Study Protocol   April 17, 2023   IRB approved 07/08/2021.

NCT 04471805   Study Title: 4 mA tDCS, Estrogen, and Leg Muscle Fatigability

This study employed a double-blind, randomized, crossover design, wherein all subjects received both active and sham stimulation during each phase of their menstrual cycle. Each of the 10 subjects completed a total of 6 visits to the lab. The first visit was a familiarization visit where maximal strength testing was performed to confirm leg dominance, and an isokinetic fatigue task (FT) was completed to familiarize each subject with the FT performed during the evaluation sessions. Additionally, subjects were asked about the start of their previous menstrual cycle, along with the projected start of their next menstrual cycle. The start of the menstrual cycle was deemed the first day of menstruation. Sessions were then scheduled to correspond with high and low points of estrogen level throughout the cycle. The early follicular phase (days 3–4) was targeted for low estrogen levels. The late follicular phase (days 9–10) and mid-luteal phase (days 18–20) were targeted for high estrogen levels. Sessions were scheduled so that each subject had both active (4 mA) and sham tDCS during each phase (early follicular, late follicular, and mid-luteal) of their menstrual cycle to fully assess the effects of stimulation. Due to the diurnal nature of estrogen, visits 2–6 were scheduled at the same time of day for each individual subject (i.e., ±2 h for each session). Testing sessions began with a blood draw and assay to assess serum estrogen levels, followed by tDCS administration (active or sham), and right and left leg FTs.

Strength testing was performed on a HUMAC NORM isokinetic dynamometer (CSMi, Stoughton, MA, USA). Before beginning the strength testing, subjects completed a warm-up exercise which consisted of 15 repetitions of knee extension and knee flexion (60◦/s, concentric/concentric). After resting for ≥30 s, three repetitions of maximal strength isometric knee extension and flexion were completed at 65◦ and 30◦ , respectively, with ≥30 s of rest between each repetition. Then, five repetitions of maximal strength isokinetic knee extension and flexion were completed at 60◦/s (concentric/concentric) with ≥30 s of rest between each set. The right leg was always tested first, followed by the left. Strong verbal encouragement was provided during each repetition to ensure maximal effort from the participants. The greatest torque value acquired during either of the strength testing tasks (isometric or isokinetic) was utilized to objectively confirm right-side dominance. Strength testing was performed on a HUMAC NORM isokinetic dynamometer (CSMi, Stoughton, MA, USA). Before beginning the strength testing, subjects completed a warm-up exercise which consisted of 15 repetitions of knee extension and knee flexion (60◦/s, concentric/concentric). After resting for ≥30 s, three repetitions of maximal strength isometric knee extension and flexion were completed at 65◦ and 30◦ , respectively, with ≥30 s of rest between each repetition. Then, five repetitions of maximal strength isokinetic knee extension and flexion were completed at 60◦/s (concentric/concentric) with ≥30 s of rest between each set. The right leg was always tested first, followed by the left. Strong verbal encouragement was provided during each repetition to ensure maximal effort from the participants. The greatest torque value acquired during either of the strength testing tasks (isometric or isokinetic) was utilized to objectively confirm right-side dominance.

The isokinetic fatigue task (FT) comprised 40 consecutive repetitions of maximal effort isokinetic knee extension and flexion (120◦/s, concentric/concentric). In visits 2–5, a 15-repetition warm-up exercise was completed before the FT, as detailed above. The FT was performed on the dominant (right) leg first and ≥5 min rest was provided to allow for adequate heart rate and respiratory recovery before starting the left leg FT. Vigorous verbal encouragement and visual feedback (i.e., per rep work bars) were given to ensure the participants were performing maximal effort contractions throughout the fatigue tasks. The largest torque from each repetition was included in the analysis.

Muscle activity throughout the strength and fatigue tasks was recorded via a wireless EMG system (Ultium-EMG, Noraxon, USA Inc., Scottsdale, AZ, USA). EMG electrodes (3M Red Dot Monitoring Electrode, Model 2560; 3M Corp., St. Paul, MN, USA; 2 cm between each 1.3 cm effective area) were secured bilaterally over the rectus femoris, vastus medialis, vastus lateralis, and semitendinosus corresponding to a 3D muscle map which followed SENIAM guidelines and was provided by the EMG software (MR 3.14, myoMUSCLE, Noraxon USA Inc., Scottsdale, AZ, USA). To increase electrode placement consistency, the same researchers placed the electrodes on the subjects during each visit. The electrode sites were prepared by shaving and cleaning the electrode site with an alcohol wipe before placing the electrodes. The electrodes and wireless transmitters were secured in place with elastic bandages and EMG data were collected at 2000 Hz.

Sessions 2–6 began with a blood draw in the Clinical Research Unit at the University of Iowa Hospital and Clinics (UIHC). Staff nurses collected 4.5 mL blood from the median cubital vein of the left arm (total volume collected per subject = 9 mL) for the estrogen assay. Samples were immediately analyzed for serum estrogen levels after the blood draws by UIHC Pathology technicians using an Electrochemiluminescence Assay (Roche Diagnostics, Basel, Switzerland).

Statistical Analysis Plan

A stimulation condition (active vs. sham) by estrogen level (high vs. low) repeated measures ANOVA was performed on the FI values and EMG activity for the right and left extensors and flexors. Post hoc analyses (paired t-tests and Bonferroni correction) and effect size (Cohen's d) were calculated to clarify significant main effects and interactions. Significance was accepted at $p \leq 0.05$. Normality and sphericity assumptions were evaluated with the Shapiro–Wilk test and Mauchly's test of sphericity for the ANOVAs. Greenhouse–Geisser corrections were used when the sphericity assumption was violated. GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) was utilized to perform the analyses.