Statistical Analytical Plan

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Skeletal muscle atrophy and dysfunction following total knee arthroplasty	3-23-22

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For <u>Aim 1</u>, using pre-surgery and 5 wk post-TKA assessments in the control group, we will assess the effect of TKA (pre- vs. 5 wk post-TKA) on muscle fiber size and function. Primary outcomes include: single muscle fiber CSA for MHC I and IIA fibers and tension and velocity for MHC I and IIA fibers, and IMF and SS mitochondrial content. We will use a general linear mixed model, with a random effect to account for observations clustered within patients (eg, single fiber CSA and function), with the model conceptualized as a multi-level analysis with single fiber values comprising level 1 variables and whole muscle or person-level covariates comprising level 2 variables (130). Covariates (eg, baseline value, etc.) can be included in the model as level 2 variables to determine if variation in any primary outcome is related to variability in these factors. Additionally, we can include sex in the model to examine whether the effects of TKA differ between men and women. In secondary analyses, we will examine whether changes in muscle fiber size or functional mediate changes in whole muscle and body function using mediation analysis, if effects of TKA are noted in the latter. Such analyses seeks to determine whether the effect of an independent variable or intervention (TKA) on some outcome variable (whole muscle/body function) can be transmitted through a third variable, referred to as a mediator (single fiber CSA or function). Variables with multiple observations per subject will be collapsed into a single mean value.

For <u>Aim 2</u>, pre- and 5 wk post-surgery data will be compared between volunteers randomized to NMES and control interventions. Our primary analysis will assess treatment effects (group X time interaction) on muscle structural and functional variables. Primary outcomes are identical to Aim 1 and will be similarly evaluated using a general linear mixed model, as described above, with group (NMES vs. control) and time (pre vs. 5wks post-TKA) as between- and within-subject factors, respectively. Potential confounding factors will be compared between groups and factors that differ can be included into the model as level 2 covariates. We assume that dropouts will be random, but will examine whether they differ from volunteers completing the study. Moreover, we can conduct sensitivity analysis on dropouts, assuming the most and least conservative responses, to assess their impact on study outcomes. Additionally, we can include sex as an additional effect in these models to determine if NMES effects differ by sex. In secondary analyses, we will also examine the effect of treatment on whole muscle/body functional measures. If there are effects of NMES to maintain whole muscle/body functionality at 5 weeks, we will examine whether changes in single fiber structural or functional outcomes partially or completely mediate whole muscle/body functional improvements with NMES using mediation analysis.