15th Annual
Muscle Health Awareness Day
May 17, 2024

Program and Abstracts

Muscle Health & Research Centre

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HOUDLABS

15th Annual Muscle Health Awareness Day Program
Friday May 17, 2024
Muscle Health Research Centre (MHRC)
Life Science Building South Lobby and Room 103, York University

8:15 – 8:55 Registration, poster mounting, and light breakfast

Session 1: Skeletal Muscle, Cardiorespiratory and Cardiovascular Physiology (8:55-10:30)

Session Chair: Dr. Tara Haas, MHRC

8:55-9:00 – Dr. Christopher Perry, *Director of the MHRC, York University*
Welcome and Introduction

9:00-9:30 – Dr. Nicholas Dumont, *Associate Professor, Université de Montréal*
Underlying mechanisms of muscle stem cell myopathies and therapeutic avenues

9:30-10:00 – Dr. Daniel Keir, *Assistant Professor, University of Western Ontario*
Peripheral chemoreflex contributions to exercise hyperpnea: are they important?

10:00-10:30 – Dr. Robert Bentley, *Assistant Professor, University of Toronto*
Cardiovascular Contributions to Skeletal Muscle Oxygen Delivery

10:30 – 11:15 Poster Viewings and Break (Life Science Building South Lobby)

Session 2: Lived Experiences in Sport Participation and Athletic Rehabilitation for Older Adults (11:15-12:10)

Session Chair: Dr. Michael Paris, MHRC

11:15-11:45 – Dr. Julia Creet, *Professor, York University, Athlete and Filmmaker*
The Aging Athlete

11:45-12:10 – Michael Modica, *PhD Candidate, York University, Certified Athletic Therapist*
Navigating the Roadblocks: Rehabilitation Strategies for Mature Athletes

12:10 – 2:00 Catered Lunch (Life Science Building South Lobby); 12:50-2:00 Poster Presentations

Session 3: CSEP- sponsored Exercise, Nutrition and Appetite Symposium (2:00-3:55)

Session Chair: Dr. Olasunkanmi Adegoke

2:00-2:15 – Zach Weston, *CEO, Canadian Society for Exercise Physiology*
Applied Exercise Science from the lab to the podium: Integrating foundational science for the exercise professional

2:15-2:45 – Dr. Tom Hazell, *Associate Professor, Wilfrid Laurier University*
Appetite (dys)regulation and exercise: implications for obesity

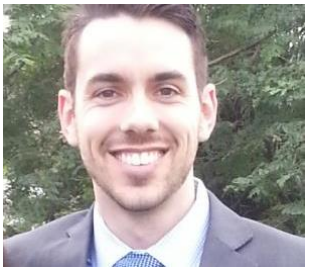
2:45-3:15 – Dr. Lora Giangregorio, *Professor, Schlegel Research Chair in Mobility and Aging, University of Waterloo*
New exercise guidelines and research on exercise for fracture prevention

3:15-3:45 – Dr. Andrea Josse, *Associate Professor, York University*
Whole-food dairy and exercise for musculoskeletal health: acute and chronic effects

3:45-3:55 – Poster Awards Presentation

3:55- 4:00- Concluding Remarks

15th Annual Muscle Health Awareness Day
Speaker Research Profiles



Dr. Robert Bentley, University of Toronto

Robert Bentley completed his PhD in cardiovascular physiology in the School of Kinesiology & Health Studies at Queen's University in 2016. His doctorate was followed by a postdoctoral position, during which he conducted research in the laboratories of KPE Professor Jack Goodman and Associate Professor Susanna Mak of the Faculty of Medicine at the University of Toronto. His overarching research goal is to is to understand how individuals match oxygen delivery to oxygen demand to help inform strategies and interventions to improve exercise performance, exercise tolerance and quality of life across the health spectrum.

Dr. Julia Creet, York University

JULIA CREET, B.A., History, University of Victoria, M.A. History and Philosophy of Education, University of Toronto, Ph.D., History of Consciousness, UC Santa Cruz. Prof. Julia Creet is a leading international scholar in Cultural Memory Studies having been involved in the development of the field since the 1990s. Prof. Creet's research projects are broadly interdisciplinary spanning the Humanities and the Social Sciences including the history of the Holocaust, literary studies, film studies, archival studies, public history, data privacy and direct-to-consumer genetics.

Memory and Migration: Multidisciplinary approaches to memory studies, co-edited with Andreas Kitzmann (UTP 2010, reissued in paper in 2014) is held by 925 libraries worldwide (Worldcat) making it one of the foundational texts in the field of Memory Studies. H.G. Adler: Life, Literature, Legacy, (Northwestern UP 2016) co-edited with Sara Horowitz and Amira Dan, won the Jewish Thought And Culture Award from the Canadian Jewish Literary Awards. Her forthcoming The Genealogical Sublime (University of Massachusetts Press, 2019) is a crossover academic/trade book that traces the cultural, historical and corporate histories of the longest, largest, and most profitable genealogy databases in the world.

	In 2017, Julia Creet received a York Research Leader Award in part for her leadership in public engagement. In addition to her scholarship, Creet has also produced and directed two documentary films. MUM: A Story of Silence (38 min 2008) is a personal documentary about a Holocaust survivor who tried to forget. That engagement with family history led to a documentary investigating the cultural and technological zeitgeist of genealogy itself. Data Mining the Deceased: Ancestry and the Business of Family (56 mins 2017, HD) has aired multiple times on TVO to over 300,000 viewers and is now streaming on demand in Canada, the UK, the US, India and Australia. Creet's nonfiction and journalism has featured in The Conversation, The National Post, Reader's Digest, Toronto Life, Exile, Border/Lines and West Coast Line.
	**Dr. Nicolas Dumont,** Universite de Montreal Dr. Nicolas Dumont obtained his PhD at the Université Laval where he studied the regulatory network between inflammatory cells and skeletal muscles. He did his post-doctoral training at the Ottawa Hospital Research Institute in Dr. Michael Rudnicki's lab, where he studied muscle stem cell defects in Duchenne Muscular Dystrophy. Dr. Dumont became an assistant professor at the Université de Montréal in 2016, and he established his lab at the Sainte-Justine hospital research center. His research program is divided in 3 axes: 1) characterizing the intrinsic mechanisms regulating muscle stem cell fate decision during myogenesis, 2) characterizing the impact of rare genetic variants on muscle stem cell function, and 3) investigating novel therapeutic avenues targeting defective muscle stem cells to mitigate muscular dystrophies. Dr. Dumont holds a FRQS Junior-2 award, and his lab is funded by grants from the CIHR, NSERC, ThéCell network, Stem Cell Network, Orphan disease center, AFM-Telethon, and Muscular Dystrophy Canada.

Dr. Lora Giangregorio, University of Waterloo

The aim of Dr. Giangregorio's research and that of her research team is to reduce the burden of osteoporotic fractures. We use medical imaging technologies to explore bone and muscle responses to activity or neurologic impairment, and evaluate new methods for image analysis. We conduct epidemiologic studies to inform fracture risk assessment algorithms. We conduct clinical trials to investigate the effects of exercise interventions for reducing fracture risk in high risk individuals. We lead knowledge dissemination and translation activities, and implementation studies to move research on exercise for older adults into practice. For example, our research team has worked with Osteoporosis Canada to develop BoneFit, a two-day workshop for physiotherapists and kinesiologists on appropriate assessment and exercise prescription for individuals with osteoporosis. We also led the development of the Too Fit To Fracture Exercise and Physical Activity Recommendations for Individuals with Osteoporosis.

Dr. Tom Hazell, Wilfrid Laurier University

My research program aims to better understand how physical activity/exercise contributes to the regulation of appetite, its subsequent effects on energy intake, and its overall role in reducing positive energy balance and fat mass. Current funded work examines the regulation of energy intake via the specific mechanisms involved in how exercise alters appetite through the integration of peripheral signals with either orexigenic (appetite-stimulating) or anorexigenic (appetite-inhibiting) properties. Overall, we are interested in the potential for exercise intensity to improve energy balance through alterations in appetite regulation and post-exercise metabolism. With my research interests in nutrition/exercise physiology, I am also interested in the effect of different nutritional supplements or feeding strategies on exercise metabolism.

	**Dr. Andrea Josse,** York University Dr. Josse joined York University in January 2019 from Brock University where she has been an assistant professor since 2014. Her research area combines clinical nutrition and exercise physiology in the context of both health and chronic disease, and centres on lifestyle modification strategies and/or training regimens that manipulate diet and exercise to achieve a healthier body composition and/or a beneficial metabolic outcome. She is particularly interested in utilizing diet (i.e. whole foods [including dairy products], nutrients, supplements) with different modes of exercise (i.e. aerobic, resistance, plyometric) to facilitate changes in body weight, body composition, strength and bone turnover in different populations across the lifespan.
	**Dr. Daniel Keir,** University of Western Ontario Daniel Keir's integrative cardiorespiratory research lab studies how the cardiovascular, respiratory, sympathetic and muscle metabolic systems respond, interact, and adapt to environments, activities, and conditions that challenge oxygen availability and carbon dioxide removal in health, chronic disease and across the lifespan. Of specific interest are integrative physiological responses to exercise, hypoxia (low O2), hypercapnia (high CO2), and their combination.
	**Michael Modica, PhD Candidate,** York University Michael is currently pursuing his PhD in Kinesiology and Health Science at the WHIPR lab Investigating varsity athlete concussion reporting in university and collegiate settings. With the purpose of bringing actionable recommendations to help identify effective and manageable concussion reporting strategies that can be implemented within postsecondary institutions. Michael has over 10 years experience as an athletic therapist with national and international experience. Having been an athletic therapist for professional teams such as the Toronto Argonauts and the TFC Academy, Michael has a deep understanding and experience treating a diverse range of athletic injuries.

Zach Weston, Canadian Society for Exercise Physiology

Zach Weston is a clinician, educator and health system administrator. As an entrepreneur, he has founded and operated several health science companies leveraging MedTech in the Kitchener-Waterloo, Guelph and Toronto communities.
Since 2003, he has taught courses in entrepreneurship and kinesiology/exercise physiology within the Faculty of Science, as well as entrepreneurial methods/business model development within the Lazaridis School of Business and Economics.
Currently, he also holds the role of Manager of Health System Performance and Clinical Innovation at the Waterloo Wellington Local Health Integration Network (LHIN).
He earned a BSc in Kinesiology and MSc in Exercise Physiology from the University of Waterloo and an MBA at Laurier. He is a registered clinical exercise physiologist with the American College of Sports Medicine and the Canadian Society for Exercise Physiology.

Abstract Title: The Effect of Biological Sex on Ventilatory Neural Drive During Hypercapnia

Authors: Ghazal Adibmoradi[1], Christine A Darko[1], Parsa Shekarloo[1], Heather Edgell[1,2], Devin B Phillips[1,2]

Author Affiliation:

1 School of Kinesiology and Health Science, Faculty of Health, York University 2 Muscle Health Research Centre, York University

Previous research has shown that the hypercapnic ventilatory response (HCVR) is similar or lower in healthy pre-menopausal females, when compared to age-matched males. However, females are more likely to develop significant respiratory mechanical constraint in conditions of heightened ventilatory drive (e.g., CO2 rebreathing or exercise) due to smaller lung size. The mechanical constraint would blunt the increase in ventilation, and thus, commonly used measurements of ventilatory output by expired gas may underestimate the HCVR in females. The proposed study will compare the neural ventilatory drive responses during a modified Duffin CO2 rebreathing protocol in young healthy females and males. We hypothesize that ventilatory drive during CO2 rebreathing will be similar between the sexes, despite a blunted ventilation in females. This cross-sectional study will include 15 young healthy females (age 18-40 years) and 15 age-matched males. All participants will complete a modified Duffin hypoxic CO2 rebreathing protocol to estimate the HCVR. Diaphragmatic electromyography (EMGdi) will be recorded continuously using a customized esophageal catheter. Neural ventilatory drive will be determined by quantifying the maximal EMGdi burst upon inspiration. Throughout CO2 rebreathing test, expired gas data will be acquired to determine ventilation. The physiological cause of the blunted HCVR in females is not fully understood and therefore quantifying the electrical activation of the diaphragm by EMG allows for determination of the efferent neural ventilatory drive from the medulla, independent of the prevailing ventilatory output as assessed using expired gas. Should females have a similar or elevated EMG response to hypercapnia, but a lower ventilatory output, compared to males, these data would provide novel evidence that respiratory mechanical constraint may lead to underestimations in the HCVR. An abnormally high HCVR is common in cardiovascular disease (CVD). It is entirely plausible that in some females with CVD, the HCVR may be underestimated, which may have prognostic implications.

Abstract Title: Calcification and fibrosis differs across muscle types in a mouse model of Duchenne Muscular Dystrophy.

Authors: Ihtisham Ahmed, Shahrzad Khajehzadehshoushtar, Laura N Castellani, Carmen J Haines, Catherine A Bellissimo, Christopher GR Perry

Author Affiliations: School of Kinesiology & Health Science, Muscle Health Research Centre, York University, Toronto, ON, Canada

Introduction: Duchenne muscular dystrophy (DMD) is characterized, in part, by muscle inflammation, fibrosis and calcification. However, the degree to which this is influenced by fibre type is unknown, and there remains an unmet need to develop therapies targeting calcification. The purpose of this study is to determine 1) the degree to which calcification and fibrosis occur in relation to each other in skeletal muscle with different fibre-type compositions from D2.*mdx* mice, and 2) the potential to alleviate this histopathology by inhibiting protease-activated receptor 1 (PAR1), which regulates inflammation, with parmodulins (ML-161, NRD-21; Function Therapeutics, USA).

Methods: In this pilot study, D2.*mdx* male mice were treated with vehicle (20% DMSO, 20% PEG, 60% PBS), 10 mg/kg ML-161, or 10 mg/kg NRD-21 (n=2-5/group) by subcutaneous injections from 4-28 days of age. Mice were assessed for muscle tissue calcification and fibrosis in the quadriceps (Quad), soleus (Sol) and tibialis anterior (TA).

Results: Calcification was greatest in the TA, somewhat detectable in the Quad, and not detectable in the type I-rich Sol. Fibrosis was most notable in the Quad and TA with less fibrosis noted in the Sol. Calcification and fibrosis in similar locations in most muscles. Parmodulins had no effects on calcification or fibrosis, although calcification was almost completely absent in the TA from mice treated with ML-161.

Summary: Calcification is heterogeneous across muscle types whereby the Sol appears resistant despite the presence of fibrosis. In Quad and TA, calcification and fibrosis occur in the same locations. More research is required to determine whether parmodulins prevent calcification in a fibre type-specific manner in Duchenne muscular dystrophy.

Abstract Title: Intramuscular glycogen phosphorylase inhibition blunts sarcoplasmic reticulum calcium release in skeletal muscle

Authors: Nathaniel J. Andrews3, Rasmus Jensen2, Niels Ørtenblad1, Arthur J. Cheng3

Author Affiliations: 1Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark. 2Research Center for Applied Health Science, University College South Denmark, Odense, Denmark. 3School of Kinesiology & Health Science, Muscle Health Research Centre, York University, Toronto, ON, Canada

Intramuscular glycogen is a primary energy source for fuel in skeletal muscle and depletion of glycogen is associated with exercise-induced fatigue caused by decreased sarcoplasmic reticulum (SR) Ca^{2+} release. However, it is unknown if acute pharmacological inhibition of glycogen utilization can cause decreased sarcoplasmic reticulum calcium release without depleting muscle glycogen content. We hypothesized that inhibiting glycogen phosphorylase activity within the skeletal muscle using the drug CP-316819 would result in decreased SR Ca^{2+} release due to decreased localized SR triad [ATP], whereby decreased [ATP] would inhibit ryanodine receptor opening. Mouse flexor digitorum brevis muscle was enzymatically dissociated and loaded with Indo-1 to measure myoplasmic free [Ca^{2+}] ([Ca^{2+}]$_i$). Our results indicate that inhibiting glycogen phosporylase via CP-316819 results in decreased SR calcium release at all stimulation frequencies (15 – 150Hz), it blunted total SR calcium release assessed with supraphysiological [5mM] caffeine treatment, and it reduced resting [Ca^{2+}]$_i$. These results may suggest that skeletal muscle utilizes glycogen at a localized level to regulate SR Ca^{2+} release.

Abstract Title: The HOMET1D Trial – Skeletal muscle health changes in response to exercise training and disuse in Type 1 Diabetes

Authors: Aditya N Brahmbhatt(1) , Emma S Juracic(2), Kayla R Bulyovsky(2), Dinesh A Khumbare(3), Irena A Rebalka(2), Christopher G R Perry(1), Thomas J Hawke(2)

Author Affiliations: (1) School of Kinesiology and Health Sciences, Muscle Health Research Centre, York University, Toronto, ON Canada. (2) Faculty of Health Sciences, McMaster University, Hamilton, ON Canada. (3) University Health Network, Department of Medicine, Division of Physical Medicine and Rehabilitation, Toronto, ON Canada

Type 1 diabetes mellitus (T1D) is an autoimmune disorder in which the destruction of ß-cells in the pancreas results in insulin deficiency and hyperglycaemia. T1D subjects show increased skeletal muscle (SKM) fatiguability and decreased strength which could be explained by altered mitochondrial metabolism. Indeed, we have previously found altered mitochondrial bioenergetics and ultrastructure in young and middle- to older-aged males and females with T1D who exceeded the currently recommended guideline of 150 mins/week. This suggests that higher exercise intensity/volume is warranted to improve and maintain their muscle mitochondrial health relative to healthy counterparts. Furthermore, detraining/disuse caused reversal of the benefits provided by exercise have been well characterized in other populations, but not in those with T1D. Therefore, the aim of this study is to investigate the effects of i) a 12-week exercise program that fits the current guidelines, ii) 1-week of detraining, iii) and subsequent 4-weeks of re-training on mitochondrial carbohydrate and fat oxidation in young (18-30 years) and older (45-65 years) females and males with and without T1D. Biopsies of the vastus lateralis are sampled at baseline and after 12-weeks of exercise training, 1-week of detraining, and 4-weeks of re-training. Using permeabilized muscle fibre bundles, high resolution respirometry is used to assess the degree to which mitochondria oxidize carbohydrate (pyruvate) vs fatty acid (palmitoyl CoA). Currently, there are 8 control (4 male and 4 female) and 8 T1D (3 male and 5 female) subjects under observation. Our preliminary baseline data suggests that submaximal and maximal pyruvate-malate supported (PM) and palmitoyl CoA (PCoA) stimulated state III respiration was slightly lower and higher, respectively, in young T1D subjects compared to their control counterparts when sexes are grouped together. This study will provide novel insight into the role of exercise and disuse on SKM health and function in those with T1D.

Abstract Title: PARASPINAL MUSCLE CONTRACTILE VARIABILITY IN CANINE INTERVERTEBRAL DISC EXTRUSION PATIENTS

Authors: K. Josh Briar (1), Alex Chan (2), Fiona James (2), Francesca Samarini (2), Stephen H. M. Brown (1)

Author Affiliations: (1)-Human Health and Nutritional Sciences, (2)-Ontario Veterinary College, University of Guelph, Guelph Ontario

Spinal degeneration and deformity alter the natural structure of the spine, affecting muscle function. Chondrodystrophic canines demonstrate a naturally occurring model of this condition. Previous literature has linked poor paraspinal muscle health to low-back disorders; however, the lengthy and variable progression makes obtaining biomechanical and physiological muscle contractile data difficult. A wide range of paraspinal muscle functional capability has been reported in surgical patients with spinal degeneration and deformity; however, within-patient observations into single muscle fibre contractile function have not been conducted. Therefore, the purpose of this study was to explore within-patient paraspinal muscle single fibre contractile variability in canines being treated for intervertebral disc extrusion. During canine hemilaminectomy surgery (n = 16), a biopsy of the paraspinal muscles was extracted at the surgical level, chemically permeabilized, and single fibres were isolated. Single muscle fibres were placed at a sarcomere length of 2.5µm into a pCa[4.5] bath to induce maximal contraction where specific force, active modulus, and rate of force redevelopment (KTR) were measured. An average of 20 fibres were tested per biopsy; mean values and the relative (coefficient of variation) and absolute (standard deviation) variability were calculated, and correlation analyses were performed. A statistically significant strong negative relationship ($r=-0.766$, $p=0.001$) between specific force and relative variability within the muscle was observed indicating that as mean force generating ability increases there is a corresponding increase in the relative variability of force generating capability amongst fibres within the muscle. A similar relationship exists for active modulus ($r=-0.741$, $p=0.001$), which can be thought of as a proxy representation of the number of bound cross-bridges during contraction. Finally, a strong negative correlation also exists between the KTR mean and relative variability for ($r=-0.895$, $p<0.0001$). Conversely, a strong positive relationship between absolute variability and mean specific force ($r=0.568$, $p=0.022$), modulus ($r=0.650$, $p=0.006$), and KTR ($r=0.680$, $p=0.004$) was observed indicating that as the mean contractile capability increases, absolute within-muscle variability also increases. The source and the impact of the absolute and relative within-muscle contractile variability will continue to be explored.

Abstract Title: A ketogenic diet does not affect glucose homeostasis or muscle insulin response in rats regardless of fish oil content.

Authors: Joshua M. Budd, Nicole M. Notaro, Blair Macleod, David M. Mutch and David J. Dyck

Author Affiliations: University of Guelph

The ketogenic diet (KD) is extremely high in fat and low in carbohydrates. Evidence suggests that KDs promote weight loss and improve glucose metabolism in humans and rodents who are metabolically unhealthy. However, research in otherwise healthy rodents suggest that KDs impair glucose homeostasis. Considering KDs are also consumed by healthy huans, further investigation into the efficacy of KDs in this population is necessary before it can be holistically recommended. Moreover, while skeletal muscle is the primary location of insulin-stimulated glucose uptake, little research has investigated the effect of KDs on this process. All investigations into the efficacy of KDs in glucose homeostasis are also confounded by the use of mice under cold stress, which affects glucose homeostasis. Finally, most experimental KDs are predominantly composed of saturated and monounsaturated fatty acids, with almost no omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs). Evidence supports a beneficial role for the n-3 LC-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on glucose homeostasis in the context of a metabolic challenge. To our knowledge, however, no study has examined whether the inclusion of EPA and DHA affects the impact of a KD on glucose homeostasis. The objective of this study was to examine the impact of a KD on whole-body glucose tolerance and the skeletal muscle insulin response in rats, and to determine if altering the fatty acid composition of a KD with fish oil could improve metabolic parameters. Male Sprague Dawley rats were pair-fed one of a low-fat diet, high-fat diet, KD, or a KD supplemented with fish oil for 8 weeks. No significant differences in whole-body glucose tolerance, skeletal muscle insulin signaling, or skeletal muscle insulin-stimulated glucose uptake were detected between the dietary groups. Our findings suggest that KD feeding, with or without supplementation of fish oil, does not affect whole-body glucose homeostasis or skeletal muscle insulin response under pair-feeding conditions.

Abstract Title: Retinoblastoma-like protein 2 (Rbl2) is indispensable for muscle stem cell differentiation

Authors: Lucas Campagna and Anthony Scimè

Author Affiliations: Molecular, Cellular and Integrative Physiology, Stem Cell Research Group, Faculty of Health, York University, Toronto, Ontario, Canada, M3J 1P3

Background: Impaired skeletal muscle (SM) regeneration is a predominant feature of SM during aging and muscle wasting diseases. This is attributed to dysregulated muscle stem cell (MuSC) fate decisions to self-renew or commit to differentiate. Thus, to improve SM regenerative potential, mechanisms that influence MuSC fate decisions need to be further elucidated. Individuals with retinoblastoma-like protein 2 (Rbl2) mutations display severe developmental delays and musculoskeletal abnormalities. The goal of these experiments was to characterize Rbl2 during MuSC fate decisions to determine its role in SM regeneration. Methods: Myofibers with their associated MuSCs were isolated from mouse extensor digitorum longus muscle and observed for Rbl2 protein sub-cellular localization during MuSC fate decisions. MuSC fates were tracked by immunostaining with the MuSC specific marker Pax7 and the myogenic commitment marker MyoD. Rbl2 was stained and assessed for overlap with nuclear or mitochondrial stains using microscopy. For SM regeneration experiments, mice were subjected to femoral artery ligation for 8 days to induce lower-limb ischemia and subsequent muscle damage. Tibialis Anterior muscle was extracted, sectioned and immunostained for Rbl2. Finally, targeted deletion of the Rbl2 gene in C2C12 myoblasts (Rbl2KO) was achieved using CRISPR-Cas9. Rbl2KO and control were differentiated and assessed for their differentiation and myofusion indexes. Results: Rbl2 protein was absent from MuSCs during quiescence but expressed in the nucleus during commitment and in the mitochondria during differentiation. Rbl2 protein expression in the mitochondria of differentiating MuSCs was confirmed in vivo by immunohistochemistry of regenerating fibers. Additionally, Rbl2KO had an approximately 19.5-fold reduction in the number of myoblasts that differentiated and an approximately 8-fold reduction in the average myofusion index compared to control. Conclusions: An impaired differentiation potential and reduced myotube size in Rbl2KO suggests that Rbl2 is crucial for either permitting or instructing the differentiation program. Furthermore, Rbl2 may be regulating the myogenic differentiation program through a mitochondrial role. The requirement of Rbl2 in myogenic differentiation provides a potential therapeutic target for improving SM regeneration.

Abstract Title: The effects of cardiac cachexia on the myogenic capacity of satellite cells

Authors: Jack Campbell, Stephanie Tobin, Neil Emery

Author Affiliations: Trent University

Decreased perfusion caused by chronic heart failure results in systemic inflammation, gastrointestinal complications, and increased protein catabolism, all of which contribute to a loss of body weight and muscle mass in a condition termed cardiac cachexia. MuSCs experiencing the chronic inflammation present within the cachexic muscle tissue begin their differentiation process normally by developing into myoblasts but fail to complete the process. The degree to which this dysfunctional MuSC behaviour is responsible for muscle wasting in cachexia is unclear and no mechanism has been reported. Therefore, we aimed to investigate the effects of the inflammatory cardiac cachexia muscle environment on the ability of MuSCs to progress through their differentiation process to form new muscle fibers and the phenotypes of mature muscle fibers. Over the course of eight weeks the drug monocrotaline was used to induce cardiac cachexia in mice. Whole body weight, food intake and stool output were measured weekly, and organ mass (lungs, heart, muscles, fat, intestines) were measured upon termination. We found no change in food intake or stool output, but lung mass was increased in both sexes. Whole body weight and fat and skeletal muscle mass were reduced in males only. Additionally, the abundance of leukocytes (CD45+) in hindlimb muscle was measured through flow cytometry. This revealed an increased degree of inflammation and abundance of leukocytes in males who received monocrotaline but not females. Future work will quantify the number of Pax7+ cells and laminin to determine the number of MuSCs and myofiber width. RNA sequencing was performed on the gastrocnemius muscles and analysis of the sequencing data is still underway.

Abstract Title: Induction of the Nrf-2 pathway mitigates oxidative stress and enhances the mitochondrial phenotype in skeletal muscle cells

Authors: Champsi S, Hood D.A.

Author Affiliations: York University

Abstract text (no figures are permitted): Loss of mitochondrial content, reduced respiration, and excessive ROS production are all hallmarks of skeletal muscle deterioration, which are consistently observed with aging. Although exercise is an established intervention proven to enhance mitochondrial health and cellular homeostasis, certain populations are unable to perform physical activity due to their health status. This highlights the importance of investigating novel compounds that can improve mitochondrial health and mitigate muscle loss. Our candidate for this role is Sulforaphane (SFN), a nutraceutical that has been extensively studied for its powerful antioxidant effects in cancer cells. While this agent has exhibited beneficial anti-cancer properties, its effects on muscle health have been poorly characterized. The objective of this study was to examine the effect of SFN when combined with chronic exercise in muscle. To evaluate this relationship, C2C12 myotubes were electrically stimulated to model “exercise in a dish” and treated with SFN for various timepoints. Following treatment, myotubes were collected for subsequent analysis, including western blotting for proteins, flow cytometry for ROS emission, oxygen consumption, and transcriptional activity assays. SFN upregulated key antioxidant proteins, including catalase, glutathione reductase, and NQO1. This was accompanied by a significant reduction in cellular and mitochondrial ROS. Mitochondrial respiration was enhanced, exhibiting a marked increase in basal respiration, ATP production and maximal respiration. Signaling to mitochondrial biogenesis was also examined, utilizing a PGC-1α promoter-luciferase reporter construct. Our results indicated a 47% increase in transcriptional activity, demonstrating a strong drive towards mitochondrial biogenesis. This was further supported by nuclear cytosolic fractions, which revealed a significant increase in the nuclear translocation of SP1 and CREB1, transcription factors known to enhance PGC-1α promoter activity. Chronic contractile activity (CCA) revealed no differences in mitochondrial content between “exercised” cells and non-exercised SFN treated cells, indicating that SFN activates signaling cascades similar to exercise in this myotube contraction model. Our data demonstrate that SFN induces cellular and mitochondrial adaptations that are observed with training, including an elevation in antioxidant capacity, mitochondrial respiration, and biogenesis. This mitochondrial enhancement may be useful as a therapeutic strategy to improve muscle mitochondrial phenotypes in aging and disease.

Abstract Title: Ethnic Variations in Cardiovascular Disease (CVD) Risk Factors: Implications on Prevalent CVD and CVD Mortality

Authors: Queenie Cheung, Dr. Jennifer L. Kuk

Affiliations; York University

This study investigates ethnic differences in cardiovascular disease (CVD) risk factors, and how they relate with prevalent CVD and CVD mortality rates. Data from the National Health and Nutritional Examination Survey (NHANES) 2011-2020 were analyzed, with a focus on diverse ethnic differences (i.e. White, Black, Asian, Hispanic) in the association between CVD risk factors and prevalent CVD and CVD mortality. This study revealed that while obesity is consistently associated with increased odds of prevalent CVD risk in all ethnic group (White (OR=3.55), Black (OR= 4.28), Asian (OR= 4.16), and Hispanic (OR=2.36, $p<0.05$)), the association between obesity and CVD mortality was only significant in the Other ethnic group (HR= 2.26). For physical activity, only White (OR=1.31) and Black (OR= 1.45) were at higher odds for prevalent CVD and only White were at a significantly higher risk for CVD mortality (HR=2.15, $P<0.05$). With hypertension, the association between hypertension and prevalent CVD was not significantly different by ethnicity ($P>0.3$), but the association between hypertension and CVD mortality was only significant for White (HR=3.17) and Hispanics (HR = 3.72) . Future efforts should address the potential differences in how CVD risk factors may relate to CVD morbidity and mortality and develop targeted tailored interventions that consider these potential differences.

Abstract Title: Reprogramming of Muscle Stem Cell Fate Decisions In Vivo by High Fat Feeding or Lack of Hormones

Authors: Mark A. Danesh, Jaryeon Lee, Justin Hsiung, Lucas Campagna, Alexandra Pislaru, Christian Martone, Tara L. Haas and Anthony Scimè

Author Affiliations: Molecular, Cellular and Integrative Physiology, Faculty of Health, York University and Department of Biology, York University

Muscle stem cell (MuSC) fate choices have profound implications for skeletal muscle health. In muscle complications, such as sarcopenia, the balance between MuSC self-renewal and commitment is flawed. Evidence suggests that in sarcopenia, skeletal muscle adapts to an increasingly changing microenvironment that can affect the behaviour of MuSCs. However, little is known about fate choices being inherently programmed by muscle adaptation in quiescent MuSCs. To investigate this, we impacted the skeletal muscle microenvironment by altering gonadal hormone levels and/or providing a high fat diet (HFD) in a sex dependent manner. We used mice that were divided into 8 groups, differing between sex, gonadectomy, and HFD. Their myofibers from the extensor digitorum longus were assessed ex vivo in a time course and stained with MuSC markers, Pax7 and MyoD, to follow fate choices. We found that at 4-hrs post-isolation, HFD and/or ovariectomy delayed the activation of MuSCs in both sexes. Additionally, HFD resulted in a significant increase in MuSC commitment in females at 48- and 72-hrs. Analysis of cell number per MuSC cluster revealed that HFD or ovariectomy impaired proliferation in females. However, these differences in commitment and proliferation were not observed in the males. Importantly, differences in fate decisions and proliferation illustrate an intrinsic reprogramming of the quiescent MuSC by diet and lack of gonadal hormones. Therefore, for regenerative medicine therapies, the reprogramming of quiescent MuSCs provides a novel approach to correct the imbalance of fate decisions found in different muscle complications.

Abstract Title: The effect of biological sex on ventilatory responses during CO2 rebreathing

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Introduction: The central chemoreceptors, located on the medulla within the brainstem, are activated by an increase in arterial carbon dioxide (CO2) tension. It is well known that total ventilation rises in direct response to central medullary chemoreceptor activation (e.g., increased CO2 tension [termed hypercapnia]). Previous research has shown that the hypercapnic ventilatory response (HCVR) is lower in healthy pre-menopausal females, when compared to age-matched males. However, detailed components of ventilation, including breathing pattern and lung volume data during hypercapnia have not been reported. Thus, this study compared detailed components of ventilation during a standardized CO2 rebreathing protocol in young healthy females and males. Methods: Following baseline lung function testing, 8 young healthy females and 9 age-matched males completed a modified Duffin CO2 rebreathing protocol. Throughout rebreathing, ventilation, breathing pattern (tidal volume and breathing frequency) and end-tidal partial pressure of CO2 (PETCO2) were continuously acquired by expired gas analysis. Inspiratory capacity maneuvers were completed at 2-minute intervals throughout rebreathing to determine operating lung volume, specifically end-inspiratory lung volumes (EILV). Results: Females were shorter and had smaller lung volumes, compared with age-matched males (both $p<0.005$). The ventilatory response to progressive increases in PETCO2 was significantly lower in females than males, secondary to a lower tidal volume ($p<0.001$) and higher breathing frequency ($p<0.001$). Despite a lower ventilation, EILV was higher at given ventilations in females versus males (both $p<0.005$). Conclusion: During the rebreathe test, females adopted a rapid and shallow breathing pattern and ventilated at a higher EILV than males reflecting increased mechanical load at higher ventilations. Females had a blunted HCVR compared with males, which suggests decreased central medullary chemosensitivity. However, increased EILV provide novel evidence that the mechanical load of the respiratory system is greater in females than males during progressive hypercapnia. The blunted EILV in females, which was not observed in males, was likely an attempt to mitigate increases in mechanical load during hypercapnia. Future work interrogating airway mechanics and neural drive during hypercapnic conditions that allow for observation of mechanical constraints are needed to better understand the interrelationship between operating lung volumes and ventilatory regulation differences between the sexes.

Abstract Title: SkQ1 treatment in a mouse model of ovarian cancer prevents early- and late- stage skeletal muscle weakness while modulating skeletal muscle calcium release.

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Introduction: Muscle weakness and wasting are defining features of ovarian cancer-induced cachexia, a disease that currently has no treatment. How ovarian cancer induces myopathy remains incompletely understood partly because of limited preclinical models that do not sufficiently recapitulate the disease in women. As skeletal muscle mitochondrial stress occurs in ovarian cancer, we determined the ability of a mitochondrial-targeted enhancer drug SkQ1 to prevent weakness and atrophy in a novel mouse model of this condition.

Methods: C57BL/6J female mice were injected with $1x10^6$ epithelial ovarian cancer (EOC) cells underneath the ovarian bursa, generating a metastatic model of ovarian cancer. Mice received the mitochondrial-enhancing drug SkQ1 in their drinking-water (EOC-SkQ1) or standard drinking-water (EOC-Vehicle) while cancer developed for ~40 (Early-Stage; no apparent metastasis) and ~80 (Late-Stage; robust metastasis) days. Control mice were age matched, injected with saline and provided standard drinking-water.

Results: SkQ1 had heterogenous effects on complex I-stimulated mitochondrial H_2O_2 emission (mH_2O_2). In the tibialis anterior (TA), mH_2O_2 was increased in Late-Stage EOC-Vehicle mice vs control but decreased in EOC-SkQ1 mice vs EOC-Vehicle. Interestingly, in the diaphragm, mH_2O_2 was increased at Late-Stage in both EOC groups vs control, however, EOC-SkQ1 mH_2O_2 was further increased vs EOC-Vehicle. TA weakness and atrophy occurred in Early-Stage EOC-Vehicle vs control. SkQ1 partially preserved force production at Early-Stage in TA and diaphragm, and at Late-Stage in diaphragm, but did not prevent atrophy in either muscle. High-force muscle weakness linked to lower calcium release in Flexor Digitorum Brevis in EOC-Vehicle vs control was completely prevented by SkQ1 at Late-Stage. SkQ1 did not affect primary tumour size.

Conclusion: These findings suggest that SkQ1 treatment can improve force production independent of atrophy in mice injected with EOC. Moreover, these improvements are associated with lower mH_2O_2 and improved calcium handling albeit, these relationships are heterogenous across muscles. Thus, mitochondrial-targeted therapeutics in ovarian cancer-induced cachexia warrants further investigation, particularly in the context of muscle weakness.

Abstract Title: The Impact of Glial Derived Neurotrophic Factor and Prostaglandin E2 on Fibro-Adipogenic Progenitors

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Background: Peripheral nerve trauma induces skeletal muscle atrophy and fibro-fatty infiltration (FFI). The duration of denervation determines the potential for muscle recovery. Fibro-adipogenic progenitor cells (FAPs) are muscle resident stem cells that differentiate to fibroblasts and adipocytes, mediating FFI. FAPs are critical for muscle repair, but undergo a phenotypic switch with persistent denervation, resulting in pathogenic FFI. The cellular and molecular mechanisms regulating FAPs phenotypic switch remain incompletely defined. Glial derived neurotrophic factor (GDNF) dynamics correlate with FFI in muscle post denervation, with increased GDNF associated with greater FFI. The presence of the bioactive lipid Prostaglandin E2 (PGE2) has been demonstrated to have a negative regulation on fibrosis in direct muscle injury but is poorly studied in long-term denervation. The effect of GDNF and PGE2 on FAPs differentiation has not been studied. Given their role in FFI in skeletal muscle, it suggests these factors may regulate denervation-mediated FAPs differentiation and pathogenesis.
Objectives: Determine the role of GDNF and PGE2 on FAPs proliferation and differentiation Methods: Utilizing the rat tibial nerve transection model, the gastrocnemius muscle was denervated, with the contralateral limb serving as an internal control. FAPs were isolated at 5 and 12-weeks post injury, representing reversible and irreversible denervation injury respectively. FAPs were cultured and treated with varying concentrations of GDNF and PGE2, and mRNA and protein expression for FAPs proliferation and differentiation was assessed.
Results: GDNF stimulation of cultured FAPs led to a dose dependent increase in adipogenic (perilipin-1) and fibrogenic (SMA and Col1a1) mRNA expression, with significant increases observed at 100ng/mL of GDNF ($P<0.05$), along with a 10% higher perilipin-1 protein expression relative to controls ($P<0.05$). Denervated FAPs exhibited elevated PTGS2 (gene encoding PGE2 biosynthesizing enzyme), and decreased 15-PGDH (PGE2 degrading enzyme) mRNA levels at 5-weeks post denervation, indicating the presence of PGE2 at this timepoint. The reverse was seen at 12-weeks. Stimulation of healthy FAPs with 1000nM PGE2 demonstrated a 400-, 200- and 100-fold decrease in SMA, col1a1 and Perilipin-1 mRNA expression respectively. 100nM of PGE2 inhibited adipogenic differentiation in FAPs isolated from 12-week denervated gastrocnemius.
Conclusion: GDNF promotes FAPS proliferation and fibrogenic/ adipogenic differentiation in a dose dependent manner. FAPs PGE2 secretion is increased at 5-wks but resolves by 12-wks. Exogenous PGE2 on cultured FAPs inhibits fibrogenic and adipogenic differentiation. Together these data suggests that GDNF and PGE2 may be two potential factors driving FAPs phenotypic switch.

Abstract Title: Influence of combined voluntary exercise and pharmacological PPARβ/δ activation on skeletal muscle contractile function and fatigability in the rodent model of Barth syndrome

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Background: Barth syndrome (BTHS) is an X-chromosome linked disease caused by mutated tafazzin, resulting in reduced remodelled cardiolipin (CL) and, in turn, impaired mitochondrial form and function. A hallmark of BTHS is skeletal muscle myopathy that leads to muscle weakness and exercise intolerance. Endurance and resistance exercise interventions have demonstrated some improvements in skeletal muscle function, although complementary treatment strategies may yield further benefits. Activation of peroxisome proliferator activated receptor (PPAR)β/δ improves skeletal muscle metabolic function, but it is unclear if this translates to improved contractile function. Recent work in our lab using the rodent model of BTHS, the Taz knockdown (TazKD) mouse, demonstrated modest improvements in the rates of soleus muscle contractile force generation and relaxation, and fatigability with pharmacological activation of PPARβ/δ. Past research investigating PPARβ/δ activation has found that exercise may potentiate PPARβ/δ activation-mediated improvements in skeletal muscle contractile function. It is hypothesized that pharmacological activation of PPARβ/δ coupled with exercise will improve skeletal muscle contractile function and fatigue resistance in TazKD mice. *Methods:* 4–5-month-old TazKD and wildtype littermate male mice, with free access to running wheels, received GW501516 (5 mg/kg/day), a PPARβ/δ specific agonist, or vehicle through intraperitoneal injections for 4 weeks. Voluntary wheel running, non-wheel activity, soleus contractile function and fatigability (*in vitro* contraction), and body composition (dual x-ray absorptiometry) were quantified. *Results:* Four weeks of PPARβ/δ activation coupled with voluntary wheel running improved soleus contractile kinetics similar to GW501516 administration alone, but fatigability did not improve. There was an increase in soleus rate of relaxation, but no potentiation of peak twitch force or reduction in time to peak twitch when GW501516 was combined with exercise. Additionally, GW501516 appeared to protect against decreases in lean mass over the experimental period, and to a greater extent within TazKD mice. *Conclusion:* This study demonstrates the potential of PPARβ/δ activation coupled with exercise as a therapeutic strategy for improving skeletal muscle function, specifically rates of relaxation, in BTHS. Future work will focus on *in vivo* exercise tolerance and fatiguability along with skeletal muscle metabolic adaptations that may play a mechanistic role in this therapeutic strategy.

Abstract Title: Analysis of glucose levels and hormone responses to acute and repeat dosing of a somatostatin receptor 2 antagonist (ZT-01) in male and female type 2 diabetic (T2D) rats

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Introduction: Glucose management in diabetes is challenged by the inability to precisely deliver the correct amount of insulin, exposing individuals to high (hyperglycemia) and low (hypoglycemia) blood sugar levels on a recurring basis. While several medications exist to treat hypoglycemia, no medications exist to prevent it. Zucara Therapeutics is developing ZT-01, a SSTR2a, as the first preventative therapeutic approach by modulating the counterregulatory system with a somatostatin receptor 2 antagonist (SSTR2a) to normalize the body's ability to use endogenous glucagon as a defense against hypoglycemia. The work outlined here will test several vigorous approaches including prolonged treatment and sex differences to determine the therapeutic potential of ZT-01 for hypoglycemia prevention in T2D.Methods: A male (M) and female (F) high-fat-fed (HFF), low dose streptozotocin model of T2D was characterized using glucose and insulin tolerance tests. The effect of SSTR2a (0.3- 3 mg/kg ZT-01) for hypoglycemia prevention was assessed in M and F rats with 12 U/kg insulin-induced hypoglycemia (aspart) in single or repeated challenges, with acute or repeated dosing.Results: Acute treatment with SSTR2a displayed increased glucagon response ($p<0.05$) and protection against hypoglycemia in M and F rats (survival curve analysis, $p<0.05$ for both). With repeated treatment in M rats, we noted improved glycemia with lower HbA1c vs controls (4.3 ± 0.9 vs $5.3 \pm 0.8\%$, $p<0.05$) after 2-weeks. Glucagon response to hypoglycemia across all challenges was 9.7 ± 3.1 vs 22.6 ± 4.8-fold over baseline, with vehicle and ZT-01, respectively. When assessed for sex differences during hypoglycemia, ZT-01 was found to cause a greater increase in glucagon in F than M rats.Conclusion: Both acute and daily dosing of SSTR2a resulted in increased glucagon during repeated hypoglycemic challenges, reduced hypoglycemia exposure in initial challenges in M rats and may also improve overall glycemia. We also observed apparent sex-related differences to the hormonal responses to hyper- and hypoglycemia, and SSTR2a may increase glucagon responses more so in F rats with T2D. This research may have significant implications in optimizing glucose levels to prevent loss in muscle mass from hyper/hypoglycemia and ensure overall improved muscle health.

Abstract Title: High-intensity interval training via electrical stimulation increases fatigue resistance in mouse skeletal muscle through enhanced sarcoplasmic reticulum Ca2+ release

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High-intensity interval training (HIIT) is a time-efficient alternative to traditional endurance training, offering comparable or superior changes in physiological, performance, and health-related markers. Previous studies have shown improved fatigue resistance after four weeks of HIIT via electrical stimulation (ES). Fatigue resistance is characterized by a sustained ability to maintain muscle force generation over time, with muscle contractile force primarily regulated by myoplasmic free Ca2+ concentrations ([Ca2+]i). It remains unknown how HIIT-ES results in adaptations to intracellular Ca2+ handling via training-induced alterations in sarcoplasmic reticulum (SR) Ca2+ release. We hypothesize that preserving SR Ca2+ release during repeated contractions increases fatigue resistance in mouse skeletal muscle fibres after four weeks of HIIT. Mice underwent HIIT-ES of the plantar flexors via tibial nerve stimulation every second day for four weeks with either submaximal (20 Hz) or maximal (100 Hz) stimulation in the exercised leg, or no stimulation in the contralateral (control) leg. HIIT-ES involved 60 repeated contractions in 30 seconds followed by a 4-minute rest between sets, totalling 6 sets (3 min total exercise time), mimicking human HIIT protocols. After four weeks, plantar flexion torque-frequency curves and fatigue tests were performed. The flexor digitorum brevis (FDB) muscles, which were also activated by tibial nerve stimulation, were isolated and enzymatically dissociated to obtain intact single muscle fibres to measure [Ca2+]i transients. Other muscles were isolated to quantify protein changes associated with [Ca2+]i transients and mitochondrial content. HIIT-ES increased in-vivo fatigue resistance more than controls, with 100 Hz training demonstrating greater fatigue resistance than 20 Hz training. Overall, there was greater SR Ca2+ release in the HIIT-ES than in the control leg during repeated contractions, with greater preservation of SR Ca2+ release at 100 Hz than at 20 Hz HIIT-ES. Resting [Ca2+]i was better maintained after HIIT-ES than controls and was greater following 100 Hz than 20 Hz HIIT-ES. This study demonstrates that [Ca2+]i transients are improved with HIIT, which explains the increased muscle fatigue resistance after training. Furthermore, it may demonstrate the benefits of electrical stimulation-induced HIIT as a time-efficient training technique for individuals who are immobile or have injuries preventing conventional training methods.

Abstract Title: Examining Intrinsic Mitochondrial Respiration in Females and Males

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The effect of biological sex on rates of intrinsic mitochondrial respiration *i.e.,* mitochondrial respiration normalized to a marker of mitochondrial content, independent of differences in training status, has not been fully elucidated. In addition, whether females and males exhibit differences in creatine sensitivity is unknown. The purpose of this investigation was to examine if females and males with similar peak oxygen uptake ($\dot{V}O_{2peak}$) normalized to fat-free mass (FFM) demonstrate different rates of intrinsic mitochondrial respiration and creatine sensitivity. We hypothesized that females and males would not exhibit statistically significant differences in rates of submaximal or maximal pyruvate and malate (PM)-supported, adenosine diphosphate (ADP)-stimulated, intrinsic mitochondrial respiration. Biopsies of the *vastus lateralis* were obtained from 12 males (age: 22 ± 3 years; $\dot{V}O_{2peak}$: 55.9 ± 5.7 mL/min/kg FFM; Body Mass Index [BMI]: 23.0 ± 1.5 kg/m^2) and 12 females (age: 22 ± 2 years; $\dot{V}O_{2peak}$: 57.5 ± 6.6 mL/min/kg FFM; BMI: 23.6 ± 2.2 kg/m^2) in the rested, fasted state. Mitochondrial respiration was analyzed via high-resolution respirometry in both the presence and absence of 20 mM creatine, and intrinsic mitochondrial respiration was quantified as oxygen flux normalized to total electron transport chain subunit protein content. Creatine sensitivity index values were determined as the rate of mitochondrial respiration in the 20 mM creatine condition divided by the rate of respiration in the non-creatine condition. Rates of PM-supported as well as maximal complex I- and complex I and II-supported, ADP-stimulated intrinsic mitochondrial respiration were not statistically different between females and males in both the non-creatine (P=0.8424) and 20 mM creatine (P=0.3329) conditions. Likewise, creatine sensitivity did not statistically differ between females and males (P=0.8664). Our results suggest that rates of submaximal and maximal ADP-stimulated intrinsic mitochondrial respiration are not statistically different between females and males with similar $\dot{V}O_{2peak}$ normalized to FFM.

Abstract Title: Robust right ventricular fibrosis in a mouse model of Duchenne muscular dystrophy is prevented by the anti-inflammatory adiponectin receptor agonist ALY688

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Introduction: Duchenne muscular dystrophy (DMD) is caused by X-linked recessive mutations in the DMD gene, which encodes for the subsarcolemmal protein, dystrophin. Occurring predominantly in males, cardiomyopathy is the leading cause of mortality and is associated with inflammatory, fibrotic, and mitochondrial stress. Current standard of care for DMD - immunosuppression via glucocorticoid administration - has demonstrated some efficacy for prolonging ambulatory function and slowing disease progression. However, given the many unwanted side effects that accompany glucocorticoids, there is value in developing alternative therapies that address inflammation and other cellular dysfunctions in DMD. The adiponectin-receptor agonist ALY688 has previously demonstrated anti-inflammatory and anti-fibrotic properties in other disease models. Here, we determined the degree to which ALY688 influences early disease effects on chamber-specific fibrosis, macrophage polarization shifts (an index of the inflammatory state), and mitochondrial stress responses in dystrophin deficient mice. Methods: 4-week-old male D2.mdx mice were injected daily from 7-28 days of age at 15 mg/kg or with saline (VEH-treated) and were compared to age-matched wildtypes (DBA/2J; WT). Results: histopathological assessments identified elevations in left atrial (+67%) and right ventricular (RV) (+154%) collagen in VEH, as well as increased RV cardiomyocyte cross sectional area (+15%) vs WT, which were prevented by HD. In RV, pyruvate (NADH; Complex I)-supported mitochondrial H2O2 emission (mH2O2) were increased in VEH (+59%) but did not change with ALY688. ADP-stimulated respiration supported by pyruvate was lower in VEH (-44%) but was rescued by HD. Similar changes in RV fatty acid-supported respiration were observed in VEH and HD across a range of [ADP]. In non-fibrotic LV, which served as a control to RV, pyruvate-supported mH2O2 was increased in VEH (+25%) vs WT but was rescued by ALY688. ADP-stimulated respiration supported by pyruvate or fatty acids did not change between groups. Flow cytometry revealed significant elevations to RV-specific F4/80+Ccr2+ ‘pro-inflammatory’ macrophages in VEH vs WT that were prevented by ALY688. Conclusion: RV fibrosis in D2.mdx is related to lower mitochondrial pyruvate and fat oxidation and increased complex I-stimulated mH2O2. Prevention of RV fibrosis by ALY688 is linked to shifts in macrophage polarization towards an anti-inflammatory phenotype and mitochondrial reprogramming.

Abstract Title: Exploring the relationship between muscle/strength metrics and hip geometry in men and women aged 50 and older with low bone mass.

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Introduction: Osteoporosis is a skeletal disease characterized by a rapid loss of bone mass and a degradation of bone architecture, thereby increasing fracture risk. Hip fractures in particular pose the greatest risk to quality of life and economic burden of all osteoporotic fractures, and are projected to increase in frequency over the course of the next 16 years. Currently the most common method of assessing bone health is via a DXA scan, in which areal bone mineral density (aBMD) is measured. While this is useful in assessing fracture risk, it does not capture other important attributes of bone strength such as its' micro-architecture or geometry. Hip structural analysis (HSA) software does allow the evaluation of bone geometry, which has been illustrated to differ in populations depending on indices of muscle mass and physical activity. Though a comprehensive analysis of the relationship between measures of hip geometry and measures of lower limb muscle/strength in a high-risk population has yet to be completed. Therefore, it is the purpose of this investigation to determine the relationship between measures of lower limb muscle/strength and measures of hip geometry in an at-risk population of men and post-menopausal women aged 50 and above. Methods: This investigation entails a secondary analysis of the baseline data of the Finding the Optimal Resistance Training Intensity For Your (FORTIFY) Bones trial, which is a multicentered, superiority randomized controlled trial. The study includes men and post-menopausal women aged 50 and above, with a low bone mass and/or a high risk of fracture. The proposed statistical analysis includes several multivariable linear regressions in which the relationship of section modulus, cross sectional moment inertia and buckling ratio to lower limb muscle mass, knee extension strength and 30 second chair stand test score at 3 key regions of the hip will be evaluated. Multivariable linear regressions will account for covariates of age, sex, BMI and physical activity. Significance: The findings of this work will inform investigators for logical next steps of future research, which may be to discern whether improving measures of lower limb muscle/strength can lead to improvements in hip geometry within participants.

Abstract Title: Skeletal Muscle GCN5 in Metabolism – Friend or Foe?

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Skeletal muscle is the largest organ in the body and plays a central role in supporting whole body energy metabolism in response to metabolic stresses. By taking up circulating glucose and providing important gluconeogenic substrates, skeletal muscle buffers both post-prandial and fasting blood glucose levels. PGC-1α is a major positive regulator of skeletal muscle metabolism and is inhibited by the lysine acetyltransferase GCN5 (Kat2a). Therefore, inhibition/ablation of GCN5 may be beneficial in diseases such as obesity and diabetes by permitting PGC-1α activity. Previously, others have hypothesized that loss of GCN5 would increase mitochondrial content and improve metabolism in healthy mice. However, alone this has not been the case. We hypothesized that GCN5 ablation/inhibition in skeletal muscle would improve fat oxidation during high-fat diet-(HFD) feeding and gluconeogenesis during acute fasting. Skeletal muscle specific GCN5 ablated mice (GCN5 skm-/-) were fed a high fat diet or exposed to 48 hours of fasting. Body composition, metabolic substrate oxidation, and cardiometabolic outcomes were monitored. Additionally, cultured myotubes were treated with GCN5 inhibitors and examined for metabolic reprogramming. With HFD-feeding, we observed no differences in body mass, body composition, tissue mass, energy intake, or energy expenditure in GCN5 skm-/- mice. However, we did observe a relative preference for glucose versus fat oxidation. Treatment of cultured myotubes with GCN5 inhibitors increased basal extracellular acidification rates and was associated with a decrease in the expression of Pdk4 – a negative regulator of glucose oxidation. These findings suggest that GCN5 activity contributes to increasing fat oxidation and reducing glucose oxidation. In fasted mice, we observed no difference in body mass, tissue masses, or blood glucose. Interestingly, in quadriceps from fasted mice and nutrient-starved myotubes, there was increased atrophy and autophagy gene expression, but this was significantly attenuated in GCN5 ablated/inhibited tissues. This suggested that GCN5 may be protective of skeletal muscle and skeletal muscle metabolism. Altogether and in contrast to previous studies, these experiments suggest that GCN5 may contribute to/regulate fat oxidation and muscle wasting during metabolic stress. These results suggest that the requisite role of GCN5 in skeletal muscle metabolism may be misunderstood and needs revisiting.

Abstract Title: Characterizing the involvement of the peripheral respiratory chemoreflex at different intensities below the respiratory compensation point.

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Located in the carotid bodies, peripheral chemoreceptors detect falls in the arterial pressure of oxygen (PaO_2) or rises in arterial hydrogen ion concentration ($[H^+]$) to then elicit a ventilatory response to restore such values in the blood. To evaluate the extent to which the peripheral chemoreflex is assisting with the breathing response to exercise at different intensities, we tested the effect of acute hyperoxic PO_2 exposure on the absolute change in minute ventilation ($\Delta\dot{V}_E$) and alveolar ventilation ($\Delta\dot{V}_A$) at rest, and during four increasing sub-maximal exercise intensities below the respiratory compensation point (RCP). Eighteen participants (9 females) performed baseline evaluations and a maximal exercise test on visit 1, and a step-incremental exercise test at two moderate (MOD_1 & MOD_2) and two heavy (HVY_1 & HVY_2) intensities for visits 2 to 4 involving one visit with arterialized-venous blood sampling, and two visits with hyperoxic gas administration. Exercise was performed on a cycle ergometer while collecting arterialized blood gas measurements and breath-by-breath measurements of end tidal CO_2 ($P_{ET}CO_2$), end tidal O_2 ($P_{ET}O_2$), tidal volume, breathing frequency, and $\dot{V}_E$ using a metabolic cart and pneumotach. One-way ANCOVA with $[H^+]$ as the covariate was used to determine the effect of exercise intensity on the ventilatory contribution from the peripheral chemoreceptors. The average $\Delta\dot{V}_E$ (n=18) at rest was -0.3 ± 1.0 $L{\cdot}min^{-1}$, -2.7 ± 1.6 $L{\cdot}min^{-1}$ at MOD_1, -4.2 ± 1.8 $L{\cdot}min^{-1}$ at MOD_2, -5.8 ± 2.5 $L{\cdot}min^{-1}$ at HVY_1, and -6.6 ± 2.8 $L{\cdot}min^{-1}$ at HVY_2 (p=0.569). The average $\Delta\dot{V}_A$ (n=15) at rest was -0.35 ± 1.4 $L{\cdot}min^{-1}$, -2.2 ± 1.6 $L{\cdot}min^{-1}$ at MOD_1, -3.3 ± 1.9 $L{\cdot}min^{-1}$ at MOD_2, -4.1 ± 2.9 $L{\cdot}min^{-1}$ at HVY_1, and -4.5 ± 2.7 $L{\cdot}min^{-1}$ at HVY_2 (p=0.534). Based on the findings, the increasing change in ventilation due to hyperoxia can be explained by the increasing $[H^+]$ contribution. The chemoreceptors are contributing to the hyperpneic response to exercise below RCP and the intensity-dependent rise is explained by an increased humoral stimulus arising from progressive elevations in arterial $[H^+]$.

Abstract Title: Age-related blunting of serial sarcomerogenesis and mechanical adaptations following 4 weeks of maximal eccentric resistance training

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During adult aging, muscles atrophy, which is partly accounted for by a loss of sarcomeres in series. Serial sarcomere number (SSN) is associated with aspects of muscle mechanical function including the force-length and force-velocity-power relationships; hence, the age-related loss of SSN contributes to declining performance. Training emphasizing muscle lengthening (eccentric) contractions increases SSN in young healthy rodents. However, the ability for eccentric training to increase SSN and improve mechanical function in old age is unknown. Therefore, the purpose of this study was to investigate the sarcomerogenic response to eccentric training in old as compared to young rats. We hypothesized that old rats would experience a smaller magnitude of serial sarcomerogenesis than young, which would correspond to smaller beneficial adaptations in mechanical function. Ten young (9 months) and 11 old (33 months) male Fisher344/BN F1 rats completed 4 weeks of maximal isokinetic eccentric plantar flexion training. Pre- and post-training, the plantar flexors were assessed for maximum isometric torque, the passive torque-angle relationship, and the torque-angular velocity-power relationship. Following post-training testing, rats were sacrificed, and the soleus, lateral gastrocnemius (LG), and medial gastrocnemius (MG) were harvested for SSN assessment via laser diffraction, with the untrained leg used as a control. In the untrained leg/pre-training, old rats had lower SSN in the soleus (–9%), LG (–7%), and MG (–14%), lower maximum torque (–27 to –42%), power (–63%), and shortening velocity (–35%), and greater passive torque (+62 to +191%) than young. Following training, young exhibited 4-8% increases in SSN of the soleus and MG, whereas old had no change in soleus SSN, only a 2% increase in MG SSN, and 4% SSN loss in the LG. Furthermore, young rats exhibited a 13% increase in maximum isometric torque, while old rats had further reductions in maximum isometric torque (–35%), shortening velocity (–46%), and power (–63%), and further increased passive torque (+24 to +51%). Altogether, maximal eccentric training induced serial sarcomerogenesis and improved mechanical function in young rats, while old rats exhibited dysfunctional remodeling that led to further impaired muscle mechanical performance. Supported by NSERC.

Abstract Title: Sex-specific myocardial remodelling and dysfunction post-splenectomy in wistar rats.

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BACKGROUND: Cardiovascular disease (CVD) is the leading cause of death globally, and is often characterized by hypertrophic and fibrotic remodelling of the myocardium that contributes to systolic and diastolic dysfunction (i.e. impaired contraction, relaxation and filling). Research efforts are aimed at understanding cardiac risk factors, to reduce this substantive burden. Intriguingly, splenectomy is associated with an increased risk of cardiovascular events, suggesting the spleen may represent a previously unknown role in the development of CVD. The spleen supports optimal cardiovascular function through its regulation of blood volume and pressure, breakdown of cardiotoxic lipoproteins, and capacity to modulate inflammation following cardiac insult. Yet, how the spleen regulates myocardial structure and function is unknown.

Thus, the aim of this work is to characterize the role of the spleen in maintaining left ventricle (LV) structure and function. As biological sex plays a crucial role in the presentation and etiology of CVD, we will study splenectomy in both males and females. It is hypothesized that splenectomy will induce hypertension, LV remodelling and dysfunction, given the spleen's established roles maintaining optimal cardiovascular function.

METHODS: 7-week-old wistar rats were randomly allocated to undergo sham or splenectomy surgery, and LV structure and function were evaluated by echocardiography and invasive hemodynamics at 9-weeks post-surgery. Histological assessment of cardiomyocyte cross-sectional area with wheat-germ agglutinin, and interstitial fibrosis with picrosirius red were used to assess hypertrophy and fibrosis.

RESULTS: Splenectomy increased LV wall thickness in both sexes, in the absence of changes in cardiomyocyte cross-sectional area. This indicates that LV wall thickening cannot be directly attributed to hypertrophy, and instead could reflect incomplete relaxation of the myocardium.

There was a sex-specific effect of splenectomy on fibrotic remodelling and diastolic function; females exhibited increased interstitial fibrosis, and elevated end-diastolic pressure, which indicates increased LV stiffness. There were no changes in systolic function in either sex.

CONCLUSION: This study identifies for the first time, a sex-specific role for the spleen in maintaining healthy cardiac structure and function. Future work aimed at elucidating the mechanisms by which this occurs could uncover new targets for the treatment of myocardial remodelling and dysfunction in CVD.

Abstract Title: Investigating peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) gene expression following an acute force-matched high-intensity interval exercise compared with moderate-intensity exercise performed in mouse plantar flexor muscles in-vivo.

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It is known that mitochondrial biogenesis via increased PGC1-α gene expression is exercise intensity-dependent, with higher intensity exercise leading to greater mitochondrial biogenesis. We sought to determine whether the acute mechanism triggering increased PGC1-α gene expression with high- (HIIE) vs. moderate-intensity interval exercise (MIIE) pertains to differences in cytoplasmic Ca^{2+} flux due to increased sarcoplasmic reticulum (SR) Ca^{2+} release with HIIE, causing increased Ca^{2+}/calmodulin-dependent Protein Kinase (CaMK) activation compared with MIIE. We used a novel paradigm in female mice in-vivo (n=8) where the electrically evoked plantar flexion force output between HIIIE vs. MIIE were equivalent at the start of exercise despite differences in the stimulation frequency between HIIE (100Hz) vs. MIIE (20Hz) causing greater SR Ca^{2+} release in the HIIE protocol. Repeated contractions were performed by stimulating the tibial nerve, and the cohorts were divided into the MIIE group (n=4) where max 20 Hz force production occurred at optimal length, and the HIIE group (n=4), where the foot was plantar flexed during 100 Hz stimulation to force match to the MIIE cohort. Despite greater total work performed in the MIIE vs. HIIE group, our findings showed that there was no difference in the increase in PGC1-α gene expression in gastrocnemius muscle at 3h post-exercise following HIIE compared to MIIE.

Abstract Title: The effect of cytokinins (CKs) on skeletal myogenesis

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Myogenesis is a crucial process in embryonic development, postnatal growth, and adulthood, involving the formation and maturation of muscle tissue from precursor cells called myoblasts. Disruption of myogenesis can lead to various muscle disorders. Cytokinins (CKs) are adenine-derived signaling molecules. In mammals, these molecules are primarily synthesized through the tRNA degradation pathway. This is initiated by the enzyme TRIT1, which adds an isopentenyl group to the N^6 of A37 (i^6A37) of specific subset of tRNAs, and upon tRNA degradation the isopentyl adenosine (iPR) could be released. Recent studies have uncovered the potential functions of CKs in mammalian cells and in regenerative medicine, including skeletal muscle differentiation. iP is the building block for downstream CK types and may play a role in muscle cells, similar to its role in endothelial cells, mobilizing catabolic processes downstream of AMPK signaling pathway.

C2C12 cells were used as a myogenic model in the present study. Cell Counting Kit-8 and BrdU assays were performed on cells cultured with different concentrations of CKs for 24h in growth medium. In another experiment, the growth medium was replaced with differentiation medium, and cells were differentiated under the desired treatments for 144h before the CCK8 assay. To evaluate the differentiation process quantitatively, we analyzed the effects of cytokinins by assessing differentiation and fusion indices as well as the expression levels of Myogenin (MyoG) and Muscle Creatine Kinase (MCK) using RT-qPCR.

Under growth conditions, iPR primarily affected cell viability without directly affecting DNA synthesis or proliferation, whereas kinetin riboside exhibited cytotoxic effects by reducing both proliferation and viability. Throughout myogenesis, cells exhibited variable viability as they progressed through the differentiation stages, particularly within the non-synchronized cell population, and fluctuations in cell viability were evident under differentiation conditions. Although both kinetin riboside and iPR hindered myoblast differentiation, differences in their fusion indices suggested distinct mechanisms of action. The significant decrease in MyoG and MCK mRNA levels in iPR-treated cells confirmed the negative effect of iPR on myoblast differentiation. Future research will focuse on total RNA sequencing of C2C12 cells post-iPR treatment to elucidate its impact on gene expression dynamics during myogenesis.

Abstract Title: Exploring the relationship between mitochondrial-linked cell death and muscle atrophy during ovarian cancer progression

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Introduction: The mechanisms of muscle atrophy during ovarian cancer remain unknown. While much research has focused on protein turnover in skeletal muscle, less is known regarding the role of cell death pathways, particularly those that are linked to mitochondrial dysfunction. The purpose of this ongoing study is to 1) explore whether mitochondrial reactive oxygen species-mediated apoptosis and necroptosis contribute to skeletal muscle atrophy during ovarian cancer and 2) determine whether a mitochondrial-targeted antioxidant prevents ovarian cancer-induced atrophy. Methods: Transformed murine ovarian surface epithelial cells (EOC) from C57BL/6J mice were injected beneath the ovarian bursa of syngeneic adult female mice and compared to PBS-injected controls (PBS-control, n=24). Tumors were allowed to progress for 40 and 80 days (purpose 1). Following EOC injections, half of the mice received the mitochondrial-targeted antioxidant SkQ1 in drinking water (EOC-40-SkQ1 n=12, EOC-80-SkQ1 n=30), while the rest received standard water (EOC-40 n=12, EOC-80 n=30, purpose 2). Results: Type IIb fibers cross-sectional areas were decreased in gastrocnemius and quadriceps muscles of 80-day EOC groups, indicating atrophy. Pyruvate-supported (NADH, Complex I-stimulated) mitochondrial H_2O_2 emission was elevated in the mixed gastrocnemius (MG) muscles of both 80-day EOC groups. However, upon normalization to electron transport chain content, an index of mitochondrial abundance, SkQ1 decreased the heightened H_2O_2 emission observed in 80-day EOC. In control MG, few fibres were susceptible to calcium stress-induced mitochondrial permeability transition (mPT) – a major event linked to apoptosis. However, mPT was more common as cancer progressed (PBS vs all EOC groups). Conclusion: Muscle atrophy in ovarian cancer coincides with increased mitochondrial H_2O_2 emission, which was mitigated by SkQ1 treatment. Ongoing work will determine whether EOC-induced atrophy involves necroptosis or apoptosis, as evidenced by increased mPT with tumor progression, and will assess the impact of SkQ1 on these processes despite its inability to influence cross-sectional area in MG.

Abstract Title: Effect of ketogenic diet on hepatic cholesterol metabolism

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In recent years the ketogenic diet (KD) has gained popularity for its weight-loss effects and benefits in the treatment of several metabolic diseases. The KD generally provides ~80% of total calories from fats, 15% from proteins, and 5% from carbohydrates. Although the ketogenic diet has shown to be medically beneficial, concerns arise that an increase in dietary saturated fat intake could increase cholesterol levels and elevate the risk of cardiovascular diseases. Currently, little is known about how the KD affects the molecular mechanisms that regulate cholesterol metabolism. This is particularly relevant in the liver, an organ that produces large amounts of cholesterol daily. Thus, this study was designed to investigate how the KD regulates crucial molecular steps involved in endogenous cholesterol production (HMG-CoA reductase and SREBP-2) and uptake (PCSK9 and LDLr) by the liver. For that, male Wistar rats were fed one of the following diets: standard rat chow diet (SC, 60% carbohydrate,13% fat, and 27% protein), a high-fat sucrose-enriched diet (HFS, 20% carbohydrate, 60% fat, and 20% protein), or a KD (0% carbohydrate, 80% fat, and 20% protein). Subsequently, liver tissue was extracted and used for the determination of gene expression by real-time PCR and protein content by western blotting. Blood samples were also collected to measure circulating cholesterol levels. We found that neither plasma cholesterol levels nor HMGCR and SREBP-2 expression in the liver differed among the dietary interventions. However, the KD significantly reduced liver PCSK9 content and expression in comparison to SC and HFS diets. This could have potentially enhanced cholesterol uptake via the LDLr, although no significant changes were detected in total liver LDLr content. It is possible that a higher number of LDLr recycled to the cell membrane in KD-fed rats and facilitated the clearance of circulating cholesterol. Thus, membrane versus cytoplasmic distribution of LDLr will be determined next. In conclusion, the KD altered key steps that regulate hepatic cholesterol metabolism and prevented plasma cholesterol levels from increasing, despite its elevated saturated fat content.

Abstract Title: Myocardial infarction causes transient pulmonary inflammation that leads to pulmonary edema in mice

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Background: Following a myocardial infarction (MI), patients suffer from pulmonary edema, which decreases quality of life and survival. Pulmonary edema can develop as a consequence of pulmonary hypertension (a common pathological sequalae of heart failure), or from increased permeability of the alveolar-capillary barrier (from pulmonary inflammation). Following an MI, pulmonary edema is commonly attributed to pulmonary hypertension, secondary to left ventricle dysfunction. However, patients also develop pulmonary edema in the absence of pulmonary hypertension, demonstrating that the response of the lung following an MI is relatively unknown. Therefore, the purpose of this study was to investigate the mechanism(s) driving fluid accumulation in the lungs post-MI. We hypothesize that pulmonary inflammation would be apparent post-MI, but transient, and would contribute to the development of pulmonary edema. Methods: The left anterior descending coronary artery was permanently ligated in male CD-1 mice. At 3, 7, or 14 days following surgery, mice underwent echocardiography and cardiac catheterization of the left and right ventricles to evaluate cardiac structure and function. Next, mice either underwent bronchoalveolar lavage for visualization of alveolar cells and lungs were frozen for molecular analysis, or lungs were weighed, dried, and re-weighed to determine water content. Results: MI mice had impaired left ventricle systolic and diastolic function by 3 days post-surgery, whereas pulmonary edema (i.e., increased lung wet/dry weight) was present at 7 days. Pulmonary hypertension was not observed at any time point following the MI (i.e., right ventricle pressure was not significantly elevated), and therefore cannot be used to explain the development of pulmonary edema. However, there was an increase in leukocytes in the bronchoalveolar fluid of mice 3 days post-MI, that decreased at the later timepoints. Additionally, RNA sequencing analysis revealed an upregulation of inflammatory cytokines and pathways of leukocyte migration and chemotaxis in the lung at 3 days post-MI. Conclusion: Overall, we show that pulmonary inflammation is a consequence of an MI which precedes and contributes to the development of pulmonary edema. This work provides insight into potential therapies that directly target the lungs following an MI to improve patient survival and quality of life.

Abstract Title: Understanding a Myriad of Cardiac Actin Mutations and Their Role in Contraction Regulation

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Abstract text (no figures are permitted): Cardiomyopathies are a type of heart disease associated with the dysfunction of the ventricular myocardium, leading to reduced cardiac output and progressive heart failure. The main forms are dilated cardiomyopathy (DCM), characterized by atrophy of the ventricular myocardia, and hypertrophic cardiomyopathy (HCM), marked by hypertrophy of the ventricular myocardia. Mutations in cardiac sarcomere proteins, including α-cardiac actin, are a common cause of cardiomyopathies. The focus of this work lies in characterizing mutations within the tropomyosin binding region of the ACTC1 gene. These mutations are believed to lead to hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) by disrupting the regulation of contraction. Variations in this central protein actin can impact the binding of myosin, the force-generating protein, as well as the regulatory proteins troponin and tropomyosin, all essential for sarcomere contraction. Thus, understanding these variants will help uncover the molecular mechanisms driving DCM and HCM, enabling personalized treatments. The Sf9-baculovirus expression system utilizes insect cells to express wildtype and variant human recombinant actin, which can be used in a variety of assays such as the in vitro motility assay, ATPase assay, and tropomyosin binding assay, to determine the properties and disease mechanism of each variant cardiac actin. This research aims to delineate activity differences between variant α-cardiac actin and wildtype proteins to advance individualized treatments strategies, a promising approach to mitigate the global impact of cardiomyopathies.

Abstract Title: The Effects of Aging on Mitochondrial Stress Responses in Skeletal Muscle

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Skeletal muscle is characterized by losses of mass and strength with age. Mitochondria are the energy-producing organelles within muscle, and they appear to deteriorate with age. The Integrated Stress Response (ISR) is defined as a molecular mechanism that serves to restore mitochondrial homeostasis and functioning. We have investigated the ISR within aged muscle in an attempt to understand its role under basal conditions, and in response to the stress of exercise. We hypothesize that the ISR will exhibit an exaggerated response to exercise with age to restore homeostatic conditions. We studied the ISR and its effects in muscle from young (4-6 months) and aged (20-22 months) mice at rest and following an acute exhaustive exercise treadmill protocol compared to a sedentary counterpart. Our results showed that muscle of aged mice exhibited increased components of the ISR both basally and under stress. Acute exercise was able to increase AMP kinase signaling toward mitochondrial biogenesis in young and aged mice, however, aged mice had a blunted signaling response compared to young animals. ISR proteins ATF4 and CHOP were also elevated in aged mice. Additionally, aged muscle showed an increase in lysosomal proteins associated with mitochondrial clearance, suggesting signs of a greater mitophagy flux. These data demonstrate that muscle of aged mice exhibits an enhanced stress response both at rest, and during exercise. Aged muscle also showed attenuated signaling toward mitochondrial biogenesis, and when combined with the potentially higher mitophagy flux, results in a decline in mitochondrial content, with possible implications for the maintenance of muscle mass. Understanding these mechanisms and the exercise effect on the restorative capabilities of mitochondria can be an important step towards maintaining mitochondrial and skeletal muscle health in aged individuals.

Abstract Title: A novel protocol for ex-vivo MuSC protein expression in myofibers

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The use of plasmid vector transfection to over-express cDNA has been a well-established method to explore the role of proteins in a cellular context, such as myoblasts. However, unlike myoblasts, the transfection of muscle stem cells (MuSCs) in situ to study their function and fate decisions has never been shown. Herein, we have developed an approach to transfect plasmids into MuSCs using an ex-vivo approach on isolated myofibers from the extensor digitorum longus (EDL) muscle. In our approach we optimized for myofiber number, transfection reagent, and plasmid cDNA concentration. Using this transfection methodology, we examined the impact of the transcriptional co-repressor retinoblastoma like 1 (Rbl1, p107) protein on MuSC function. Accordingly, we transfected p107 genetically deleted (p107KO) MuSCs on myofibers with p107 which is forced to localize in the mitochondria, with and without green fluorescent protein (GFP), as a fluorescent tag. At 72 hours post myofiber isolation, MuSCs that expressed GFP were immuno-stained with markers for Pax7 and MyoD to analyze fate decisions. Compared to the transfected control cells (GFP only), we found that p107 expression in the mitochondria did not express Pax7. This suggests that mitochondrial function of p107 forced commitment and not self-renewal. Our transfection findings strongly supports a role for p107 in MuSC fate decisions that can be potentially manipulated in regenerative medicine applications, thereby providing a possible avenue in rescuing myopathies such as sarcopenia.

Abstract Title: AMPK is required for skeletal muscle mass maintenance in cancer cachexia through the regulation of mitochondrial dynamics and fibrosis

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Cancer cachexia, a comorbid syndrome that presents in concert with several types of cancers, is characterized by a loss of skeletal muscle mass, adipose tissue, and metabolic dysfunction. As 20% of cancer-related deaths are due to cachexia, mitigating muscle loss during cancer treatment is crucial for patient survival. AMP-activated protein kinase (AMPK) is an intracellular energy sensor that regulates signaling pathways including autophagy, mitochondrial biogenesis, and fatty acid metabolism, all of which are typically involved in skeletal muscle health. Previous work has shown increases in AMPK subunit expression and identified AMPK to play a role in glucose metabolism during cachexia, yet alternative influences of AMPK on cachectic severity have yet to be elucidated. Thus, the purpose of this study is to address the role of AMPK on mitochondrial function and biogenesis and skeletal muscle morphology during cachexia. 8-week-old male and female wild-type (WT) mice or AMPK muscle knockout (mKO) mice received a single subcutaneous injection of either saline (PBS) or Lewis lung carcinoma cells (LLC; 1 x 106 cells) in the right flank to generate a model of cancer cachexia. All animals were monitored for 28-days, followed by functional tests performed prior to tissue harvest. Cancer (LLC) decreased tumour-free body weight, grip strength, and extensor digitorum longus (EDL) contractility in WT-LLC mice. mKO-LLC mice displayed an additional 9-14% reduction in muscle mass in the EDL, gastrocnemius, and quadriceps compared to WT-LLC ($p<0.05$). Grip strength was further reduced by 14% in mKO-LLC mice, however ex-vivo assessment of EDL function saw resistance to fatigue in comparison to WT-LLC counterparts ($p<0.05$). Mitochondrial respiration was reduced as a consequence of genotype in mKO-PBS mice and of cancer in WT- and mKO-LLC mice, however mKO-LLC muscle did not demonstrate any further impairments in respiration in comparison to WT-LLC muscle ($p<0.05$). Cancer increased inflammatory, atrophic, and autophagic protein expression irrespective of genotype. Skeletal muscle fibrosis was increased by 43% in WT-LLC mice in comparison to WT-PBS and further increased by 17% in mKO-LLC ($p<0.05$). Collectively, these preliminary data suggest that AMPK is involved in regulating skeletal muscle quantity and quality during cancer cachexia.

Abstract Title: Non-persistent pulmonary remodeling following myocardial infarction

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Myocardial infarction (MI) remains a leading global cause of death. Despite the existence of pulmonary edema, dyspnea, and exercise intolerance post-MI, how the lungs respond is largely unknown. Indeed, pulmonary edema post-MI increases capillary pressure surrounding the alveoli, which may cause compression and drive structural changes. Therefore, our objective is to examine structural characteristics of the lung post-MI. We hypothesize that MI will cause reductions in alveolar cross-sectional area (CSA) accompanied by an increase in fibrosis. MI was induced by permanent ligation of the left anterior descending coronary artery. Pulmonary rales, indicative of pulmonary edema, were auditorily assessed each day post-MI. At 3-, 7-, and 14-days post-MI, cardiac function was assessed by echocardiography, following which animals were perfusion fixed with formalin, and lungs were excised and processed for histological analysis. Slices of the lung embedded in paraffin wax were sectioned at 5um, then stained using hematoxylin and eosin or picrosirius red, to examine changes in alveolar CSA and quantity of lung fibrosis, respectively. Results were analyzed using one-way ANOVA; a p-value <0.05 was considered statistically significant. Echocardiography revealed left ventricle chamber dilatation, evident as an increase in end-systolic dimension and end-diastolic dimension at all timepoints. Stroke volume decreased, while heart rate remained unchanged, resulting in reductions in cardiac output at all time points. At 3-, 7-, and 14-days, the incidence of pulmonary rales was 27%, 21%, and 0% respectively. Alveolar CSA decreased after 3-days ($p=0.0049$) and returned to sham levels at 7- and 14-days. The quantity of lung fibrosis was unchanged at all timepoints. Here we show MI causes transient structural changes in the lungs with no fibrotic remodeling; thus, further research is warranted to investigate the incipient mechanism behind these changes and how they might impact lung function post-MI. Current treatments are predominantly cardiac-centric, yet we have provided evidence to show that lung structure, and possibly function, is affected post-MI. By better understanding how the lungs respond post-MI, new therapeutics targeting the lung can be developed, ultimately improving the quality of life for MI patients whose chief complaint remains dyspnea and exercise intolerance.

Abstract Title: THE CHARACTERIZATION OF TAZ PROTIEN-PROTEIN INTERACTIONS IN MUSCLE

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Hippo signaling serves as a critical regulator of cell proliferation, differentiation, and apoptosis. Recent studies by the McDermott lab have characterized the Hippo signaling downstream target protein TAZ as a repressor of myogenic differentiation. Since TAZ lacks inherent DNA binding ability itself, its function and activity change depending on its protein interactions. Previous research investigated a proteomic screen of TAZ interacting proteins in muscle cells. Employing a GFP-nanoTrap affinity purification coupled with LC-MS/MS protein identification we documented a list of novel and well characterized TAZ protein interactions in a myogenic context. Notably, we identified the pro-myogenic methyl transferase protein, CARM1 as one of TAZ's interacting partners. Further investigation into this interaction revealed the functional role of the TAZ and CARM1 interaction on the Hippo signalling pathway. Utilizing the (HOP/HIP) gene reporter assay we observed that CARM1 repressed TAZ transcriptional activator function, promoting TAZ Ser89 phosphorylation, leading to the cytoplasmic sequestration of TAZ. The TAZ and CARM1 protein interaction was further investigated in the dysregulated context of Embryonic Rhabdomyosarcoma cells (RD) and interactions and function were maintained, however overall, Hippo signaling was found to increase compared to control C2C12 myoblasts. Further, Mass Spectrometry analysis was conducted and revealed TAZ as a substrate of CARM1 methylation at amino acid Arg 77 in a PGPR*LAGG consensus peptide resulting in enhanced TAZ Ser89 phosphorylation. These finding underscore the dual role of CARM1 in regulation of TAZ in both proliferative and differentiation pathways during myogenesis.

Abstract Title: The Impact of Fundamental Movement Skills Assessments on the Association with Physical Activity During Guided Active Play and Health-Related Fitness During Early Childhood

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Background: Development of fundamental motor skills (FMS) are necessary during childhood and adolescence to support participation in adult sport-specific and lifelong physical activity (PA). During early childhood the association between FMS proficiency (gross motor competence) with PA outputs and health-related fitness (HRF) is reported to be indeterminant, and/or weak to moderate. Methods used to report FMS (locomotor-LOC; object control-OC) using the Test of Gross Motor Development-2 (TGMD-2) may explain the varied relationships. These include using standardized scores and percentiles with reduced variability and uneven distribution between percentile ratings and/or using inappropriate linear analysis of variance and Pearson coefficients for categorical variables (FMS subtypes). This study examines the associations between normative FMS TGMD-2 percentile outputs and FMS classifications (L<33% and H>66%) from grouped individual raw MS scores (GRPIND) with classifications for PA outputs using a guided active play (GAP) cooperative games (COOP) format and classifications of health-related fitness (HRF) variables for children (5-7yrs). Methods: Children (n=20; 6.3±0.8 years) recruited from a summer day camp participated in 1hr.d-1; 4days.wk-1 GAP sessions. Accelerometry determined vectors were used to quantify PA by estimating energy expenditure (EE) and percent time at moderate-vigorous PA (%MVPA). Measurements included height, body mass, vertical jump (VJ), grip strength (STR), estimated aerobic power (VO2max) and FMS (TGMD-2). Chi-Square (X2) and cross-tabulation analysis was performed on L and H classifications between FMS, with PA outputs and HRF. Results: Significant associations between OC classifications, prepared from GRPIND, with GAP (COOP) PA outputs for EE ($X^2=24.68$; 4, $p<0.05$) and %MVPA ($X^2=16.85$; 4, $p<0.05$) were observed, but not with normative TGMD-2 outputs. For HRF, Chi-Square statistics between LOC classifications, prepared from GRPIND were associated with VJ ($X^2=19.21$; 4, $p<0.05$) and STR ($X^2=15.34$; 4, $p<0.05$), but not with the normative TGMD-2 outputs. Conclusion: Results elucidate the significant associations between FMS with PA and HRF when reporting GRPIND for LOC and OC subtypes. The grouping of individual raw scores for LOC and OC skills has advantages for research studies interested in understanding the influence of PA outputs and/or HRF on the development and proficiency of FMS during early childhood.

Abstract Title: Time-dependent effects of cold-water immersion on intramuscular temperature and human skeletal muscle function

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Cold water immersion (CWI) is a post-exercise intervention that has become widely popular due to its proposed benefits on improving the recovery of exercise performance. This modality is assumed to be effective in improving post-exercise skeletal muscle recovery with previous studies alluding to benefits while others associating negative effects. One potential explanation for the discrepancies in the literature on the effectiveness of CWI as a recovery modality may be due to inconsistencies in the duration of CWI employed, which will have implications on the intramuscular temperature and its effects on muscle contractile function. Thus, the purpose of this study was to investigate time- and intramuscular temperature-dependent changes in human skeletal muscle function with 1h of 10°C CWI per most used temperature. We further aimed to replicate post-exercise CWI to determine whether increasing intramuscular temperature with exercise or passive heating are sufficient to attenuate cooling-dependent impairments in muscle function. Using a randomized crossover study design, nine participants (8 M, 1 F) were recruited to partake in 1h CWI at 10°C of their lower leg to assess the time effect of intramuscular cooling. Three separate visits consisting of 1) 1h CWI at 10°C without exercise, 2) non-fatiguing exercise followed by 1h post-exercise CWI at 10°C (Exercise + CWI), and 3) passive pre-heating with no exercise followed by 1h CWI at 10°C (Passive + CWI). Our results showed that acute CWI at 10°C impaired peak power after 20 min of CWI, whereas prolonged CWI at 10°C (> 30 min) also impaired sarcolemmal membrane excitability and maximal voluntary isometric strength. Furthermore, increasing intramuscular temperature with exercise as well as passive heating could mitigate the cooling-dependent impairments in muscle contractile function caused by prolonged post-exercise CWI.

Abstract Title: Investigating capillary density reduction in skeletal and cardiac muscle in mice with ovarian cancer

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Introduction: Cancer cachexia is a condition of involuntary weight loss due to the progressive loss of adipose tissue, and skeletal and cardiac muscle, termed muscle wasting. Recent evidence demonstrates that cancer-induced muscle impairments occur prior to muscle atrophy. Similarly, pre-existing data obtained from our laboratory demonstrates that capillary rarefaction in the heart precedes any evidence of cardiac muscle atrophy in ovarian cancer. However, it is unknown whether this cancer-induced pathology is a cardiac specific phenomenon, or if capillary rarefaction occurs in skeletal muscle as well. Thus, our objective was to evaluate whether capillary density decreases in skeletal muscle and whether this capillary rarefaction precedes muscle atrophy in ovarian cancer. We then compared the results to cardiac muscle data.

Methods: We used an orthotopic, syngeneic mouse model of epithelial ovarian cancer (EOC). Female C57BL/6 mice were injected with 1.0×10^6 transformed ovarian epithelial cells (ID8) under the ovarian bursa. In this model, metastatic lesions and abdominal ascites develop at 60 days post-surgery, representing clinical stage III EOC. At 75 days of tumour development, we performed histological analyses of tibialis anterior (TA) muscle to determine whether ovarian cancer induces capillary rarefaction. Formalin-fixed, paraffin-embedded TA muscle was sectioned and stained with wheat germ agglutinin and isolectin B4 to quantify myocyte cross-sectional area (CSA) and capillary density, respectively.

Results: In TA muscle, capillary density decreased (4.7 ± 0.6 in EOC compared to 5.7 ± 0.1 in shams; $p=0.009$) with no evidence of muscle atrophy (CSA of 1074 ± 213.8 μm^2 in EOC compared to 948.3 ± 160.5 μm^2 in shams; $p=0.35$) by histological assessments. This aligns with our observations in cardiac muscle, where capillary density decreased before any evidence of cardiomyocyte atrophy. The extent of capillary rarefaction in skeletal muscle was compared to cardiac muscle data by expressing the decline in capillary density as a percent change from shams. Capillary density decreased by 18.0% in skeletal muscle compared to 5.3% in cardiac muscle.

Conclusion: We demonstrate that capillary rarefaction precedes muscle atrophy in skeletal muscle, revealing that this cancer-induced pathology is common among both skeletal and cardiac muscles in ovarian cancer.

Abstract Title: Investigating the role of estrogens and exercise in attenuating fat infiltration and fibrosis in a VCD-induced model of ovarian failure in mice: A proposal

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Menopause is characterized by a reduction in estrogens, which has been shown to impair muscle function and increase the amount of fatty infiltration and fibrosis in skeletal muscles. Changes to muscle composition may be influenced by many factors such as hormonal status, metabolic conditions, as well as physical activity. Previous studies using the ovariectomy model in rodents have shown that estrogen deficiency decreases the function of key lipid metabolism enzymes, increasing fatty infiltration in skeletal muscles, notably in Type I fibers, but not in Type II fibers. Additionally, estrogen deficiency has been linked to increased extracellular matrix (ECM) components contributing to increased muscle fibrosis. This proposal seeks to extend these findings by exploring the morphological changes in the SOL and EDL muscles using a chemically-induced ovarian failure model in mice. This model uses VCD to induce gradual ovarian failure, to more closely mimic the natural trajectory of menopause experienced in humans than the ovariectomy model. Additionally, a high fat diet (HFD) model will be implemented to stimulate metabolic stress, thereby exacerbating the potential for fatty infiltration and fibrotic remodelling. Moreover, this study will also investigate the restorative potential of reintroducing estrogens and the protective effects of high-intensity interval training (HIIT) against these changes as previous findings indicate that HIIT can activate beta-oxidation pathways and induce metabolic adaptations beneficial for muscle health. Thus, we hypothesize that HIIT can mimic estrogen's restorative effects and counteract the adverse, compounded effects induced by both ovarian failure and a HFD. Our outcome measures will include muscle structural composition evaluation via histology using Hematoxylin and Eosin (H&E) stain, and quantification of ECM accumulation and fat infiltration with Picosirius Red and Oil-O Red staining, respectively. Non-histological measures will include anatomical cross-sectional area (CSA), assessments of muscle length and wet weight. These comprehensive assessments will help us understand the structural integrity and composition changes in the SOL and EDL muscles as an effect of ovarian failure, HFD, estrogen replenishment and HIIT, setting the stage for mechanistic investigations and implications on muscle function.

Abstract Title: Investigating the role of CARM1 in skeletal muscle regeneration and repair

Authors: Hooriya A. Masood, Sean Y. Ng, Andrew I. Mikhail, Vladimir Ljubicic

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Introduction: Skeletal muscle is highly plastic and capable of regeneration and repair following injury. The regenerative process is primarily driven by myogenic stem cells, termed satellite cells. Protein arginine methyltransferases (PRMTs) are enzymes that catalyze the methylation of arginine residues on target molecules to modulate cellular mechanisms in the body. PRMT4, also known as coactivator-associated arginine methyltransferase 1 (CARM1), is highly expressed in skeletal muscle. Notably, CARM1 specifically found in satellite cells is necessary for myogenic repair, through the methylation of paired box transcription factor Pax7, which then induces Myf5 expression during satellite cell activation. However, the requirement of skeletal muscle CARM1 for muscle regeneration and repair remains unknown. Therefore, this project investigates the role of skeletal muscle-specific CARM1 expression in the regenerative response to acute muscle trauma. We hypothesize that the absence of CARM1 in skeletal muscle will attenuate regeneration and repair. Methods: The tibialis anterior (TA) and gastrocnemius (GAST) muscles in one hindlimb of 12-week-old, adult, male and female wild-type (WT) and CARM1 skeletal muscle-specific knockout (mKO) mice (n = 6 - 10) were injected with a 10 μM cardiotoxin solution. The contralateral hindlimb was injected with saline to serve as an uninjured control. Muscles were harvested at 7, 14, and 21 days post-injury (DPI) and processed for morphological and biochemical analyses. Results: Histological assays of TA muscles revealed a reduction in myofiber cross sectional area and minimum feret diameter at 7 DPI, followed by a gradual recovery over time in WT and mKO mice. Both genotypes displayed a significant increase in centrally nucleated fibers at 7 DPI, which decreased at 14 and 28 DPI. No changes were seen in metrics of fibrosis. Protein markers of regeneration in GAST muscles, including myogenin and embryonic myosin heavy chain, peaked following injury at 7 DPI and declined throughout the time course in both genotypes. Summary: Our preliminary results suggest that while CARM1 in satellite cells is essential for skeletal muscle regeneration and repair of skeletal muscle, CARM1 in the whole muscle is not required for these processes. This ongoing study will provide insight into the role of CARM1 in skeletal muscle plasticity.

Abstract Title: Skeletal muscle AMPK regulates neuromuscular biology

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Endurance exercise is pleiotropic lifestyle intervention that elicits multisystemic benefits partly due to the activation of AMP-activated protein kinase (AMPK). This kinase is a heterotrimeric energy sensor that influences key cellular pathways including mitochondrial biology, autophagy, and fuel utilization. However, the role of AMPK in determining skeletal muscle phenotype, as well as its retrograde effects on the neuromuscular junctions (NMJ) and innervating motoneurons remains undefined. Thus, this study aimed to examine the function of skeletal muscle AMPK in neuromuscular biology under homeostatic conditions and after exercise training. First, we generated novel, HSA-Cre-driven skeletal muscle-specific b1b2 AMPK knockout (mKO) mice to assess the importance of AMPK during basal maintenance of the neuromuscular system. Our data highlight increased instability of the NMJ as indicated by ~2-11-fold greater ($p<0.05$) expression of denervation markers including *Chrna1*, *Chrng*, and *Myog* in TA muscles of mKO mice relative to WT. Additionally, AChR turnover was significantly blunted in the absence of AMPK due to reduced ($p<0.05$) deposition of new AChRs. Nevertheless, we did not observe any overt morphological abnormalities in axons of the sciatic nerve. Next, to elucidate AMPK-specific effects following exercise training, we utilized a novel tamoxifen (TMX)-inducible skeletal muscle-specific b1b2 AMPK knockout (imKO) mouse model, as germline deletions result in severe exercise intolerance. We treated WT and imKO mice with TMX at 16 weeks of age after which they were allocated into a sedentary group or progressive treadmill training for 6 weeks. Exercise significantly improved muscular function (i.e., cage hang and exercise capacity) in WT and imKO. Interestingly, WT mice outperformed imKO animals in all functional measures, regardless of training status. We noted a significant training-induced increase in CI+II ADP-stimulated respiration in WT animals only, but OxPhos protein content was augmented with exercise in WT and imKO mice. Similar to mKO mice, temporal deletion of AMPK augmented the expression of denervation markers, which were completely normalized to WT levels following the training intervention. Concomitantly, exercise induced a reduction ($p=0.059$) in synaptic nuclear domain in imKO mice. Collectively, our data demonstrate that skeletal muscle AMPK is a key regulator of neuromuscular biology and is required for exercise training-evoked mitochondrial functional remodeling.

Abstract Title: Analysis of Myopathies and Disease Mechanisms Using Zebrafish as a Model

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Actin is a highly conserved protein found in all eukaryotic cells. It plays crucial roles in various cellular processes, notably cellular contractility. Within the sarcomere, interactions between actin filaments, myosin motor proteins, along with regulatory thin filaments troponin and tropomyosin orchestrate the generation of contractile forces. Mutations in genes encoding sarcomere proteins, such as the Alpha cardiac actin 1 (ACTC1) gene have been implicated in cardiovascular diseases such as cardiomyopathy.

in human patients. Patients with congenital heart disease may also manifest non-cardiac related congenital abnormalities, with musculoskeletal abnormalities being the most prevalent. To investigate the effects of mutations in the ACTC1 gene, a transgenic CRISPR/Cas9 zebrafish line can be used as a model to study cardiac disease progression during key stages of development. The transparent appearance of larvae allow for clear visualization of both the pericardial cavity as well as the tail muscle, displaying phenotypes of cardiomyopathy and musculoskeletal myopathy. Birefringent light analysis on tail muscles show disorganized tail muscle in variant larvae when compared to the wildtype counterparts throughout early development. Brightfield imaging

of the pericardial sac with subsequent heart rate analysis show a difference in heart rate between variant and wildtype larvae. Phenotype analysis provides a crucial platform for correlating observable traits with underlying genetic and protein expression patterns, aiding in the comprehensive understanding of biological mechanisms and disease processes. Overall, zebrafish provide a powerful model system for elucidating fundamental aspects of heart development, function, and disease, offering valuable insights into muscle disorders in humans.

Abstract Title: Regulation of MEF2A Function by Actin Signalling in Skeletal Muscle

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Abstract text (no figures are permitted): Myocyte Enhancer Factor 2 (MEF2) is a transcriptional regulatory complex that is encoded by four genes, mef2a-d. MEF2 is part of the MADS (MCM-1, Agamous, Deficiens and Serum Response Factor) family of DNA binding proteins. MEF2 proteins are involved in numerous organ systems, with a particular emphasis on muscle development including cardiac, skeletal, and smooth muscle, controlling cellular differentiation and proliferation. MEF2 proteins are involved in tightly regulating the expression of myogenic factors and act as co-factors to myogenic regulatory proteins, controlling the expression of muscle-specific genes. Dysregulation of MEF2 has been implicated in cardiac hypertrophy and cancer, signifying its importance. Actin signalling pathways have been shown to regulate gene expression, including the expression of serum response factor (SRF) target genes, a protein that is part of the same MADS family as MEF2A. Additionally, a recent interactome screen from the McDermott Lab found that MEF2A interacts with actin binding proteins Ezrin, Radixin and Moesin. In this study, I aimed to determine whether actin dynamics regulated MEF2A function. Ectopic expression of non-polymerizable actin mutations repressed MEF2A activity, as determined through a gene reporter assay. Using the same assay, addition of Moesin also repressed MEF2A activity. Collectively, this data illustrates a potential interaction between MEF2A and actin dynamics in skeletal muscle cells.

Abstract Title: Inflammatory cytokines and regulators following high-intensity interval running and cycling in adolescents.

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BACKGROUND: High-intensity interval exercise (HIIE) has been linked to positive responses of the immune system and derived neurotrophic factor (BDNF) by upregulating and inhibiting anti-inflammatory (e.g., muscle-derived IL6 and IL10) and pro-inflammatory cytokines (e.g., TNFα), respectively. Although part of this effect is regulated by adipokines, such as leptin and adiponectin, no studies have examined the effects of different modes of HIIE on all these markers, especially in children. PURPOSE: The purpose of this study was to compare the response of cytokines, adipokines and BDNF to high-intensity interval running (HIIR) versus cycling (HIIC) in female adolescents. METHODS: Eleven adolescent females at post-peak height velocity, aged 15 to 19 years, performed two trials (HIIR and HIIC) in random order. Each participant performed a progressive incremental test for each mode of exercise to determine the >90% max workload for the trials. The trials consisted of 8 bouts of 1 min of running or cycling with 1 min of rest in between. Blood samples were collected pre-exercise, 5 and 60 min- post-exercise. IL6, IL10, TNFα were measured in serum, while adiponectin, leptin and BNDF were measured in plasma. RESULTS: There were no significant mode-by-time interactions found for the measured markers. Exercise-induced increases (time effect) from pre to 5 min post-exercise were observed in IL6 ($p=0.004$, $p\eta^2=0.272$), IL10 ($p=0.004$, $p\eta^2=0.319$), TNFα ($p<0.001$, $p\eta2=0.462$) and BDNF ($p<0.001$, $p\eta^2=0.400$). A significant reduction in leptin was observed only between 5 min and 60 min post-exercise (time effect, $p=0.013$, $p\eta^2=0.196$). Significant correlations were observed between changes in TNFα and IL-6 ($r=0.460$, $p=0.031$), IL-10 ($r=0.702$, $p<0.001$), and BDNF ($r=0.444$, $p=0.038$). No correlation was found with the adipokines. CONCLUSION: In female adolescents, high-intensity interval exercise induces interdependent changes in cytokines and BDNF, along with an independent leptin response, and no effect of exercise mode (i.e., running versus cycling).

Abstract Title: Development of a high through put actomyosin in Vitro motility assay

Authors: Noah Presley

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Cardiovascular diseases are the leading cause of deaths globally, accounting for 32% of mortalities and posing an immense economic burden of $21.2 billion annually in Canada alone. Among these is a disease of the ventricular myocardium known as cardiomyopathy, predominantly appearing as hypertrophic or dilated cardiomyopathy, whose development is often linked to genetic mutations in genes encoding sarcomere proteins. Current treatments for hypertrophic cardiomyopathy target symptom relief and have limitations in treatment efficacy, along with many negative side effects. Treatment options for dilated cardiomyopathy are also limited and a large precent of affected individuals end up in need of heart transplants within five years of diagnosis. The in vitro motility assay is a widely employed assay used for studying disease development in cardiomyopathies, as well as novel medications capable of targeting disease mechanism. While this assay has the ability to assess the force and calcium sensitivity of contraction in sarcomere proteins, the assay faces challenges in lengthy data acquisition and analysis and is therefore considered low throughput. To address the bottleneck in cardiomyopathy drug development, this project aims to adapt the in vitro motility assay to a high-throughput assay with automated data collection and analysis. This involves the translation to a static well configuration requiring a new form of surface functionalization as well as automation of analysis and image capturing. With a successful automated assay, it is possible to expedite characterization of variant sarcomere proteins linked to this disease and identify potential therapeutic compounds targeting hypertrophic and dilated cardiomyopathies' molecular disease mechanism, a crucial step towards eliminating the burden of this disease.

Abstract Title: Should You Sprint to an Ice Bath? Post-Sprint Interval Exercise Cold-Water Immersion Results in Modest Acute Muscle Recovery While Not Impacting Prolonged Recovery or Next-Day Performance

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Cold-water immersion (CWI) has emerged as one of the most popular post-exercise recovery interventions for avid exercisers. It has been suggested that CWI can help accelerate the recovery of muscle fatigue following exercise. However, little scientific evidence exists to support such claims, especially following high-intensity interval exercise (HIIE). Therefore, to investigate the use of CWI following HIIE, 10 young recreationally active individuals were recruited to participate in a randomized cross-over study involving repeated all-out contractions of the ankle dorsiflexor muscles followed by 10 min of passive recovery or CWI. Following the recovery interventions, neuromuscular, functional, and intramuscular temperature assessments were performed throughout the acute (≤1-h) and prolonged (≤24-h) stages of recovery. The results suggest that CWI increased low-frequency force compared to passive recovery immediately following the recovery intervention ($p<0.05$), thus offering a modest improvement in skeletal muscle recovery. However, low-frequency force remained depressed in both conditions throughout the 24-h recovery period following the initial bout of HIIE ($p<0.05$), suggesting that regardless of the recovery intervention, prolonged low-frequency force depression was apparent. Lastly, no differences in performance were seen in a second HIIE bout performed 24-h later. Although modest improvements were seen in the acute phase of recovery following CWI, these improvements were not sustained enough to impact either recovery at prolonged stages (>1-h) or next-day performance.

Abstract Title: Are muscle-derived extracellular vesicles involved in the metabolic effects of resistance training?

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Rationale: Skeletal muscle secretes extracellular vesicles (EVs) containing molecular cargo capable of altering the metabolism of different tissues, such as cytokines (myokines) which can regulate cellular pathways. It has been shown that chronic resistance exercise training (RET) enhances whole-body and skeletal muscle insulin sensitivity; however, the mechanisms governing this effect remain unclear. Evidence also suggests that muscle-derived EVs enhance whole-body and tissue-specific insulin action, but this has yet to be investigated in the context of RET. Furthermore, the role of insulin in EV secretion from muscle and the influence of RET remains unclear. Purpose: To determine the effects of 10 weeks of RET on EV secretion from slow and fast-twitch skeletal muscle and its association with insulin sensitivity in female rodents. Methods: Twenty female Sprague-Dawley rats will be divided into two groups: sedentary (SED, n=10) and resistance-trained (RET, n=10). RET rodents will undergo 10 weeks of RET consisting of progressively overloaded vertical weighted ladder climbing. Following the 10 weeks, all animals will undergo an intravenous glucose tolerance test to assess insulin sensitivity. Analysis: Slow-twitch and fast-twitch muscles will be incubated in culture media for 24h with or without insulin. EVs will be isolated from media using ExoQuick-TC. EV marker content and EV concentration will be assessed via western blot and a Bradford or BCA protein assay, respectively. Data will be analyzed using a two-way analysis of variances with training and fiber type as factors. Associations between EV secretion and insulin sensitivity will be determined using correlational analysis. Implications: The results from this study will provide valuable insight into the effects of RET on muscle-derived EVs and insulin sensitivity. Future studies will aim to understand the physiologic effects of these EVs and their potential for disease treatment.

Abstract Title: Developing an in vivo animal exercise assay to detect exercise behaviours in mice

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Introduction: Exercise is vital for preventing disease and promoting longevity. Over time, the body improves its maximal exercise capacity through training adaptations such as an increase in VO2 max. In sports, athletes may use prohibited substances that increase their physical performance. Although there are many detection methods available, there is still a need for more methods as new doping agents are continually being developed. Therefore, the development of pre-clinical assays could serve as a useful platform for testing performance enhancing agents. There is a need to design and test a non-invasive, yet sensitive animal-based assay, that improves resolution for differentiating exercise performance in a regular cyclometer (which presents a single value from a summary of dynamic data collected over time) and offer circadian analyses. This work could help increase doping detection rates (that improve exercise tolerance) before they reach human competition. Methods: Using a hand-built cyclometer programmed through the raspberry pi computer, voluntary wheel running behaviours in mice were recorded for 10 consecutive days. The pi cyclometer (featuring a Hall Effect sensor and neodymium magnets attached on the running wheels) will detect changes to wheel rotation, speed, acceleration, and distance (on a minute-minute basis) and publish the data to a server in real-time. To compare capabilities, running wheels will also be equipped with the VDO M2.1 WR Cycling Computer to track distance which will be manually recorded once a day. Results: In our sample size of n=24, the main findings include that body weight was inversely correlated to voluntary wheel running distance over 10 days, and the running behaviours using the VDO showed a gradual increase in running distances, whereas the pi cyclometer showed a decrease in running distance, speed and acceleration. Conclusion: Although the VDO is more reliable, there was no insight given on their exercise behaviours within 24 hours to compare circadian data making the pi cyclometer more superior. This head-to-head comparison shows our dynamic circadian cyclometer offers validity for collecting mouse running data, but further needs optimizing in accuracy. Future work will include differentiating exercise performance in doped and un-doped states.

Abstract Title: A Protein Interactome Screen of HIPPO Signaling Effector TAZ

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Background: HIPPO signaling is important for growth, differentiation, and overall cell homeostasis in striated muscle. Transcriptional co-activator with PDZ-binding domain (TAZ) is highly regulated by the HIPPO pathway. TAZ is widely recognized as an activator of proliferation in muscle precursor cells; however, its role in muscle cell differentiation is less known. Previous work by the McDermott lab has shown a repressive role of TAZ in muscle cell differentiation. TAZ cannot bind DNA; it must interact with DNA-binding proteins to regulate gene transcription. Therefore, there is a need to determine proteins that interact with TAZ, as these interactions facilitate its co-regulatory effects on gene expression. **Purpose**: Our aim is to identify potential TAZ-interacting proteins and their corresponding molecular and biological roles, and further biochemically and functionally characterize these interactions and their implications on TAZ function in a myogenic context. **Methods**: We conducted a proteomic interactome screen of TAZ-interacting partners following a FLAG immunoprecipitation (FLAG-IP) of a nuclear-enriched fraction from human embryonic kidney (HEK293T) cells. The FLAG-IP consisted of: 1) an experimental sample (nuclear-enriched fraction alone), and 2) an out-competition control (nuclear-enriched fraction + competitive peptide). The samples then underwent on-bead digestion and subsequent analysis using the TimsToF Pro 2 with liquid chromatography-tandem mass spectrometry. Subsequently, Gene Ontology (GO) analysis was used to categorize the molecular and biological relevance of the identified proteins from the interactome screen. **Results**: The interactome screen revealed 57 candidate proteins including 33 unique proteins found only in the experimental sample, and 24 proteins with ≥3-fold enrichment in the experimental sample compared to control. The GO analysis provided a comprehensive list of biological processes and pathways enriched in this screen including, but not limited to, transcriptional regulatory activity, HIPPO signaling, cell differentiation, and gene expression. **Conclusion**: Our proteomic interactome screen revealed a list of unique and enriched candidate TAZ-interacting proteins. Our research provides insight into understanding the molecular underpinnings involved in striated muscle development, proliferation, and differentiation, which may help to shed light on how these processes can become dysregulated in severely debilitating myopathies.

Abstract Title: Effect of Poly-ADP-Ribosylation Signaling on Muscle Satellite Cell Fate and Function

Authors: Kejzi Saraci, Alex Green, Keir J. Menzies

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Skeletal muscle is often under physiological and metabolic stress, which can result in damage. This damage is repaired through the activation of quiescent muscle stem cells (aka satellite cells) that are located on the periphery of the muscle fiber and beneath the basal lamina. Following damage, satellite cells will proliferate then fuse and repair the damaged muscle fibers, while others will undergo a process of self-renewal to replenish the satellite cell population. How this process of satellite cell proliferation/differentiation/self-renewal occurs is still not well known. Poly-ADP-Ribosylation proteins (PARPs) covalently add ADP-ribose polymers to target proteins through a process known as PARylation, and Poly (ADP-ribose) glycohydrolase (PARG) reverses this process, maintaining balance. The most active PARylation proteins are PARP1 and PARP2. PARylation plays an important role for apoptosis, DNA damage repair, and transcription. Despite the important role PARylation and dePARylation play in the cell, there has been little research as to their role within satellite cells. To assess this, inducible muscle stem cell specific knockout (iMSKO) models of PARP1, PARP2 and PARG have been generated. The tibialis anterior of these mice have been injected unilaterally with cardiotoxin to induce muscle damage. The contralateral limb and the limb from an uninjured littermate will be used as controls. These tissues will be cross-sectioned and stained with myogenic markers to assess satellite cell fate as 0, 7, 14 and 21 days post injury. These data will be verified by performing similar staining of cultured extensor digitorum longus muscle fibers isolated from each mouse models. Early data suggests that the absence of PARG in satellite cells ablates muscle regeneration, while the absence of PARP1/2 activity appears to reduce satellite cell self-renewal.

Abstract Title: Mitochondrial Localization of Retinoblastoma like-1 (Rbl1) Drives Muscle Stem Cell Fate Decisions Through Fragmentation of the Mitochondrial Network

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Muscular diseases such as sarcopenia and muscular dystrophy trace their onset to the dysfunction of the intrinsic regenerative capacity of skeletal muscle tissue. Maintenance of skeletal muscle integrity is credited to the function of resident adult muscle stem cells (MuSCs). To effectively serve their function, MuSCs, once activated, commit to one of two potential fates—population maintenance via self-renewal or differentiation into new muscle. Crucially, loss of balance between these two fates attenuates muscle regeneration. Thus, understanding the mechanisms dictating fate decision pathways offers the potential to generate novel therapies for muscular diseases. Previous laboratory members have established a non-nuclear non-canonical role of retinoblastoma like-1 (Rbl1), a member of the retinoblastoma family of proteins, influencing MuSC fate decisions, as well as oxidative phosphorylation through Sirtuin 1 (Sirt1) deacetylase, which is dependent on the cellular NAD+/NADH ratio. Hence, we hypothesize that the ability of Rbl1 to influence MuSC fate decisions might function through the manipulation of the mitochondrial network that influences OXPHOS efficiency. To test this idea, we established an ex vivo mouse muscle fiber culture approach to follow MuSC behaviour. Myofibers were grown in culture media designed to activate or deactivate Sirtuin activity. This was done directly via Sirtuin activators and inhibitors and indirectly through altered culture media glucose concentrations which manipulated the cellular NAD+/NADH ratio. Using confocal microscopy and three-dimensional rendering software, we observed high Rbl1 mitochondrial localization, as well as fragmentation of the mitochondrial network, in MuSCs on myofibers grown in Sirtuin deactivating conditions. Sirtuin activating conditions yielded the opposite results, with less Rbl1 mitochondrial localization and greater integrity of the mitochondrial network. Further, increased OPA1, an inner mitochondrial fusion protein, expression levels were found in Rbl1 genetically deleted myoblast progenitor cells offering a possible mechanism for the Rbl1-dependant changes in mitochondrial dynamics. Therefore, altering the localization of Rbl1, and thus mitochondrial fusion and fission, offers a potential avenue to restore balance to muscle stem cell fate decisions which are often disrupted in degenerative muscle diseases.

Abstract Title: Isolation of a persistently quiescent muscle satellite cell population.

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Abstract text (no figures are permitted): While studies have identified characteristics of quiescent satellite cells, their isolation has been hampered by the fact that the isolation procedures result in the activation of these cells into their rapidly proliferating progeny (myoblasts). Thus, the use of this cell population for therapeutic strategies (myoblast transplants, regenerative medicine) or industrial applications (cellular agriculture) has been impeded by the limited proliferative and differentiative capacity of the myogenic progeny. Here we identify a subpopulation of satellite cells isolated from mouse skeletal muscle using flow cytometry that are highly Pax7 positive, exhibit a very slow proliferation rate (~7 day doubling time), and are capable of being maintained in culture for months without a change in phenotype. These cells can be activated from their quiescent state using a p38 inhibitor or by exposure to freeze-thaw cycles. Once activated, these cells proliferate at a much higher rate (~23 hour doubling time), have reduced Pax7 expression (vs. quiescence) and differentiate into myotubes with a high degree of efficiency. Furthermore, these cells withstand freeze-thawing readily without a significant loss of viability. The results presented here provide researchers with a method to isolate quiescent satellite cells, allowing for more detailed examinations of the factors affecting satellite cell quiescence/activation and providing a cell source that has a unique potential in the regenerative medicine and cellular agriculture fields.

Abstract Title: In silico modelling of actin R312C/H substitutions reveal the structural mechanisms differentiating HCM and DCM-causing actin variants

Authors: Karl Steffensen, Michael Jones, John Dawson

Author Affiliations: University of Guelph

Actin plays a central role in cellular processes through interactions with actin binding proteins (ABPs). In the sarcomere, actin's interactions with myosin, tropomyosin, and troponin regulate contraction, with amino acid substitutions disrupting these interactions and resulting in diseases like hypertrophic (HCM) and dilated cardiomyopathy (DCM). Actin amino acid substitutions R312C/H have been found in patients with HCM and DCM, respectively. Previously, we characterized the R312C/H variants biochemically, observing altered interactions with myosin, troponin, and tropomyosin. R312H actin exhibited increased actomyosin activity under low calcium conditions, and both variants displayed decreased calcium sensitivity in vitro, contrary to the prevailing hypothesis that HCM and DCM are linked to increased and decreased calcium sensitivity, respectively. It was hypothesized that factors other than changes in calcium sensitivity differentiate between the onset of HCM and DCM. Understanding how actin variants result in different forms of cardiomyopathy is critical for guiding the development and application of treatments for heart disease. Here, in silico modeling revealed structural mechanisms behind altered actin:ABP interactions in vitro. Notable changes to structural properties and conformations occurred in the DnaseI binding loop (D-loop) and 222-230 helix in both variants. While both variants displayed decreased flexibility in D-loop residues, simulations predicted that: the D-loop of R312C moves closer to the binding sites of myosin and troponin which stabilizes those interactions, increasing actomyosin force output; the D-loop of R312H moves away from myosin and troponin binding sites, destabilizing those interactions which decreases actomyosin force output and alters troponin's regulation of myosin binding under low calcium conditions. Both variants exhibited large changes in the position of the 222-230 helix that would interfere with tropomyosin's ability to access the open state, decreasing activity under high calcium conditions, in line with decreased calcium sensitivity in vitro. Predictions from molecular dynamics simulations therefore support the hypothesis that factors such as actomyosin force output and the stability of interactions with regulatory proteins, rather than changes to calcium sensitivity, drive the differentiation between HCM and DCM and must be considered in the development of clinical treatments for heart disease.

Abstract Title: Does Resistance Training Restore Pancreatic Islet Function/Morphology in Type One Diabetes?

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Rationale: Resistance training (RT) exercise is beneficial for those living with type one diabetes mellitus (T1DM) and is well documented that RT improves glucose tolerance and insulin sensitivity. Evidence suggests that RT positively affects pancreatic beta-cell mass and function in healthy humans and rodents. Secreted factors from muscle (myokines, metabolites, miRNA's and exosomes) are noted to promote islet beta-cell function and survival ex vivo. Secreted factors from muscle are upregulated with RT and limited data is available on how these factors affect pancreatic islet function and morphology in vivo. Purpose: To determine the effects of RT on the preservation/restoration of pancreatic beta cell function and morphology in insulin treated T1DM. Methods: Forty Sprague-Dawley rats will be randomly divided into four groups: Control sedentary (CS, n=10), diabetic sedentary (DS, n=10), control resistance trained (CRT, n=10), diabetic resistance trained (DRT, n=10). Diabetes will be induced in DS and DRT groups with seven consecutive low dose streptozotocin injections (20 mg/kg/day) and blood glucose will be maintained at 4-9 mmol by implanting a subcutaneous insulin infusion pump. CRT and DRT will undergo 10-weeks of RT consisting of progressively overloaded vertical weighted ladder climbing. After 10-weeks all animals will be sacrificed to harvest their tissues. Analysis: Blood will be collected to measure c-peptide concentrations. Pancreata will be fixed for immunohistochemical staining. Islet number, size and hormone content will be quantified using light microscopy and ImageJ software. A Ki67 stain and TUNEL stain will be conducted to measure beta-cell proliferation and apoptosis respectively. A 2-way analysis of variance will be conducted with diabetes and training as factors. Implications: Results from this study will provide insight on the effects of RT on pancreatic islet function and morphology in rodents with T1DM. Future studies will examine possible mechanisms for this phenomenon by examining the endocrine functions of muscle.

Abstract Title: Contractile and Metabolic Function of Engineered Human Skeletal Muscle Microtissues

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Human skeletal muscle microtissues are emerging as a powerful tool to accelerate drug discovery, providing human-relevance data that is quickly becoming a critical asset in clinical trial applications. However, functional and metabolic assay parameters used for engineered muscle conventionally attempt to mimic those for evaluating native human skeletal muscle. They have yet to be optimized for microtissues. Therefore, the goal of this study was to define the optimal electrical field stimulation (EFS) parameters to elicit peak contractile force and culture conditions to evaluate insulin- stimulated glucose uptake of engineered microtissues. Muscle constructs were fabricated onto an opposing pair of microposts in the 96-well MyoTACTIC culture platform using three human primary myoblast lines. In response to EFS between needle electrodes, contraction deflects the microposts proportional to developed force. With a 5 V electrical field, pulse durations used for native muscle (0.1-1 ms) failed to elicit contraction of constructs. Instead, pulse durations of 60 ms were required to elicit peak force (40-79 μN) during twitch contractions. In contrast to peak tetanic force elicited at 20-50 Hz for native human muscles, peak tetanic

force of constructs (112-247 μN) occurred at 7 Hz. A new parameter, the dynamic oscillation of force, captures trends during rhythmic contractions, while quantifying the duration-at-peak force provides an extended kinetics parameter. Our findings indicate that muscle microtissue constructs contract and relax more slowly than native muscle, implicating under-developed excitation-contraction coupling. However, microtissues demonstrate superior muscle cell differentiation over traditional petri dish culture. We report that the ratio of insulin-regulated glucose transporter type 4 to insulin-independent glucose transporter type 1 was ~14x

higher in microtissues than 2D myotubes. While physiological glucose levels were adequate to form microtissues capable of EFS induced post deflection, supraphysiological insulin and amino acid levels were necessary. Following differentiation, microtissues cultured for four days without added insulin showed an upregulation of glucose uptake in response to insulin stimulation. Our findings illustrate that physiological EFS parameters and culture conditions mask the functional and metabolic potential of muscle constructs. To maximize the utility of human skeletal muscle microtissues in developing new therapies, it is imperative to optimize assay conditions.

Abstract Title: Does serial sarcomerogenesis contribute to the repeated bout effect following muscle damaging exercise?

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Abstract text (no figures are permitted): Neuromuscular function is impaired following an unaccustomed bout of eccentric exercise. However, through the repeated bout effect (RBE), the muscle is protected from damage following a subsequent bout of eccentric exercise. In addition to other proposed mechanisms, it has been speculated that the addition of sarcomeres in series (e.g., sarcomerogenesis) reduces mechanical strain on muscle fibers during active lengthening. However, whether sarcomeres are added following muscle damage is unknown. Furthermore, the protective effects of sarcomerogenesis have yet to be determined. Therefore, we investigated the protective effects of sarcomerogenesis following eccentric exercise-induced muscle damage, on a subsequent bout of eccentric exercise. Using an in-vivo set up, N=25 Sprague-Dawley rats will undergo 3 sets of 50 eccentric contractions at 900°/s with 5 minutes of rest in between sets to induce muscle damage and impairments in mechanical function. Following the initial eccentric exercise bout, and the assessment of mechanical function, N=10 rats will be sacrificed, and the soleus, and medial gastrocnemius (MG) will be harvested to assess serial sarcomere number via laser diffraction with the un-exercised leg used as a control. Furthermore, N=5 rats will be sacrificed for assessment of muscle damage via transmission electron microscopy. After a 2-week recovery period, the remaining N=10 rats will complete an identical second bout of eccentric exercise to assess whether muscle damage was reduced, and impairments in mechanical function attenuated due to the RBE. Consistent with previous literature, we expect significant muscle damage and impaired mechanical performance following the initial bout of eccentric exercise. Furthermore, upon recovery from the initial bout of eccentric exercise, we expect to observe an increase in serial sarcomere number. Therefore, we expect to observe a robust RBE following the second bout of eccentric exercise, indicated by decreased muscle damage and protection from impaired mechanical performance. These results would confirm, for the first time, that sarcomerogenesis contributes to the RBE. Supported by NSERC.