outlined in Section 3.1, Protection of Human Subjects Section. Protocols for reported AEs and SAEs are outlined above. All unexpected AE and SAEs will be monitored until resolved. A detailed summary of all AEs will be prepared weekly by the research staff and relayed to the PI. Study procedures will follow as much as possible the FDA's Good Clinical Practice Guidelines (www.fda.gov/oc/gcp). All requests by subject's physicians and other medical providers will be referred directly to PI.

DSMP Administration
The PI will be responsible for ensuring participants' safety on a daily basis.

The PI will examine the database for missing data, unexpected distributions or responses, and outliers. We will report results at the end of the trial.

DSM Board
A DMSB is not required as the intervention is considered minimal risk and the minimal duration of the trial.

Confidentiality will be maintained during all phases of the trial including monitoring, preparation of interim results, review, and response to monitoring recommendations.

ClinicalTrials.gov Requirements

In accordance with Public Law 110-85, the proposed trial will be registered with ClinicalTrials.gov. Applicable requirements regarding results reporting will be adhered to.