This study utilized a single-blind, randomized, SHAM-controlled, cross-over design. Each participant attended three experimental sessions, with sessions 1 (strength testing and treadmill familiarization) and 2 (first tDCS/PET Scan session) separated by at least 3 days to allow for ample recovery after strength testing and sessions 2 and 3 (first and second tDCS/PET sessions) separated by at least 7 days to allow the tDCS effects to subside. The experimental protocol is shown in Figure 1. During Session 1, subjects were consented and then filled out the Patient Determined Disease Scale (PDDS), which is strongly correlated with and is considered an alternate assessment to the Expanded Disability Disease Status Scale (EDSS) and the fatigue severity scale (FSS) questionnaires. The subjects then completed isokinetic strength testing to objectively determine their more-affected (weaker) leg. Subsequently, the subjects walked on a treadmill to self-select a comfortable walking pace, which was utilized in experimental sessions 2 and 3. At the beginning of Session 2 and 3, blood glucose, height, and weight were measured, and an IV catheter was inserted to facilitate FDG administration. Prior to Sessions 2 and 3, the subjects fasted for a minimum of 6 h and blood glucose was required to be ≤ 200 mg/dL to proceed with FDG administration and the PET scanning. The subjects then sat comfortably in a chair and received 20 min of SHAM or tDCS (3 mA; stimulation condition was randomized) targeting the motor cortex corresponding to their more-affected leg, as determined in the strength testing (see below). The subjects then rested for 10 min to allow for optimal stimulation effects. After this rest period, the subjects walked on a treadmill for 20 min at the speed determined in Session 1. Two minutes into the walking, ~10 ± 10% mCi of FDG was injected via IV injection. Immediately after the 20-min walking task was completed, the subjects underwent positron emission tomography/computed tomography (PET/CT) imaging.

Data Analysis and Statistics

Twenty regions of interest (ROIs) were drawn on the CT scan from each session (SHAM and active) by the same investigator to locate the lower limb skeletal muscles. The muscles that comprise the knee extensors (rectus femoris, vastus medialis, vastus intermedius, and vastus lateralis) and knee flexors (semimembranosus, semitendinosus, long head of the biceps femoris, short head of the biceps femoris, gracilis, and sartorius) were identified via visual inspection in the upper leg, and the plantar flexors (gastrocnemius, soleus, peroneus longus, peroneus brevis, flexor digitorum longus, flexor hallucis longus, and tibialis posterior) and dorsiflexors (tibialis anterior, extensor digitorum longus, and extensor hallucis longus) were distinguished in the lower leg. Figure 2 displays a representative upper leg CT image with ROIs identified, a corresponding PET image, and the CT and PET images co-registered. As a result of FDG uptake occurring during the treadmill task, glucose uptake (GU) values closely reflect FDG uptake during the task. For each ROI, standardized uptake values (SUVs) were calculated based on the injected FDG dose and subjects' body weight. Despite the fasted state of the subjects, SUVs may be affected by varying insulin levels during Sessions 2 and 3. Therefore, SUV data were analyzed without normalization and as values normalized to the liver activity as a reference tissue. Moreover, SUV asymmetry indices (AIs) were calculated to determine the magnitude of asymmetry between the more- and less-affected legs with a previously used equation: ((less-affected side − more-affected side)/((0.5) × (less-affected side + more-affected

side)) × 100). An AI value ≥ 10% was considered asymmetric. The relative distribution ((standard deviation / mean) × 100) of GU values in PET image voxels within each muscular ROI was calculated as an index of spatial glucose uptake heterogeneity (GUh). The data were analyzed using PMOD Version 4.001 (PMD Technologies LLC, Zurich, Switzerland).

Statistical Analysis

Mean ± standard deviation SUVs and GUh for each muscle group (i.e., knee extensors, knee flexors, plantar flexors, and dorsiflexors) were calculated for each subject, and statistical analyses were performed for each muscle group. Normality assumptions were evaluated by way of histograms, Q-Q plots, and the Shapiro–Wilk test. Because these assumptions were met, paired t-tests were performed for each participant to compare the muscle groups of each leg between conditions (e.g., left knee extensors during SHAM vs. left knee extensors during tDCS). Significance was accepted at $p < 0.05$, and Cohen's d effect size was calculated for all significant results. Analyses were performed using GraphPad Prism 8.1.2 (GraphPad Software, San Diego, CA, USA).