STATISTICAL ANALYSIS PLAN

STUDY TITLE: ISCHEMIC CONDITIONING IMPROVES WALKING FUNCTION POST STROKE

DATE OF DOCUMENT: 7/26/2019

4.4 Statistical Design and Power

All statistical analysis will be over seen by Dr. Sergey Tarima, Ph.D. (Co-I). We will start with exploring the distributional form of the primary study outcome, self-selected walking speed (Aim 1), and the secondary outcomes of strength and time to fatigue of the knee extensor muscles (Aim 2), percent brachial and popliteal artery flow mediated dilation and VO_2 Peak (Aim 3), and finally the tertiary outcomes (Aim 1: clinical measures of strength, balance, walking distance, walking kinematics; Aim 2: motor unit firing rate, hyperemic blood flow; Aim 3: heart rate variability). If an outcome is not normally distributed, we will use a variance stabilizing transformation to make linear models applicable. We plan to enroll 120 subjects in our study: 90 stroke survivors and 30 healthy adults frequency matched to stroke survivors on their age and gender. Assuming a possible attrition rate of 16.67% or the use of a nonparametric test, we (pessimistically) described power for the study at 25 subjects per group and optimistically at 30. Among 90 stroke survivors, 30 will be randomized to the Treadmill only group ("TM"), 30 to Treadmill and IC group ("TM+IC"), and 30 to IC only group ("IC"). All 30 control subjects will receive treadmill training plus IC (TM+IC).

Randomization of stroke survivors to the three intervention groups will be performed by Dr. Tarima using randomized block designs (randomly chosen block sizes of 3, 6 and 9). This number accounts for possible dropouts and the use of non-parametric methods. Dr. Tarima will work directly with Jennifer Nguyen, the clinical research assistant who will be un-blinded to the group assignments and will be performing the IC interventions on the study volunteers. When a stroke survivor is enrolled, he or she will receive a sequential number which will have a randomization schedule assigned to it (i.e. TM, IC, or TM + IC), and this will be communicated to Ms. Nguyen.

Data analysis for each aim is separated into confirmatory and exploratory parts. Power analysis is completed for confirmatory parts and type I error is controlled separately for each specific aim. P-values for exploratory analyses will be declared significant if <5%. <u>It is important to note all preliminary data used for power analysis was from two-weeks of IC intervention only, and we would expect effect sizes to be larger both with the longer, 4-week IC period and with the addition of gait training.</u>

Statistical Analyses Aim 1 (confirmatory part): Three group pairwise comparisons (IC + Treadmill Training; IC Only; Treadmill Training Only) of 4-week change in walking speed in stroke survivors will be performed with Analysis of Variance (ANOVA). We plan to compare self-selected walking speed of three groups of stroke survivors to the healthy group of control subjects on an *exploratory basis only* (two sample t-tests). To account for 3 pairwise tests Bonferroni adjustment will be applied to lower the cutoff for significance to 1.67%. Alternatively, Kruskal Wallis and Mann-Whitney tests will compare changes in walking speed at 4-weeks if the data is not normally distributed.
Statistical Analyses Aim 1 (power calculations for planned analyses): 25 subjects per group will allow detecting 1.0 SD difference in means with 85% power, and 30 per group 0.9 SD difference with 84% power. On log scale the SD of walking speed is estimated at 0.15 m/s from our pilot data. Thus, we expect to be able to detect a 14-16% change in walking speed depending on sample size (25-30 per group) and applicability of normality assumption (t- or Mann-Whitney test).

Statistical Analyses Aim 2 (confirmatory part): We plan to compare knee extensor muscle strength and fatigability between the three patient groups (and to the healthy control group on an exploratory basis). To account for 6 tests (2 outcomes, 3 comparisons), Bonferroni adjustment will be applied to lower the cutoff for significance to 0.83%. 4-week change will be compared between the stroke survivor intervention groups with ANOVA and the comparisons versus healthy controls will be completed with two sample t-tests. Alternatively, Kruskal Wallis and Mann-Whitney tests will compare changes in walking speed if the data is not normally distributed.
Statistical Analyses Aim 2 (power calculations for planned analyses): 25 subjects per group will allow detecting 1.1 SD difference in means with 87% power, and 30 per group 1.0 SD difference with 87% power. On log scale the SD of strength and time to fatigue are estimated at 0.08 and 0.5, respectively, from our pilot data. Thus, we expect to be able to detect an 8-9% change in strength and 65-73% change in time to fatigue.

Statistical Analyses Aim 3 (confirmatory part): We plan to compare FMD and VO_2 Peak between three patient groups (and to the healthy control group on an exploratory basis). To account for 6 tests (2 outcomes, 3

comparisons), Bonferroni adjustment will be applied to lower the cutoff for significance to 0.83%. 4-week change will be compared between stroke survivor intervention groups with ANOVA and the comparisons versus healthy controls will be completed with two sample t-tests. Alternatively, Kruskal Wallis and Mann-Whitney tests will compare changes in walking speed if the data is not normally distributed.
Statistical Analyses Aim 3 (power calculations for planned analyses): 25 subjects per group will allow detecting 1.1 SD difference in means with 87% power, and 30 per group 1.0 SD difference with 87% power. On original scale the SD of FMD percent is estimated at 3.6% and the SD of VO_2 Peak is 2.8% from our pilot data. Thus, we expect to be able to detect a 3.6-4.0% difference in FMD and 2.8-3.1% difference in VO_2 Peak.

Statistical Analyses Aim 1, 2, and 3 (exploratory part): The secondary outcome measures are (1) leg strength, (2) time to muscle fatigue, (3) FMD, and (4) VO_2 peak. The confirmatory tests will be extended to regression modelling controlling for the effect of baseline values, severity of stroke, patient's age, sex, level of physical activity and time since stroke. A parsimonious model will be chosen with the smallest Akaike Information Criterion (AIC). In the case of non-normal outcomes we will use median regression. Interactions with baseline values, severity of stroke, patient's age, sex, level of physical activity and time since stroke will be tested for significance. Statistical significance will be declared if p-value is less than 5%. Because outcomes will be assessed at multiple occasions (baseline and after sessions 1, 6, 12 and 1-month post IC) for every study participant, a mixed effects ANOVA will be used to compare over time profiles (possibly on logarithmic scale) between the groups. The functional form of overtime association of primary and secondary outcomes will be modelled by a linear, quadratic, piecewise linear, and a categorical time variable. The chosen modelled overtime trend (possibly different for different outcomes) will be used to compare groups. We are not expecting missing data in addition to possible dropouts. If dropouts are ignorable (missing at random and the data follow a chosen parametric model), then the optimality of likelihood inference is preserved. If needed, the effect of ignorability assumption will be explored with a sensitivity analysis (breaking-point analysis).