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| **Protocol Title:** | Exercise Interventions for Muscular Dystrophies |
| **Protocol Type:** | IACUC |
| **Approval Period:** | 10/08/2020-10/07/2023 |
| **Important Note:** | **This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.** |

**c.** Could a smaller, less sentient mammalian species or a non-mammalian species (e.g. fish, invertebrates) substitute for the mammals in any of the experiments planned? Indicate below if such substitution is or is not possible and provide a narrative on how you came to your conclusion.

There are c.elegans, drosophila, and zebrafish models of several muscular dystrophies (Gaud et al. 2004, Kreipke et al. 2016, Widrick et al. 2016). These models are useful for mechanistic studies and drug screens. However, skeletal muscle anatomy and physiology of c. elegans, drosophila and zebrafish differ significantly from mammalian skeletal muscle, due to which, c. elegans, drosophila and zebrafish models cannot adequately replace mouse models for exercise studies.

**i. Describe the biological characteristics that make each species, strain and sex selected the most appropriate for this project. If you will use transgenic, knockout or knockin animals, describe the unique feature(s) of each. Cost is not an acceptable consideration.**

The goal of our studies is to understand how muscular dystrophies develop in humans, and to use this knowledge to develop therapies that can reduce disease severity. For these studies we must study mammalian muscle that is similar to human muscle in its genetics, proteomics, anatomy and physiology. We therefore choose to use mice and rats for our studies as they give us a good estimate of changes that occur in human muscle.

**Mice:** Our experiments with mice make use of a variety of strains, because they offer the opportunity to examine different mutant mice that model myopathies and muscular dystrophies that affect humans. Due to structural and functional similarities with human muscle, the data collected from control and mutant mice give us insights into cellular and molecular level changes that occur in healthy and dystrophic human muscle following perturbations, such as exercise (Roche, Lovering et al. 2010, Roche, Ru et al. 2012). Such data cannot be obtained from less sentient animal models, in vitro models or computer models. The use of male mice is preferred since male mice produce higher contractile forces than their strain and age matched female counterparts and respond more predictably to injurious exercise, thus reducing experimental error. We will include female mice if the need to compare males versus females arises.

**Mouse Strains:**

C57BL/6. Wild type strain for BLAJ mice. Are dysferlin-sufficient. No known muscle pathology.
BLAJ. Dysferlin-deficient strain. Model human dysferlinopathies. Generated by introgressing A/J dysferlin mutation into C57BL/6J strain. Spontaneous retrotransposon insertion within intron 4, causing aberrant splicing of the dysferlin gene. Early onset, limb-girdle muscular dystrophy
A/J. Dysferlin-deficient strain. Model human dysferlinopathies. Spontaneous retrotransposon insertion within intron 4, causing aberrant splicing of the dysferlin gene. Late onset, limb-girdle muscular dystrophy. Good model for sub-clinical stage of human dysferlinopathies.
CRAJ. Control strain for A/J. Dysferlin-sufficient and same genetic background as A/J. No known muscle pathology.
mdx. Dystrophin-deficient. Model Duchenne Muscular Dystrophy. Show high susceptility to exercise-induced muscle injury. C-to-T transition (resulting in a termination codon) at position 3185 within exon 23 of the dystrophin muscular dystrophy gene (*Dmd*) on the X chromosome.
B10.ScSnJ. Control strain for mdx. Dystrophin-sufficient. No known muscle pathology.

**d.** Could a different animal model or different animal procedure that involves (1) less distress, pain, or suffering, or (2) fewer animals substitute for any proposed animal model or animal procedure planned? Indicate below if such replacement is or is not possible, and provide a narrative on how you came to your conclusion:

Less sentient animal models cannot mimic the effects of exercise on human muscle and are therefore not suitable. The animal procedures that are used to study adaptations to exercise in muscle are performed under general anesthesia, so the animals are free from distress, pain and suffering during the procedure itself. Following the exercise protocols, animals are likely to experience pain similar to post-exercise muscle soreness in humans. We do not provide pain relief for exercise-induced muscle soreness since humans are not routinely treated with analgesic drugs for muscle soreness. Animals are also likely to experience some post procedural pain due to a needle that must be transiently placed through the head of the tibial bone for limb stabilization during exercise (25 - 30 Gauge for mice). We will treat this pain by applying EMLA (lidocaine 2.5% + prilocaine 2.5%) or lidocaine cream (2-5%) over the site of needle insertion, before and after the exercise protocol. The procedures are confined to the left ankle dorsiflexors and the left tibia and therefore do not affect feeding, drinking and grooming. In the 17+ years of experience that the PI has in performing these procedures in mice and rats, the PI has observed that animals recover within a few minutes after these procedures; and feed, drink and groom normally thereafter. Animals do not show measurable alterations in gait (example: lameness)

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and mobility (Tang, Lovering et al. 2009).

**e.** Does the proposed research unnecessarily duplicate previous work? Indicate below if the proposed work unnecessarily duplicates previous work and provide a narrative on how you came to your conclusion:

The proposed work does not duplicate previous work. Our earlier work on exercise for muscular dystrophies has received extramural funding for many years due the novelty and significance of our work. The currently proposed experiments build on our earlier work and therefore do not unnecessarily duplicate prior work.

**2. Indicate the METHOD(S) used to determine the group size of animals needed for this study.**

Note: The Guide states that whenever possible, the number of animals requested should be justified statistically. A power analysis is strongly encouraged to justify group sizes when appropriate. Please provide this information.

**a.** X Group sizes determined statistically. State what statistical analysis was performed and give the power function. The variance may be estimated from similar previously published studies. Software such as that available at www.poweranalysis.com or www.statistics.com may be helpful.

For a 10% difference in muscle contractile force, to be statistically significant at alpha level 0.05, with a statitical power of 0.80, we require 6-12 animals per group (https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html).

**b.** X Group sizes based on quantity of harvested cells or amount of tissue required. Elaborate. (Note: A statement such as "The study requires 50 experiments" is not sufficient.)

Of the 12 animals studied per strain per time point, tissue from 4 animals each will be used to generate fixed tissue (histology), unfixed tissue (histology and gene expression) and homogenized tissue (biochemistry)

**c.** Pilot study or preliminary project, group variances unknown at present. Minimal number of animals should be requested. You must provide justification for the number of animals you are requesting. State the basis for your request.

**d.** Other Elaborate and justify criteria used to determine group size.