

# UROP Proposal

## **Title of Proposal**

The Effects of Metformin on Muscle Recovery

## **Problem/Topic of Research or Creative Work**

As individuals grow older they become more prone to experience injury or illness leading to muscle disuse, atrophy, and insulin resistance. Older adults (those older than 65 years) are at a disadvantage in the speed and integrity with which their muscle and metabolism recover after a disuse event. This disadvantage results in incomplete recovery, as most adults find themselves unable to regain the strength and energy that they possessed before their period of inactivity. Furthermore, incomplete recovery following disuse places the older populations at greater risk of developing metabolic diseases, prolonged physical decline, and increases mortality.

A possible treatment for these conditions may be found in the drug metformin, which is currently employed to combat Type 2 Diabetes, but has shown promise in treatment of age-related diseases (cancer, dementia, heart disease). Though metformin's effect on muscle recovery during rehabilitation is unknown, current research suggests that the drug may target skeletal muscle and prevents muscle atrophy during periods of inactivity in mouse studies. Moreover, the amino acid, leucine, has been shown to increase muscle protein synthesis (Drummond, 2008). Therefore, this research aims to deduce the cellular and molecular effects of a metformin plus leucine combined therapy on muscle and metabolism recovery after a disuse event. These data will bring us closer to additional treatment options to older adults to enhance muscle and metabolic recovery following a period of physical inactivity (surgery, illness, injury).

## **Relevant Background/Literature Review**

One-third of older adults (those older than 65) will encounter an extended period of inactivity due to hospitalization leading to muscle atrophy and inadequate recovery. These disuse events increase the risk of older adults experiencing metabolic diseases and decreased physical function. Overall, these consequences may reduce the quality of life and lead to a greater risk of mortality in the older populations. To combat these undesirable effects, a combination of the drug metformin and the amino acid leucine may be used to assist in adult recovery after disuse events.

Research conducted in mice displays a cooperative behavior between metformin and leucine. Leucine, one of the branch-chained amino acids, increases the synthesis of proteins and the growth of skeletal muscle in both mice and humans. The combined by metformin and leucine to date has shown an increase in skeletal muscle glucose uptake and in life expectancy in C-elegans. However, little is known about the influence of metformin and leucine on muscle and metabolic recovery in aged mice.

While metformin currently exists as a popular regulator of glucose homeostasis for Type 2 Diabetes and as a treatment for age-related diseases such as cancer and dementia, the drug's effect on glucose homeostasis and muscle size recovery during aging is not yet known.

However, metformin has been shown to increase the amount of lean tissue present in adults with Type 2 Diabetes when compared with healthy adults not taking metformin (Musi, 2002). Dr. [REDACTED]'s preliminary data has also demonstrated an ability to reduce the extent of muscle atrophy experienced by mice during a period of hindlimb immobilization but its independent or combined effect with leucine has not been examined during recovery from muscle disuse.

We believe a combination of metformin and leucine will amplify muscle size and increase glucose regulation during recovery from disuse (Fu, 2015). The mechanisms that these outcomes might occur are by limiting skeletal muscle pro-inflammatory and protein breakdown pathways and increasing satellite cells (muscle stem cells); all of which metformin and leucine have been linked to. If these hypotheses can be proven, the use of metformin in conjunction with leucine may be used to revitalize the way in which muscle and metabolism recovery can be treated in adults after disuse events in older humans (such as injury, sickness, or surgery).

### **Specific Activities to be Undertaken and Timeframe for Each Activity**

The study that I am helping out with is currently being supported by a NIH grant. An idea of the overall study design is provided below as a brief summary of the required experiments: Old mice will be randomly assigned to 3 groups; metformin (300mg/kg/d in drinking water) in combination with leucine (at 25g/kg food/d), treatment with METF (300mg/kg/d), or LEU (25g/kg food/d). Mice within each treatment group will be randomly assigned to ambulatory control (CON) or 14 days of hindlimb unloading (HU) to simulate a period of physical inactivity, followed by either 7 days (Rec7d) or 14 days (Rec14d) of recovery. Mice assigned to CON will be able to freely ambulate in their cage and have free access to food and water. These mice will not undergo HU. For the HU mice population, mice will undergo hindlimb unloading as conducted regularly by other members of the [REDACTED] lab. Following day 14 of HU, the mice in the recovery groups (Rec) will be removed from the suspension apparatus and housed in individual cages. These mice will then be allowed to ambulate freely until their specified recovery time period of analysis (Rec7d, Rec14d) is concluded. At either HU or the respective recovery time points, mice will be fasted (4h) before being humanely euthanized. Soleus, gastrocnemius, and EDL hindlimb muscles will be rapidly dissected, weighed, and used immediately for analysis.

My particular assignments on this project will be to: 1) help with the dissection of muscles following these specific experiments, 2) prepare muscle tissue for analysis (specifically, to section muscle tissue on a cryostat and place on glass slides for analysis), 3) immunofluorescently stain such slides to assess fiber size and satellite cells present in the muscles, and 4) to quantify images to assess changes in myofiber size and satellite cells across the muscles of the various treatment groups.

I have been helping with Dr. [REDACTED]'s lab since Fall 2018 and therefore have familiarized myself with many of the methods, lab projects, and have worked closely with the other lab personnel dedicated to the [REDACTED] lab. Therefore, I feel I will be proficient and effective in the analysis of data and completion of the various components necessary for this lab project.

### **Relationship of the Proposed Work to the Expertise of the Faculty Mentor**

Over the last 10 years, Dr. [REDACTED]'s lab has been focused on how disuse and physical inactivity affects muscle and metabolism in aging. In addition to the muscle disuse studies the lab conducts in rodents, the lab also conducts human studies to demonstrate the effects of physical inactivity on muscle function in a way more pertinent to the human race. Dr. [REDACTED]'s ultimate goal is to develop therapeutics to limit muscle atrophy and metabolic issues during disuse periods in older adults and to accelerate recovery following these disuse events. Thus, the proposed project is right in line with the goals of the lab. In fact, Dr. [REDACTED]'s lab is conducting a clinical study to see if metformin therapy can keep muscles healthy during bed rest in older adults. A nice future addition to this human study would be

to add leucine in combination to metformin to see if a metformin-leucine combination could reap further benefits for bed-ridden older adults. The data I collect from this study will assist with the lab's goal to discover new and methods to assist rehabilitation for older individuals.

### **Relationship of the Proposed Work to Student's Future Goals**

I was very interested in Dr. [REDACTED]'s lab because of his research focus on aging muscle responses to physical inactivity and recovery. My long-term goal is to become a physical therapist, which relies heavily on the idea of helping individuals rehabilitate after surgeries or injuries. I love the idea of helping others heal and return to their original state of health, so they may continue to live their lives in a comfortable and meaningful way. Due to the toll aging takes on the body, many individuals requiring physical therapy are older adults, often requiring therapy due to a surgery that has caused at least a few days' worth of inactivity. Dr. [REDACTED]'s work is aligned perfectly to support my desire to help improve the rehabilitation process and thus the lives of others. Furthermore, the research will help better prepare me to understand the mechanisms associated with muscle dysfunction following disuse events. Overall, I am excited to be part of a process that may uncover a new tool that could be used to offset muscle and metabolic decline during the recovery process.

### **References**

Drummond, M.J. and B.B. Rasmussen, Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Curr Opin Clin Nutr Metab Care*, 2008. 11(3): p. 222-6. PMID: PMC5096790

Fu, L., et al., Leucine amplifies the effects of metformin on insulin sensitivity and glycemic control in diet-induced obese mice. *Metabolism*, 2015. 64(7): p. 845-56.

Musi, N., et al., Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes*, 2002. 51(7): p. 2074-81.