Official Title: *Prevention of Skeletal Muscle Adaptations to Traumatic Knee Injury and Repair*

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STATISTICAL ANALYSIS PLAN

Sample size estimates: A primary goal of the proposed studies is to demonstrate, using measurements at cellular and sub-cellular levels, that early institution of NMES prevents deterioration in skeletal muscle fiber size and function in patients with traumatic knee injury throughout the post-injury and early, post-surgical periods. In Aim 1, primary outcomes consist of: 1) single muscle fiber cross-sectional area (CSA)(MHC I and II fibers), 2) tension (MHC I and IIA fibers) and 3) velocity (MHC I and IIA fibers). In calculating samples size estimates for single fiber measurements, we assume that, for each patient, ~200 fibers will be evaluated for single muscle fiber CSA, with the goal of obtaining 100 MHC I and 100 MHC II fibers and ~25-30 fibers for mechanical assessments, with the goal of obtaining 10 MHC I and 10 MHC IIA fibers. The remaining fibers assessed for mechanical measures will likely be comprised of mixed MHC isoform fibers (IIA/IIX) and will be too few for analysis without analyzing prohibitively large numbers of fibers/patient. These fibers also constitute a relatively small fractional percentage of the total number of fibers in patients. Our estimates for relative changes in single muscle fiber CSA and function are based on data from our laboratory and others at the single fiber (1) and whole muscle (2) levels in patients experiencing ACL rupture and reconstruction, as well as data from our laboratory in another orthopedic surgical population (Fig 2). From these data, we expect reductions at 3 weeks post-surgery in single fiber CSA of -30% and -40% for MHC I and II fibers (Fig 2), respectively, and in tension (-50%) and velocity (-30%) in MHC IIA fibers (Fig 2), in patients randomized to control treatment. Based on our observed effects of NMES (Fig 2) to mitigate muscle fiber atrophy and dysfunction in total knee arthroplasty (TKA) patients, we estimate that reductions in these variables will be prevented or diminished (CSA: 0% and -10% for MHC I and II fibers, respectively; tension in MHC IIA fibers: -40%; velocity in MHC IIA fibers: +20%).

To obtain data to estimate samples sizes based on these relative differences, we used a larger data set of knee OA patients (n=16) assessed for identical measurements proposed in this application (single fiber CSA and function). Mixed linear models were developed to estimate both between cluster variances and intraclass correlation coefficients (ICC), where samples were obtained at times before and after treatment, with clusters of fibers available for each volunteer at each time point. Cluster variance and ICC estimates were 1,030,622 and 0.337 for MHC I CSA; 1,047,066 and 0.383 for MHC II CSA; 254 and 0.263 for MHC IIA tension and 576 and 0.610 for MHC IIA velocity, respectively. These data were then used in a two group repeated measures analysis of variance model to estimate power for group, leg and group X leg interaction effects. Using relative differences detailed above and variance estimates from our mixed model analyses, we estimated that an n=10/group would detect significant group X leg interaction effects for MHC I CSA and MHC IIA tension and velocity, with powers in excess of 80% (87%, 99% and 99%, respectively) at a significance level of 5%. We chose these three variables because they represent important determinants of whole muscle size and function. More specifically, as the most prevalent fiber type in muscles (3, 4), MHC I CSA is the most important factor determining whole muscle size. Similarly, given that MHC IIA fibers are the most prevalent fast-twitch, type II fiber in human muscle, it disproportionately determines the contractile characteristics of whole muscle because of its large power output (2, 5). Power output is dependent on both the force producing capacity and contractile velocity of the constituent fibers (ie, power=force X velocity). Thus, we have included both force and velocity in our sample size estimates. Of note, we did not focus on MHC IIAX fiber function because, unlike our studies in older adults, where these fiber types are much more prevalent, far fewer of these fibers are present in younger adults (4), who will comprise the majority of our ACL injured population. Assuming ~20% dropout rate, based on our prior work (6-8), we will enroll 12 individuals/group with the expectations of a final sample size of 10/group.

In Aim 2, our primary outcome is knee extensor isokinetic torque. Using our data in ACL reconstructed patients (Fig 1), we anticipate reductions in isokinetic muscle strength at 6 months post-surgery of -30% vs. uninjured leg in volunteers randomized to the control group (10). Given the effect of NMES to prevent atrophy and contractile dysfunction at the cellular level (Fig 2) and preliminary evidence from others showing that NMES maintains whole muscle size and strength post-surgery (11), we predict that loss of isokinetic strength will be minimal at 6 months (~5% loss or 95% of uninjured) in the NMES group. Based on these differences, a sample of n=10/group would detect a group X leg interaction effect with 80% power and a significance level of 0.05. We have not performed sample size estimates on secondary outcome variables, as it is unlikely that our sample size and length of follow-up will be sufficient to detect effects of NMES on these factors. In anticipation of observing salutary effects of NMES on muscle size and function (Aim 1), analyses of these secondary outcome variables will provide preliminary data to inform power calculations for future randomized controlled clinical trials to interrogate broader clinical efficacy of NMES to mitigate skeletal muscle structural and

functional adaptations to traumatic knee injury and surgical intervention, with the goal of mitigating long-term knee pathology (10).

Statistical analytical approach: Our primary goal is to assess the effect of group assignment (NMES vs. control) on skeletal muscle fiber size and contractile function 3 wks post-surgery using a mixed model, with group and leg (injured vs. non-injured) as fixed effects and subject ID and fiber number as random effects to account for the pairing of legs and clustering of fibers within each volunteer, respectively, as generally described (21). Primary outcomes include: MHC I and II fiber CSA and MHC IIA tension (force/CSA) and velocity. Any confounding factors differing between groups (eg, physical activity level, time between injury and surgery, tourniquet time during surgery) can be included as covariates, although most factors should be balanced by randomization.

The goal of this analysis is to determine whether dynamic muscle strength is better preserved in the injured limb in the NMES group compared to controls 6 mos post-surgery. As in Aim 1, a mixed model will be used, with group and leg effects and subject ID as a random effect. As strength in the injured leg will be compared to the contralateral, uninjured leg, we will monitor strength in the contralateral leg throughout the study (Fig 3) to use as a covariate if any group differences are found. Other covariates that may differ between groups include crutch use, compliance with rehabilitation/activity restrictions and time between injury and surgery. Secondary analysis will evaluate changes in SLH, quadriceps muscle CSA, JSW, MRI outcomes and KOOS, SF-36 and IKDC scores.

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