

14th Annual
Muscle Health Awareness Day
May 19, 2023

Program and Abstracts



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14th Annual Muscle Health Awareness Day Program

Friday May 19, 2023

Life Science Building South Lobby and Room 103, York University

8:15 – 9:00 Registration, poster mounting, and light breakfast

Session 1: Physiology and pathology of muscle and bone (9:00-10:35)

Session Chair: Dr. Christopher Perry

9:00-9:05 – Dr. David Hood, York University

Welcome and Introduction

9:05-9:35 – Dr. Ewan Goligher, University of Toronto

Ventilator-induced diaphragm in the critically ill: mechanisms, outcomes, and opportunities for intervention

9:35-10:05 – Dr. David MacLean, Northern Ontario School of Medicine University

Cancer, chemotherapy and exercise: New insights using rodent models

10:05-10:35 – Dr. Panagiota (Nota) Klentrou, Brock University

The bone response to exercise: what can blood markers tell us?

10:35 – 11:30 Poster Presentations and Break (Life Science Building South Lobby)

Session 2: Muscle metabolism and protein turnover (11:30-12:30)

Session Chair: Dr. Ola Adegoke

11:30-12:00 – Dr. Tyler Churchward-Venne, McGill University

Reemerging role of ketone bodies as regulators of skeletal muscle protein turnover

12:00-12:30 – Dr. Jamie Melling, Western University

The Effect of Exercise on Skeletal Muscle Metabolism and Insulin Resistance Development in Type 1 Diabetes

12:30 – 2:00 Catered Lunch (Life Science Building South Lobby);

1:30-2:00 Poster Presentations

Session 3: Imaging tools and sex differences in physiology (2:00-3:40)

Session Chair: Dr. Peter Backx

2:00-2:30 – Dr. Heather Edgell, York University

Sex differences in the cardiorespiratory response to reflex activation

2:30-3:00 – Dr. Michaela Devries-Aboud, University of Waterloo

Sex-based differences in muscle metabolism

3:00-3:30 – Dr. Amy Kirkham, University of Toronto

Magnetic Resonance Imaging as a Novel Tool to Uncover Cardiac and Skeletal Muscle Determinants of Exercise Intolerance

3:30-3:40 – Poster Awards Presentation, Concluding Remarks

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4	Parashar Bhatt	Characterization of Temporal Cardiac Inflammatory Dynamics Using a Novel Volume Overload Model To Assess Correlation with Atrial Fibrillation Vulnerability in CD1 mice	<i>York University</i>
5	Lucas Campagna	The mitochondrial function of p130 modulates differentiation of skeletal muscle stem cells	<i>York University</i>
6	Nadia Cartwright	Identifying unique anti-inflammatory effects of individual short chain fatty acids on lipopolysaccharide stimulated skeletal muscle cells	<i>University of Guelph</i>
7	Sabrina Champisi	Characterizing the role of Sulforaphane in Mitochondrial Function and Content in Skeletal Muscle Cells	<i>York University</i>
8	Bridget Coyle-Asbil	The pathophysiological mapping of cardiac structure and function in mice with epithelial ovarian cancer	<i>University of Guelph</i>
9	Daniel Da Eira	Sucrose-enriched and carbohydrate-free high-fat diets distinctly affect insulin-stimulated glucose metabolism and metabolic flexibility in rat oxidative and glycolytic muscles	<i>York University</i>
10	Benjamin Dalton	Time-course changes in neuromuscular function during and following ovarian failure: A mouse model of early to late-stage menopause	<i>University of Guelph</i>
11	Mark Danesh	The Contribution of Pericytes to Hallmark Features of Ischemic Myopathy	<i>York University</i>
12	Luca Delfinis	Early muscle weakness during colorectal cancer progression precedes atrophy but later recovers within quadriceps in association with mitochondrial function compensations	<i>York University</i>
13	Luca Delfinis	Early muscle weakness and mitochondrial stress in mice with ovarian cancer are reversed during later stages of cancer progression in tibialis anterior but not diaphragm	<i>York University</i>

14	Christina Doherty	The Impact of Sensory Protection and Glial Derived Neurotrophic Factor on Fibro-Adipogenic Progenitors	<i>University of Toronto</i>
15	Mohamed Elghobashy	Hypoglycemia is not associated with fatigue during single-legged endurance exercise	<i>York University</i>
16	Luke Flewwelling	Effects of Menopause on Fatigue Resistance and Calcium Transients in Skeletal Muscles of Mice	<i>York University</i>
17	Emily Fraschetti	The influence of post-exercise milk consumption on exercise-induced bone turnover and inflammatory cytokine responses	<i>York University</i>
18	Beata Friesen	The Effect of Ovariectomy on the Repeated Bout Effect	<i>University of Toronto</i>
19	Shivam Gandhi	Adiponectin-receptor agonism prevents right ventricular cardiac fibrosis, hypertrophy, and mitochondrial stress responses in the D2.mdx Duchenne muscular dystrophy mouse model	<i>York University</i>
20	Madison Garibotti	Exploring the relationship between skeletal muscle function and mitochondrial stress responses in an experimental autoimmune myositis rodent model	<i>York University</i>
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22	Avery Hinks	Do ultrasonographic measurements of fascicle length accurately depict serial sarcomere number?	<i>University of Guelph</i>
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24	Justin Hsiung	p107 mitochondrial function regulates satellite cell self-renewal by reprogramming metabolism through OPA1	<i>York University</i>
25	Emma Hubbard	VCD-induced ovarian failure impairs muscle power in mice but impairments are partially reversed with high intensity interval training	<i>University of Guelph</i>
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MHAD14 Abstracts

The Heart as a Novel Source of Erythropoietin

R. Alshamali., J.P. Marrow., M.J. Platt., Keith R. Brunt., and J.A. Simpson.

1. *University of Guelph, Guelph, Ontario, Canada.* 2. *Dalhousie University, Saint John, New Brunswick, Canada.*
3. *IMPART Investigator Team, Canada.*

Background: Erythropoietin (EPO) is widely viewed as a glycoprotein produced by the kidney to stimulate erythropoiesis (i.e., red blood cell production). EPO assays detect increased serum levels of EPO following an acute myocardial infarct (AMI). However, the source of elevated serum EPO levels is attributed solely to the kidney, despite emerging data that *Epo* mRNA expression can occur in non-renal cells/tissues. Thus far, non-renal sources of EPO have received little consideration. We hypothesize that the heart is a source of EPO production in organ specific hypoxia (i.e., AMI).

Methods: To assess whether EPO is produced from the heart post-AMI, CD1 male mice were subjected to a coronary artery ligation surgery to induce myocardial infarction. Left ventricle and kidney tissue was collected at 12 hours, 4 weeks, and 18 weeks post AMI surgery and snap frozen at -80 °C for qPCR analysis. Left ventricle hematocrit was measured at 2, 4, and 18 weeks. To detect differences in EPO mRNA expression between Sham and AMI mice, we used a one-way ANOVA with Dunnet's post-hoc test. Data was considered significant when $p < 0.05$.

Results: After an AMI, *Epo* mRNA expression was significantly elevated (100-fold increase) in infarcted areas of the heart. Importantly, *Epo* mRNA expression in the kidney was decreased post-AMI. Further, left ventricular hematocrit was measured at baseline, 2-week post-AMI, 2-week sham, 4-week post-AMI, 4-week sham, 9-week sham, 9-week post-AMI. Hematocrit levels were elevated at 2-weeks post-AMI, with no changes to sham mice.

Conclusion: Here we show that the heart, is a physiologically relevant source of EPO after an AMI. This has major implications on the prognostic and diagnostic value of EPO in AMI patients. Future work will focus on determining whether cardiac-derived *Epo* mRNA is translated into protein and secreted from the heart into serum of AMI patients.

PDCD4 Depletion Increases Myotube Diameter and Anabolic Signaling in Parallel with Reduced MuRF1 Expression

Termeh Ataie, Stephen Mora, and Olasunkanmi A.J. Adegoke
York University

Post-transcriptional downregulation of the tumor suppressor, Programmed Cell Death Protein 4 (PDCD4), has been shown to promote tumorigenicity in a myriad of cancers, including colon, breast, and lung cancer. As a tumor suppressor, PDCD4 functions as an inhibitor of mRNA translation and protein synthesis. However, its function in skeletal muscle has remained ambiguous. Within the past two years, PDCD4 has been characterized in skeletal muscle as a downstream substrate of the Mammalian Target of Rapamycin Complex 1/S6 Kinase Beta-1 (mTORC1/S6K1) pathway. However, there is limited knowledge regarding the regulation of PDCD4 and its effects on skeletal muscle. Therefore, the objective of this study was to investigate the effect of PDCD4 depletion on myotube morphology, anabolic signaling, and protein synthesis/breakdown. L6 myotubes were treated with either scrambled or PDCD4 siRNA for 48h. We observed improved morphology, characterized by larger myotube diameter (36%), and higher myofibrillar protein content of MHC-1 (502%), troponin (143%), and tropomyosin (266%) following PDCD4 depletion. Moreover, increased p-Aktser473 and p-S6K1thr389 was found following PDCD4 knockdown, indicative of enhanced anabolic signaling. These changes in anabolic signaling occurred in parallel with reduced myotube protein synthesis. However, PDCD4 depletion decreased the expression of the skeletal muscle E3 ubiquitin protein ligase MuRF1, but had no effect on autophagic markers, namely beclin-1, LC3B, and p62. There was also a tendency for reduced accumulation of ubiquitinated proteins in starved myotubes.

Our findings suggest that PDCD4 plays a role in regulating myotube morphology and myofibrillar protein abundance. Targeting this protein could be a promising approach to better preserve skeletal muscle in conditions/diseases associated with skeletal muscle atrophy.

The influence of sex on skeletal muscle fiber-specific characteristics linked to aerobic energy metabolism

Celine Bailleul, N Hodson, S Abou Sawan, DA Kumbhare, DR Moore, JB Gillen

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INTRODUCTION: Sex differences in skeletal muscle characteristics may contribute to the increased fat oxidation reported during moderate-intensity endurance exercise in females compared to males. For example, females have been demonstrated to have increased skeletal muscle mitochondrial content and capillarization at a whole-muscle level, which may be explained by a higher proportionate area of slow-oxidative type I muscle fibers in female skeletal muscle. However, it is possible that oxidative capacity of type I and type II muscle fibers is elevated in females versus males, but this has not been explored. The purpose of this study was to investigate the influence of sex on fiber-specific indices of mitochondrial content and capillarization in human skeletal muscle. **METHODS:** Resting skeletal muscle biopsy samples from the vastus lateralis were collected from untrained females (n=14; 23±5yr, 23.3±3.2kg/m²) and males (n=13; 23±4yr, 23.1±2.4kg/m²). Type I, IIa and IIx fiber distribution and cross-sectional area were determined via immunofluorescent analyses. Type I and II fiber-specific mitochondrial content and capillary density were quantified via COX IV pixel intensity and number of CD31-labelled capillaries per fiber square millimeter, respectively. **RESULTS:** Compared to males, females had increased proportionate area of type I fibers (42±12% vs 29±10%, p=0.01) and decreased proportionate area of type IIa fibers (39±6% vs 49±13%, p=0.02). Males, but not females, had increased capillary density of type I vs. type II fibers (364±88 vs. 280±66 capillaries/mm², p<0.05), owing to a 15% larger cross-sectional area of type IIa vs. type I fibers in males only. However, there were no differences between sexes in the mitochondrial content or capillary density of type I or type II fibers (p>0.05). **CONCLUSION:** Our findings demonstrate that while muscle fiber composition and size differs between sexes, oxidative potential of type I and type II muscle fibers is largely similar between males and females.

Characterization of Temporal Cardiac Inflammatory Dynamics Using a Novel Volume Overload Model To Assess Correlation with Atrial Fibrillation Vulnerability in CD1 mice

Parashar Bhatt, Robert Lakin, Mihir Parikh, Dana Sherrard, Ryan Debi, Peter Backx

York University, Muscle Health Research Center

Atrial fibrillation (AF) is the most common supraventricular arrhythmia characterized by random atrial excitation leading to asynchronous atrial contraction and very rapid and irregular ventricular pumping. AF predisposes to stroke and promotes heart failure, with incidence increasing with age. AF is promoted by cardiovascular disease and especially heart valve malfunction, which has been linked with atrial fibrosis, hypertrophy (enlargement) and altered electrical properties. These changes are invariably accompanied by elevations in myeloid immune cells and increased oxidative stress, as observed in both AF patients and animal models. To investigate the potential connection between myeloid immune cell increases and AF structural remodelling, we have generated and characterized a novel model of surgically induced valvular insufficiency (volume overload) called aortic regurgitation (AR). We further analyzed time dependent chamber-specific immune cell inflammatory dynamics with AR. Morphometric measurements show an initial dip in cardiac hypertrophy 3 days post-AR (3D: 11.16 g/mm² vs SHAM: 15.49 g/mm²; P = 0.014), followed by a progressive increase with AR compared to SHAM animals. After 4 weeks, AR animals developed key AF hallmarks such as atrial fibrosis (14.0±2.6% vs. 5.3±1.6%; P<0.05) and increased AF inducibility (P<0.05) as well as increased atrial macrophage numbers (143±19 vs. 39±11 cells/mm²) compared to SHAM animals. To assess time course dynamics of immune cell responses to AR, we measured total leukocyte (CD45+) and myeloid immune cell numbers (macrophages/monocytes – F4/80+) using immunochemistry. These results revealed an upward trend in the left atria 1-week post-AR (~ 2-fold for CD45

and F4/80) followed by a decrease back to baseline levels 2-weeks post-AR relative to SHAM animals. Similar results were observed in the left ventricle. Flow cytometry reveals an expansion of “M2” reparative/homeostatic non-classical monocytes (CD45+CD11b+Ly6G-CD64+F4/80-/loLy6C-CCR2-) and resident tissue macrophages (CD45+CD11b+Ly6G-CD64+F4/80med/hi), with negligible involvement of neutrophil (CD45+CD11b+Ly6G+) and lymphoid cells (CD45+CD11b-CD11c-). Taken together, our data aligns with previous findings in AF patients showing associations between innate myeloid immune cells and AF and demonstrates a complex temporal time course of myeloid cell changes in response to volume overload. Future work will aim to further dissect myeloid immune cell phenotype and role in AF through drug and genetic interventions.

The mitochondrial function of p130 modulates differentiation of skeletal muscle stem cells

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Skeletal muscle is a highly plastic tissue, maintaining an ability to regenerate throughout life in the absence of disease. Underlying the ability to regenerate are resident stem cells termed skeletal muscle stem cells (MuSCs), which are required for healthy maintenance of muscle. Upon injury, MuSCs receive activation signals from their environment for the choice to generate further copies (self-renewal) so as to expand the stem cell pool, or commit to becoming muscle (differentiation). Dysfunctional MuSC fate choices for self-renewal versus differentiation lead to poor muscle quality, as in sarcopenia during aging and in neuromuscular disorders. Understanding the mechanisms involved in MuSC fate choices remain to be fully elucidated that is crucial for developing potential therapeutic approaches. Governance of fate choices can be attributed to the metabolic profile of the MuSC. The relationship between metabolism and MuSC fate decisions forms the framework of this study. We found that Rbl2 (p130), a member of the retinoblastoma transcriptional co-repressor family is involved in determining MuSC fate decisions for differentiation through a mitochondrial function. We assessed MuSC fate decisions by culturing myofibers for up to three days ex vivo that were isolated from mouse extensor digitorum longus muscle. In order to evaluate differentiation, we cultured MuSCs from myofibers. p130 was found to be present in the mitochondria of the differentiating cells, but absent during proliferation. In the mitochondria, p130 might affect fate decisions by increasing reactive oxygen species (ROS), which is known to be necessary for the differentiation of other stem cell types. Altering MuSCs to favor activation and/or differentiation through p130 mitochondrial function will promote maintenance of skeletal muscle.

Identifying unique anti-inflammatory effects of individual short chain fatty acids on lipopolysaccharide stimulated skeletal muscle cells

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Microbial metabolites produced from dietary precursors have the capacity to influence extra-intestinal tissue function, including skeletal muscle via the gut-muscle axis. Short chain fatty acids (SCFA) are produced by microbial fermentation of non-digestible carbohydrates and to a lesser degree from non-digested protein. Once absorbed into the host blood stream, SCFA (and other microbial-derived metabolites) have been shown to exert metabolic effects, including the suppression of lipid accumulation in adipose tissue and modulation of glucose transporter expression in skeletal muscle. Additionally, SCFA have been shown to downregulate the expression of inflammatory transcription factors within various immune cell populations, thereby suggesting anti-inflammatory effects; however, this has not been examined in skeletal muscle cells. Furthermore, SCFA types may exert differential effects, despite being frequently assumed to all function similarly. Therefore, we examined the effect of individual SCFA at a physiologically relevant levels (2.5 mM of acetate, propionate, or butyrate) for 24 hours on L6 myotube inflammatory mediator mRNA expression and protein secretion. To recapitulate obese

skeletal muscle conditions rat L6 myotube cultures were also stimulated with the concentration of lipopolysaccharide (LPS) that mimics obese circulating levels (10 ng/mL). LPS stimulation increased inflammatory mediator gene expression compared to unstimulated control cell cultures, independent of SCFA type. In response to LPS, the magnitude of the anti-inflammatory effects of each SCFA was related to carbon number, wherein butyrate reduced mRNA expression of TNF α , IL-6 and MCP-1 compared to control-LPS stimulated cultures ($P<0.05$). Propionate exerted an intermediate effect, reducing mRNA expression of TNF α and MCP-1 ($P<0.05$), whereas acetate had no effect on inflammatory mediator expression. Secreted protein levels of MCP-1 and IL-6 exhibited a similar outcome to gene expression, wherein acetate had no effect, whereas only butyrate reduced IL-6 secretion and both butyrate and propionate reduced MCP-1 secretion in response to LPS ($P<0.05$). Furthermore, in response to LPS stimulation phosphorylated STAT3 cellular protein level was reduced only by butyrate, whereas all three SCFA types reduced phosphorylated NF κ Bp65 cellular protein levels compared to LPS-treated cells alone ($P<0.05$). Collectively, these data demonstrate that individual SCFA exert differential anti-inflammatory effects in response to LPS stimulation in skeletal muscle cells.

Characterizing the role of Sulforaphane in Mitochondrial Function and Content in Skeletal Muscle Cells

Sabrina Champisi, Neushaw Moradi & Dr. David Hood

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Despite recent advance in muscle research, muscle atrophy remains a major concern for aging, muscle disuse and disease. These conditions are characterized by a loss of mitochondrial content and function, further contributing to poor muscle health and subsequent atrophy. This highlights the importance of investigating novel therapeutic strategies that maintain and refresh the mitochondrial pool to preserve muscle mass and ameliorate atrophy. Sulforaphane (SFN) is a nutraceutical that has demonstrated extraordinary promise for the treatment of numerous disorders. SFN mainly accomplishes this by inducing activation of the Nrf-2-ARE mediated antioxidant pathway. Despite these promising characteristics, the role of SFN in mitochondrial adaptations in skeletal muscle remains undetermined. Therefore, our aim was to establish the effects of SFN treatment on mitochondrial function and content in skeletal muscle cells. Mature myotubes were treated with 10uM SFN for 24hrs and 48hrs, and immunoblotting was performed to analyze protein content. After 24hrs, SFN dramatically increased Nrf-2 expression and downregulated the negative regulator of Nrf-2, Keap1. Nuclear-cytosolic fractions revealed an 8-fold increase in Nrf-2 nuclear localization, consequently promoting the expression of downstream antioxidant markers HO-1 and NQO1. The Increase in antioxidant genes were sustained after 48hrs. Moreover, upregulation of TFAM was observed, suggesting an increase in drive towards mtDNA replication and transcription of mitochondrial proteins. This was accompanied by an increase in COX4, a critical component of the ETC, following 48hrs of treatment. Preliminary flow cytometry data further corroborated the observed increase in mitochondrial content through MitoTracker Red (MTR) staining. Mitochondrial function was assessed using Seahorse analysis. Following both 24hrs and 48hrs after treatment, there was a marked increase in basal respiration, ATP production, maximal respiration, and spare respiratory capacity, indicating that mitochondrial performance was improved. Together, these data suggest that SFN improves mitochondrial function and increases the drive towards mitochondrial biogenesis in skeletal muscle cells. This demonstrates the merit of SFN basally and presents an interesting avenue to evaluate if chronic contractile activity can work synergistically with SFN to improve the mitochondrial pool even further.

The pathophysiological mapping of cardiac structure and function in mice with epithelial ovarian cancer

Bridget Coyle-Asbil¹, Leslie M. Ogilvie¹, Madison Pereira¹, Kathy Matuszewska¹, Luca J. Delfinis², Shivam Gandhi², Keith R. Brunt³, Christopher G. R. Perry², Jim Petrik¹, Jeremy A. Simpson¹

1University of Guelph, Guelph, CA, 2York University, Toronto, CA, 3Dalhousie Medicine New Brunswick, Saint John, CA

Introduction: Cancer cachexia, characterized by the progressive loss of skeletal muscle mass (i.e., atrophy), negatively impacts patient quality of life and correlates with poor patient prognosis. While skeletal muscle atrophy and weakness are well characterized in cancer patients, the progression of cancer-induced cardiac atrophy has received little attention. Preliminary work from our lab has shown that cardiac dysfunction, measured by invasive hemodynamic assessment, precedes the development of metastatic disease in a mouse model of epithelial ovarian cancer (EOC). Yet, the time course of changes in cardiac structure and function throughout the progression of ovarian cancer remain unknown. Thus, our objective was to map the pathophysiological changes in cardiac structure and function throughout the progression of EOC. We hypothesize that EOC will cause systolic and diastolic dysfunction independent of changes in cardiac structure and mass. Methods: To investigate the impact of ovarian cancer on cardiac structure and function, we used an orthotopic, syngeneic mouse model of EOC. This model was generated by injecting transformed murine ovarian surface epithelial cells (ID8; 1.0×10^6) or saline (surgical sham) directly under the ovarian bursa of syngeneic mice. In this model, by 60 days after tumour cell injection, large primary tumours and numerous secondary lesions are readily apparent. Echocardiography and invasive hemodynamic assessment were used to examine changes in left ventricle (LV) structure and function throughout the progression of EOC at days 45, 60, 75, and 90 post-tumor induction (PTI). Morphometric assessments at each timepoint were used to determine the presence of cardiac atrophy. Results: Echocardiography showed that EOC mice had preserved LV function with no changes in structural dimensions at days 45, 60 and 75 PTI. However, by day 90 PTI, LV wall thickness decreased, suggesting evidence of cardiac atrophy. Conversely, hemodynamic assessment showed progressive impairments in both systolic and diastolic function throughout the progression of EOC. Morphometric assessment showed profound atrophy at 90 days PTI, but preserved cardiac mass at all other timepoints compared to shams. Conclusion: Together, this work demonstrates that in a mouse model of EOC, there are progressive impairments in both systolic and diastolic function. By advanced stage disease (90-days), cardiac atrophy becomes evident, yet there are no other changes in LV dimensions. Here we show that cardiac dysfunction occurs early in the progression of EOC and in the absence of cardiotoxic cancer therapies. Any improvements in patient cardiac health may help to improve patient quality of life and tolerance to cancer therapies.

Sucrose-enriched and carbohydrate-free high-fat diets distinctly affect insulin-stimulated glucose metabolism and metabolic flexibility in rat oxidative and glycolytic muscles

Daniel Da Eira, Shailee Jani, Mateja Stefanovic and Rolando B. Ceddia

School of Kinesiology and Health Science, Muscle Health Research Centre, York University

The objective of this study was to determine whether an obesogenic, high-fat, sucrose-enriched diet (HFS) or a carbohydrate-free ketogenic diet (KD) exert distinct effects on fat, glucose, and ketone metabolism in oxidative and glycolytic skeletal muscles. To investigate this, male Wistar rats were fed either a HFS or a KD for 16 weeks. Subsequently, soleus (Sol), extensor digitorum longus (EDL) and epitrochlearis (Epit) muscles were extracted to measure palmitate oxidation, insulin-stimulated glucose metabolism, and markers of mitochondrial biogenesis, ketolytic capacity, and cataplerotic and anaplerotic machinery. Sol, EDL, and Epit muscles from KD-fed rats preserved their ability to elevate glycogen synthesis and lactate production in response to insulin, whereas all muscles from rats fed the HFS diet displayed blunted responses to insulin. Maintenance of metabolic flexibility by the KD was accompanied by muscle fiber type-specific adaptive responses. This was characterized by the Sol muscle from KD-fed rats enhancing mitochondrial biogenesis and ketolytic capacity without elevating its rates of FA oxidation in comparison to HFS feeding. Conversely, in the Epit muscle, rates of FA oxidation were increased, whereas the ketolytic capacity was markedly reduced by the KD in comparison to HFS feeding. In the EDL muscle, the KD also increased rates of FA oxidation, although it did so without altering its ketolytic capacity when compared to HFS feeding. In conclusion, even though obesogenic and ketogenic diets have elevated contents of

fat and alter whole-body substrate partitioning, these two dietary interventions are associated with opposite outcomes with respect to skeletal muscle metabolic flexibility.

Time-course changes in neuromuscular function during and following ovarian failure: A mouse model of early to late-stage menopause

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Removal of the ovaries impairs muscle function in animal models, but the effects of gradual ovarian failure on muscle contractility are unknown. The occupational chemical 4-vinylcyclohexene diepoxide (VCD) has offered an experimental design that keeps the ovaries intact while gradually reducing ovarian estrogens. We used a VCD-induced ovarian failure CD1 mouse model (VCD: n=10, control: n=8) to investigate the effect of gradual ovarian failure on time-course changes in neuromuscular function of the plantar flexors across the peri-, early-, and late menopause stages. VCD mice were injected with 160 mg/kg/day of VCD for 15 days. Neuromuscular assessments of the plantar flexors were then performed in vivo at 40, 80, 120, and 176 days following injection. A torque-frequency relationship was constructed (10-200 Hz). The frequency required to produce 50% of peak torque (F50) and the ratio of TQ10 to TQ200 (TQ10:200) were then determined. A torque-velocity-power relationship was constructed using isotonic contractions across a range of loads (10-80% of peak torque), then fitted to the Hill equation to determine the maximum velocity (Vmax) and power (Pmax), as well as curvature (a/P0). Following the final mechanical testing session (Day 176), muscle fibres were isolated from the flexor digitorum brevis. The calcium-frequency relationship was determined by measuring myoplasmic free Ca²⁺ transients across frequencies of 10-200 Hz (identical to the in-vivo torque-frequency assessment). There were no interactions of group × time for any measures (p>0.05). However, there was an effect of group for TQ10 such that the VCD group produced ~18% less torque compared to controls (p=0.04). The VCD group trended towards producing less torque than controls at TQ15, TQ20, TQ80, and TQ100 (p=0.051-0.063, η^2 =0.20-0.22). No effect of group was observed for F50 or TQ10:200 (p>0.05). Sarcoplasmic reticulum (SR) Ca²⁺ release was similar between groups at each stimulation frequency, as well as the ratio of SR Ca²⁺ release produced at 10:200 Hz (p>0.05). VCD produced ~23% less power than controls (p=0.02), with no difference in Vmax or a/P0. It appears that ovarian failure negatively affects the ability to produce power and this was likely due to impairments in torque, rather than velocity as Vmax was unaffected. The reduction in torque observed in the VCD group appears to be unrelated to SR Ca²⁺ handling, suggesting cross-bridge based mechanisms may be responsible.

The Contribution of Pericytes to Hallmark Features of Ischemic Myopathy

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Background: Key features of ischemic myopathy include fibrosis and adipocyte accumulation. To improve the ischemic condition, it is important to understand the cells that contribute to its progression. In vitro studies have suggested that skeletal muscle pericytes can differentiate into multiple cell types, including adipocytes and fibroblasts. Thus, the goal of these experiments was to quantify the extent and time course of association of pericytes with fibrosis and fat deposition in ischemic muscles. **Methods:** Mice that produce Discosoma Red (DSred) fluorescent protein under the control of a pericyte-specific NG2 promoter underwent femoral artery

ligation to induce severe lower-limb ischemia, which triggers muscle damage and a regenerative response. At days 4, 8 or 14 post-ligation, ischemic and non-ischemic TA muscles were extracted, then longitudinally sectioned and stained with markers of different cell types to visualize overlap with DSred using confocal microscopy. Results: Fibrotic markers platelet derived growth factor alpha (PDGFR α) and type 1 collagen were increased significantly in ischemic muscles at all timepoints, and at days 8 and 14, respectively. DsRed+ cells often co-localized with PDGFR α and type 1 collagen. Interstitial lipid accumulation was prevalent at 8- and 14- days post-ligation time points. At the 8- and 14-day timepoints, approximately 40% of large lipid droplets (detected by Bodipy) were located within DSred+ cells which were identified as adipocytes through Plin1 expression. Some of these DsRed+ adipocytes were positive for the adipogenic factor peroxisome proliferator-activated receptor γ (PPAR γ). This supports the idea of pericyte involvement in fibrosis and adipogenesis. In contrast, we found that cardiotoxin-injected mice contained relatively few adipocytes, most of which were not DsRed+. This suggests that ischemia is required for pericyte contribution to adipocyte formation. Finally, no overlap was observed between DSred and Pax7, indicating that DsRed cells do not appear to become satellite cells. Conclusion: Our findings support the idea that skeletal muscle pericytes contribute substantially to the pathological features of fibrosis and fat accumulation in ischemic myopathy. This may reveal pericytes as a therapeutic target to improve the outcome for conditions such as critical limb ischemia. Funded by NSERC and CIHR.

Early muscle weakness during colorectal cancer progression precedes atrophy but later recovers within quadriceps in association with mitochondrial function compensations

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Muscle weakness and wasting are defining features of cancer-induced cachexia. Mitochondrial stress occurs before muscle atrophy in certain muscles, but the heterogeneity between muscles and across time remains unclear. This investigation compared the effects of cancer on quadriceps and diaphragm muscle force, atrophy, and mitochondrial function at 2 and 4 weeks of tumour growth. Colon-26 (C26) carcinoma cells or phosphate-buffered saline (PBS) were injected in the hind flank of 8-week-old male CD2F1 mice. Tumours developed for 2 or 4 weeks. At 2 weeks, small tumours had no effect on body or muscle mass, while specific force production was lower in both quadriceps and diaphragm vs control. Pyruvate-supported ADP-stimulated mitochondrial respiration was lower in quadriceps, while mitochondrial H₂O₂ emission was elevated in diaphragm without changes in ETC protein contents in both muscles. At 4 weeks, the presence of large tumours corresponded to lower tumour-free body mass, muscle mass, and cross-sectional area of quadriceps and diaphragm fibres. Specific force production was the same as control in the quadriceps but remained lower in diaphragm. Mitochondrial respiration was increased in both muscles vs control across a range of [ADP] despite lower ETC protein content in quadriceps and unchanged contents in diaphragm. These findings indicate muscle weakness precedes atrophy in quadriceps and diaphragm in the C26 model of cancer cachexia. Early weakness is associated with heterogeneous mitochondrial responses whereby pyruvate oxidation is reduced in quadriceps and H₂O₂ emission is elevated in diaphragm. Muscle-specific compensations in force production and mitochondria occur thereafter which suggests the effect of cancer may not be shared amongst all muscles. Exploring the heterogeneity of cachexia across time and muscle type may reveal distinct mechanisms that confer unique sensitivities, or resistances, to cancer.

Early muscle weakness and mitochondrial stress in mice with ovarian cancer are reversed during later stages of cancer progression in tibialis anterior but not diaphragm

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Muscle weakness and wasting are defining features of cancer cachexia and are associated with decreased survival. In the C26-colorectal subcutaneous cancer model, muscle mitochondrial stress and weakness precedes atrophy. However, this relationship has not been explored in an orthotopic, syngeneic mouse model of epithelial ovarian cancer (EOC). Furthermore, mouse models of ovarian cancer cachexia have been understudied in the literature. The aim of this investigation was to explore the impact of EOC on diaphragm and tibialis anterior (TA) muscle function during ovarian cancer progression. Muscle force production was evaluated using the force-frequency relationship and mitochondrial respiration were measured by high-resolution respirometry in permeabilized muscle fibres. Transformed murine ovarian surface epithelial cells from C57BL6 mice (ID8; 1.0×10^6) were injected directly under the ovarian bursa of syngeneic mice and developed for 45-, 75- and 90-days post-inoculation while control mice (sham) were injected with PBS and examined at 75 days. At 90 days post-inoculation, mice exhibited a reduction in tumour-free body mass, grip strength, volitional wheel running distance and several hindlimb muscle weights compared to sham. Muscle force production and pyruvate-supported ADP-stimulated mitochondrial respiration initially decreased in diaphragm and TA at 45 and 75 days. However, by 90 days TA force production increased compared to 45 and 75 days while diaphragm force continued to decrease. Moreover, diaphragm creatine-independent respiration (ADP/ATP diffusion model) was restored to sham levels while creatine-dependent respiration (adenylate-phosphocreatine phosphate shuttling model) remained lower vs sham. Interestingly, TA respiration was restored in both conditions. These data suggest mitochondrial pyruvate oxidation and force production in both muscles are reduced early in EOC. However, in advanced stages, adaptive restoration of pyruvate oxidation and force occurs in the TA but not the diaphragm. Our ongoing research focuses on clarifying how these muscle-specific and time-dependent mitochondrial responses to ovarian cancer relate to cachexia.

The Impact of Sensory Protection and Glial Derived Neurotrophic Factor on Fibro-Adipogenic Progenitors

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Abstract text (no figures are permitted): Background/Rational: Peripheral nerve trauma causes atrophy and fibro-fatty infiltration (FFI) of skeletal muscle. Duration of denervation determines whether re-innervation can reverse these muscle sequelae. Fibro-adipogenic progenitor cells (FAPs) are muscle resident stem cells that differentiate to fibroblasts and adipocytes, which mediate FFI. Sensory protection (SP), whereby a sensory nerve is anastomosed to denervated muscle, partially mitigates FFI and correlates with a decrease in muscle Glial Derived Neurotrophic factor (GDNF), suggesting that GDNF may regulate denervation-mediated FAPs recruitment and differentiation. Objective: Determine the role of sensory protection and GDNF on FAPs recruitment and differentiation. Method: Rats underwent tibial nerve transection of the right hindlimb to denervate the gastrocnemius followed by no nerve repair, sensory protection of the denervated muscle (with surgical transposition of the sural nerve) or immediate repair of the tibial nerve, and were assessed 5 & 12-weeks post denervation. The left limb served as an internal control. Flow cytometry/FACS assessed gastrocnemius FAPs expression. To assess the impact of GDNF on FAPs differentiation, healthy sorted FAPs and adipose derived stem

cells were treated with GDNF (1ng-45ng/mL) in vitro and assessed for fibrogenic and adipogenic differentiation. Results: FAPs increased at 5 & 12-week in denervated gastrocnemius. The FAPs increase was mitigated by sensory protection. Muscle receiving immediate nerve repair showed minimal FAPs increase. 15ng/mL of GDNF induced adipogenic differentiation in FAPs, with a 4-fold increase in perilipin-1 expression compared to FAPs cultured without GDNF ($P<0.001$). 15ng/mL of GDNF did not induce FAPs fibrogenic differentiation. 45ng/mL GDNF was not sufficient to induce adipogenic differentiation in adipose-derived stem cells. Conclusions: Sensory protection decreases GDNF expression, FAPs recruitment and mitigates fibro-fatty degradation of denervated muscle. GDNF increases FAPs adipogenic differentiation. Together these data suggest that sensory protection of denervated muscle results, in part, from a decrease in GDNF-mediated stimulation of FAPs adipogenic differentiation.

Hypoglycemia is not associated with fatigue during single-legged endurance exercise

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Endurance exercise performance is determined by the energy demand of the exercise being met by energy supply, with one such energy supply being carbohydrates consumed as food that increases glucose availability to be used by the neuromuscular system during exercise. However, glucose has not been previously deemed as a rate limiting cause of exercise-induced fatigue. Thus, the goal of this research is to identify whether fatigue during voluntary endurance exercise performed in humans is associated with fatigue-associated changes in interstitial glucose levels assessed with a continuous glucose monitor (CGM), and further we aim to distinguish whether fatigue-induced changes in interstitial glucose levels are due to central (neural) or peripheral (intramuscular) factors. The fatigue protocol consisted of paced isokinetic single-leg knee extensions at a contraction intensity of 20% of the maximal voluntary contraction (MVC) torque, resembling submaximal endurance exercise. The magnitude of central and peripheral fatigue was evaluated on an intermittent basis using a combination of low and high frequency stimulations and the interpolated twitch technique (ITT) to discern the central and peripheral origins of fatigue. Participants, through a randomized cross-over design, ingested carbohydrates in the form of simple sugars in one condition and a flavour-matched placebo in another condition while performing the endurance exercise task. This was all performed while tracking in regular time intervals the live interstitial glucose readings generated by the CGM. The study shows that fatigue is not associated with hypoglycemia but is rather associated with a decrease in exercise-stimulated elevations in interstitial glucose. Additionally, time to task failure was increased from 92.1 min (± 19.5 SD) to 145.7 min (± 28.6) due to carbohydrate ingestion. Carbohydrate ingestion also reduced the onset of central and peripheral fatigue. In summary, carbohydrate ingestion improves endurance exercise performance by providing additional glucose availability for the brain and skeletal muscles to extend fatigue resistance.

Effects of Menopause on Fatigue Resistance and Calcium Transients in Skeletal Muscles of Mice

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Many previous studies on menopause have surgically removed the ovaries from mice, inducing rapid changes in hormone levels. on skeletal muscle function across different stages of menopause. In the current study we used a 4-vinylcyclohexene diepoxide (VCD) -induced ovarian failure mouse model to investigate the effect of fatigue resistance following menopause, whereby VCD treatment keeps the ovaries intact while progressively reducing circulating ovarian hormones over 120 days. We tested in-vivo muscular fatigue on the plantar flexors at day 176 of menopause by determining the number of contractions required to decrease force by 60% of the initial value. We also investigated the possible involvement of altered myoplasmic free calcium $[Ca^{2+}]_i$ transients with repeated electrical stimulation in isolated intact single muscle fibres from the flexor digitorum brevis (FDB) muscle. Our results showed that there was no treatment effect on fatigability in the in-vivo experiments. Similarly, the isolated

muscle fibres had no treatment effect on the fatigue-induced changes in sarcoplasmic reticulum Ca^{2+} release. However, in the isolated muscle fibres, resting $[\text{Ca}^{2+}]_i$ levels were non-significantly greater in the menopause group than the control group, indicating a potentially decreased ability to reuptake Ca^{2+} via the sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) pump. This research demonstrates that gradual ovarian failure does not lead to significant changes in fatigability of skeletal muscles following menopause.

The influence of post-exercise milk consumption on exercise-induced bone turnover and inflammatory cytokine responses

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Following high-intensity exercise, there are well-characterized increases in bone resorption markers and inflammatory cytokines. Post-exercise milk consumption may favourably alter these responses. Using a crossover design, we compared the acute effects of milk consumption (MILK) vs. a carbohydrate beverage (CHO) on bone and inflammatory biomarkers following combined high-intensity resistance and plyometric exercise in healthy, young females ($n=13$; age= 20.3 ± 2.3 y). Participants consumed either 550mL (20g protein) of 0% skim white milk or isoenergetic CHO (maltodextrin and water) at 5min and 1h post-exercise. Venous blood was obtained pre-exercise, 15min, 75min, 24h, and 48h post-exercise. Serum bone biomarkers (carboxyl-terminal crosslinking telopeptide of type I collagen [CTX], receptor activator nuclear factor kappa-b ligand [RANKL], sclerostin [SOST], osteoprotegerin [OPG], osteocalcin [OC]) and cytokines (interleukin [IL]-1 β , IL-6, IL-10, and tumor necrosis factor-alpha [TNF- α]) were measured. There were main time effects for RANKL, SOST and OC, which were lower, and the OPG/RANKL ratio, which was higher 75min post-exercise vs. pre-exercise. Area under the curve of the %change revealed a lower relative CTX response to exercise in MILK vs. CHO ($p=0.03$). Regarding inflammation, there was an interaction for the absolute concentration of IL-10, between 24 and 48h with IL-10 decreasing and increasing in the MILK and CHO conditions, respectively ($p=0.018$). There was a main time effect for IL-6, whereby the concentration was greater at 15min post-exercise vs. other timepoints ($p=0.017$). The relative concentrations of IL-1 β ($p=0.049$) and IL-10 ($p=0.028$) were lower in MILK vs. CHO at 48h. We also investigated the relationship between markers by assessing whether the %change in cytokines at 15min predicted the %change in bone markers at 75min post-exercise, using multiple linear regression. The models included %body fat, and %change for each cytokine at 15min. For CHO, IL-6 ($\beta=-1.06$) and %body fat ($\beta=6.54$) predicted the %change in CTX. IL-6 and IL-10 predicted the %change in OPG ($\beta=-0.85$ and $\beta=1.49$) and SOST ($\beta=-0.57$ and $\beta=0.59$; $p<0.05$ for all). For MILK, IL-6 predicted the %change in SOST ($\beta=-0.808$; $p=0.037$). Post-exercise milk consumption may positively influence bone resorption and inflammation following high-intensity exercise compared to CHO, and there are relationships between inflammatory and bone biomarkers following differential post-exercise nutrition.

The Effect of Ovariectomy on the Repeated Bout Effect

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Preconditioning skeletal muscle (SKM) with a few, non-damaging lengthening contractions prior to completing a second, more damaging bout of LCs is the phenomenon termed the repeated bout effect (RBE). LCs have been demonstrated to stimulate an increase in heat shock protein (HSP) content, thus it is posited that HSP25 and HSP72 may play a role in the RBE. Since ovarian hormones have been identified as important factors in conferring SKM protection and mediating the increase in HSPs following LCs, the removal of ovarian hormones through ovariectomy (OVX) may impact the RBE. In order to investigate the relationship between OVX and the RBE, TA muscles from ovary intact (OVI) and OVX rats were subjected to either no preconditioning or preconditioning with 15LCs, followed 24hrs later by 60LCs. A trend was observed in which peak and active torque maintenance was improved with preconditioning in both OVX and OVI animals; however, OVI animals tended to maintain

torque beyond OVX animals over 60LCs. OVI animals demonstrated a trend whereby HSP25 content and immune cell content increased proportionally with the number of lengthening contractions, while OVX animals demonstrated a trend in which preconditioning blunted HSP25 content and immune cell infiltration following 60LCs compared to non-preconditioned TA muscles. Finally, a trend was observed in which preconditioning blunted HSP72 expression following 60LCs in TA muscles from both the OVI and OVX animals compared to non-preconditioned TA muscles. Morphologically, preconditioned TA muscles demonstrated less discernable damage compared to non-preconditioned TA muscles in both the OVI and OVX groups. In conclusion, preconditioning appeared to mount a stress response in both OVI and OVX skeletal muscle; however, OVI animals demonstrate an improved response compared to OVX animals.

Adiponectin-receptor agonism prevents right ventricular cardiac fibrosis, hypertrophy, and mitochondrial stress responses in the D2.mdx Duchenne muscular dystrophy mouse model

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Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder perpetuated by mutations to the DMD gene, which encodes for dystrophin protein. Cardiomyopathy is the leading cause of mortality in DMD patients. Cardiac mitochondrial stress responses represent contributors to inflammatory and fibrotic outcomes in DMD. The degree to which fibrosis occurs across cardiac chambers is unknown. Purpose: the relationship between cardiac fibrosis and mitochondrial metabolism warrants investigation to address clinical therapy development. The adiponectin-receptor agonist ALY688-SR has demonstrated beneficial roles along both inflammatory and fibrotic pathways. Objective: determine the degree to which ALY688-SR administration in 4-week-old D2.B10-DMDmdx/2J (D2.mdx) mice influences chamber-specific fibrosis and corresponds to altered mitochondrial stress. Methods: D2.mdx mice were injected daily beginning at 7 days of age for 3 weeks at 15 mg/kg body weight (high dose; HD) or with saline (VEH-treated). Mice were compared to age-matched wildtypes (DBA/2J; WT). Results: histopathological assessments identified large elevations in left atrial and right ventricular (RV) collagen in VEH (+385%), which were both completely prevented by HD. Minimum Feret Diameter revealed that VEH and HD had elevated cardiomyocyte size (hypertrophy) compared to WT in both ventricles, but was only protected in RV by HD. In RV, pyruvate (NADH; Complex I)-supported mH₂O₂ emission assessed across a range of [ADP] was increased in VEH (+88%) vs WT but did not change with HD. ADP-stimulated respiration supported by pyruvate was lower in VEH (-44%) vs WT but was completely rescued by HD. Similar effects were observed in the RV when stimulating mitochondria with fatty acid oxidation substrates across a range of [ADP]. In LV, mH₂O₂ emissions supported by pyruvate were not different between groups. However, ADP-stimulated respiration supported by pyruvate was increased in VEH (36%) and HD (46%) vs WT whereas HD had no effect vs VEH. Inflammatory (IL-10) and fibrotic (TGF- β 1, α -SMA) molecular signatures varied between intraventricular layers and were largely unaltered 24-hours post-drug administration. In summary: RV fibrosis in D2.mdx is related to lower mitochondrial pyruvate and fatty acid oxidation and increased complex I-stimulated mH₂O₂ emissions. Prevention of fibrosis and hypertrophy in the RV by ALY688-SR may involve partial mitochondrial reprogramming.

Exploring the relationship between skeletal muscle function and mitochondrial stress responses in an experimental autoimmune myositis rodent model.

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Myositis is a rare autoimmune disorder that causes skeletal muscle inflammation and weakness. A previously identified increased in muscle mitochondrial H₂O₂ emission (mH₂O₂) in patients and an experimental autoimmune myositis (EAM) mouse model was observed using a mixed glutamate (NADH) and succinate (FADH₂) substrate protocol. However, the degree to which skeletal muscle mH₂O₂ is generated from forward or reverse electron transfer pathways during myositis remains unknown. 12–16-week-old Female BALB/c mice were assessed at 21, 28, and 49 days (d) post final injection as follows: 21d, 3 injections at day 0, 7, 14; 28d and 49d, 4 injections at day 0, 7, 14, 21. Treatment animals received in-lab purified rabbit myosin stored in glycerol with Freund's Complete Adjuvant (CFA) for the first injection, and Freund's Incomplete Adjuvant (IFA) for the remaining three injections to enhance the immune response to myosin. Two separate control groups received either a glycerol saline (GS) vehicle or a glycerol CFA/IFA (GCI) vehicle. To assess skeletal muscle function in situ tibialis anterior (TA) force production and in vitro diaphragm force production is evaluated using the force-frequency relationship. Muscle tissue was collected and used to prepare permeabilized muscle fibers for assessments of complex I (pyruvate)- supported mitochondrial respiration in both the TA and the diaphragm using high-resolution respirometry. Additional permeabilized muscle fibers were used for assessments of complex I forward (NADH; pyruvate/malate) and reverse electron transfer (FADH₂; succinate) supported H₂O₂ emission, under state II conditions (no ADP). ADP was then titrated (state III) to permit forward electron flow to complex IV in all substrate conditions and to determine the ability of ADP to attenuate H₂O₂. In the TA at day 21, a decrease in force production as a result of myosin injections was seen relative to both controls ($p = <0.0001$), this weakness was recovered by day 28. Mitochondrial measures were assessed only at day 28, demonstrating a bi-directional effect under state III conditions of myosin on mH₂O₂ through reverse vs forward electron transfer. A decrease in mitochondrial respiration as a result of myosin injections was observed when compared to GS control in the presences of creatine ($p=0.0048$). No differences in state II H₂O₂ emission in response to any substrate for both tissues assessed. In the diaphragm at day 21, a surprising increase in force production for both the adjuvant and myosin group compared to the GS group ($p= 0.002$; $p=0.010$), which was reversed by day 28. In diaphragm fibers at day 28, under state III conditions, forward electron transfer supported mH₂O₂ was increased in the GCI control compared to the GS control ($p=0.0267$), demonstrating an impaired ability of ADP to attenuate H₂O₂ emission as a result of adjuvant injections. ADP had no effect on reverse electron transfer supported H₂O₂ emission in the presences or absences of creatine (ADP/ATP cycling conditions). While myosin lowered force production in TA, this EAM mouse model also demonstrates adjuvant effects on cage hangtime, diaphragm force and mitochondrial bioenergetics that were more common than myosin effects. These results suggest this common EAM model may not be appropriate for assessing muscle responses in myositis.

The Effects of Exercise Dose on Cardiac Responses and Atrial Fibrillation

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Atrial fibrillation (AF) is a supraventricular tachyarrhythmia strongly associated with cardiovascular disease (CVD) and sedentary lifestyles. Despite the abundant benefits of regular exercise, AF incidence for professional endurance athletes is proportionate to patients with CVD. To assess the relationship between exercise dose and AF, we compared the effects of strenuous endurance training on mice by varying daily swim durations (120, 180 and 240 minutes divided by two sessions daily). After receiving the same cumulative work associated with swimming (estimated from O₂ consumption measurements), all swim-trained groups showed similar elevations

($P<0.04$) in skeletal mitochondria content and left ventricular dilation ($P<0.03$). By contrast, inducible AF increased ($P<0.0001$) progressively with daily exercise dose without markedly affecting atrial refractoriness and action potential durations ($P>0.05$). Associated with an exercise dose dependency are enhanced ($P<0.0001$) bradycardia, pronounced ($P<0.003$) hypertrophy, elevated ($P<0.0007$) fibrosis and increased ($P<0.0001$) macrophage accumulation in the atria, without corresponding changes in the ventricles. Our results demonstrate that elevations in the daily amounts of strenuous exercise promote progressively adverse atrial-specific remodelling leading to increased AF susceptibility.

Do ultrasonographic measurements of fascicle length accurately depict serial sarcomere number?

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Ultrasound-derived measurements of fascicle length (FL) are often used to infer increases (e.g., with chronic stretch or training) or decreases (e.g., with aging) in the number of serially aligned sarcomeres (serial sarcomere number; SSN). Despite the widespread use of ultrasound imaging to assess FL adaptations, whether ultrasound can truly approximate SSN adaptations has not been investigated because measurements of SSN in humans are invasive and costly. Further complicating these assessments, previous research indicates the accuracy of ultrasound-derived FL may depend on the region of muscle and the joint angle at which images are obtained. To address these concerns, our experiments involved both ultrasound-derived FL, and dissected FL and SSN from the rat hindlimb. Using 3D-printed casts, we immobilized the right hindlimb of 15 male Sprague-Dawley rats in dorsiflexion for 2 weeks to stretch the soleus and induce sarcomerogenesis (increasing SSN). The left soleus served as a control. Ultrasound images of the soleus were obtained with the ankle at 90° and full dorsiflexion for both hindlimbs pre and post-cast, and 6 FL measurements were obtained proximal to distal from each image. Following post-cast ultrasound measurements, legs were fixed in formalin with the ankles at 90°. Muscles were then dissected, and 6 fascicles were teased out for measurement of dissected FL, and measurement of sarcomere lengths via laser diffraction to calculate SSN. Ultrasound detected an 11% increase in FL ($P<0.01$) that was consistent across joint angle and muscle region. These adaptations were partly reflected by SSN measurements, with a 6% greater soleus SSN in the casted leg than the un-casted leg ($P<0.01$), and no regional differences. This SSN increase appeared to be driven by a 6% increase in FL measured from dissected fascicles ($P<0.01$), as sarcomere length did not differ ($P=0.71$). Weak but significant relationships were observed between ultrasound-derived FL at 90° and FL of dissected fascicles ($R^2=0.32$, $P<0.01$). Ultrasound-derived FL at 90° also related weakly to SSN ($R^2=0.36$, $P<0.01$), and this relationship was lessened for ultrasound-derived FL at full dorsiflexion ($R^2=0.21$, $P=0.01$). Our results showed that ultrasound-derived FL measurements overestimated an increase in SSN by ~5%, and that ultrasound measurements obtained at a neutral joint angle better predict SSN than measurements obtained in a stretched position. Past, present, and future studies should exercise caution when concluding a large magnitude of sarcomerogenesis from ultrasound-derived measurements of FL, and a correction factor may be warranted to more closely approximate the actual architectural changes.

SWAP Guidelines for conducting a Systematic Review with a Perspective: a novel approach for secondary analysis of published data

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Introduction: Secondary data analysis, also known as desk research, involves the post-hoc analysis of published data to investigate a novel scientific question or increase the power of statistical evidence. A systematic review and meta-analysis, often employed in clinical research, is a form of secondary analysis that synthesizes results from several homogeneous publications.

Unfortunately, a method for mining data from heterogeneous studies in support of a novel investigation is not established, despite its potential to drive scientific discovery. The implementation of this approach will demand new strategies and practices. Thus, a set of guidelines describing this method of secondary analysis is needed.

Methods: We propose an approach for secondary analysis that employs a systematic review (SR) methodology with the inclusion of a perspective lens (i.e., the novel scientific question to be investigated). The Systematic Review with a Perspective (SWAP) guidelines will serve as a resource for researchers to conduct this form of secondary analysis consistently, much like existing guidelines for traditional systematic reviews (e.g., PRISMA, SWiM, Cochrane handbook). The SWAP guidelines incorporate many of the same principles as these established tools but are not limited to the synthesis of studies investigating the same scientific question; they have been adapted to focus the systematic review methodology to conduct original research.

Results: Our proposed analytic method has been previously employed to explore alterations in cardiac health of the Spontaneously Hypertensive Rat model spanning approximately 7 decades of publications. This unique study was a longitudinal investigation into changes in cardiac function that could not have been accomplished with primary data collection.

Conclusion: The proposed SWAP guidelines provide instructions for search strategy development, study screening, data extraction and analysis, as well as drafting of the manuscript. Implementation of this novel approach will save researchers the valuable time and resources necessary to collect primary data, provide the opportunity for retrospective investigations, and will ultimately lead to further scientific discovery.

p107 mitochondrial function regulates satellite cell self-renewal by reprogramming metabolism through OPA1

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Skeletal muscle health is dependent on myogenic stem cells known as satellite cells (SCs). When activated, they become myogenic progenitors (MPCs) that differentiate into new muscle, or undergo self-renewal to replenish their population. The dysregulation of SC fate decisions is strongly implicated in the deterioration of muscle regeneration capacity, evidenced in neuromuscular degenerative diseases (NMD). Thus, it is pivotal to improve our understanding of the control mechanisms that dictate fate decisions to improve muscle regeneration potential. Our lab has recently published a role for Rb11 (p107) in controlling myogenic progenitor cell metabolism through a mitochondrial function. Now we find that SCs from p107 genetically deleted (p107KO) mice display higher rates of self-renewal. Intriguingly, p107 may influence SC fate decisions by changing the cellular metabolic profile. Our data shows that p107KO MPCs and activated myofiber SCs have a significantly increased mitochondrial fusion index marked by enhanced interconnectivity. The mitochondrial network phenotype in p107KO cells is associated with upregulation of the mitochondrial fusion protein OPA1. The mitochondrial function of p107 in fate choices is further highlighted through increased mitochondrial interconnectivity when p107 is sequestered in the cytoplasm. However, when p107 is mitochondrially localized, the mitochondrial network is fragmented. Taken together, these novel findings propose a function for p107 in regulating mitochondrial remodeling to promote SC self-renewal. Thus, p107 presents an innovative target to regulate SC self-renewal that would 1) provide more stem cells for regenerative medicine therapies and 2) offer a therapy against muscle degeneration for individuals suffering from NMD.

VCD-induced ovarian failure impairs muscle power in mice but impairments are partially reversed with high intensity interval training

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Menopause is known to impair muscle function. The occupational chemical 4-vinylcyclohexene diepoxide (VCD) induces gradual ovarian failure over 120 days and therefore approximates the natural trajectory of menopause over an accelerated timeline. This study used the VCD model of menopause to investigate the effects of ovarian failure on muscle function and the potential of exercise to mitigate impairments. Sexually mature female CD-1 mice were assigned to one of three groups: control, VCD-sedentary, or VCD-training. Once ovarian failure was achieved in the VCD-treated groups, the VCD-training group underwent 8 weeks of uphill high intensity interval training on a treadmill. Mice were sacrificed 176 days after injection, representing late menopause. To assess the effects of ovarian failure on maximal muscle force and power, single muscle fibres from the soleus (SOL) and extensor digitorum longus (EDL) muscles were dissected, chemically permeabilized, and tied between a force transducer and a length controller. A series of isotonic load clamps were applied following maximal activation (pCa 4.5). Absolute force was 19.2-20.6% and 29.7-40.6% lower in the VCD-sedentary group compared to the control and VCD-training groups for the SOL and EDL, respectively ($p < 0.05$). However, specific force (force/cross-sectional area) did not differ ($p > 0.05$). Accordingly, EDL cross-sectional area was lower in the VCD-sedentary group compared with the control and VCD-training groups ($p < 0.05$). For peak power, there was an interaction of group \times muscle ($p < 0.05$), as well as main effects of group and muscle, such that EDL muscles from the VCD-sedentary group were 45.4% less powerful compared to controls, and VCD-sedentary fibres produced significantly less power than controls across muscles ($p < 0.0001$). Higher peak power was achieved in EDL fibres compared to SOL fibres regardless of group ($p < 0.0001$). Power appeared to be greater in the VCD-training group than the VCD-sedentary group for both muscles, but these differences did not reach significance ($p > 0.05$). These findings indicate that impairments in muscle power following ovarian failure are likely driven by decreased force-generating capacity rather than changes in contractile velocity. Additionally, as indicated by the greater menopause-induced power loss for the EDL compared with SOL muscles, impaired muscle function owing to menopause may be fibre-type specific. This work helps to inform the mechanisms through which menopause affects the dynamic components of muscle function and indicates that exercise may be an effective treatment tool to combat these deleterious changes.

Investigating NLRP3 Inflammasome Activation in response to Chronic Denervation and Exercise Training

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Mitochondria are dynamic organelles that respond and adapt to a wide variety of stimuli which ultimately impact skeletal muscle health and functioning. The NLRP3 inflammasome complex is a component of the innate immune system which plays a vital role in the recognition and response to damage-associated molecular patterns (DAMPs), which can be produced through intracellular disruptions such as mitochondrial dysfunction. To investigate the role of the NLRP3 inflammasome complex and its response to changes in mitochondrial quality, we assessed the impact of chronic muscle disuse and exercise training on its activation. Mouse hindlimb muscle was collected from mice that underwent 7 days of chronic hindlimb muscle denervation and was used to investigate the effect of muscle disuse and the associated maladaptive mitochondrial adaptations. In addition, mouse gastrocnemius tissue was extracted from mice that performed 4.5 weeks of swim training consisting of 240 minutes per day to identify the impact of chronic endurance exercise on the activation of the NLRP3 inflammasome complex. Western blotting, cytochrome C oxidase (COX) activity, and qPCR analyses were used to identify the expression of key NLRP3 inflammasome proteins, mitochondrial markers, and mtDNA content. Following 7 days of denervation, there was

a significant reduction in COX activity ($p < 0.01$), which is indicative of reduced mitochondrial content. There was an accompanying significant increase in both NLRP3 and procaspase-1 protein expression ($p < 0.05$, $p < 0.001$). However, the ratio of immature: total caspase-1 was not affected. There were also no significant changes in whole muscle mtDNA content within the hindlimb muscle. In contrast, there was a decrease in the protein expression of NLRP3, procaspase-1, and active caspase-1 (53%, 30%, and 48%, respectively) in response to chronic endurance training, alongside a 26% increase in COX activity in comparison to the matched sedentary controls. These data demonstrate that mitochondrial content is inversely related to the activation of the NLRP3 inflammasome complex and downstream signaling in response to both beneficial and maladaptive mitochondrial adaptations within skeletal muscle.

Pulmonary edema leads to unexpected lung remodeling and is the sole cause of early exercise intolerance following myocardial infarction

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Background: Myocardial infarction (MI), one of the leading causes of death worldwide, decreases exercise capacity, which has a profound impact on patient quality of life and survival. Post-MI, the strongest predictor of mortality is the development of pulmonary edema, yet its pathophysiology has been largely uninvestigated in clinical and pre-clinical research. Furthermore, while exercise rehabilitation has emerged in the past decade as being important for patient recovery, exercise intolerance is largely attributed to the heart, and the impact pulmonary edema has on the lungs post-MI remains unknown. Therefore, our objective is to examine the pathophysiology of pulmonary edema post-MI and its impact on exercise capacity. We hypothesize that, post-MI, pulmonary edema will cause transient lung remodeling, resulting in hemoglobin desaturation and contributing to exercise intolerance.

Methods: MI was induced by permanent ligation of the left anterior descending coronary artery, and cardiac function was assessed by invasive hemodynamic analysis and echocardiography. Pulmonary edema was evaluated by the detection of audible rales, and lung remodeling was evaluated by histology. Hemoglobin saturation was recorded using a pulse oximeter at rest and during exhaustive treadmill testing, where exercise tolerance was assessed with and without supplemental oxygen. To achieve a complete temporal assessment of pulmonary alterations, animals were sacrificed at 1-, 2-, 4-, 9-, and 18-weeks post-MI. Results were analyzed using a two tailed t-test or one-way ANOVA where appropriate, and a p-value < 0.05 was significant.

Results: MI caused systolic and diastolic dysfunction at all time points, and transient pulmonary edema that peaked 1-week post-MI. Surprisingly, this was followed by profound pulmonary inflammation and fibrosis, lasting up to 4-weeks post-MI. Within the first 4 weeks following the MI, mice exhibited exercise-induced hemoglobin desaturation. While exercise intolerance was observed at all time points, exercise capacity was restored with supplemental oxygen at 1-, 2-, and 4- weeks post-MI, but not at 9 and 18 weeks.

Conclusion: Here we show MI causes pulmonary edema and profound lung remodeling, which in the first 4 weeks, is the sole cause for exercise intolerance. These findings emphasize the need for research focused on clarifying the interaction between cardiovascular and pulmonary systems post-MI, and determining the mechanism causing pulmonary edema and inflammation. We highlight that early exercise intolerance can be ameliorated by supplemental oxygen, which can easily be employed as a method for rehabilitation, and that there is an unmet clinical need for the treatment of pulmonary edema post-MI. Developing therapeutic strategies targeting the pulmonary system post-MI will not only increase exercise capacity and improve quality of life, but also decrease risk of mortality in a large patient population.

Refined Approach for Characterizing Cardiomyopathy-Associated Actin Variants to Optimize Novel Drug Dosage

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Cardiomyopathies are a form of heart disease associated with dysfunction of the ventricular myocardium. This dysfunction can cause a reduction in cardiac output, eventually leading to progressive heart failure. Two of the main forms of cardiomyopathies are dilated (DCM) and hypertrophic (HCM), where DCM is characterized by thinning of the left ventricular walls and an increase in left ventricular volume. Oppositely, HCM is characterized by thickening of the ventricular walls and a reduction in left ventricular volume. Mutations in sarcomere proteins are one of the most common causes of cardiomyopathies, with many actin mutations implicated in this disease. Characterization of these variant sarcomere proteins allows for determination of the molecular mechanisms causing HCM and DCM, and personalized treatment targeting these alterations. To characterize the impact of novel drugs on actin variant-based cardiac dysfunction, in vitro motility assays using fluorescent microscopy are conducted to characterize wildtype-actin sliding velocities. Human wildtype recombinant ACTC1 is produced in Sf21 insect cells using baculovirus containing the sequence of interest, while bovine cardiac β -myosin is purified from tissue. This work will contribute to the comprehensive characterization of activity disparities between the variant ACTC1 and wildtype proteins. This is of particular importance when considering the administration of novel drugs targeting the molecular mechanisms of cardiomyopathy. By measuring sliding velocities as an indirect indicator of cardiac power output, we can elucidate the activity profiles of novel cardiomyopathy drugs and determine optimal doses specific to each variant protein through dose response curves. These preliminary steps mark a substantial stride towards formulating individualized treatment strategies tailored to the causal genetic mutations, representing a highly promising achievement in alleviating the global burden imposed by this disease

Alterations in S6K1-IRS-1 signaling is uncoupled from whole-body insulin sensitivity in animals treated with leucine or ketoisocaproic acid

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Plasma levels of branched-chain amino acids and their metabolites, branched-chain ketoacids are increased in insulin resistance, a condition that can lead to type 2 diabetes mellitus. Questions remain as to whether elevated levels of these metabolites cause or are a consequence of insulin resistance. We previously showed that leucine and its metabolite KIC suppressed insulin-stimulated glucose uptake in L6 myotubes by increasing S6K1 activation and phosphorylation of an inhibitory serine IRS-1 residue. Leucine gavage has been shown to reduce whole-body insulin sensitivity. The objective of this study is to analyze the effect of KIC gavage on skeletal muscle and liver insulin signaling and whole-body insulin sensitivity. We hypothesize that KIC gavage will reduce whole-body insulin sensitivity and alter skeletal muscle and liver insulin signaling. Five-week-old male Sprague-Dawley rats were starved for 24 hours. Rats were then gavaged with 0.75ml/100g of water, leucine (22.3g/L) or KIC (30g/L) twice, ten minutes apart, and then either sacrificed at different timepoints post-gavage (0.5-3h). A different set of rats were starved for 6 hours and then injected with insulin an hour after the first of two gavages 10 minutes apart. Basal blood glucose levels and post insulin injection (10-120min after injection) blood glucose levels were measured. KIC and leucine had no effect on whole-body insulin sensitivity. This was consistent with no change in ph-Akt (S473) phosphorylation from leucine and KIC gavage. However, IRS-1 (S612) phosphorylation was significantly ($p<0.0001$) increased 30 minutes after leucine gavage in skeletal muscle and liver, but this was alleviated by the 60-minute mark, while KIC had no effect. S6K1 (Thr389, $p<0.001$) and S6 (S235/6, $p<0.0001$) phosphorylation were significantly increased only after 30 minutes of leucine gavage in skeletal muscle and liver, while KIC had no effect. Despite increased S6K1 threonine phosphorylation and IRS-1 serine phosphorylation in skeletal muscle and liver in response to leucine gavage, there was no effect on whole-body insulin sensitivity. Interestingly, KIC did not activate S6K1-IRS-1 pathway as seen in our L6 myotubes. Thus, although insulin resistant states exhibit increased plasma leucine and KIC levels, their supplementation does not reduce insulin sensitivity, suggesting that their increased levels are a consequence of insulin resistance and not causative.

The effects of gradual ovarian failure on time-course changes in muscle contractility: A mouse model of perimenopause and menopause

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Menopause is associated with impaired muscle contractility. The temporal and mechanistic bases of this dysfunction are unknown. Using a mouse model, we identified the effects of gradual ovarian failure on single muscle fiber contractility throughout early (i.e., perimenopause) to late-stage menopause. 30 mice were injected with 4-vinylcyclohexene diepoxide (VCD) to induce ovarian failure, following the injections mice were sacrificed on specific days ranging from perimenopause to late-stage menopause: D60, D120, D134, and D176. The soleus (SOL) and extensor digitorum longus (EDL) muscles were dissected and chemically permeabilized for single muscle fiber mechanical testing. For absolute force produced by the SOL there was an interaction of group \times time, with the VCD group producing 38% less force than CON at D134. There was also an effect of time within VCD such that absolute force was 40% greater at D60 than D120, as well as D120 was 33% and 39% greater than D134 and D176, respectively. When normalized to CSA specific force was 37% lower in VCD than CON at D134, but there were no differences at any other timepoint. For instantaneous stiffness there were no interactions or main effects for SOL, implying that a lower proportion of strongly-bound crossbridges may be driving the decrease in specific force observed at D134 between VCD and CON. For absolute force produced by the EDL, there were no interactions or effects of group, however there was an effect of time, such that, across groups, force produced at D60 was ~41% greater than at D176. When normalized to CSA there were no interactions or effects for specific force. For instantaneous stiffness there were main effects of time and group, such that within the VCD group, stiffness was 48% and 41% greater at D120 than D134 and D176, respectively. The increase in stiffness with no changes in specific force implies that the proportion of crossbridges remains the same, although they are likely in a weakly-bond state. In our mouse model of menopause, there was a fibre type dependent effect, with the SOL showing greater impairments than the EDL. This divergence across muscles highlights the importance of better approximating the natural trajectory of menopause on neuromuscular function.

Does fasting alter mRNA expression of novel regulators of mitochondrial biogenesis in human skeletal muscle?

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Different models of fasting have been suggested as promising interventions able to elevate health span, induce mitophagy, and enhance mitochondrial health, with the potential ability to prevent and combat age-related diseases like sarcopenia. However, data from studies investigating fasting-induced responses in human skeletal muscle are contradictory to the notion that fasting is an effective intervention to improve mitochondrial biogenesis. It is unknown whether fasting with and without increased whole-body energetic stress affects the expression of novel genes (*PPAR β* , *NR1D1*, *Perml*, *TFEB*, *ESRR γ* , and *NR4A1*) involved in the regulation of mitochondrial biogenesis in human skeletal muscle. Thus, we tested the hypothesis that fasting induce changes in these 6 emerging regulators of mitochondrial biogenesis in skeletal muscle and that these changes are amplified when whole-body energetic stress is increased. Leg muscle biopsies were obtained from nine healthy recreationally active males before, during (4 hours), and after (8 hours) two supervised fasts performed with (FAST+EX) or without (FAST) two hours of arm ergometer exercise (~400 kcal of added energy expenditure) to increase whole-body energetic stress. qPCR was performed on samples from each exercise session. Main effects of time ($p < 0.05$) were observed for *NR4A1* and *NR1D1* mRNA with post hoc revealing decreases in gene expression for both at mid and post. No effect of

group or interaction ($p > 0.05$) were observed for these 2 genes. We failed to observe significant effect of time, group, or interaction effects (all $p > 0.05$) for *PPAR β* , *PERM1*, *TFEB* or *ESRR γ* mRNA. These results indicate that: 1) there was no activation of these genes in human skeletal muscle during the first hours of fasting and 2) elevated whole-body energetic stress does not appear to maximize fasting-induced changes in mRNA expression of the novel regulators of mitochondrial biogenesis. Therefore, our findings suggest that fasting likely does not induce mitochondrial biogenesis via these genes. Future investigations using diverse types of fasting (intermittent, time-restricted, etc.) and multiple mitochondrial biogenesis markers (content and function) are required to determine whether fasting is an effective intervention to improve and/or induce mitochondrial biogenesis in human skeletal muscle from different populations (healthy, age-related diseases, neurodegenerative diseases, etc.).

Sex Differences in Cachexia Outcomes, Anabolic Signalling and BCAA Metabolism following Chemotherapy

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Affecting nearly 80% of cancer patients is cachexia, a body and muscle-wasting syndrome. Poor nutritional status, tumour related factors and chemotherapy contribute to cachexia. Although negative effects of chemotherapy on skeletal muscle are documented, the majority of studies are completed in male animals. Although the branched-chain amino acids (BCAAs) activate anabolic signalling in skeletal muscle and have been shown to reduce some of the effects of cachexia, BCAA nutritional support does not reverse cachexia and few studies have investigated the effects of chemotherapy on skeletal muscle BCAA metabolism. The objective of this study is to compare the effects of chemotherapy on cachexia outcomes and BCAA metabolism between male and female mice. Three-month-old CD2F1 male and female mice were treated with the chemotherapy drug cocktail folfiri (50mg/kg 5-fluorouracil (5FU), 90mg/kg Leucovorin and 24mg/kg CPT11) (drug) or vehicle (10% DMSO in saline) for 6-weeks. Within sex, drug treatment led to reductions in body and skeletal muscle weight. Between sex, drug-treated female mice lost more body and gastrocnemius weight compared to drug-treated males. Within sex, drug treated animals exhibited reductions in anabolic signalling, specifically S6K1 and S6. Between sex, drug-treated female mice showed greater loss of anabolic signalling compared to drug-treated males. Within sex, the drug cocktail reduced amino acid transporters SNAT1 and LAT1, while reducing skeletal muscle BCAA concentrations. Drug-treated female mice exhibited greater loss of LAT1 compared to male-drug treated mice. However, male-drug treated mice had greater decreases in BCAA concentrations compared to female drug-treated mice. Minimal differences were found within sex for key enzymes involved in BCAA metabolism. However, the activity of branched-chain alpha-ketoacid dehydrogenase complex (BCKD) was decreased following drug treatment. Lastly, within sex, drug treatment showed elevated levels of BCAA concentrations in the liver, but only male drug-treated mice showed elevated plasma BCAAs. Our data suggests a link between muscle atrophy and decreased BCAA metabolism during chemotherapy. Drug-treated female mice had worsened outcomes for body, skeletal muscle weight and anabolic signalling compared to drug-treated male mice. However, drug-treated males exhibited greater decreases in BCAA concentrations compared to drug-treated females. These findings suggest that altered BCAA availability and metabolism may contribute to muscle wasting differently in males and females during chemotherapy.

Epithelial ovarian cancer causes cardiac dysfunction and myofilament dysregulation

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Introduction: The intersections between cancer and cardiovascular disease have become increasingly relevant in the care of both patient populations. To date, the cardio-oncology field has focused on how cancer treatment influences cardiac health. Indeed, many cancer therapeutics exert toxic effects on the cardiovascular system. However, emerging evidence shows that cancer can impact cardiac structure and function in therapy-naïve patients. This suggests that cancer itself may impair cardiovascular function, yet the underlying mechanisms

remain unknown. Thus, we investigated cardiac structure and function in mice with early-stage epithelial ovarian cancer (EOC). We hypothesized that EOC will cause cardiac dysfunction independent of myocyte atrophy. **Methods:** We used an orthotopic, syngeneic mouse model of ovarian cancer. Briefly, murine ovarian epithelial cells from C57BL/6 mice (ID8; 1.0x10⁶), or saline for surgical shams, were injected under the ovarian bursa. In this model, 60 days after tumor induction, mice form large ovarian masses and numerous peritoneal lesions – consistent with clinical features of stage III (advanced) EOC. To determine whether cardiac abnormalities precede advanced disease, at 45 days post-tumor induction, mice were anesthetized, and left ventricular (LV) structure and function were assessed by echocardiography and invasive hemodynamics. Formalin-fixed hearts were stained with wheat germ agglutinin for cardiomyocyte cross-sectional area (CSA) or picrosirius red for interstitial fibrosis. To measure mitochondrial H₂O₂ emission, LV permeabilized fiber bundles were treated with pyruvate and malate to stimulate complex I to generate maximal rates of H₂O₂ emission. Then, ADP was introduced into the buffer, where data are expressed as a percentage of maximal levels. Myofilament activity was measured with an actomyosin MgATPase assay and protein phosphorylation was determined by SDS-PAGE with Pro-Q Diamond phosphoprotein staining. **Results:** EOC mice showed systolic and diastolic dysfunction, with no evidence cardiac atrophy or changes in interstitial fibrosis. Mitochondrial H₂O₂ emission increased, providing evidence of elevated reactive oxygen species (ROS) production in tumor-bearing mice. While there were no observed differences in myofilament ATPase activity, phosphorylation of regulatory myosin light chain 2 (MYL2) and cardiac myosin binding protein C (cMyBP-C) decreased, and tropomyosin (Tpm) phosphorylation increased in EOC mice. **Conclusion:** We show that EOC impairs cardiac function and alters the myofilament phospho-proteome – proteins critical for regulating cardiac physiology. Interestingly, the observed dephosphorylation of MYL2 and cMyBP-C and hyperphosphorylation of Tpm are consistent with features of heart failure. Impairments in cardiac function preceded myocyte atrophy, which often coincides with dysfunction in patients and models of cancer. EOC increased ROS levels in the myocardium, elucidating a possible mechanism whereby tumors promote cardiac stress and inflammation, leading to functional deficits.

Elucidating the role of Poly-ADP Ribosylation in Mature Skeletal Mass, Function and Homeostasis

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Poly-ADP-ribose polymerase-1 (PARP1/ARTD1) is an enzyme which cleaves NAD⁺ to covalently attach multiple ADP-Ribose moieties to target proteins in a process known as PARylation. In contrast, Poly-ADP-ribose glycohydrolase (PARG) is responsible for dePARylating proteins. This study is done to tease out the specific role of PARP1, PARG and the effects of PARylation on muscle metabolism and health in healthy mice. We have developed tamoxifen driven Cre-loxP system based inducible mature muscle-specific Parp1 and Parg knockout mouse models i.e., Parp1-iMKO and Parg-iMKO using human skeletal actin as a promoter (HSA). At 12 weeks of age, baseline measurement of muscle function such as grip strength, grip endurance and neuromuscular function were taken before tamoxifen treatment for gene deletion. Loss of Parp1 and Parg was validated two weeks post tamoxifen treatment using biochemical assays. Body weight was assessed weekly and muscle functional assessments for grip strength, grip endurance and neuromuscular function were measured monthly for 16 weeks prior to tissue collection. Total energy expenditure and locomotor activity were monitored between 11 to 12 weeks post tamoxifen treatment in both the cohorts and the WT animals. The body composition, metabolic activity and functional assessment tests suggested no differential regulation in the muscle function or energy metabolism in the Parp1 and Parg - iMKO mouse cohorts compared to the control animals. The findings from this study may help in delineating the role of PARylation in whole body metabolism by solidifying the phenotype of muscle following induction of increased or decreased levels of PARylation.

Investigating the effects of Pannexin-1 (PANX1) channel inhibition on contractile function in skeletal muscle

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Pannexin (PANX) hemichannels are pore-forming proteins expressed in many tissues and are implicated in diverse physiological functions. In musculoskeletal tissues, the Pannexin 1 (PANX1) isoform is the most abundant, though the expression of the three variants vary across the developmental timeline. PANX1 permeability permits the diffusion of molecules across the plasma membrane, including ATP, which interacts with extracellular purinergic receptors and may modulate intracellular processes in an autocrine fashion. Presently, we are investigating the role that PANX1 channels may play in modulating the mechanical function of skeletal muscles via acute changes in intracellular Ca²⁺ homeostasis and excitation-contraction coupling. Although the mechanosensitivity of PANX1 channels has been previously established in response to evoked contractions, the mechanism by which PANX1 may act as a modulator of contractile function is unclear. Moreover, there is little understanding about how skeletal muscle phenotype and sex differences may influence the relative expression of PANX1. Intact soleus (SOL) and extensor digitorum longus (EDL) muscles from male and female C57BL/6 mice (~12-16 weeks) were studied in vitro, with and without treatment with 100µM Spironolactone, a pharmacological inhibitor of PANX1. All muscles in the control (CON) and spironolactone (SPN) groups were subjected to a standardized contractile protocol, including both isometric and concentric stimulations that were customized to each muscle type. The sixty repeated isokinetic contractions (1/s) included a brief stimulation (~100 ms) where muscle length was shortened from 1.05 to 0.85 of optimal length (Lo) at ~40% of the maximal shortening velocity (V_{max}) for each muscle type. Isometric twitch and tetanic forces were measured prior to and following the repeated dynamic contractions to quantify baseline contractile function, fatigue, and the potentiation of low frequency twitch responses. Preliminary findings include a depressive effect of SPN treatment on maximal force production in EDL muscles vs. CON but not in SOL muscles. Consistent with this was a ~15% decrease in repeated dynamic force production for EDL muscles in the SPN group. However, a similar degree of potentiation of the contractile response was apparent in EDL muscles from both groups during dynamic stimulation in spite of the depression in maximal tetanic force output. These preliminary findings will be followed by subsequent analysis of PANX1 protein expression in homogenates from EDL and SOL muscles from both male and female mice. The current work seeks to validate the efficacy of PANX1 inhibitors for use in intact muscle preparations, and to determine whether differences in PANX1 expression across muscle phenotypes may be associated with their general contractile function in mice.

The influence of 17β-Estradiol on myosin regulatory light chain phosphorylation and in vitro concentric force potentiation of C57BL/6 mouse fast twitch skeletal muscle

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Menopause is associated with an increased susceptibility to losses of skeletal muscle mass and strength, increasing the risk of falls, disability, and mortality of post-menopausal females. Estrogen hormones have been implicated in influencing skeletal muscle contractile function and specifically, the interactions between myosin and actin that facilitate contraction. Myosin regulatory light chain (RLC) phosphorylation (RLC-p) modulates contractile function via posttetanic potentiation (PTP), i.e., the short-term increase in muscle force observed following stimulation, a phenomenon that is highly characteristic of the mammalian fast-twitch skeletal muscle phenotype. The purpose of this study was to examine the influence of ovarian hormone deficiency and 17β-Estradiol (E2) replacement on PTP of concentric twitch force and myosin RLC-phosphorylation, of mouse fast twitch skeletal muscle. To this end, 4-month-old female wildtype C57BL/6 mice were allocated to the ovariectomized (OVX), ovariectomized with E2 replacement (OVX+E), or sham-ovariectomized (SHAM) condition groups (n=8 mice). Extensor digitorum longus (EDL) muscles were surgically extracted from mice of each condition and mounted for

in vitro contractile experiments at 25° C. Brief tetanic stimulation significantly increased myosin RLC phosphorylation and concentric twitch force ($p < .05$) of muscles from OVX, OVX +E, SHAM mice, with no significant differences in PTP between conditions ($p > .05$). In contrast to this, specific force was significantly less of muscles from OVX mice compared to OVX+E and SHAM mice in both the unpotentiated (~ 25% less) and potentiated (~ 29% less) state ($p < 0.001$). Muscle power expressed relative to muscle wet weight was significantly increased following brief tetanic stimulation across conditions, and absolute power generation was ~ 40% greater of muscles from OVX+E and SHAM mice compared to that of OVX mice ($p < .001$). With respect to fatigue, a significantly greater drop in relative tetanic force amongst 4 consecutive tetani (2.5s apart over 10s) was observed of muscles from OVX mice compared to that of OVX+E and SHAM mice ($p < 0.05$). Our data are contrary to the primary hypothesis that E2 influences PTP and myosin RLC-phosphorylation in adult mouse fast twitch skeletal muscle but may suggest an influence of OVX and E2 on skeletal muscle quality. While findings utilizing the OVX mouse model are not directly transferrable to that which occurs during the gradual ovarian senescence during human menopause, these findings support continued investigation into the influence of ovarian hormones on skeletal muscle function and fatigue susceptibility.

Sex Differences in Heart Rate Variability during Metaboreflex Activation

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Group IV muscle afferent nerves relay metabolic feedback about the exercising muscle to the brainstem, in order to stimulate increases in sympathetic activity for cardiorespiratory adjustment to meet the demands of exercise. Women have been shown to experience blunted metaboreflex activation compared to men (Jarvis et al., 2011; Joshi & Edgell, 2019; Minahan et al., 2018; Samora et al., 2020), which may derive from altered autonomic balance. Since heart rate variability (HRV) estimates sympathetic and parasympathetic outflow, this study aims to determine if there are sex differences in HRV during metaboreflex activation. High frequency (HF) power and greater RR interval variability (SDRR) represent cardiac parasympathetic outflow, whereas the ratio between low frequency (LF) and HF power (LF/HF) represents cardiac sympathetic outflow. Thirty-two young, healthy men ($n=16$) and women ($n=16$) were recruited to perform 2mins of isometric handgrip at 40% of their maximal voluntary contraction followed by 3mins of post-exercise circulatory occlusion (PECO). ECG was continuously recorded, and HRV analysis was determined using 3mins of ECG at baseline or PECO. In response to PECO, heart rate (Women 71 ± 12 bpm vs. 73 ± 13 bpm; Men 68 ± 12 bpm vs. 72 ± 13 bpm; $p=0.026$) and mean arterial pressure (Women 84 ± 8 mmHg vs. 93 ± 10 mmHg; Men 85 ± 9 mmHg vs. 97 ± 11 mmHg; $p<0.001$) increased equally in both sexes. There were no significant differences in SDRR, root mean square standard deviation, and total power due to sex or PECO (all $p>0.07$). At all timepoints and compared to men, women had 1) significantly lower LF power (Women 19 ± 10 nu vs. 24 ± 16 nu; Men 42 ± 21 nu vs. 49 ± 17 nu; $p<0.001$), 2) higher HF power (Women 78 ± 9 nu vs. 74 ± 16 nu; Men 57 ± 20 nu vs. 50 ± 17 nu; $p<0.001$), 3) higher proportion of RR intervals differences greater than 50ms (Women $60 \pm 21\%$ vs. $58 \pm 22\%$; Men $39 \pm 16\%$ vs. $35 \pm 20\%$; $p=0.003$), regardless of time ($p>0.2$), and 4) lower LF/HF (Women 0.26 ± 0.17 vs. 0.41 ± 0.42 ; Men 1.01 ± 0.90 vs. 1.34 ± 1.30 ; $p=0.002$). Unlike previous findings, no sex differences were observed in the pressor response to metaboreflex activation. HRV did not differ in response to PECO in both sexes; however, women had greater cardiac parasympathetic dominance than men.

Sex-specific differences in a murine model of cardiac cachexia

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While several sexual dimorphisms have been linked to heart failure (HF) incidence and severity, sex-specific differences in cardiac cachexia remain elusive. In this study, we used a mouse model to analyze body weight,

muscle mass, and gene expression to characterize the phenotype associated with cardiac cachexia. To test the hypothesis that a sexually dimorphic phenotype exists in this disorder, male and female C57BL/6 mice were injected with either Monocrotaline (MCT) or saline for 8 weeks. Body weight was recorded weekly throughout the study and normalized to tibia length. We performed treadmill fatigue testing to assess exercise tolerance. Upon euthanasia, the heart, tibialis anterior (TA), gastrocnemius, and soleus were dissected, weighed and frozen. We performed histological analyses for the heart and TA and quantified the myofiber CSA. Analysis of raw body weight over the study period displayed a significantly greater weight in control than MCT-treated males from weeks 4-8. Conversely, no significant differences were found for muscle and heart weights or exercise tolerance for either male or female groups. In the female group, there was a decreased TA myofiber CSA in response to MCT. Gastrocnemius muscles were subject to genetic analysis, and no significance was seen in MyoD expression between either group. These results suggest that while a systemic effect may be occurring in response to MCT, muscle involvement remains inconclusive. Similarly, the lack of cardiac hypertrophy in the MCT-treated groups suggests that further research should be pursued using differential doses of MCT to optimize this model for inducing HF.

Influence of gene expression and muscle atrophy in a murine model of cardiac cachexia

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While several sexual dimorphisms have been linked to heart failure (HF) incidence and severity, sex specific differences in cardiac cachexia remain elusive. In this study, we used a mouse model to analyze body weight, muscle mass, and gene expression to characterize the phenotype associated with cardiac cachexia. To test the hypothesis that a sexually dimorphic phenotype exists in this disorder, male and female C57BL/6 mice were injected with either Monocrotaline (MCT) or saline for a total of 8 weeks. Body weight was recorded weekly throughout the study and normalized to tibia length. Upon euthanasia, the heart, tibialis anterior, gastrocnemius, and soleus were dissected, weighed and frozen. Analysis of raw body weight over the study period displayed a significantly greater weight in control than MCT-treated males from weeks 4-8. Similarly, male control normalized body weight was significantly greater than male MCT mice at weeks 7 and 8 only. Lastly percentage body weight change of male control mice was greater than male MCT from weeks 6 through 8. No significance was seen between female control and female MCT mice for these measurements. Conversely, no significant differences were found for muscle or heart weights for either male or female groups. Gastrocnemius muscles were subject to molecular analysis and no significance was seen in MyoD expression between either group. These results suggest that while a whole-body effect may be occurring in the male MCT mice, muscles are not involved. Similarly, the lack of heart hypertrophy in the MCT-treated groups suggests that further research should be pursued to determine the correct dosage of MCT that is suitable for inducing heart failure.

The Effects of Resistance Exercise Training on Insulin Resistance Development in Female Rodents with Type 1 Diabetes

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The etiology of insulin resistance (IR) development in type 1 diabetes (T1D) remains unclear; however, impaired glucose metabolism in skeletal muscle may play a role. While IR development has been established in male T1D rodents, female rodents have yet to be examined in this context. Resistance exercise training (RT) has been shown to improve IR and is associated with a lower risk of hypoglycemia onset in T1D compared to aerobic exercise. Additionally, the molecular mechanisms mediating RT-induced improvements in insulin sensitivity remain unclear. Therefore, the purpose of this study was to investigate the effects of RT on IR development in female T1D rodents. Forty Sprague-Dawley 8-week-old female rats were divided into four groups: control sedentary (CS;

n=10), control trained (CT; n=10), T1D sedentary (DS; n=10), T1D trained (DT; n=10). Multiple low-dose Streptozotocin injections (20 mg/kg each day for 7 consecutive days) were used to induce T1D. Blood glucose levels were maintained in normal range (4-9mmol/L) with intensive insulin therapy (one implanted insulin pellet; 2IU/day). CT and DT underwent weighted ladder climbing 5 days/week for 6 weeks. Intravenous glucose tolerance tests (IVGTT) were conducted on all animals following the 6-week period. Results demonstrate that DS animals exhibited significantly increased weekly blood glucose measures compared to all groups including DT ($p<0.0001$), despite similar insulin dosage levels. This was concomitant with a significant increase in insulin-adjusted area under the curve following IVGTT in DS ($p<0.05$), indicative of a reduction in insulin sensitivity. Both DT and DS exhibited greater serum insulin concentrations compared to CT and CS ($p<0.05$). DS animals also exhibited significantly greater glycogen content in white gastrocnemius muscle compared to all groups ($p<0.05$) whereas DT and DS animals exhibited greater p-Akt:Akt ratio in white vastus lateralis muscle compared to CS and CT ($p<0.05$). These results indicate that female rodents with T1D develop poor glycemic control and IR which can be attenuated with RT, possibly related to differences in intramyocellular glycogen content. This data supports the negative role of elevated muscle glycogen content on insulin sensitivity in T1D and the potential role of RT in ameliorating these metabolic changes.

The transcription factor ATF4 is responsive to mitochondrial stress following an acute bout of contractile activity

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Skeletal muscle relies on efficient mitochondria to produce energy and support its metabolic flexibility. The quality of the mitochondrial pool is regulated by coordinated mitochondrial quality control (MQC) processes. One MQC pathway that has garnered interest is the integrated stress response (ISR), which is activated in response to a variety of stimuli. The transcription factor ATF4, the main effector of this pathway, serves to ameliorate cellular stress by upregulating cytoprotective genes, such as CHOP and ATF5. Recent literature has shown that the ISR is activated upon mitochondrial stress to improve organelle function and health. However, it is unknown how rapidly ISR activation takes place in response to acute exercise-induced stress in vivo. To investigate this, we used a mouse model of in situ hindlimb muscle contractions at 0.25-1 tetani/second for a total of 9 mins and extracted the muscle or allowed for a 1-hour recovery period. We observed robust 2-fold increases in the mRNA expression of ATF4 and CHOP, however, ATF5 expression was unchanged in response to acute stimulation. These increases were further enhanced following the recovery period, and they appeared to be independent of transcriptional activation, as assessed using electroporation of an ATF4 promoter-reporter plasmid. The total protein expression of ATF4 was unchanged, but contractile activity increased ATF4 localization to the nuclear fraction. While ATF5 protein remained unchanged, CHOP protein increased by 1.6-fold with contractile activity and returned to control levels following recovery. Additionally, the initial stages of ISR activation, reflected by the ratio of phosphorylated to total-eIF2 α protein, was increased in response to our acute bout of stimulation in the recovery phase. RNA sequencing analysis of mouse muscle following an acute exhaustive bout of exercise also confirmed the increase in related ISR genes following exercise. Our data suggest that acute contractile activity initiates processes that promote ISR activation at an early stage, including 1) upregulation of ATF4 mRNA, and 2) initiation of ATF4 translation, and 3) translocation of ATF4 to the nucleus for downstream transcriptional regulation of ISR-responsive genes.

How Different Insulin Administration Routes Effect Liver Glycogen Levels after an Aerobic Exercise Bout in Male Rats with Type 1 Diabetes

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In a non-diabetic individual, their beta cells of the pancreas secrete insulin when receptors (primary ones located in the liver) signal for its production. The secretion of this insulin inhibits glucagon release, ensuring glucose stays within the cell. This pathway also promotes glucose storage as glycogen in the liver. People with type 1 diabetes cannot make insulin endogenously, so rely on exogenous insulin such as insulin patches. These patches deliver insulin subcutaneously, directly into the bloodstream. This delivery method does not resemble the physiologic insulin secretion pathway. Through exogenous insulin, receptors in the liver are not activated as significantly. Furthermore, the physiologic inhibition of glucagon secretion by insulin is impaired, resulting in portal vein glucagon-to-insulin ratio increasing. A small increase in this ratio dramatically reduces hepatic glycogen accumulation. This is a significant issue because mobilization of hepatic glycogen is the primary mechanism that counters hypoglycemia. Thus, decreased hepatic glycogen might be an important predisposing factor for the development of severe hypoglycemia in people with type 1 diabetes. The omental pouch is an attractive alternative site for insulin administration because it is highly vascularized with portal venous drainage which more closely resembles the native physiological insulin secretion pathway that regulates blood glucose levels. The current project will analyze whether subcutaneous insulin administration compared to omental pouch insulin administration has any effect on liver glycogen levels in male rats with type 1 diabetes. The paper will also investigate whether the location of insulin administration affects the drop in blood glucose levels experienced by type 1 male diabetic rats post moderate aerobic exercise. Throughout the 12 week study, weekly blood glucose samples will be taken. Insulin levels will also be measured twice via an enzyme-linked immunosorbent assay (ELISA). Finally, the rat's livers will be analyzed for glycogen content and glycogen synthase levels using a glycogen assay and a western blot, respectively.

Investigating the role of LOX and LOXL enzymes in Atrial Fibrillation Pathogenesis

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Atrial fibrillation (AF) is the most common supraventricular arrhythmia characterized by random atrial excitation leading to asynchronous atrial contractions and very rapid and irregular ventricular pumping. AF predisposes to stroke and heart failure. AF incidence increases with age and is promoted by cardiovascular disease, especially heart valve malfunction. AF is associated with fibrosis, hypertrophy and altered electrical properties (remodeling) as well as elevations in innate immune response and oxidative stress in the atria.

Although cardiac fibrosis is invariably associated with elevated collagen gene transcription and collagen production, preliminary studies failed to identify an increase in collagen gene transcription levels in two mouse models of AF (i.e. volume overload induced by aortic regurgitation (AR) or chronic endurance exercise). Since AF is strongly associated with aging and aging-related fibrosis is linked to elevated lysyl oxidase (LOX) activity (a collagen stabilizing enzyme) without elevations in collagen gene transcription, I am examining the role of LOX in our AR model of AF. We aim to characterize the cellular origins of the LOXL isoforms and determine their expressional patterns through immunohistochemistry.

Knowing there is an innate immune response with AF and that remodeling, increased AF susceptibility and elevated F480⁺ (macrophages and monocytes) cells are seen at 4-weeks post AR, we wanted to explore the expression of the LOXL isoforms from F480⁺ cells in all chambers of the heart. At 4-weeks ~76% of F480⁺ cells in the LA are expressing LOXL3, with no change compared to naïve values (P>0.999). No difference was observed

relative to naïve values in the LV, RA, and RV ($P > 0.999$). LOXL3 expression was also seen in non-immune cell (F480⁺CD45⁻). The total count of colocalized cells in the LA, noted by the overlap of F480⁺/LOXL3⁺ signaling, increased at 1-week (105.1 ± 8.34 colocalized cells (cc) / mm²), dip at 2-weeks (68.85 ± 18.15 cc / mm²) and increase again at 4-weeks with chronic volume overload (119.71 ± 17.54 cc / mm²) compared to naïve hearts (102.57 ± 4.24 cc / mm²) ($P < 0.0001$). This is contrary to the LV in which there is a steady decline through 4-weeks ($P > 0.999$), and the RA ($P < 0.0001$) and RV ($P = 0.3935$) in which there is a steady incline through 4-weeks. We can conclude that each of the chambers has a different response to chronic volume overload regarding LOXL3 expression from F480⁺ cells. As well, that there may be a time dependent F480⁺ cell phenotypic switch, consistent with previous hypertension studies; currently being explored.

Examining the sex differences in substrate utilization during threshold-based exercise prescription

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The traditional “relative percent method” (RPP) of prescribing exercise (eg. % of maximal oxygen uptake [%VO₂max], % of max work rate [%WRmax], etc.) elicits sex specific amounts of metabolic stress and ratios of carbohydrate to fat oxidation that result in a lower steady-state respiratory exchange ratio (RER) in females [1,2,3,4]. In this study we examined sex-differences during threshold-based exercise prescribed (TBP) using lactate threshold (LT) and critical power (CP) [5]. LT is the workload beyond which blood lactate increases rapidly, past 2.0 mmol/L [5]. CP is the workload that represents the highest oxidative metabolic rate that can be sustained and the intensity at which the accumulation of metabolic biproducts reaches steady state [5]. The purpose of this study is to determine whether using TBP eliminates differences in substrate utilization during exercise between sexes, specifically at an intensity anchored halfway between LT and CP ($\Delta 50$). 13 females (22.0 ± 2.6 years; VO₂max 41.7 ± 5.2 ml/kg/min) and 13 males (22.0 ± 2.5 years; VO₂max 47.3 ± 4.5 ml/kg/min) completed preliminary VO₂max, LT and CP tests and were then prescribed exercise at $\Delta 50$ for a 45 minute ride. Baseline sex-differences between levels of oxidative capacity, fiber type proportion, capillarization, LT and CP were tested and analyzed. RER between females and males was not significantly different when exercising at $\Delta 50$ indicating similar substrate utilization between sex when prescribed exercise using TBP. There was no significant difference between the LT and CP thresholds as a percent of max in males and females. However, LT and CP as a percent of WRmax relative to percent of fat free mass (%FFM) were significantly higher in female than males. This aligns with previous literature as CP is positively correlated with lean muscle mass and muscle cross sectional area, although the significance of the sex differences is unclear [6,7]. In conclusion, our preliminary results show that TBP exercise may eliciting similar substrate utilization and adaptations in both sexes.

Time-dependent changes to skeletal muscle macrophage redox homeostasis in Myositis

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Introduction: Myositis is a rare autoimmune mediated disease, resulting in muscle weakness and dysfunction. While myositis has been associated with an increase in muscle fibre mitochondrial reactive oxygen species H₂O₂ emission, the role of this stress response in muscle-resident macrophages (a prominent immune cell) has yet to be explored. The purpose of this research is to elucidate the muscle and time dependent relationship between myositis-induced myopathy and changes in macrophage mitochondrial superoxide production and total cellular reduced glutathione, and to observe any differences in the immune response to the model. **Methods:** 12-16 week old female BALB/c mice received multiple injections with rabbit skeletal muscle-derived myosin and were assessed at 21, 28

and 49 days (d) post final injection as follows: 21d, 3 injections at day 0, 7, 14; 28d and 49d, 4 injections at day 0, 7, 14, 21. Control groups were comprised of either a vehicle (glycerol+saline- GS), or a vehicle and an adjuvant (glycerol+Complete/Incomplete Freund's Adjuvant (CFA/IFA) – GCI). The experimental group (M) received rabbit-derived skeletal muscle myosin emulsified with CFA/IFA to induce a model of experimental autoimmune myositis (EAM). These adjuvants were used to stimulate a stronger immune response to the myosin antigen. Following tissue collection, flow cytometry was used to assess macrophage content and characteristics (superoxide production and total glutathione content) in two hindlimb muscles. Results: At 49d, resident macrophages from the gastrocnemius demonstrated a lower proportion of monobromobimane (mBBR; reduced glutathione tag) mean fluorescence intensity (MFI) in M relative to GS (-84.97%, $p=0.005$). There were no differences in MitoSOX fluorescence (superoxide probe) between any groups. Additionally, there were no differences in macrophage MitoSOX and mBBR fluorescence in the soleus muscle. Analysis of muscle and organ wet weights did not reveal significant differences between the control and experimental groups. Conclusion: The findings suggest that the reduction in GSH-positive cells is due more to adjuvant-induced inflammation rather than a response to myosin itself. Remaining analyses will relate these findings to the time-dependent and muscle-specific force and histological responses to the adjuvant and myosin antigen.

Redox-mediated protein glutathionylation is regulated by mitochondria to promote muscle stem cell function

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Skeletal muscle contains resident stem cells (MuSCs), which reside in a quiescent state. Upon injury MuSCs activate, enter the cell cycle, proliferate and ultimately differentiate and fuse to repair damaged muscle tissue. Although the mechanisms that dictate a quiescent versus active state are not fully elucidated, we recently uncovered that mitochondrial structure and function are implicated. Specifically, physiological mitochondrial fragmentation and an associated increase in mitochondrial reactive oxygen species (mtROS) occurs at the onset of MuSC activation. Here we provide evidence that redox-mediated signaling may occur through induction of protein glutathionylation (PSSG) as a post-translational modification. Presently, the mechanism by which mtROS mediate intracellular signaling to influence MuSC biology remains undefined. Our studies show that upon mitochondrial fragmentation in MuSCs, there is a redox-associated increase in glutathione synthesis and PSSG. We explored the kinetics of PSSG during the onset of MuSC activation and uncovered a time-dependent increase in PSSG. To elucidate whether this increase in PSSG was required for MuSC activation, we inhibited PSSG with ethacrynic acid, an inhibitor of glutathione-S-transferase (GST-i) and found a blunted induction of the myogenic commitment marker, MyoD, in Pax7+ MuSCs. To uncover whether PSSG is related to mitochondria morphology, we utilized mice lacking the mitochondrial fusion gene OPA1 (OPA1-KO). Interestingly, MuSCs from OPA1-KO have elevated basal PSSG, and GST-I reduced the percent of MyoD+/Pax7+ cells, which is higher at baseline in OPA1-KO MuSCs. Cumulatively, these findings indicate that mitochondrial fragmentation elevates PSSG in MuSCs, a redox-mediated PTM that is integral for their activation

The role of TFE3 in mediating skeletal muscle mitochondrial adaptations to exercise training

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TFE3 is a transcription factor that activates the expression of lysosomal genes involved in the clearance of dysfunctional mitochondria, termed mitophagy. With exercise, TFE3 is presumed to optimize the mitochondrial pool through the removal of organelles via lysosomes. However, the molecular mechanisms of the involved

pathways remain unknown. Therefore, the effects of TFE3 on exercise-induced mitochondrial turnover are yet to be understood. Wild-type (WT) and whole-body TFE3 knockout (KO) mice were subjected to 6 weeks of voluntary wheel running as an endurance training regimen. This was followed by a 45-minute bout of in situ stimulation of the sciatic nerve innervating hindlimb muscles to evaluate muscle fatigue and contractile properties. Mitochondrial function and lysosomal protein content was assessed. A subset of animals was treated with colchicine to measure autophagy and mitophagy flux. We hypothesized that loss of TFE3 would impair skeletal muscle mitochondrial adaptations to endurance training, reducing mitochondrial function and impairing muscle performance during in situ stimulation. TFE3 KO mice presented increased fat mass and decreased relative muscle weights compared to WT mice. Fatigability during stimulation was reduced with training in WT animals, as seen by a 13% increase in percent of maximum force at 5 minutes of stimulation, and a 30% increase at 30 minutes. Permeabilized fiber oxygen consumption was also improved with training. Concurrent with improved muscle and mitochondrial function, COX activity and COX I protein expression were increased in trained WT animals compared to untrained animals, signifying an increase in mitochondrial content. These adaptations were abolished with the loss of TFE3. Surprisingly, untrained TFE3 KO animals experienced a greater induction of mitophagy flux with acute stimulation compared to their WT counterparts. Training blunted this mitophagic response in KO mice. Our results suggest that the loss of TFE3 compromises beneficial training adaptations leading to improved muscle endurance and mitochondrial function, and potentially induces a greater sensitivity to mitophagy induction. This work will further our understanding of the role of TFE3 in mitochondrial mechanisms underlying training adaptations and may establish this transcription factor as a potential therapeutic target.

Notes

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