To Fly or Not to Fly? An Observation of the Effects of Simulated Microgravity on the Flight Muscles of the House Cricket, *Acheta domesticus*

Kaitlyn C. Leonard
Wingate University
Faculty Mentor: Acchia N. J. Albury
Wingate University

ABSTRACT
Microgravity is an environmental factor known to induce muscular atrophy. Muscular atrophy is characterized by a degradation of muscle proteins and the loss of muscle mass. In the house cricket, Acheta domesticus, the flight muscles degrade naturally through an endocrine regulated process known as flight muscle histolysis. Flight muscle histolysis in the house cricket begins on Day 2 and is complete by Day 3. Environmental factors have been known to alter the normal time course of histolysis; therefore, we proposed to investigate the effects of simulated microgravity on flight muscle histolysis within the house cricket. Results indicated that during simulated microgravity there was a significant loss in muscle mass and enzymatic function by Day 1. Therefore, these findings suggest that under simulated microgravity conditions, the time course of flight muscle histolysis in the house cricket, Acheta domesticus deviates from the normal course observed within the control group and as reported by Oliver et al. (2007).

Mechanical stimuli, such as gravitational force, are essential for the normal development and maintenance of cellular structure and function (Benavides Damm et al., 2013). Microgravity is defined as less than 1-G, which is comparable to the force of gravity experienced on Earth, but less than zero-G that is associated with complete weightlessness (Freed and Vunjak-Novakovic, 2002). Studies have shown that microgravity may directly or indirectly lead to alterations in cellular morphology, metabolism and function of tissues within an organism (Freed and Vunjak-Novakovic, 2002). Gravitational force is essential for the development and normal function of skeletal muscles; hence, the neuromuscular system is directly impacted during actual (space flight) and simulated (grounded-based models) microgravity (Di Prampero and Narici 2003; Benavides Damm et al., 2013).

Exposure to microgravity results in substantial changes in muscle structure and function, including muscle atrophy, altered muscle phenotype and reduced metabolic capacity. Muscle atrophy is characterized by significant loss in muscle mass, protein content, fiber diameter, force production and fatigue resistance (Fitts et al., 2000; Jackman and Kandarian, 2004). During actual and simulated microgravity, muscle atrophy is due to an imbalance between the rate of protein synthesis and degradation. Studies have provided consistent evidence that programmed cell death (PCD) pathways are activated or accelerated during skeletal muscle atrophy induced by microgravity (Kandarian and Jackman, 2006), which may account for the increase in protein degradation.

The disruption of the neuromuscular system during microgravity has continued to be studied with an emphasis placed on the

alterations/adaptations of the skeletal muscles, especially muscle atrophy. Due to their structural similarities, several model systems, including rodents and crustaceans have been proposed as alternatives to large mammalian models. Hence, we proposed to investigate the effects of simulated microgravity on the house cricket, *Acheta domesticus* as model to study the impact of microgravity on muscles predestined to die. The house cricket exhibits age-dependent flight muscle histolysis, which is induced by juvenile hormone and is as an example of PCD (Oliver et al., 2007). Flight muscle histolysis occurs within the first few days after adult emergence and accounts for the loss of flight in winged-adults. The normal time course of flight muscle histolysis has been established; it begins 48 hours after adult emergence (Day 2) and is complete by Day 3 (Oliver et al., 2007).

Despite being an endocrine regulated event initiated by juvenile hormone, studies have indicated that environmental factors such as climate, photoperiod and availability of resources may disrupt the normal time course of histolysis (Edwards, 1969). The influence of environmental factors on the time course of histolysis suggests that under microgravity conditions deviations from the normal time course of histolysis may be observed. Therefore, we postulate that the normal time course of flight muscle histolysis in the house cricket, *A. domesticus* may be altered during simulated microgravity.

METHODS

<u>Insects:</u> Juvenile crickets were obtained from the commercial breeder (Armstrong Cricket Farm, Glenville, GA). Crickets were reared at 30°C with a 14:10 (light:dark) photoperiod. Crickets were provided with distilled water and fed Fluker's High Calcium Cricket Food (Fluker Farms, Port Allen, LA). Crickets were maintained in a large colony and adult crickets were isolated upon adult emergence; adult female crickets were retained for experimentation and male organisms were discarded. Once the adults were isolated they were designated as Day 0 (Oliver et al., 2007).

<u>Treatment Groups:</u> Adult crickets were maintained in smaller colonies by age, Day 1 being 24 hours after adult emergence. Day 2, 3 and 4 were 48, 72 and 96 hours respectively after adult emergence. Crickets designated as controls were maintained in small colonies and reared under the ideal conditions described above. Clinorotation was the method utilized to simulate microgravity within the experimental group. Clinorotation has been used as an effective method to simulate microgravity, as it eliminates the directional cues associated with gravity; therefore, altering the organism's perception of gravity (Di Prampero and Narici, 2003). A benchtop clinostat was used to stimulate microgravity (simulated microgravity is measured at <30 rpm; the benchtop clinostat was recorded at 17 rpm). Day 0 crickets were placed in 50 mL conical tubes with Fluker's Orange Cube Complete Cricket food (Fluker Farms, Port Allen, LA) to supply both food and water. Crickets were subjected to clinorotation for 24, 48, 72 or 96 hours and then removed to obtain treatments for the respective ages (Day 1, 2, 3 and 4).

<u>Lactate Dehydrogenase (LDH) Assay:</u> LDH activity was used to assess whether the muscle tissue was functional. Frozen DLMs were homogenized in potassium phosphate buffer (50 mM; pH 7.5); homogenates were separated by centrifugation and supernatant retained. Total protein concentration was determined for paired DLMs by the bicinchoninic acid method (BCA Assay; Pierce, Rockport, IL). LDH activity was measured for 10 μg total protein from DLMs (n = 4) using β-NADH as described by Bergmeyer et al. (1965). The reaction mixture (total volume 1 mL) contained 100 μL of NADH (1.2 mg/mL), 800 μL of potassium phosphate buffer and 100 μL of sample (10 μg of protein from DLM homogenates). LDH activity was initiated by adding 25 μL sodium pyruvate (0.35 mg/mL) to 250 μL of the reaction mixture.

The oxidation rate of the NADH was measured at 340 nm at 1 minute intervals for 16 minutes by the PowerWave 340i plate reader (Bio-Tek Instruments, Inc., Winooski, VT). One unit of enzymatic activity is defined as the amount of enzyme which changes the O.D. of NADH, by 0.001 in 1 minute.

Tissue Wet Weight Measurements: Oliver et al., (2007) described that there was a loss in muscle mass associated with flight muscle histolysis; hence, we collected wet weight measurements to obtain the mass of the dorsolongitudinal muscles (DLMs) to determine histolysis. Crickets were anesthetized on ice prior to experimental manipulation. DLMs were excised from crickets by ventral dissection. Paired muscles were rinsed with cricket ringer (154 mM NaCl, 8 mM KCl, 8 mM CaCl, 4 mM $MgCl_2$) and blotted dry to remove adherent fat body. Muscle mass was measured for paired DLMs (n = 9) using an analytical balance (Sartorius, Elk Grove, IL) and reported in mg/paired DLMs. DLMs were then frozen and stored at -50°C for biochemical assays.

Data Analysis: Results were reported as means ± SE. Data were analyzed using ANOVA and SNK test in XL Stat, a component of Excel. Statistical significance was determined if $p < 0.05$.

RESULTS

LDH Assay: Preliminary data collected show that the control and experimental groups experienced a decline in enzymatic activity from Day 1 to Day 3 (see Figure 1). The LDH levels exhibited by Day 1 experimental were similar to the levels exhibited by Day 3 control.

Time Course of Histolysis: An ANOVA confirmed differences in muscles loss for the control group [$F(4, 40) = 15.81$, $p<.0001$]. Flight muscle histolysis is characterized by a significant loss in muscle mass by Day 3 (Oliver et al., 2007). The results obtained for the control group aligns with the normal pattern of histolysis (see Figure 2). There was no significant loss in muscle mass from Day 0 to Day 1 ($p = 0.817$). There was a 27% loss in muscle mass from Day 1 to Day 2 ($p = 0.011$), and a 21% loss in muscle mass from Day 2 to Day 3 ($p = 0.010$). There was no significant loss in muscle mass from Day 3 to Day 4 ($p = 0.799$).

An ANOVA confirmed differences in muscles loss for the experimental group [$F(4, 40) = 20.228$, $p<.0001$]. The experimental group exhibited a different pattern of muscle mass loss compared to the control group (see Figure 2). There was a 23% loss in muscle mass from Day 0 to Day 1 ($p = 0.001$). No significant loss in muscle mass was observed from Day 1 to Day 2 ($p = 0.099$), Day 2 to Day 3 ($p = 0.149$), or Day 3 to Day 4 ($p = 0.169$).

CONCLUSIONS

Flight muscle histolysis is an endocrine regulated event, which results in the degradation of the flight muscles (Oliver et al., 2007). Studies have indicated that the normal time course of histolysis may be interrupted by environmental and/or external factors (Edwards, 1969). Our results demonstrate that under simulated microgravity conditions the normal time course of flight muscle histolysis in the house cricket, A. domesticus appears to be accelerated.

Microgravity has been shown to induce muscle atrophy in healthy skeletal muscles not predestined to die by activating and accelerating PCD pathways (Kandarian and Jackman, 2006). Day 1 muscles subjected to simulated microgravity (experimental group) expressed similar levels of LDH activity as Day 3 control, muscles that had undergone flight muscle histolysis. These preliminary results led to the examination of the time course of flight muscle histolysis under simulated microgravity conditions. During flight muscle histolysis there was a 27% loss in

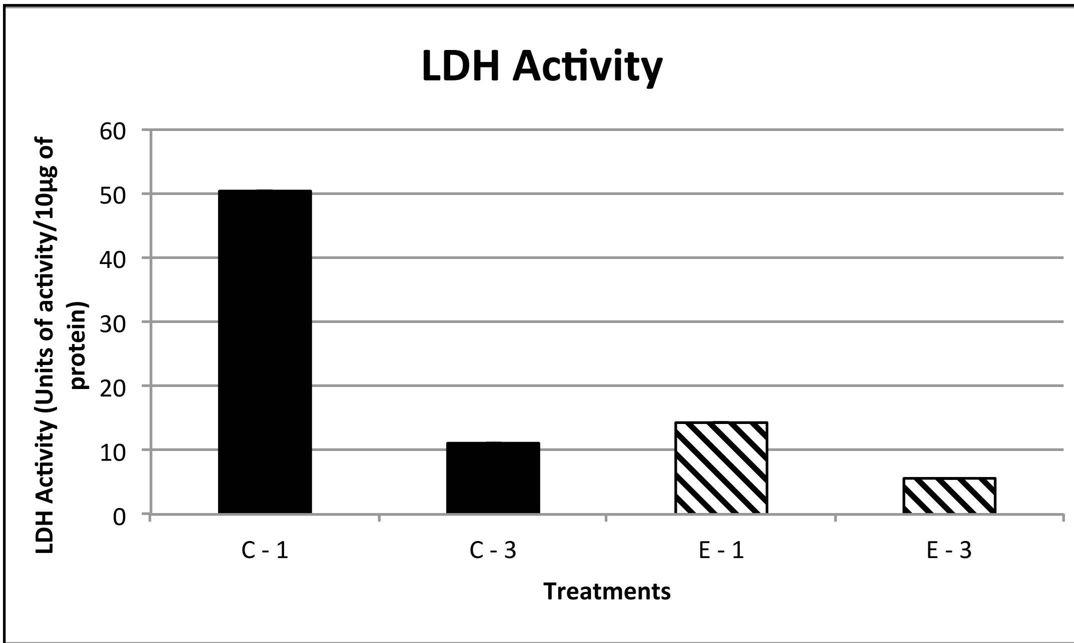

Figure 1: LDH Assay Results; means are presented.

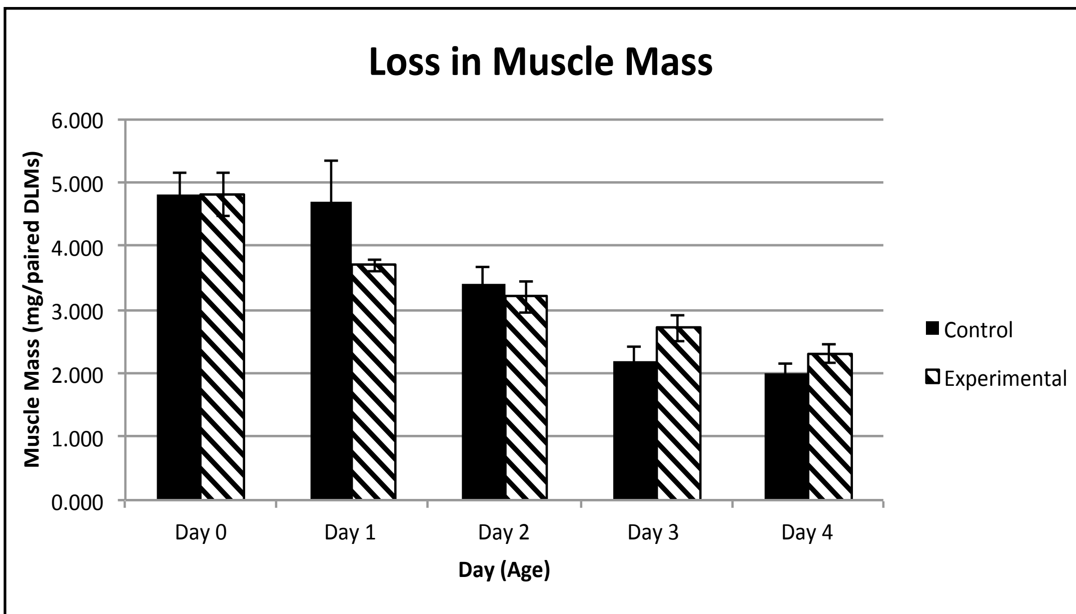

Figure 2: DLM Wet Weights of the Experimental and Control Groups from Day 0 to Day 4; means and standard errors are presented.

muscle mass from Day 1 to Day 2, indicating the initiation of PCD. There was a 21% loss in muscle mass from Day 2 to Day 3 with no significant loss in muscle mass after Day 3 indicating that the process of flight muscle histolysis was complete by Day 3. These findings coincide with the Oliver et al. (2007) study. However, during simulated microgravity there was a 23% loss in muscle mass from Day 0 to Day 1, with no subsequent loss in muscle mass thereafter. These results suggest that flight muscle histolysis is complete by Day 1 under simulated microgravity conditions. The results from the LDH assay indicate that there was a 72% difference in LDH activity between the Day 1 control group and Day 1 experimental group.

In summary, flight muscle histolysis in the house cricket, Acheta domesticus is an example of endocrine regulated PCD that begins on Day 2 and is complete by Day 3 and accounts for the loss of flight in winged-adults. Hence, this system can be an excellent model to study the impact of microgravity on muscles predestined to die. During simulated microgravity, there is significant loss in muscle mass and decline of enzymatic activity that occurs on Day 1, with no significant reduction thereafter. These findings suggest that the normal time course of flight muscle histolysis in the house cricket is altered during simulated microgravity. While the findings of this research provide insight on the effects of simulated microgravity on flight muscle histolysis, the implications are farther reaching. The house cricket may prove to be an ideal model system to study the impact of environmental pressures such as microgravity to gain insight on how environmental pressures may impact the underlying mechanisms of muscular degenerative diseases.

FUTURE WORK

Previous studies have identified the loss of muscle mass as an indicator of flight muscle histolysis in the house cricket, A. domesticus. However, we plan to follow through with biochemical analyses to confirm flight muscle histolysis. We are currently conducting acid phosphatase assays to measure lytic activity. In addition, flight muscle histolysis is an endocrine regulated event; therefore, evaluating the relationship between the environmental stress of microgravity and the potential impact on hormone regulation during flight muscle histolysis may prove essential to better understand the alterations seen in the time course of histolysis.

ACKNOWLEDGMENTS

We would like express our gratitude to Wingate University for the support of our project through the Creative Investigative Partnership Symposium (CIPS). We would also like to offer special thanks to Dr. Rush Oliver (Benedict College) for all of his support and expertise, as well as Dr. Debra Davis for her contributions throughout the duration of our project. Finally, we would like to thank Brooklyn Boston, Maria Ortiz, Samantha Bourn and Walter Loza who were extremely instrumental in animal care and data collection.

REFERENCES

Bergmeyer, H., Bernt, E. and Hess, B. Lactic Dehydrogenase. In: Methods of Enzymatic Analysis. New York: Academic Press 1965; 736 – 743.

Benavides Damm, T., Richard, S., Tanner, S., Wyss, F., Egli, M. and Franco-Obregón, A. Calcium-dependent deceleration of the cell cycle in muscle cells by simulated micro gravity. The FASEB J. 2013; 27: 2045 – 2054.

Di Prampero, P. and Narici, M. Muscles in Microgravity: from fiber to human motion. J. Biomech 2003; 36(3): 403 – 412.

Edwards, F. Environmental control of flight muscle histolysis in the bug *Dysdercus interme dius.* J. Insect Physiol 1969; 15(1): 2013 – 2020.

Fitts, R., Riley, D. and Widrick J. Physiology of a microgravity environment invited review: Microgravity and skeletal muscle. J. Appl Physiol 2000; 89: 823 – 839.

Freed, L. and Vunjak-Novakovic, G. Spaceflight bioreactor studies of cells and tissues. Adv Space Biol Med 2002; 8: 177 – 195. Review.

Jackman, R. and Kandarian, S. The molecular basis of skeletal muscle atrophy. Am J Physiol. 2004; 287: C834 – C843.

Kandarian, S. and Jackman, R. Intracellular signaling during skeletal muscle atrophy. Muscle Nerve 2006; 33: 155 – 165.

Oliver, R., Albury, A. and Mousseau, T. Programmed cell death in flight muscle histolysis of the house cricket. J Insect Physiol 2007; 53: 30 – 39.