10th Annual
Muscle Health Awareness Day
May 24, 2019

Program and Abstracts

Muscle Health & Research Centre

Adaptation • Development • Metabolism • Disease

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Date: May 24, 2019

To: All Participants

From: David A. Hood, MHRC Director

David A. Hood, PhD

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Canada Research Chair
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Welcome to the 10th Annual Muscle Health Awareness Day

The Muscle Health Research Centre at York University welcomes you to MHAD10, our 10th annual "*Muscle Health Awareness Day*", designed to bring together scientists, faculty members, graduate students and post-doctoral fellows to discuss issues related to skeletal and cardiac muscle physiology, metabolism, adaptation, development and disease.

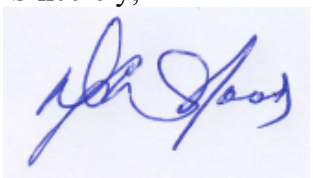
We are pleased to welcome 8 great speakers for MHAD10. The focus this year is on 1) muscle and exercise metabolism, 2) neuromuscular physiology and pathology, and 3) exercise and disease.

Our goal is to give graduate students a chance to present their work in an informal, yet educational manner. This year we have 60 poster presentations to showcase, offering plenty of opportunity to network among faculty members and trainees.

Every year we try to improve MHAD, so any suggestions that you might have for improvement are appreciated. In addition, if you know of any colleagues in the area who would be interested in speaking at MHAD in the future, please let us know.

We thank all of our speakers, presenters, volunteers and sponsors for their participation, and for helping to make this a successful, yearly event. Enjoy MHAD10!

Sincerely,



David A. Hood, PhD
Director, Muscle Health Research Centre

10th Annual Muscle Health Awareness Day Program
Friday May 24, 2019
Life Science Building South Lobby and Room 103, York University

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

Session 1: Muscle and Exercise Metabolism (9:00-10:35)

Session Chair: Dr. Gary Sweeney

9:00-9:05 – Dr. David Hood, *York University*
Welcome and Introduction

9:05-9:35 – Dr. David J. Dyck, *University of Guelph*
Skeletal Muscle Insulin Response - the Role of Adipose and Stomach-Derived Factors

9:35-10:05 – Dr. Keith Dadson, *University Health Network*
Understanding the p53/mdm2 relationship in the heart

10:05-10:35 – Dr. Mireille Khacho, *University of Ottawa*
Muscle stem cell maintenance by mitochondrial retrograde signalling in health and aging

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

Session 2: Neuromuscular Physiology and Pathology (11:30-12:30)

Session Chair: Dr. William Gage

11:30-12:00 – Dr. Clark Dickerson, *University of Waterloo*
Caught in a bad neighborhood: muscle fatigue and the origins of rotator cuff damage

12:00-12:30 – Dr. Geoffrey A. Power, *University of Guelph*
The history-dependence of force and implications on neuromuscular function

12:30 – 2:00 Catered Lunch (Life Science Building South Lobby);
1:30-2:00 Poster Presentations

Session 3: Exercise and Disease (2:00-4:00)

Session Chair: Dr. Emilie Roudier

2:00-2:30 – Dr. Ali Abdul-Sater, *York University*
How to train an immune system: The role of exercise in regulating inflammation

2:30-3:00 – Dr. Marina Mourtzakis, *University of Waterloo*
Characterizing muscle wasting syndromes using multi-modal approaches

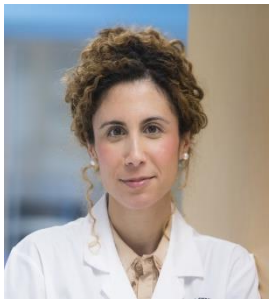
3:00-3:30 – Dr. Paul Oh, *University of Toronto*
Exercise for the prevention and management of cardiovascular disease

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

10th Annual Muscle Health Awareness Day Speaker Profiles

Dr. Keith Dadson, University Health Network

Dr. Keith Dadson is a Post-Doctoral Fellow in Molecular Cardiology at the Toronto General Research Institute, University Health Network. He is working under the supervision of Dr. Phyllis Billia. His research focuses on identifying pathways that lead to recovery of heart function following cancer therapy induced cardiotoxicity, and methods to stimulate cardiomyocyte cell-cycle re-entry to repair the heart following myocardial infarction. He completed his PhD at York University under the supervision of Dr. Gary Sweeney.



Dr. Mireille Khacho, University of Ottawa

Dr. Mireille Khacho is an Assistant Professor in the Department of Biochemistry, Microbiology and Immunology. Her research focuses on the mechanisms by which mitochondria regulate stem cell function and longevity in order to develop therapeutic strategies that enhance tissue regeneration during aging and degenerative diseases. She is also studying the metabolic regulation of muscle stem cells function and muscle regeneration.

Dr. Paul Oh, University of Toronto

Dr. Paul Oh is a Senior Scientist and Research Division Head at the Toronto Rehabilitation Institute. He is also the Medical Director of the Cardiovascular Prevention and Rehabilitation Program. His research focuses on how exercise affects cardiovascular health and on ways of optimizing exercise interventions. He is identifying ways to make cardiac rehab even more effective for people who are recovering from various forms of heart disease and/or surgery. He has found that the right volume and intensity of exercise are important to maximize gains in function, minimize risk factors and ultimately change cardiovascular health. Dr. Oh is also working to extend the successful model of cardiac rehabilitation to other at-risk populations

Dr. Geoffrey A. Power, University of Guelph

Dr. Geoffrey Power is Assistant Professor in the Department of Human Health and Nutritional Sciences in the College of Biological Sciences (CBS) at the University of Guelph. He is the Director of the Neuromechanical Performance Research Lab. His research uses various in vitro, in vivo, in situ and whole human techniques to investigate muscle function and neuromuscular control of movement across the lifespan.

	**Dr. David J. Dyck,** University of Guelph Dr. David Dyck is a Professor in the Department of Human Health and Nutritional Sciences at the University of Guelph. Dr. Dyck's research focuses in the regulation of fat and carbohydrate metabolism in skeletal muscle, with a particular emphasis on the dysregulation that occurs in obesity and diabetes. He studies the effects of adipokines on muscle lipid and carbohydrate metabolism, and how muscle becomes resistant to their effects in obesity models. The interaction of diet and exercise is also a point of interest in terms of the muscle's response to various hormones including insulin, leptin and adiponectin.
	**Dr. Ali Abdul-Sater,** York University Dr. Ali Abdul-Sater is an Assistant Professor in the School of Kinesiology and Health Science at York University. His research identifies novel regulators of inflammation and understanding the molecular mechanisms through which these regulators control innate immunity and the inflammatory response. Dr. Abdul-Sater investigates the molecular mechanisms through which different exercise regimens regulate the immune response. Understanding the disparate roles of TRAF1 in controlling chronic inflammatory and autoimmune diseases.
	**Dr. Marina Mourtzakis,** University of Waterloo Dr. Marina Mourtzakis is Associate Professor, Associate Chair, Applied Research, Partnerships and Outreach at the University of Waterloo. Her research focuses on the interrelationship between nutrition, exercise, body composition, and the effects of these factors on muscle metabolism in healthy people as well as patients with cancer. Furthermore, her research examines potential underlying mechanisms of this problem to develop rehabilitative approaches to counter, and potentially prevent, muscle loss by integrating concepts of nutrition, protein metabolism and muscle physiology.
	**Dr. Clark Dickerson,** University of Waterloo Dr. Clark Dickerson is Professor and Canada Research Chair in Shoulder Mechanics in the Department of Kinesiology at the University of Waterloo. He is the Associate Director, (Research) of the Centre of Research Expertise for the Prevention of Musculoskeletal Disorders. His research focuses on identifying, quantifying, and reducing work-related stresses in the shoulder through mathematical modeling and experimentation. This can then be used together to improve the safety and usability of workspaces and other man-machine interfaces, thereby reducing the frequency and severity of occupational shoulder injuries.

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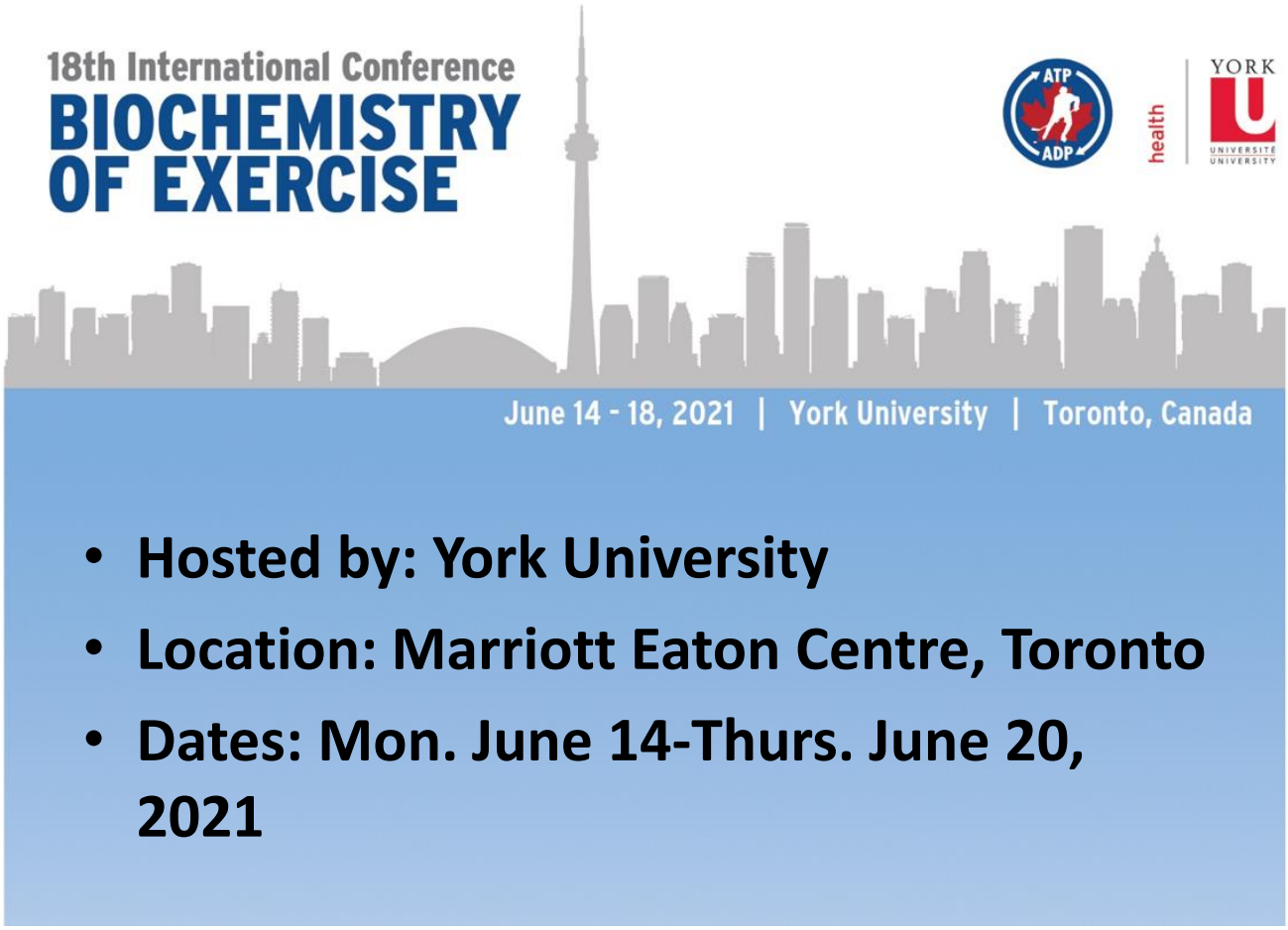

IBEC 2021 Theme: Exercise for Health, Adaptation and Rejuvenation

List of Symposia Topics	
Molecular control of muscle mass: beyond mTOR?	Cytoskeleton and extracellular matrix as regulators of muscle metabolism
Mitochondrial turnover and exercise	Exercise and the immune system
Muscle regeneration/stem cells Exercise and injury	Exercise and bone turnover during aging
Molecular control of angiogenesis with exercise	Exercise therapy for muscle metabolic diseases
Inter-organ cross talk and exosomes	Exercise and cancer
Muscle regulation of glucose uptake, metabolism in health/disease	Regulation of substrate utilization during exercise
Epigenetic regulation of gene expression	Regulation of fatigue during exercise
Exercise, Bioenergetics, and oxidative stress	Sedentarism and dysfunctional integration of metabolic control
Calcium regulation of muscle function in health and disease	Regulation of peripheral blood flow
Exercise and aging rejuvenation for muscle	Exercise therapy for cardiac pathophysiology

Poster Presentation Abstract List

Poster Number	First Author (Surname)	Abstract Title	University Affiliation
1	**Afshar**	A 96-well culture platform enables longitudinal analyses of engineered human skeletal muscle microtissue strength	*University of Toronto*
2	**Avrutin**	Exercise frequency mediates adaptations in lean tissue mass following 24 sessions of exercise in women currently or recently treated for breast cancer	*University of Waterloo*
3	**Baker**	The role of mitochondrial Opa1 in satellite stem cell function and muscle regeneration	*University of Ottawa*
4	**Barazi**	Effect of Circadian Rhythm on Cardiac Adaptations to Exercise	*York University*
5	**Bellissimo**	The mitochondrial-enhancing drug Olesoxime improves quadriceps mitochondrial function and force recovery but not diaphragm function in a mouse model of Duchenne muscular dystrophy	*York University*
6	**Bhattacharya**	Metabolic regulation of myogenic stem cell fates	*York University*
7	**Braun**	SERCA activity is impaired in left ventricles from young and old tafazzin deficient mice	*Brock University*
8	**Brunetta**	Dietary nitrate attenuates HFD-induced glucose intolerance in association with reduced epididymal adipose tissue inflammation and mitochondrial ROS emission	*University of Guelph*
9	**Cervone**	Ghrelin prevents palmitate-induced impairments in oxidative skeletal muscle insulin action	*University of Guelph*
10	**Cheema**	Mitochondrial Motility in Autophagy	*York University*
11	**Da Eira**	High-fat diet enhances triglyceride recycling, impairs UCP1-mediated thermogenic activity, and causes insulin resistance in rat brown adipocytes	*York University*
12	**Dibe**	Exercise training desensitizes the liver to the effects of epinephrine.	*University of Guelph*
13	**El Osta**	Developing a Fluorescent Binding Assay Between α-Cardiac Actin and Tropomyosin from Bovine Cardiac Tissue	*University of Guelph*
14	**Erlich**	Chronic contractile activity and Retinoic Acid operate independently to stimulate mitochondrial turnover in C2C12 cells	*York University*

15	**Frendo-Cumbo**	Atg16L1 Knockout Induces Insulin Resistance Through Proteasomal IRS1 Degradation	*University of Toronto*
16	**Gandhi**	Exercise and Dairy have Distinct Effects on Indices of Liver and Systemic Lipid Metabolism	*University of Guelph*
17	**Gheorghiu**	SNARE Expression and Promoter Regulation in Cardiomyocytes	*York University*
18	**Gill**	Measuring Sternocleidomastoid Muscle Volume with Phase-Contrast MRI	*York University*
19	**Grafham**	The Capacity for Skeletal Muscle to Repair After Exercise-Induced Muscle Damage in Young-Adults with Type 1 Diabetes Mellitus	*McMaster University*
20	**Gupta**	Identification of novel microRNAs (miRs) regulating skeletal muscle regeneration in sustained ICUAW.	*University of Toronto*
21	**Hamstra**	Low dose lithium feeding improves murine left ventricular SERCA function by regulating SERCA2a and phospholamban expression.	*Brock University*
22	**Hogan**	Remodeling of the Extracellular Matrix Following Cardiotoxin-induced Muscle Damage in Aged Mice	*University of Windsor*
23	**Jacyniak**	A Preliminary Investigation of Cardiac Cell Proliferation Following a Puncture Injury to the Heart of the Leopard Gecko (Eublepharis macularius)	*University of Guelph*
24	**Jafari**	Mechanistic Insights into The Effects of Exercise on Inflammatory Responses.	*York University*
25	**Jani**	Oxidative and glycolytic muscles develop insulin resistance despite displaying fiber type-specific patterns of intracellular glycerolipid accumulation	*York University*
26	**Johnston**	Thoracolumbar co-contraction: a predisposing mechanism for back pain associated with large chest size?	*York University*
27	**Jones**	Analysis of the T-class -cardiac actin (ACTC1) variant R312C related to hypertrophic cardiomyopathy	*University of Guelph*
28	**Kirsh**	Dietary Sodium Nitrate Increases the Content of Both SERCA and PLN in the Hearts of Healthy Rats	*University of Guelph*
29	**Lakin**	Atrial arrhythmias and adverse atrial remodeling induced by exercise requires soluble tumor necrosis factor alpha (TNFa) derived from atrial myocardium	*York University*
30	**Larkin**	Effects of High Dose Radiation on Juvenile Skeletal Muscle Development and Satellite Cell Dynamics	*University of Ottawa*

31	**Larrigan**	The role of mitochondrial ROS and protein glutathionylation in myogenic lineage commitment	*University of Ottawa*
32	**Lloyd**	Effect of localized radiation exposure on skeletal muscle inflammation and fibro/adipogenic progenitors in juvenile mice	*University of Ottawa*
33	**Logan**	Dietary Assessment of York University Women's Varsity Hockey Team	*York University*
34	**Madu**	Disruption of Branched-chain α-ketoacid dehydrogenase (BCKD) Enhances Myofibrillar Protein abundance in Myotubes	*York University*
35	**Marrow**	Exogenous erythropoietin and novel splice variant preserve cardiac function in vivo in a murine model of myocardial infarction	*University of Guelph*
36	**Medak**	Female mice are protected against acute olanzapine-induced hyperglycemia	*University of Guelph*
37	**Mora**	Myotube Morphology and Protein Metabolism are Negatively Regulated by Chemotherapy Drugs	*York University*
38	**Moyle**	The LINC complex modulates satellite cell response to injury via cell fate regulation	*University of Toronto*
39	**Nardone**	Comparison of Non-Invasive Peripheral Vascular Function to Invasive Measures of Coronary Function in Patients with Suspected Coronary Microvascular Dysfunction	*York University*
40	**Newport**	Photoperiod influence on fat accumulation in Peromyscus leucopus	*Trent University*
41	**Ng**	Neuromuscular biology of skeletal muscle-specific AMPK knockout mice	*McMaster University*
42	**Noonan**	Age-related changes in human single muscle fibre passive elastic properties are sarcomere length dependent	*University of Guelph*
43	**Ogilvie**	Investigating Sex-Specific Mechanisms of Diastolic Dysfunction in a New Model of Heart Failure with Preserved Ejection Fraction	*University of Guelph*
44	**Ojehomon**	Investigating the Role of ACTC Genes in Zebrafish Development	*University of Guelph*
45	**Oliveira**	Mitochondrial Maintenance in Aged Human Cardiac Muscle	*York University*
46	**Petrick**	Sodium nitrate supplementation prevents cardiac dysfunction and attenuates left ventricular mitochondrial ROS emission in high-fat diet fed mice	*University of Guelph*
47	**Pooni**	Reproducibility in the cardiometabolic responses to high-intensity interval exercise in adults with type 1 diabetes	*York University*

48	**Ramos**	Microtubule disorganization is associated with mitochondrial dysfunction in Duchenne muscular dystrophy	*York University*
49	**Salvin**	Progressive Suppression of Autophagy with Denervation	*York University*
50	**Savinova**	The Effect of a High-Fat Diet on Glucose Tolerance in Mice Lacking Endothelial-Derived Erythropoietin	*University of Guelph*
51	**Shamshoum**	Acute olanzapine-induced hyperglycemia is exacerbated in female AMPK beta 1 mice	*University of Guelph*
52	**Sidhu**	ADPr-actin trimer: A short-F-actin complex for determining atomic structures of F-ABP complexes	*University of Guelph*
53	**Taqvi**	Guided Active Play Tracks Children's Physical Activity Participation	*York University*
54	**Te**	Enabling skeletal muscle repair and functional recovery following denervation-induced injury using ultrasound mediated gene delivery (UMGD)	*University of Toronto -St. Michael's Hospital*
55	**Teng**	"Characterizing alpha-cardiac actin variants associated with cardiomyopathy."	*University of Guelph*
56	**Tripathi**	Regulation of myogenic gene transcription by Smad7: b-catenin complex.	*York University*
57	**Turnbull**	Fatty Acid-Induced Hepatocellular Carcinoma Growth is Mediated by Decreasing Mitochondrial H2O2 Emission Coupled to Increased Glutathione Levels	*York University*
58	**vanLieshout**	The Impact of Acute Exercise on Skeletal Muscle-Specific Coactivator-Associated Arginine Methyltransferase 1 Knockout Mice	*McMaster University*
59	**Webber**	DCA Can be used to Prevent Olanzapine-Induced Weight Gain	*University of Guelph*
60	**Whitley**	Low dose lithium supplementation reduces muscle inflammation and serum creatine kinase in mdx mice	*Brock University*

MHAD10 Complete Abstract List

A 96-well culture platform enables longitudinal analyses of engineered human skeletal muscle microtissue strength

Mohammad E. Afshar[1,2,#,] Haben Y. Abraha[1,2,#,] Mohsen A. Bakooshli[1,2,#,] Sadegh Davoudi [1,2], Nimalan Thavandiran[1,2], Kayee Tung[3], Henry Ahn[3], Howard Ginsberg[1,3,4], Peter W. Zandstra[1,2,5], and Penney M. Gilbert[1,2,6,7]

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[3] Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Canada,

[4] Department of Surgery, University of Toronto, Toronto, Canada,

[5] Michael Smith Laboratories and the School of Biomedical Engineering, University of British Columbia, Vancouver, Canada,

[6] Department of Biochemistry, University of Toronto, Toronto, Canada,

[7] Department of Cell and Systems Biology, University of Toronto, Toronto, Canada,

[#] These authors contributed equally to the work

Three-dimensional (3D) in vitro models of human skeletal muscle mimic aspects of native tissue structure and function, thereby providing a promising system for disease modeling, drug discovery or pre-clinical validation, and toxicity testing. Widespread adoption of this research approach is hindered by the lack of an easy-to-use platform that is simple to fabricate and yields arrays of human skeletal muscle micro-tissues (hMMTs) in culture with reproducible physiological responses that can be assayed non-invasively. Here, we describe a design and methods to generate a reusable mold to fabricate a 96-well platform, referred to as MyoTACTIC, that enables bulk production of 3D hMMTs. All 96-wells and all well features are cast in a single step from the reusable mold. Non-invasive calcium transient and contractile force measurements are performed on hMMTs directly in MyoTACTIC, and unbiased force analysis occurs by a custom automated algorithm, allowing for longitudinal studies of function. Characterizations of MyoTACTIC and resulting hMMTs confirms the reproducibility of device fabrication and biological responses. We show that hMMT contractile force mirrors expected responses to compounds shown by others to decrease (dexamethasone, cerivistatin) or increase (IGF-1) skeletal muscle strength. Since MyoTACTIC supports hMMT long-term culture, we evaluated direct influences of pancreatic cancer chemotherapeutics agents on contraction competent human skeletal muscle fibers. A single application of a clinically relevant dose of Irinotecan decreased hMMT contractile force generation, while clear effects on fiber atrophy were observed histologically only at a higher dose. This suggests an off-target effect that may contribute to cancer associated muscle wasting, and highlights the value of the MyoTACTIC platform to non-invasively predict modulators of human skeletal muscle function.

Exercise frequency mediates adaptations in lean tissue mass following 24 sessions of exercise in women currently or recently treated for breast cancer.

Egor Avrutin, Amanda G Pfeiffer, Schuyler Schmidt, Lisa Bos, Caryl Russell, Marina Mourtzakis.
Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada.

Background: In individuals with a cancer diagnosis, body composition changes such as fat mass gain or lean tissue loss are often observed over the course of treatment. Lifestyle modification, such as exercise training or diet interventions, may help mitigate some of the negative side effects of cancer treatment. In breast cancer patients, exercise is a safe and effective means to improve body composition, physical function, and quality of life. The main objective of our study was to explore the effects of exercise frequency on lean tissue mass and muscle strength in women currently or recently treated for breast cancer.
Methods: We recruited 33 women with a breast cancer diagnosis (of any cancer stage or type), who are currently undergoing or recently completed treatment. The participants completed 24 supervised group exercise classes. Classes were held 2x/week and consisted of 60 minutes of mixed aerobic and strength intermediate intensity exercises. The participants were evaluated at baseline and upon completion of 24 exercise sessions, we examined body composition using Dual Energy X-ray Absorptiometry (DXA) and bilateral maximal isometric torque for knee extension and elbow flexion using a force transducer. Session attendance was recorded to calculate the average exercise frequency for each participant.
Results: Exercise session frequency varied between participants in our cohort (median attendance frequency 1.4x/week, range: 0.75-2 sessions/week). To determine the effects of exercise frequency the cohort was split into 2 groups, above and below the median exercise frequency. Two factor repeated measures ANOVA was used to determine whether attendance frequency mediates the adaptations in lean tissue mass or strength. While we did not observe a main effect of the exercise intervention on lean tissue mass, there is a statistically significant interaction. Participants in the high frequency group but not low frequency group increased whole body lean tissue mass, and limb specific lean tissue. Additionally, we observed an effect of exercise on strength, but it was not mediated by exercise frequency.
Conclusions: Our study demonstrates that exercise frequency mediates the effects of exercise training on lean mass in women with breast cancer.

The role of mitochondrial Opa1 in satellite stem cell function and muscle regeneration.

Nicole Baker[1], Sarah Larrigan1, Damian Chwastek[1], John Girgis[1], Ryo Fujita[2], Colin Crist[2], Michael Rudnicki[3], and Mireille Khacho[1].
[1]*Department of Biochemistry, Microbiology and Immunology, Ottawa Institute of Systems Biology (OISB), Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.*
[2]*Lady Davis Institute for Medical Research, Jewish General Hospital, Department of Human Genetics, McGill University, Montreal, QC, Canada.*
[3]*Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, Regenerative Medicine Program, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.*

Skeletal muscle is known for its great regenerative capacity. However, during aging and muscle degenerative diseases, there is a decline in satellite stem cells and muscle regeneration. It is known

that mitochondrial dysfunction and fragmentation are common features in aging and degenerative diseases, however, how this impacts muscle regeneration is unknown. To address the effect of mitochondrial fragmentation in satellite stem cells, we generated a knockout mouse model of the mitochondrial fusion protein Opa1, using the Pax7CreERT2 inducible system. Analysis of muscle regeneration following cardiotoxin injury reveals a defect in the muscle regenerative potential of Opa1 knockout mice, as indicated by both the size and the number of newly formed fibers. Moreover, immunofluorescent staining of injured tissue reveals a substantial decrease in the number of Pax7+ cells in Opa1 KO muscle, indicating an impairment in self-renewal capacity. Upon further analysis it was determined that the number of committing (MyoD+/MyoG+) cells were increased in Opa1 KOs, illustrating that Opa1 KO muscle stem cells are driven towards commitment at the expense of self-renewal. Interestingly, even in the absence of a regeneration event, muscle stem cells lacking Opa1 are severely depleted just one month after knockout. Furthermore, upon activation of prolonged Opa1 KO muscle stem cells using EDL myofiber cultures, these cells exhibit skewed cell fate decisions, demonstrated by an increase in committed (MyoG+) cells. Finally, using mdivi-1, a mitochondrial fragmentation inhibitor, and MitoTEMPO, a reactive oxygen species scavenger, stem cell fate defects seen in Opa1 knockouts can be restored. These data are the first to demonstrate a novel role for mitochondrial structure and function in the regulation of muscle stem cell regenerative capacity. Understanding the mechanism by which aberrant mitochondrial fragmentation affects stem cell fate can help identify novel approaches to restore muscle function during aging and muscular degenerative diseases.

Effect of Circadian Rhythm on Cardiac Adaptations to Exercise

Nour Barazi[1], Robert Lakin[1], and Peter H. Backx[1]
[1]Muscle Health Research Centre, Department of Biology, York University

Background: Circadian rhythm, the internal regulator of a system's 24 hour rhythm, is entrained by numerous time cues, or zeitgebers (ZT), with light being the most potent stimulus. Many biological processes, including heart rate (HR), exhibit a circadian rhythm. Resting HR is traditionally thought to be controlled by the fine balance between the two branches of the autonomic nervous system (ANS), the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS), both of which exert their effect on the intrinsic firing of the pacemaker of the heart, the sinoatrial node (SAN). Our studies have previously established that basal HR decreases following endurance training, with analysis of Heart Rate Variability (HRV), the beat-to-beat variation in HR, suggesting that enhanced PNS modulation of HR mediates this response. However, it has been argued that both the daily circadian fluctuations and the exercise adaptations in resting HR are a result of intrinsic remodelling in the SAN (i.e., ion channel expression) rather than extrinsic autonomic remodelling.

Purpose and Methods: The proposed research will utilize surface ECG (sECG) and pharmacological ANS blockade (atropine and propranolol) on sedentary and 6-week swim-exercised CD1 mice to investigate the extent of extrinsic and intrinsic modulation of resting HR fluctuations and exercise-induced bradycardia. sECG, and HRV analysis of the recordings, will be conducted at various time points (ZT0, ZT6, ZT18, ZT24) throughout the day to create a complete circadian profile of HR. Additionally, we will study whether exercising nocturnal mice during

their usual waking hours leads to enhanced benefits, or if circadian-specific effects on exercise are present. This will be achieved by housing mice in two separate rooms, each with a different light cycle: Standard 12-h:12-h Light-Dark (LD) cycle and reversed 12-h:12-h Dark-Light (DL) cycle. Both LD and DL mice will complete 6 weeks of swim exercise twice per day at ~70% VO2Max. For DL mice, swim training will be conducted in the dark, under dim red light, catering to their nocturnal nature and resembling their subjective day. Data will be collected and compared among the exercise and sedentary groups of each light condition at ZT0, ZT6, ZT18, and ZT24 to assess circadian effects.
Research Significance: This research will shed light on the contribution of circadian rhythm to resting HR and cardiac adaptations to exercise. The findings are applicable to the general population, especially shift workers or those with a disrupted sleep-wake cycle, and athletes who wish to optimize the benefits of their training.

The mitochondrial-enhancing drug Olesoxime improves quadriceps mitochondrial function and force recovery but not diaphragm function in a mouse model of Duchenne muscular dystrophy

C.A. Bellissimo, L. J. Deflinis, M.C. Hughes, P. Tadi, C. Amaral, A. Dehghani, C.G.R. Perry
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, ON, Canada.

Introduction Mitochondrial dysfunction is thought to be a secondary contributor to muscle weakness in Duchenne muscular dystrophy (DMD) – a severe muscle-wasting disease affecting ~1:3500 males. Olesoxime (TRO19622) is a mitochondrial-targeting compound that prevents mitochondrial dysfunction, improves motor function and increases life span in other neurodegenerative diseases. As we have previously demonstrated mitochondrial dysfunction occurs in diaphragm and quadriceps in mice with DMD, we hypothesized that olesoxime administration will improve mitochondrial bioenergetics and muscle force in these muscles.
Methods Male D2.B10-DMDmdx/2J (D2.mdx) mice received a daily oral gavage of corn oil supplemented with olesoxime (DRUG) (30mg/kg body weight) or without (VEH, vehicle) from age 10 days to 28 days. Computed tomography assessed total trunk fat, whole body lean and hindlimb muscle volume. In-situ isometric quadricep force and ex-vivo isometric diaphragm force measured force production and resistance to fatigue. High resolution respirometry assessed mitochondrial respiratory control by ADP.
Results Whole body lean volume and hindlimb muscle volume trended higher in the DRUG group (DRUG vs VEH, +8%, p=0.09 and +11%, p=0.06; N=10-12, respectively) while total trunk fat trended lower (DRUG vs VEH, -14%, p=0.07; N=10-11) but were not significantly different. There was a significant improvement in recovery 15 mins after fatigue in quadriceps (DRUG 79.4±5.4% of maximal force vs VEH 52.6±9.9% of maximal force, p=0.04; N=6-7) with no changes to twitch and tetanic force production. This improvement in quadriceps fatigue resistance was related to enhanced ADP-stimulated mitochondrial respiration (25μM ADP; +84% with DRUG vs VEH, p=0.004; N=11). In diaphragm, olesoxime had no effect on mitochondrial respiration, force production or recovery from fatigue.
Discussion &Conclusion Olesoxime improved force recovery and mitochondrial respiratory control by ADP in quadriceps but not the diaphragm. Ongoing research will examine whether

olesoxime improves histological markers of the disease (fibrosis, centralized nuclei, cross-sectional area) as well as lowers H2O2 emission and mitochondrial induction of apoptotic cell death pathways.

Metabolic regulation of myogenic stem cell fates

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Recent researches have uncovered that different metabolic pathways control stem cell maintenance and fate decisions. Importantly, the regulation of energy generation is integrated into the control mechanisms that govern muscle stem cell self-renewal and myogenic progenitor cell (MPC) differentiation. In this regard, glycolysis in the cytoplasm and oxidative phosphorylation in the mitochondria work together to regulate the yield of total ATP. In our study, we found a novel control mechanism for ATP generation in the mitochondria by an unknown function of the cell cycle regulator and transcriptional co-repressor p107. By molecular, cellular and biochemical approaches, we show that p107 is localized within the mitochondrial matrix. Here p107 interacts at the D-loop promoter region of mitochondrial DNA to repress its gene expression and ultimately mitochondrial ATP generating capacity. Moreover, p107 is regulated by the cellular NAD+/NADH ratio and NAD+ dependent deacetylase Sirt1. Activating and inhibiting Sirt activity influences p107 mitochondrial function. Indeed, p107 genetically deleted MPCs fail to respond to the NAD+/NADH ratio and Sirt1 in controlling the mitochondrial ATP generation capacity. This innovative energy control system provides insights to increase the regenerative efficacy of muscle stem cells and MPCs. This will potentially help to combat muscle wasting diseases and complications such as muscular dystrophy and sarcopenia.

SERCA activity is impaired in left ventricles from young and old tafazzin deficient mice

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Barth syndrome is a rare and severe X-linked genetic disorder leading to cardiomyopathy, skeletal myopathy, and neutropenia. It is caused by a mutation in the TAZ gene leading to reductions in tafazzin protein, and thus total cardiolipin and tetralinoleyl cardiolipin species. In turn, Barth syndrome is characterized by mitochondrial dysfunction and an increased production of reactive oxygen species (ROS). The sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) is imperative for normal cardiac function and is sensitive to oxidant damage. Here, we assessed SERCA function in left ventricles (LV) obtained from young (3-5 months) and old (10-12 months) wild-type (WT) and tafazzin deficient (TazKD) male mice. Interestingly, maximal SERCA activity was impaired in both young and old TazKD mice compared with WT, and although there was no change in SERCA2a expression, there was an increase in SERCA2a tyrosine nitration at both age groups indicative of enhanced oxidant damage to the ATPase pumps. In addition, phospholamban (PLN) is a SERCA inhibitor highly implicated in cardiomyopathy. Here, we show that PLN was decreased, and its phosphorylation increased, which we believe represents a compensatory

response aimed at improving SERCA function in the face of oxidative stress. Altogether, our findings show for the first time that SERCA function is impaired in LVs obtained from young and old TazKD mice. Since cardiac dysfunction has only been observed in old TazKD mice, our findings suggest that SERCA impairment may occur before the onset of cardiomyopathy and could potentially be a viable therapeutic target

Dietary nitrate attenuates HFD-induced glucose intolerance in association with reduced epididymal adipose tissue inflammation and mitochondrial ROS emission

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Obesity is characterized by adipose tissue hypertrophy, low-grade inflammation, and elevated mitochondrial reactive oxygen species (ROS) emission. These features are related to changes in adipose tissue function, which are linked to the development of whole-body insulin resistance. Here, we investigated the protective effects of dietary nitrate on whole-body glucose homeostasis and epididymal white adipose tissue (eWAT) in high-fat fed animals. Male C57Bl/6N mice (~20-22 weeks old) were randomly divided into three groups: Lean group fed with control diet (10% fat), diet-induced obesity (DIO) group fed with high-fat diet (HFD, 60% fat) and, DIO + nitrate group fed with HFD and supplemented with 4 mM sodium nitrate via drinking water. The groups were followed for 8 weeks. At the end of the treatment intraperitoneal glucose tolerance (ipGTT), adipocyte cross-sectional area, inflammatory markers, and mitochondrial respiration and H2O2 emission were determined. Diet-induced obesity mice presented impaired glucose homeostasis ($P<0.05$), increased body weight mass, and eWAT cross-sectional area. Moreover, within eWAT, consumption of HFD increased leucocyte infiltration, JNK phosphorylation, mitochondrial H2O2 emission, 4-HNE content, and attenuated insulin-induced Akt phosphorylation. In stark contrast, despite similar body weight gain and food intake, dietary nitrate attenuated the induction of whole-body glucose intolerance by ~30%. In addition, nitrate consumption dramatically reduced all cellular responses within eWAT tissue ~50% indicative of HFD-diet induced insulin resistance; including reducing leucocyte infiltration, JNK phosphorylation, mitochondrial H2O emission, 4-HNE content and increasing insulin-induced Akt Threonine-308 phosphorylation. As a result, DIO mice consuming oral nitrate were not different than control mice in almost any parameter assessed. Intriguingly, the positive effects of dietary nitrate appear to be independent of mitochondrial respiratory capacity, as, despite the reduction in markers of mitochondrial content, HFD resulted in compensatory increases in mitochondrial respiration, a response not influenced by nitrate consumption. In conclusion, dietary nitrate attenuates the development of HFD-diet induced insulin resistance. The positive effects on whole-body glucose homeostasis are associated with an attenuation in eWAT inflammation and redox balance, independent of mitochondrial respiratory capacity or content.

Ghrelin prevents palmitate-induced impairments in oxidative skeletal muscle insulin action

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Introduction: Ghrelin is a gastric hormone that exists as two circulating isoforms, acylated (AG) and unacylated (UnAG). Although AG is an orexigenic signal, both forms have substantial metabolic effects on insulin-responsive tissues (eg. skeletal muscle). Existing work from our lab using rat skeletal muscle (Kraft EN et al. 2019), and others using myoblasts (Han L et al. 2015) have demonstrated that ghrelin can stimulate fatty acid oxidation. Our experimental model was aimed at extending these findings and determining whether ghrelin can protect skeletal muscle (major site for insulin-stimulated glucose disposal) against acute, fatty acid-induced impairments in insulin signaling and glucose uptake.

Methods: To date, oxidative soleus muscle strips have been isolated from healthy, male Sprague-Dawley rats and incubated in vials containing pre-gassed (95% O2, 5% CO2) and warmed (30ºC) media. All muscles were equilibrated for 30min, then underwent either low palmitate (0.2mM, LP) or high palmitate (2mM, HP) treatment, with or without AG or UnAG (150ng/ml), for 4h. Exogenous palmitate oxidation was measured through captured radiolabeled 14C-palmitate in the final 2h. Subsequently, insulin-stimulated glucose uptake and insulin signaling were assessed. We first confirmed that HP impairs muscle insulin signaling in rodent soleus muscle within 4h. Compared to control, insulin significantly stimulated AKT activation (Ser473) in both palmitate conditions at 1mU/ml (LP: 2.4±0.6; HP: 1.9±0.3) and 10mU/ml (LP: 12.5±1.6; HP: 5.4±0.6) doses. The inhibitory effects of HP on AKT phosphorylation were clear at the maximal insulin concentration (LP: 12.5±1.6; HP: 5.4±0.6); therefore, this concentration was utilized for subsequent incubations to examine the potential effects of ghrelin. For glucose uptake assays, muscles were subjected to either saline or insulin (10mU/ml) for 30min. Muscles were then trimmed of their tendons, blotted, weighed and solubilized to measure the accumulation of radiolabeled 3H-2-deoxy-D-glucose tracer. For insulin signaling experiments, muscles were exposed to insulin for 10min and then snap frozen in liquid nitrogen. Western blots were used to quantify the phosphorylation (activation) of downstream insulin signaling protein AKT (Ser473, Thr308).

Results: For signaling experiments with ghrelin, when compared to control (LP-ins: 1.2±0.6 arbitrary units), 10mU/ml insulin significantly ($p<0.05$) increased the phosphorylation of AKT at its Ser473 (LP+ins: 15.3±3.23; HP+ins: 6.86±1.01) and Thr308 (LP+ins: 12.1±3.17; HP+ins: 7.7±2.05) residues during LP and HP exposures ($p<0.01$). Consistent with our pilot work, 4h of HP significantly reduced insulin's activation of AKT at Ser473 ($p<0.01$) and Thr308 ($p<0.05$). Interestingly, AG (14.8±7.6, $p<0.05$), but not UnAG (11.2±7.3, $p>0.05$) was able to preserve Ser473 AKT activation, although both isoforms protected Thr308 phosphorylation ($p<0.05$) in the presence of HP. Importantly, only UnAG's protective effect on insulin signaling manifested as a significant increase in the rate of insulin-stimulated glucose uptake (+58%; $p<0.05$) compared to HP+ins alone. In line with this, UnAG significantly increased palmitate oxidation (+78%; $p<0.001$) when compared to HP alone, perhaps accounting for its protective effect on glucose uptake.

Conclusions: UnAG may be more potent than AG in stimulating fatty acid oxidation in oxidative rat skeletal muscle. As such, UnAG may exert desirable effects towards protecting muscle insulin action during high saturated fatty acid availability. These experiments help to contribute to the interpretation of the differential effects of AG and UnAG in skeletal muscle glucose/fatty acid metabolism and current work is targeted at elucidating any potential effects of ghrelin on these metabolic endpoints in the context of obesity (i.e. following a high-fat diet).

Mitochondrial Motility in Autophagy

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Mitochondria exist as a dynamic network which becomes elongated or fragmented depending on cellular conditions. The morphology of mitochondria is regulated by fusion, fission and motility of the organelle. Mitochondrial motility is vital for redistributing the organelle within the cell and for maintaining mitochondrial function. It has been shown that oxidative stress induces mitochondrial dysfunction, resulting in fragmented morphology and stationary mitochondria, with an increase in autophagy proteins. Autophagy is a cellular mechanism through which dysfunctional components are degraded and recycled to maintain cellular homeostasis. Rapamycin, an autophagy inducer, has been shown to rescue mitochondrial myopathy through the activation of autophagy and lysosomal biogenesis. However, the effect of rapamycin on mitochondrial motility is unknown. Therefore, in this study we determined mitochondrial morphology and motility with rapamycin treatment in C2C12 myoblasts and human fibroblasts from healthy subjects, and those with the mtDNA mutation leading to MELAS. We observed a time-dependent decrease in mitochondrial motility in treated myoblasts. By 24 hrs, mitochondria become stationary and morphology was elongated with less fragmented mitochondria compared to mitochondria in control myoblasts. At 24hr, rapamycin increased mitochondrial content with an intact membrane potential, whereas chloroquine, an autophagy inhibitor, resulted in an accumulation of mitochondria with a lower membrane potential. An increase in lysosome content and function was also detected at 24hrs of rapamycin treatment. Similar findings were observed in human fibroblasts, where rapamycin increased lysosome number and function. Rapamycin also increased lysosomes in fibroblasts isolated from MELAS patients. These findings suggest that the motility of mitochondria is altered during autophagy, and that rapamycin has a potential effect on lysosomal biogenesis.

High-fat diet enhances triglyceride recycling, impairs UCP1-mediated thermogenic activity, and causes insulin resistance in rat brown adipocytes

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Brown adipose tissue (BAT) is rich in uncoupling protein 1 (UCP1) and dissipates energy through thermogenesis. However, even though BAT mass and its UCP1 content increase under conditions of diet-induced obesity (DIO), marked expansion of the WAT is not prevented, suggesting impairment of BAT-mediated diet-induced thermogenesis (DIT) in obesity. Thus, the objective of

this study was to investigate the metabolic and molecular mechanisms that regulated BAT thermogenesis in DIO. To accomplish this, rats were fed a high-fat diet (HFD) for eight weeks. Subsequently, glucose and fat metabolism and the molecular mechanisms underlying these processes were assessed in BAT adipocytes. Despite increasing BAT mass (1.3-fold), UCP1 content (2.1-fold), and isoproterenol (Iso)-induced lipolysis (1.7-fold), HFD reduced UCP1-mediated glucose (62%) and fatty acid (57%) oxidation, and abrogated insulin-stimulated glucose uptake in BAT adipocytes. Furthermore, phosphoenolpyruvate carboxykinase (PEPCK) and glycerol kinase (GyK) contents, as well as glycerol and palmitate incorporation into lipids were all significantly increased (1.8-fold, 2.1-fold, 2-fold, and 1.7-fold, respectively) in HFD BAT. This coincided with 3.6- and 3.7-fold elevations in lipoprotein lipase (LPL) and cluster of differentiation 36 (CD36), respectively, in HFD BAT adipocytes. Morphological analysis also revealed that these adipocytes were more unilocular in appearance. Altogether, these findings provide novel evidence that HFD suppresses UCP1-mediated thermogenesis, shifts metabolism toward triglyceride recycling, and induces insulin resistance in BAT adipocytes. These adaptive responses to chronic HFD are consistent with a mechanism that attenuates the contribution of BAT to DIT and favors the development of obesity and its related metabolic disorders.

Dietary Assessment of York University Women's Varsity Hockey Team

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A well-balanced diet equipped with adequate amounts of nutrients is extremely important for the maintenance of health and performance in athletes. Before, during and post exercise training, athletes need to consume high quality food sources, in order to meet increased dietary needs and support proper recovery. Athletes who fail to optimize their nutritional habits pose a risk of potentially diminishing their sport performance. In addition, this may also have a negative effect on their overall health. In this study, we set out to investigate whether university level athletes are meeting their increased dietary needs in order to optimize their performance. A self-reported 3-day dietary food log was completed by 12 York University female hockey players. Based on the food log, we analyzed macro- and micro- nutrient intakes. To objectively estimate their required intakes, we used anthropometric measures (weight, height), physical activity level, and the Harris-Benedict equation. The most striking observation is the fact that the majority of the athletes were not consuming enough calories. The athletes were also not meeting their daily carbohydrate or fat (with one outlier) requirements. In addition, 33% of the athletes were above their daily required protein intake. Regarding micronutrients, 88% of the athletes were deficient in vitamin D, 55 % in vitamin E, and 24 – 40 % in vitamin C, vitamin K and iron. Approximately 67% of the athletes were in excess of sodium. This analysis has showed that the women's varsity hockey players collectively as a group are not consuming enough calories, and this likely explains the deficiencies seen in micronutrients. Even though some of them reported higher protein intakes, such dietary proteins are likely not being efficiently used. The deficiencies reported could potentially contribute to sub-optimal performance and health in these athletes.

Exercise training desensitizes the liver to the effects of epinephrine.

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The effects of exercise training on skeletal muscle metabolism are well known and include increases in skeletal muscle insulin sensitivity and fat oxidation. The liver is the primary organ involved in the endogenous production of glucose through glycogenolysis and gluconeogenesis. Liver glucose production is increased via neural-hormonal mechanisms such as increases in catecholamines. To date, the effects of prior exercise training on the hepatic response to epinephrine have not been fully elucidated. To examine the role of epinephrine signalling on indices of liver glucose production in trained mice, male C57BL/6 mice were subject to either 12 days of voluntary wheel running (EX) or remained sedentary (SED). Epinephrine (0.5 mg/kg BW), or vehicle were injected intraperitoneally on day 12 prior to sacrifice and blood glucose was measured 15 minutes post injection. Epinephrine caused a larger glucose response in sedentary (SED-EPI) compared to exercised (EX-EPI) mice. In the liver, there was a main effect of epinephrine to increase the phosphorylation of protein kinase-A (p-PKA) substrates, which is primarily driven by increases in the sedentary, but not trained, mice. Similarly, epinephrine-induced increases in the mRNA expression of β-adrenergic receptor 1 and 2 (Adrb1/2), and glucose-6-phosphatase (G6PC) were greater in sedentary compared to trained mice. Treatment with epinephrine decreased hepatic glycogen content, however, the relative decline following epinephrine injection was significantly greater in sedentary mice. Taken together, our data suggest that prior exercise training desensitizes the liver to epinephrine. This could be beneficial in the context of training-induced sparing of glycogen during exercise.

Developing a Fluorescent Binding Assay Between α-Cardiac Actin and Tropomyosin from Bovine Cardiac Tissue

Lana El Osta and John Dawson

Cardiovascular disease is one of the leading causes of death worldwide. Among Canadians, it has been reported that seven people every minute die from heart disease or stroke A main form of cardiovascular disease is hypertrophic cardiomyopathy (HCM). HCM can be attributed to mutant forms of actin that are hypothesized to cause increased calcium sensitivity leading to hypercontractility of the cardiac sarcomere. One other protein that is a major contributor to cardiac sarcomere function is tropomyosin. Tropomyosin is naturally found as a dimer made up of two alpha-helices folded into seven parts that associate to form a coiled coil. A single tropomyosin dimer binds seven actin subunits to prevent contraction by blocking myosin-binding sites on actin. The purpose of my research is to develop a fluorescence-based assay that determines the binding efficiency/ Hill coefficient between various actin variants and tropomyosin. This could assist in determining how functionally active the actin variants are, and to what extent, by looking at a range of tropomyosin concentrations. A co-sedimentation gel-based assay has already been developed to identify such binding interactions between these two proteins, however, it is labour-intensive and costly. Therefore, we are testing a fluorescent probe, IAEDANS, to label

tropomyosin and determine the fluorescence after binding F-actin. Our progress with this assay will be presented.

Chronic contractile activity and Retinoic Acid operate independently to stimulate mitochondrial turnover in C2C12 cells

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Maintaining a healthy pool of mitochondria is crucial for the ability of a muscle cell to meet its metabolic needs. In order to maintain an optimal pool, there is a balance between mitochondrial biogenesis and mitophagy, termed mitochondrial turnover. Exercise is known to improve mitochondrial turnover by activating both processes. Moreover, research in adipocytes and liver tissue has also shown the potential of the dietary supplement retinoic acid to activate mitochondrial biogenesis. Retinoic acid has also been shown to be effective in activating autophagy in cancer cells. However, the combined effect of retinoic acid isomers and exercise in facilitating mitochondrial turnover has not been described. The purpose of this project was to evaluate the effect of retinoic acid (RA) isomers combined with exercise on mitochondrial turnover in muscle cellss. Mouse C2C12 myoblasts were plated on 6-well plates and allowed to differentiate into myotubes. Myotubes were then treated with either vehicle (DMSO), or the retinoic acid (RA) isomers 9-cis RA or All-trans RA (ATRA) in the absence or presence of electrical stimulation to elicit Chronic Contractile Activity (CCA; 4days, 3hrs/day). Mitochondrial biogenesis was assessed by examining the protein content of PGC-1α, as well as nuclear-and mitochondrially-encoded mitochondrial proteins. CCA led to a 3-fold increase in COX subunit IV expression, as well as a 2-fold increase in PGC-1α and UQCRC2. UQCRC2 was also significantly increased by 9cis-RA and ATRA treatment, but did not increase further with CCA. The mtDNA-encoded COX subunit I was modestly changed by CCA, but increased 2-fold in response to 9cis-RA or ATRA treatment. These RA isomers also increased PGC-1α by 3-fold, but had no significant impact on COX IV expression. Despite this, RA treatment increased basal respiration by 50%, as measured by Seahorse assay. When RA isomers were combined with CCA, no additive or synergistic effects were observed. To evaluate mitochondrial degradation, we examined several lysosomal and mitophagy proteins. While autophagy proteins Beclin and p62 showed no changes with CCA or RA treatment, the LC3II to LC3I ratio was reduced 2-fold with CCA, suggesting an enhanced clearance of autophagosomes. RA isomers had no effect on this ratio. The lysosomal fusion protein Lamp1 was not affected by CCA or RA treatment. Surprisingly, LAMP-2A showed a trend to decrease with RA treatment, and was not affected by CCA. However, transcription factor EB (TFEB) was increased 1.5-fold and 2-fold by 9-cis and ATRA, respectively. These effects were not increased further with CCA. While the immature form of Cathepsin D showed increases with RA, the ratio of immature to mature Cathepsin D did not change with CCA or RA treatment. Our data suggest that while RA isomers and CCA both promote mitochondrial biogenesis, RA appears to target proteins selectively, while CCA appears to have greater effects on the expression of nuclear-encoded proteins. CCA also may serve as a stimulus for the clearance of autophagosomes containing damaged cargo, while RA has an effect of lysosomal biogenesis. The lack of synergy

among these pathways suggests that they operate independently to stimulate mitochondrial turnover.

Atg16L1 Knockout Induces Insulin Resistance Through Proteasomal IRS1 Degradation

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Insulin resistance is a defining feature of type 2 diabetes, yet our understanding of the progression and development of insulin resistance is incomplete. Recently, deficient autophagy, a bulk degradation pathway, was associated with the induction of insulin resistance, although the causative mechanism remains unknown. We sought to investigate the underlying signals responsible for how deficient autophagy induces insulin resistance. We examined mouse embryonic fibroblasts lacking Atg16l1 (ATG16L1 KO MEFs), an essential autophagy gene, and observed deficient insulin and insulin-like growth factor-1 signaling. ATG16L1 KO MEFs displayed reduced protein content of Insulin Receptor Substrate-1 (IRS1), pivotal to insulin signaling, while IRS1myc overexpression recovered downstream insulin signaling. Endogenous IRS1 protein content and insulin signaling were restored in ATG16L1 KO MEFs upon proteasome inhibition. Through proximity-dependent biotin identification (BioID) and co-immunoprecipitation, we found that kelch-like proteins KLHL9 and KLHL13, which together form an E3 ubiquitin (Ub) ligase complex with cullin 3 (CUL3), are novel IRS1 interactors. Expression of Klhl9 and Klhl13 was elevated in ATG16L1 KO MEFs and siRNA-mediated knockdown of Klhl9, Klhl13 or Cul3 recovered IRS1 expression. Moreover, Klhl13 and Cul3 knockdown increased insulin signaling. Notably, adipose tissue of high-fat fed mice displayed lower Atg16l1 mRNA expression and IRS1 protein content, and adipose tissue KLHL13 and CUL3 expression positively correlated to body mass index (BMI) in humans. We propose that ATG16L1 deficiency evokes insulin resistance through induction of Klhl9 and Klhl13, which, in complex with Cul3, promote proteasomal IRS1 degradation.

Exercise and Dairy have Distinct Effects on Indices of Liver and Systemic Lipid Metabolism

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Objective. To investigate the individual and combined effects of dairy supplementation and exercise training on improving markers of hepatic and whole-body lipid metabolism in male Sprague-Dawley rats. Methods. Male Sprague-Dawley rats (n=47) were fed a high-fat, high-sugar (HFHS) diet during an 8-week feeding intervention to induce obesity. At ~12 weeks of age, the rats were assigned to one of four weight-matched, isocaloric HFHS groups for 6 weeks: 1) casein-Sedentary (casein-S), 2) casein-Exercise (casein-E), 3) dairy-Sedentary (dairy-S), and 4) dairy-Exercise (dairy-E). An exercise training protocol consisted of five days/week motorized treadmill running with speed and incline increased on a weekly basis until week three of training, where they remained at 20m/min at 5% incline for 60 mins for the remainder of the exercise intervention. Non-fat skim milk powder was used as the sole protein source in the dairy diet, while casein was the sole protein source in the control casein diet – macronutrient composition was matched between diets. **Results.** Exercise, but not dairy, altered the composition of liver triglycerides and reduced indices of lipogenesis such as the ratio of 16:0/18:2n-6 and mRNA expression of Srebp1c and Acc1, with concomitant reductions in ACC1 protein content. Exercise reduced carbohydrate, while increasing whole-body fat oxidation, which was mirrored by increases in the expression of key genes involved in hepatic oxidative metabolism such as Pparα, Cpt1a, and β-Had. Dairy and exercise were found to reduce serum triglyceride concentrations in an additive manner. **Conclusions.** Collectively, our results provide evidence that dairy and exercise exert distinct effects on whole-body and tissue-specific carbohydrate and lipid metabolism. Dairy and exercise both positively modulate systemic and hepatic lipid metabolism, but likely work via distinct mechanistic pathways.

SNARE Expression and Promoter Regulation in Cardiomyocytes

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Introduction: The role of SNARE proteins in the exocytotic release of hormones and neurotransmitters has been widely studied. Two SNARE proteins, SNAP25 and syntaxin 1A (STX1A), are widely expressed in neurons and neuroendocrine cells, and more recently, shown to be expressed in the heart. The underlying mechanisms behind their expression in the heart are largely unknown. Other groups have demonstrated the SNAP25 and STX1A gene promoters are positively regulated by HDAC inhibitors, PKA activation and SP1 activity. Furthermore, these factors can induce the expression of the SNARE proteins in fibroblasts, which do not express SNARE proteins endogenously. Our studies examined the regulation of the SNAP25 and STX1A gene promoter in the cardiac cell line, H9c2. Full-length and truncated constructs upstream of the

firefly luciferase gene were transfected into H9c2 cells. The cells were treated with the HDAC inhibitor, trichostatin A (TSA), forskolin to activate PKA, or retinoic acid to induce differentiation of H9c2 cells to a more cardiac phenotype. Preliminary data show greater activity of the -292 bp SNAP25 promoter compared to the -1517 bp full-length construct. Similarly, the -627 and -204 bp promoter constructs of STX1A were higher than the full-length -1931 bp promoter. Neither treatment with TSA, forskolin nor retinoic acid induced expression of SNAP25 or STX1A in H9c2 cells. Our results demonstrate SNARE protein expression cannot be induced in H9c2 cells. Further studies are required to determine the regulation of SNARE proteins in the heart. Keywords: promoter activity, HDAC inhibition, PKA activation, differentiation

Measuring Sternocleidomastoid Muscle Volume with Phase-Contrast MRI

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The sternocleidomastoid (SCM) muscle is a cervical flexor muscle, which passes obliquely across the side of the neck bilaterally. It is comprised of two heads that originate from the sternum and the clavicle to insert onto the mastoid process of the temporal bone behind the ear.1,2 This muscle has several functions including flexion and rotation of the head and neck, but it also serves as protection for the carotid arteries due to its position anterior to the carotid sheath.1,2 Cross sectional area measurements of the SCM muscle have been explored to some extent, however at varying cervical vertebrae levels leaving a significant gap in the literature related to the total volume of the SCM muscle. This study measured the SCM muscle volume and explored sex-related differences using a well-established model for calculating the muscle volume of the thigh muscles, 3 Imaging of the SCM muscle was conducted using Phase Contrast Magnetic Resonance Imaging (PC-MRI) of 34 healthy participants (19 females, 15 males). SCM muscle volume was first calculated as the sum of the cross-sectional area calculated over each of the cervical vertebrae levels to provide a cumulate cross-sectional area. This was then multiplied by the MRI slice thickness size to provide total muscle volume.3 The preliminary results established a dependable method to calculate the cumulative SCM volume with a high inter-rater reliability (intra-class correlation of 0.947). A significant sex difference was identified for average SCM volume calculated as an average from 3 raters for both the right and left SCM muscles ($p=0.001$, $p=0.0003$ respectively). The body mass index (BMI) adjusted SCM volume also demonstrated a significant sex difference on both the right and left sides ($p=0.000061$, $p=0.000420$ respectively). This demonstrates that significant sex-related differences exist within the SCM muscle volume bilaterally, supporting the need for whiplash-related research to take sex differences into account. In addition, a reliable method of measurement for SCM volume has been established. An impairment and/or changes in the functional capacity of the neck musculature has shown to lead to cervicogenic symptoms such as recurrent or chronic headaches4. In the future, these preliminary results will be an essential comparison group of healthy SCM muscle volume values for use with populations suffering from whiplash-related injuries.

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The Capacity for Skeletal Muscle to Repair After Exercise-Induced Muscle Damage in Young-Adults with Type 1 Diabetes Mellitus

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Background: There is strong evidence that skeletal muscle health is compromised in young-adults with type 1 diabetes mellitus (T1D). These impairments include reduced strength, insulin resistance, mitochondrial dysfunction, and decreased satellite cell content. Maintaining healthy muscle requires successful muscle repair. To date, the impact of T1D on human skeletal muscle repair has not been studied; however, attenuated repair would account for the reduced functional capacity and premature institutionalization which often characterizes those with diabetes. **Methods:** The purpose of this on-going study is to determine the effect of T1D on the recovery of skeletal muscle function, morphology, and ultrastructure following exercise-induced muscle damage (EIMD) of the vastus lateralis. Males and females (18-30 years of age) with and without T1D performed the EIMD protocol. Pre-EIMD, and at 48- and 96-hours post-EIMD, subjects gave a blood sample and vastus lateralis muscle biopsy, and performed a maximal isometric knee extension. Preliminary
Results: Relative to body weight, pre-EIMD isometric peak torque was similar between control subjects (Ctl) and those with T1D (2.93±0.29 versus 3.08±0.31 N.m/kg). Isometric peak torque was significantly reduced ($P<0.0001$, main effect for time) in both groups post-EIMD, but there were surprisingly no differences between groups immediately (Ctl: 36.41; T1D: 35.45), 48- (Ctl: 78.61; T1D: 66.41) or 96-hours (Ctl: 85.18; T1D: 71.08% of pre-EIMD peak torque) post-EIMD. Muscle biopsies from several subjects showed substantial evidence of muscle damage–degenerating and regenerating fibers, and infiltrating mononuclear cells. At 48-hours post-EIMD, there was a greater leftward shift in control subjects' minimum ferret diameter distribution, compared to subjects with T1D. The distributions continued to deviate 96-hours post-EIMD. **Discussion/Conclusion:** Contrary to previous studies in rodents and humans with T1D, our preliminary data suggests that skeletal muscle function–in terms of isometric strength and strength recovery—is not impaired in young-adults with T1D. Despite the lack of difference in muscle function, there is a less robust shift to smaller muscle fibers after muscle damage in subjects with T1D. This may be indicative of delayed muscle repair, possibly due to alterations in fiber-type or muscle ultra-structure, which are currently being analyzed. While our preliminary findings are positive (no functional impairment in those with T1D), a comprehensive understanding of muscle recovery is essential in order to maximize muscle health and ultimately, improve their healthy lifespan.

Identification of novel microRNAs (miRs) regulating skeletal muscle regeneration in sustained ICUAW

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Rationale: ICU acquired weakness (ICUAW) is a common complication of critical illness characterized by decreased skeletal muscle mass and impaired contractile function that may persist for years after ICU discharge, with few patients regaining complete physical independence. Critical illness survivors with ICUAW have a lack of skeletal muscle repair and regeneration due to the depletion and dysregulation of gene co-expression networks of skeletal muscle stem cells (satellite cells). MicroRNAs (miRs) regulate gene expression in satellite cells and myoblast proliferation and differentiation, thereby controlling the maintenance, self-renewal and regeneration of skeletal muscle. Thus, we sought to identify miRs that regulate the failure of muscle regeneration in critical illness survivors with sustained ICUAW.

Methods: From a cohort of critically ill patients, skeletal muscle strength, mass, and physical function were measured and whole-transcriptome miR and mRNA expression was determined in quadriceps muscle biopsies at Day 7 and Month 6 (6M) post-ICU discharge. We then conducted an integrated miR-mRNA analysis to identify dysregulated miR/gene pairs that were robustly correlated with sustained (6M) muscle wasting in ICUAW and evaluated their impact on myoblast proliferation and differentiation in vitro. The highest ranking, differentially expressed, miRs identified in our miRNA/mRNA analysis were selected for in vitro study. Candidate miRs were overexpressed in C2C12 myoblasts and their influence on myoblast proliferation and differentiation were subsequently determined by quantification of cell counts, Ki67 nuclear localization, histone phosphorylation, and myosin heavy chain expression.

Results: At 6M post-ICU, distinct miR expression signatures were found to separate patients with significant improvement in muscle mass from those with sustained ICUAW and persistent muscle atrophy. Eight miRs were found to regulate these differentially expressed gene signatures, including miR-490-3p and 744-5p, which we have identified as novel regulators of myogenesis. miR-490-3p overexpression significantly reduced C2C12 myoblast proliferation, and induced contact independent myoblast differentiation. miR-744-5p overexpression did not affect myoblast proliferation, but instead attenuated myoblast differentiation.

Conclusion: MicroRNA profiling identified key miRs involved in the regulation of muscle weakness at Day 7 and in the recovery of muscle mass at 6M post-ICU discharge. We identified miR-490-3p and 744-3p as novel regulators of myoblast proliferation and differentiation, respectively, which may play a causative role in the pathogenesis of sustained ICUAW.

Low dose lithium feeding improves murine left ventricular SERCA function by regulating SERCA2a and phospholamban expression.

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The sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) pump is responsible for regulating calcium (Ca2+) within muscle cells. SERCA2a is the predominant isoform seen in cardiac muscle. Its inhibitor, phospholamban (PLN), decreases SERCA's affinity for Ca2+ therefore decreasing SERCA activity. This means that changes in the SERCA2a:PLN ratio can cause calcium dysregulation, which is often seen in patients with dilated cardiomyopathy. The enzyme glycogen synthase kinase-3β (GSK3β) negatively regulates SERCA function by decreasing the SERCA2a:PLN ratio. In this study, we sought to determine whether feeding mice a low dose of lithium, which is a natural GSK3 inhibitor, would improve left ventricular SERCA function by altering the SERCA2a:PLN ratio. To this end, male wild type mice were fed low-dose lithium via their drinking water (10mg/kg/day for 6 weeks) and their left ventricles were removed and tested. Maximal SERCA2a activity did not change between lithium and control mice however, SERCA's apparent affinity for Ca2+ did significantly increase in lithium-fed mice. In this group, individual SERCA2a expression increased while PLN expression was decreased leading to a 2.0-fold increase in the SERCA2a:PLN ratio. GSK3β activity was also shown to be effectively inhibited in the lithium-fed mice. These findings suggest that low-dose lithium can improve SERCA function via alteration in the SERCA2a:PLN ratio through GSK3β inhibition which could potentially be a therapeutic strategy for dilated cardiomyopathy.

Remodeling of the Extracellular Matrix Following Cardiotoxin-induced Muscle Damage in Aged Mice

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Introduction: The regeneration process is crucial to returning muscle to normal function following damage. The extracellular matrix (ECM) plays a critical role in guiding and modulating the regeneration process, however, aberrant changes occur to the ECM with aging. The aim of this study was to investigate the changes that occur to the ECM in response to damage in aged skeletal muscle.
Methods: Young (3 month old) and aged (18 month old) C57BL/6J male mice were studied (n = 15 in each age group). Each mouse received a cardiotoxin (CTX) injection in the left tibialis anterior muscle to induce damage. Mice were sacrificed at 3, 5, or 7 days following CTX injury. Via immunohistochemistry, collagen I, IV, and fibronectin were examined as important markers of ECM remodeling while embryonic myosin heavy chain (eMHC) was used as an index of regeneration.
Results: eMHC expression in aged muscle was found to be significantly lower following damage as defined by a reduction in eMHC+ area relative to total myofibre area (main effect: $p<0.05$). At

7-days following damage, the cross sectional area (CSA) of young regenerating myofibres exceeded that of the aged regenerating myofibres (simple main effects post-hoc analysis: $p<0.05$). Collagen I content in aged muscle remained significantly greater in the necrotic regions at 3 and 5 days post-damage. Fibronectin was found to be significantly lower during all timepoints in aged compared to young ($p < 0.05$). Basement membrane thickness (ie: collagen IV positive structure surrounding each myofibre) was significantly greater in aged muscle at both 5 and 7-day timepoints (main effect: $p < 0.05$).
Discussion: Aged muscle displayed an impaired regenerative response along with accumulation of collagen I and IV, and repression of fibronectin content. These findings suggest that the reduced regenerative capacity of aged muscle is related to dysregulation of select ECM proteins.

A Preliminary Investigation of Cardiac Cell Proliferation Following a Puncture Injury to the Heart of the Leopard Gecko (Eublepharis macularius)

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A hallmark feature of cardiac self-repair is an increase in cell proliferation associated with both the wound site (local) and across the entire organ (global). Among species capable of cardiac regeneration, including zebrafish and salamanders, injuries to the heart initiate a surge in cardiomyocyte proliferation. In contrast, among species that repair cardiac injuries with a scar, rates of spontaneous cardiomyocyte proliferation are essentially unaltered in response to a lesion. Hence, cardiomyocyte proliferation in response to injury may provide an index of regenerative-competence. Here, we characterize cell proliferation following a cardiac puncture in a representative reptile, the leopard gecko (Eublepharis macularius, hereafter 'gecko'). Cardiac punctures (cardiocentesis) are a procedure used in veterinary clinics to collect blood from small lizards and snakes. Geckos readily tolerate this injury, but little is known about the structural and cellular changes that take place to repair the heart. To investigate cardiac cell proliferation, we performed double immunofluorescence with the DNA synthesis marker proliferating cell nuclear antigen (PCNA), with each of myosin heavy chain (MHC; a cardiomyocyte marker) and Vimentin (Vim; a marker of fibroblasts and endocardial cells). Prior to injury, we observed proliferating populations of both cardiomyocytes (MHC+/PCNA+) and non-cardiomyocytes (Vim+/PCNA+). One day post-cardiac puncture (dpc), the wound site is characterized by the formation of a blood clot capping the puncture, and the localized loss of MHC+ cardiomyocytes within the wound site. At 5 and 10 dpc, there is an increase in Vim+ expression within the wound site, while increasing numbers of proliferating cardiomyocytes are observed along the border of the lesion. By 14 dpc, MHC+ cardiomyocytes repopulate the wound site, restoring the original trabeculated architecture of the myocardium. Taken together, these data demonstrate that cardiac self-repair in the gecko heart is characterized by an increase in cardiomyocyte proliferation. Our findings reveal that gecko heart repair closely follows the regenerative trajectory observed in species such as zebrafish, and is distinct from the fibrotic response of mammals.

Mechanistic Insights into The Effects of Exercise on Inflammatory Responses.

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Introduction:

Despite the importance of the inflammatory response in fighting infections, prolonged or unwanted inflammation is the root cause for serious human diseases including atherosclerosis and cancer. Therefore, understanding how inflammation is regulated is crucial for preventing these diseases. The objectives of this study include conducting in-vivo and ex-vivo experiments to gain an in-depth understanding of the molecular pathways and the regulatory elements of the inflammatory response that are altered following long-term moderate exercise in mice. Furthermore, the effects of exercise on macrophage polarization to the M1 phenotype (pro-inflammatory) and M2 phenotype (anti-inflammatory and tissue repair) will be assessed.

Methods:

C57/Bl6 mice were randomly assigned to two groups: a control group where no exercise was performed, and a second group where a single bout of moderate exercise intensity (i.e. 20 m/min for 60 min) was carried out for 5 days per week for a total of 9 weeks. Bone marrow derived macrophages (BMDMs) were isolated from mice of both groups and were then stimulated with several inflammatory pathways inducers, such as Lipopolysaccharide (LPS) from Gram negative bacteria to induce Toll like receptors, the viral dsRNA mimic, poly I:C or dsDNA mimic, poly dA:dT to induce RIG-I receptors. mRNA was then extracted, and gene expression of pro- and anti-inflammatory cytokines was measured by real-time PCR. To understand how exercise is modulating the inflammatory phenotype of BMDMs, we evaluated gene expression of genes associated with M1 and M2 phenotypes by real-time PCR.

Results:

Our data demonstrate that inflammatory genes have been downregulated following moderate chronic exercise program (e.g. NF-□B mediated genes [IL1-β, TNF-α] and IRF3 mediated genes [IFN-β]). Mechanistically, genes associated with M1 phenotype were downregulated (e.g. iNOS, HIF1-α) and M2 phenotype were up-regulated (e.g. Arginase-1, IL-10), which indicates that moderate exercise training drives macrophages into the anti-inflammatory M2 phenotype.

Discussion:

Our data indicate that moderate chronic physical activity alters inflammatory response by differentially altering intracellular signalling pathways, which might lead to improved immune responses to certain infections or faster resolving of inflammation in prolonged inflammatory conditions. Future experiments will focus on additional mechanistic insights or details on how exercise is exerting these effects. We will also test how other exercise intensities and durations alter the inflammatory response.

Oxidative and glycolytic muscles develop insulin resistance despite displaying fiber type-specific patterns of intracellular glycerolipid accumulation

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Intramyocellular accumulation of glycerolipids has been linked to the development of skeletal muscle and whole-body insulin resistance. In this context, it has been hypothesized that elevated capacity to oxidize fat may protect skeletal muscles form intracellular glycerolipid accumulation and the development of insulin resistance. The rationale is that channeling fatty acids towards oxidation would leave fewer available to be converted into lipid intermediates that could otherwise cause lipotoxicity. To test this hypothesis, we investigated whether the intracellular accumulation of mono-, di-, and triacylglycerol (MAG, DAG, and TAG, respectively) differs between oxidative and glycolytic muscles, and how it correlates with high-fat (HF) diet-induced skeletal muscle insulin resistance. To accomplish that, male Wistar rats were fed either a standard chow (SC, control) or a HF diet for 8 weeks. Subsequently, we measured basal and insulin-stimulated glycogen synthesis and glucose oxidation, as well as palmitate oxidation, MAG, DAG, and TAG contents in soleus (Sol, rich in type IA fibers), extensor digitorum longus (EDL, rich in type 2A fibers), and epitrochlearis (Epit, rich in type IIB fibers) muscles. Phosphorylation of proteins involved in insulin signaling such as protein kinase B (Akt), glycogen synthase kinase-3 (GSK-3), and glycogen synthase (GS) were also assessed in these muscles. Rats fed HF for 8 weeks became hyperinsulinemic and their Sol, EDL, and Epit muscles displayed impaired ability of insulin to stimulate glycogen synthesis and glucose oxidation, to promote AktThr308/Ser473 and GSK-3□/β phosphorylation, and to cause GS dephosphorylation. Conversely, palmitate oxidation significantly increased in all muscles of HF rats, although their rates varied depending on fiber type distribution (Sol>EDL>Epit). Despite these fiber type differences in fat oxidative capacity, MAG, DAG, and TAG contents significantly increased in Sol, EDL and Epit muscles, which was consistent with insulin resistance displayed by all of them. Interestingly, MAG, DAG and TAG concentrations were lowest in Sol and greatest in the Epit muscles, whereas the EDL muscle displayed intermediate contents of these glycerolipids. These findings indicate that prolonged oversupply of lipids cannot be offset by enhanced capacity to oxidize fat. Therefore, our findings provide novel evidence that muscles with high capacity to oxidize fat tend to accumulate less MAG, DAG, and TAG, but are as vulnerable to developing insulin resistance as glycolytic muscles with much lower ability to oxidize fat under conditions of HF diet-induced obesity.

Thoracolumbar co-contraction: a predisposing mechanism for back pain associated with large chest size?

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Objective: The purpose of this study was to investigate prolonged standing induced back pain with the factor of chest size to determine if different trunk muscular co-contraction patterns exist between women with small and large chest sizes.

Methods: Sixteen young women were divided into two groups: small chest size (n=7, ~B/C cup) and large chest size (n=9, ~D/E cup). Participants completed a 2 hour prolonged standing protocol, during which 8 channels of bilateral trunk electromyography were collected. The 2-hr stand was composed of 15-minute epochs, in which back pain ratings (upper, mid, and low back) were collected using a 100mm visual analog scale. Using the normalized, linear envelope of the EMG data, a co-contraction index (CCI: Eq'n 1) was calculated for each possible pairing of muscles (n=120) at each 15-minute epoch [1,4]. General linear models were completed for all muscle pairings with main effects and interactions of chest size, pain development, and time.
Results and Discussion: All women in the large chest group and 4 of the small chest group were classified as pain developers (VAS>10mm), and exhibited coactivation of gluteus medius [1]. Global flexor/extensor activity was significantly higher for the large chest group, and the pain developers in the small chest group. The large chest group had higher co-contraction levels for 77 of the 120 possible CCI pairings. All bilateral CCIs were significantly different between chest sizes ($p<0.038$). The small group did not have any differences CCI between bilateral pairings. For the large chest group the mean CCIs for bilateral LATs, and erector spinae (T4, T9, L1) (CCI(%MVC) = 1997±176) were approximately 2 times greater, than all other pairings (CCI(%MVC) = 1150 ± 149) ($F(7,575)=10.0$, $p<0.001$).
Conclusions: The thoracolumbar region is affected by the anterior load of larger chest sizes [2,3]. Demands of the posterior active and passive structures have been highlighted by kinematic and muscular activation differences between chest sizes [2]. The women in the large group started the stand with higher levels of co-contraction for T4, T9 and L1 erector spinae and LATs and sustained higher levels throughout the stand. These high co-contractions may be a potential pattern, in addition to the known mechanism of gluteus medius, contributing to the increased pain development for larger chested women in prolonged standing [1].

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Analysis of the T-class -cardiac actin (ACTC1) variant R312C related to hypertrophic cardiomyopathy

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Cardiovascular disease is currently the leading cause of death globally. One such disease is known as cardiomyopathy, which is further subdivided into hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). The former results in a thickening of the left ventricle of the heart while the latter results in a thinning of the left ventricle. □-cardiac actin (ACTC1) is required for the contraction of the heart. Sixteen different point mutations have been identified in patients with cardiomyopathy, including one resulting in the R312C variant related to HCM progression. Since the change lies in the area of actin interacting with regulatory proteins troponin and tropomyosin, the R312C variant is categorized as a T-class variant. Since the R312C variant has not been characterized biochemically, we characterized its intrinsic stability, myosin interactions and

troponin/tropomyosin interactions compared to either wild-type recombinant (WTrec) actin or bovine cardiac actin. The R312C variant has a decreased melting temperature and increased polymerization rate. Myosin interactions were not impacted, while calcium sensitivity remained similar to WTrec. These results were unexpected since this variant is thought to interfere with regulatory protein interactions. To determine the cause for disease progression, an in vivo model may be needed to determine which protein interactions are being affected in the presence of the R312C variant. Further analysis will be performed to compare to the R312H variant which results in DCM rather than HCM. The comparison of these two variants may allow us to determine the underlying molecular difference between these two disease progressions.

Dietary Sodium Nitrate Increases the Content of Both SERCA and PLN in the Hearts of Healthy Rats

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Background: The dynamic properties of contraction-relaxation coupling in the heart requires cyclical fluctuations in cytosolic Ca2+. Intriguingly, dietary sodium nitrate (NaNO3) has been reported to increase cytosolic Ca2+ and heart contractility, and while this would necessitate a greater demand on removing Ca2+ for relaxation and passive-filling of the heart, the influence of nitrates on properties associated with relaxation have not been previously considered. Therefore, this study aimed to determine the effect of NaNO3 supplementation on parameters influencing Ca2+ homeostasis, including sarcoplasmic/endoplasmic reticulum Ca2+ -ATPase (SERCA) enzymatic properties.

Hypothesis: NaNO3 will increase SERCA content and activity in healthy rat hearts.

Materials and Methods: Sprague-Dawley Rats (n=16) were fed standard chow with or without 4 mM of NaNO3 supplemented in their water for one week. Echocardiograms were performed to determine in vivo left ventricle morphology and function. The hearts were excised, and SERCA activity assays were performed to estimate Ca2+ sensitivity. Western blots were also done to determine the content of key proteins.

Results: NaNO3 supplementation increased ($p<0.05$) SERCA content without affecting calsequestrin (CSQ) abundance. However, there was a proportional increase in phospholamban (PLN) content, a known inhibitor of SERCA, and as a result, there was no change in SERCA enzymatic properties (maximal activity or Ca2+ sensitivity). Additionally, there was no change in functional parameters of the heart assessed by echocardiograms following NaNO3 supplementation.

Conclusion: These data suggest that NaNO3 increases the content of SERCA, however, in a healthy rodent model there is no function effect on the heart due to compensatory changes in PLN-mediated inhibition.

Atrial arrhythmias and adverse atrial remodeling induced by exercise requires soluble tumor necrosis factor alpha (TNFa) derived from atrial myocardium

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Background: Intense endurance exercise is linked to atrial fibrillation (AF). We previously identified the pro-inflammatory cytokine, TNFα, as a key mediator of exercise-induced atrial remodeling and AF vulnerability in mice. However, the nature of TNFα-dependent cardiac signaling and the cardiac source of TNFα mediating exercise-induced AF remain elusive. Purpose: To determine whether TNFα-dependent atrial changes induced by exercise requires soluble TNFα derived from the atrial myocardium. Methods and Results: Adverse atrial remodeling, characterized by atrial fibrosis and inflammatory cell infiltrates, as well as increased AF susceptibility induced by six weeks of intense swim exercise were prevented when the TNFα gene was selectively ablated in the atrial myocardium of mice with floxed TNFα genes and with cre-recombinase expression under the control of the atrial-specific NPPA promoter. On the other hand, reductions in heart rate, increased vagal tone and enhanced ventricular function were unaffected by disruption of TNFα in the atrial myocardium. To determine whether the exercise-induced atrial changes involves signaling through TNFα receptors via enzymatically liberated soluble TNFα (solTNF) versus via membrane bound TNFα, we treated mice with XPRO®, a selective dominant-negative inhibitor of solTNF. XPRO® also largely prevented the adverse atrial changes induced by exercise, independent of beneficial physiological changes. Conclusions: Our results establish that exercise-induced atrial remodeling and AF vulnerability requires soluble TNFα originating from the atrial myocardium.

Effects of High Dose Radiation on Juvenile Skeletal Muscle Development and Satellite Cell Dynamics

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PURPOSE: To examine muscle development and satellite cell dynamics, throughout a time course of development, following an acute dose of localized radiation. METHODS: At 5-6 weeks of age, male CBA mice were exposed to an acute dose of radiation (16 Gy) localized to the lower left hindlimb (IRR). The right leg was used as a contralateral control (CON). Mice were sacrificed at 3, 7, 14, or 56 days following radiation. Tibialis anterior (TA) and gastrocnemius/soleus complex (GA) were removed and used for analysis. Immunofluorescence staining was completed to determine changes to muscle cross-sectional area (CSA), myonuclear content, and satellite cell dynamics.
RESULTS: Average GA myofiber CSA was reduced at 3 and 14 days post IRR ($p<0.05$ vs. CON), and trended to increase in TA at 56 days post IRR. In GA the content of Pax7+satellite cells was not different between IRR and CON; however, there was a significant reduction in the number of differentiated myoblasts (Pax7-MyoD+) in IRR compared to CON at 7 and 14 days post-radiation ($p\leq0.05$). In TA, no differences were found between IRR and CON satellite cell dynamics.

CONCLUSION: Juvenile radiation may hinder skeletal muscle development due to decreased CSA and negative impacts on satellite cell differentiation.

The role of mitochondrial ROS and protein glutathionylation in myogenic lineage commitment

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Adult stem cells play an essential role in the lifelong maintenance and repair of bodily tissues. However, a decline in stem cell populations is often observed during aging and diseases. A balance of stem cell fate decisions, of self-renewal versus commitment, is required for stem cell maintenance. However, the mechanisms that regulate stem cell fate decisions are not completely understood. It has been demonstrated that mitochondrial reactive oxygen species (mtROS) can act as signaling molecules to regulate gene expression and affect stem cell fate decisions, though the mechanism is not fully characterized. It has been previously shown that increased ROS levels can promote glutathione synthesis. Moreover, protein glutathionylation has recently emerged as an important post-translational modification in redox regulation that can influence gene expression. Thus, we hypothesized that mtROS may signal through protein glutathionylation in order to modify gene expression and promote stem cell commitment. We began investigating the existence of this mechanism in C2C12 myoblasts. Levels of mtROS were moderately increased using low-dose rotenone, a complex I inhibitor. We observed that this promoted global protein glutathionylation and increased expression of the myogenic regulatory factors MyoD and MyoG. These data suggest that there is a link between mtROS, protein glutathionylation, and expression of myogenic regulatory factors. Ultimately, this research will help us elucidate a mechanism that may promote stem cell depletion, and identify potential therapeutic targets to reverse or prevent this decline in aging and other degenerative diseases.

Effect of localized radiation exposure on skeletal muscle inflammation and fibro/adipogenic progenitors in juvenile mice

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Radiation therapy is a common treatment option for childhood cancers, and has contributed to improved outcomes and increased survival rates. However, survivors have an increased risk for debilitating late-effects of radiation therapy that contribute to poor muscle health in adulthood. Radiation induced fibrosis (RIF) is characterized by muscle atrophy, fibrosis, and impaired regeneration. Fibro/adipogenic progenitors (FAPs) coordinate with inflammatory cells, including macrophages, during muscle regeneration. Previous studies have examined the effect of radiation on adult skeletal muscle; however, little is known regarding the role of FAPs and macrophages in skeletal muscle development following juvenile radiation exposure. The purpose of this study was to examine changes in FAP and muscle macrophage content over a time-course following localized radiation (IRR). We hypothesized that radiation would contribute to long-term reductions in FAP content, and a prolonged inflammatory response. Five-week-old, male, CBA mice were exposed

to localized radiation (16 Gy) to the left hindlimb. The right leg was shielded and used as a contralateral control. Mice were euthanized at 3, 7, 14, and 56 days post-IRR. Total macrophages, M1 macrophages (pro-inflammatory; F4/80+CD206-), M2 macrophages (anti-inflammatory; F4/80+CD206+), and FAPs (PDGFRa+) were quantified in tibialis anterior (TA) and gastrocnemius/soleus (GAS) by immunofluorescence. FAP content decreased at 14- and 56-days post-IRR ($p<0.05$) in both muscles. In the TA, total and M2 macrophage content increased 7-days ($p<0.05$), and M1 macrophage content decreased 7-days ($p<0.05$) post-IRR. In the GAS, total macrophages were increased 3-days ($p<0.05$), M2 macrophages were elevated at 3- and 7-days ($p<0.05$), and M1 macrophages were reduced at 14-days ($p<0.05$) post-IRR. In developing skeletal muscle, radiation decreases FAP content at late time points and promotes a temporary pro-regenerative macrophage polarization. These data provide novel cellular targets for reducing the negative long-term consequences of radiation exposure on skeletal muscle in childhood cancer survivors.

Disruption of Branched-chain α-ketoacid dehydrogenase (BCKD) Enhances Myofibrillar Protein abundance in Myotubes

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Branched-chain amino acids (BCAAs) are essential amino acids that are important for skeletal muscle protein metabolism. As BCAAs are critical for skeletal muscle anabolism, alterations in their anabolic capacity is associated with the development of several muscle atrophic diseases such as cancer, chronic inflammatory and neurological disorders. As substrates for energy production, BCAAs are catalyzed in the mitochondria through a multi-step process to generate acetyl CoA, an intermediate for the TCA cycle. BCAA catabolism is dependent on the reversible transamination by branched-chain aminotransferase 2 (BCAT2) followed by the irreversible carboxylase activity of branched-chain ketoacid dehydrogenase (BCKD). Recent studies have shown that BCAT2 AND BCKD are essential for the differentiation of skeletal myoblasts into myotubes. Other studies have shown that BCKD deficiency leads to the buildup of BCAAs and BCKAs (BCAA metabolites) as implicated in maple syrup urine disease. This study examines the effect of BCKD depletion on L6 myotube morphology and myofibrillar protein content. On day 4 of differentiation, myotubes were transfected with scrambled siRNA (SCR SiRNA, control), BCKD siRNA or BCAT2 siRNA. By day 6 of differentiation, enhanced myotube morphology was observed in the BCKD depleted cells compared to those treated with scrambled and BCAT2 siRNA. In addition, BCKD depletion resulted in an 87% increase in myosin heavy chain (MHC) ($n=4$), and 3-fold increase in Tropomyosin ($n=4$, $p=0.0827$) by day 6 of differentiation compared to control. When compared to BCAT2 depleted myotubes, BCKD depleted myotubes exhibited a 4-fold increase in MHC ($n=4$, $p<0.05$), 35% increase in troponin ($n=4$) and a 2-fold increase in tropomyosin ($n=4$). To further investigate the increase in myofibrillar protein content, we examined signaling through mTORC1 (mechanistic target of rapamycin complex 1), a vital complex necessary for skeletal muscle anabolism. By day 6 of differentiation, phosphorylation of mTORC1's upstream activator, AKT was enhanced in BCKD depleted myotubes compared to cells depleted of BCAT2 ($n=4$,

p<0.01). In BCKD depleted myotubes, phosphorylation of mTORC1's downstream substrates ribosomal protein S6 and its kinase, S6K1 was increased by 68% and 2-fold respectively compared to BCAT2 depleted myotubes (n=4, p= 0.3439, p=0.0730). Findings from this study suggest that the depletion of BCKD enhances myofibrillar protein content and anabolic signaling in myotubes.

Exogenous erythropoietin and novel splice variant preserve cardiac function in vivo in a murine model of myocardial infarction

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Introduction: Erythropoietin (EPO) is the master regulator of erythropoiesis. Recent studies reveal that recombinant human EPO (rhEPO) also induces cytoprotection, cell proliferation, and increases myocardial contractility. Emerging data shows the existence of a functionally relevant alternative form of the EPO transcript, denoted hS4 (partially lacking exon 4), detected in cancer cells. In vitro studies suggest hS4 is neuroprotective, as it improves cell survival in a brain model of ischemic injury, however it lacks the ability to increase cell proliferation in colony forming unit assays, suggesting this splice variant would not have erythropoietic activity in vivo. Therefore, the primary objective of this work was to demonstrate the ability of rhEPO and hS4 treatments to promote cytoprotection in the heart and preserve cardiac function in a murine model of myocardial infarction (MI). It was hypothesized that in comparison to the untreated group, in vivo cardiac function would be enhanced in rhEPO- and hS4-treated MI mice without stimulating erythropoiesis.
Methods: Male CD1 mice were subjected to permanent ligation of the left anterior descending coronary artery. Mice were randomly assigned to the untreated group or to receive either rhEPO or hS4 at the time of artery ligation (administered at a dosage of 10,000 units/kg of rhEPO or equimolar concentration of hS4). At 4 weeks post-MI, cardiac structure and function were assessed using B-Mode echocardiography and invasive hemodynamics, respectively.
Results: At 4 weeks post-MI, echocardiography revealed rhEPO and hS4 increased fractional shortening, ejection fraction, stroke volume and cardiac output, with no differences in heart rate compared to the untreated group. End diastolic dimensions and pressure were significantly lower in the treated mice. Invasive hemodynamics indicated left ventricular pressure at 40 mmHg and +dP/dtmax were increased in both treatment groups compared to the untreated group. To assess erythropoietic activity, a subset of healthy mice were treated for 2 weeks with either rhEPO, hS4 or saline (vehicle control) and hematocrit was measured. Indeed, only rhEPO-treated mice showed an increase in hematocrit as compared to saline or hS4-treated mice.
Conclusions and Implications: Our data revealed rhEPO and the splice variant, hS4, improved cardiac structure and function following MI compared to untreated animals but only rhEPO had erythropoietic activity. While it is known the erythropoietic effect of chronic rhEPO dosing limits its use in clinical trials, a non-erythropoietic variant that maintains comparable cytoprotective effects may serve as a prospective drug alternative to maintain myocardial contractility in instances of MI.

Female mice are protected against acute olanzapine-induced hyperglycemia

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Olanzapine is a second-generation antipsychotic (SGA) used frequently in the treatment of schizophrenia and a growing list of off-label conditions1. Though effective in reducing psychoses, acute olanzapine treatment causes rapid increases in blood glucose that are believed to be mediated by increases in liver glucose output, skeletal muscle insulin resistance, and beta cell dysfunction. Clinical evidence has linked these effects to increased type 2 diabetes risk2. While females have been reported to be more susceptible to olanzapine-induced weight gain3, there is little known about the impact of sex on the acute glycemic response to SGAs. The purpose of this study was to determine if the acute effects of SGAs on glucose metabolism display a sexually dimorphic response in C57BL/6J mice. Age matched male and female C57BL/6J mice were treated with the SGA olanzapine (5 mg/ kg, IP) or vehicle control and blood glucose was measured at baseline, 15, 30, 60, 90, and 120 minutes post-treatment and tissues and serum harvested. These experiments were repeated, and mice underwent an insulin tolerance test or pyruvate tolerance test following 60 minutes of olanzapine treatment. Females were protected against olanzapine-induced increases in blood glucose compared to male mice with and without pyruvate co-treatment, and this occurred despite the development of severe insulin resistance. In male mice olanzapine increased the glucagon:insulin ratio whereas in female this ratio was reduced. In addition, when challenged with exogenous glucagon (1 mg/ kg IP), females were less responsive than males. Our findings provide evidence that females are protected from acute excursions in blood glucose following olanzapine administration. This was associated with a more robust insulin response in females and reductions in serum glucagon coupled with decreases in glucagon responsiveness. Further studies will investigate if the apparent protective effect in females is mediated by sex hormones.

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Myotube Morphology and Protein Metabolism are Negatively Regulated by Chemotherapy Drugs

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Cachexia, a condition prevalent in many chronically-ill patients, is characterized by weight loss and fatigue resulting from decreases in muscle mass and function. Although development of cachexia is associated with tumour burden and disease-related malnutrition, other studies have suggested a causative link between chemotherapy treatment and cachexia. In order to understand the mechanisms of chemotherapy induced cachexia, we investigated the effects of a common chemotherapy drug cocktail on myotube morphology and myofibrillar protein abundance. On day 4 of differentiation, myotubes were treated with vehicle or a chemotherapy drug cocktail (a mixture

of cisplatin (20μg/mL), leucovorin (10μg/mL), and 5- fluorouracil (50μg/mL)). Compared to myotubes treated with vehicle, those treated with the drug cocktail show dysmorphic shape and abnormalities in myotube morphology. Drug treatment also induced significant reductions in myosin heavy chain (MHC) ($n=4$, $p < 0.0001$), troponin ($n=4$, $p < 0.0001$) and tropomyosin ($n=4$, $p < 0.0223$) by day 6 of differentiation. To explore the reasons for the low abundance of these myofibrillar proteins, we examined treatment effects on mTORC1 (mammalian/mechanistic target of rapamycin complex 1). Myotubes treated with the drug cocktail showed significant reductions in the phosphorylation of mTORC1 activator AKT ($n=4$, $p < 0.0349$) and reductions in phosphorylation status of mTORC1 substrates ribosomal protein S6 ($n=3$, $p < 0.0138$) and its kinase, S6K1 ($n=3$, 0.0209). Drug treatments also led to reductions in protein synthesis ($n=4$), as well as further reductions in mitochondrial complexes cytochrome C oxidase (COX IV) and succinate dehydrogenase (SDHA) ($n=4$, $p < 0.05$). Future experiments will investigate the effect of the drug cocktail on glucose metabolism in myotubes. The above findings suggest that it is critical to identify interventions that can limit the negative effects of these drugs on muscle protein status and mitochondrial content.

The LINC complex modulates satellite cell response to injury via cell fate regulation

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A fundamental goal in muscle stem cell (MuSC) biology is to understand how a combination of physical and chemical cues results in the transition from quiescence to activation. The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex is a physical bridge between cytoskeletal networks and the nuclear envelope which acts as an intracellular force conductor. Nesprins bind to cytoplasmic f-actin and to SUN proteins, which in turn bind to Lamin A/C and Emerin of the nuclear lamina, and subsequently to chromatin. This provides a mechanism by which physical cues arising from the MuSC niche could lead to transcriptional changes that result in MuSC activation. Whilst the LINC complex has been shown to regulate myonuclear position and sarcomere assembly, a role in MuSC activation is currently unexplored. Discerning this is particularly relevant as mutations in LINC complex proteins cause Emery-Dreifuss muscular dystrophy (EDMD), a progressive muscle wasting disorder. To address this, we have performed a thorough assessment of the LINC-complex associated proteins during MuSC regeneration, and show that some components of the LINC complex are expressed in quiescent MuSCs, whilst others rapidly increase upon activation. LINC complex proteins were disrupted in MuSCs using siRNA and dominant-negative (DN) constructs and cell cycle dynamics and transcriptional assessed. Finally, Pax7tm2.1(CRE/ERT2):Tg(CMV-LacZ/eGFP-KASH2) mice expressing DN-Nesprin2 in MuSCs were subjected to rounds of myotoxic injury to assess how LINC disruption affects myogenic regeneration within the native niche. These results extend our understanding of how MuSCs respond to physical cues and give insight into the pathogenesis of EDMD.

Comparison of Non-Invasive Peripheral Vascular Function to Invasive Measures of Coronary Function in Patients with Suspected Coronary Microvascular Dysfunction

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Introduction: The purpose of this study is to compare non-invasive vascular assessments to invasive gold standard measures of coronary flow and resistance in patients with suspected coronary microvascular dysfunction (CMD), a condition driven by systemic endothelial dysfunction. We hypothesize that non-invasive measures will be associated with both coronary flow and resistance following pharmacological hyperemia. Methods: Forty-one patients with suspected CMD attended the Cardiovascular Integrative Physiology Clinic at Southlake Regional Health Centre. Patients underwent finger-based arterial tonometry (RHPAT) to non-invasively quantify microvascular endothelial function (EndoPAT). A subset of participants (n=15) also concurrently completed flow mediated dilation (FMD) of the brachial artery to assess conduit artery endothelial function. Briefly, a standard blood pressure cuff was positioned on the right arm of patients, distal to the elbow joint. Baseline recordings preceded 5 minutes of forearm ischemia, and was followed by cuff deflation, eliciting reperfusion. Within 4 months, patients underwent coronary reactivity testing using the Doppler guidewire method. Specifically, the coronary flow reserve (CFR), and the index of microvascular resistance (IMR) were calculated during pharmacologically-induced hyperemia using adenosine, then acetylcholine, then dobutamine. Prior to each stimuli, baseline measures were obtained to ensure hemodynamics results to baseline. Results: RHPAT was negatively correlated to the IMR during dobutamine ($r=-0.39$, $p=0.04$), but not the CFR ($r=0.14$, $p=0.49$). FMD was negatively correlated to the IMR during adenosine ($r=-0.64$, $p=0.01$), but not the CFR ($r=0.29$, $p=0.30$). RHPAT and FMD were not correlated to the IMR or CFR during acetylcholine. Conclusion: These preliminary results suggest that measures of non-invasive peripheral vascular function can predict pharmacologically induced changes in coronary resistance, but not coronary flow.

Photoperiod influence on fat accumulation in Peromyscus leucopus

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Circadian dysregulation has been associated with obesity, suggesting that abnormal exposure to light can impact adiposity (i.e. shift workers, light at night). In cold conditions, wild mammals rely on thermogenesis, where brown fat tissue is activated to convert its fat stores into thermal energy. It has been suggested that thermogenesis can also be activated by shortened photoperiod, an environmental cue that accompanies the cold, winter season. During the warm summer season, mammals typically store excess food energy as fat in preparation for winter. Our aim is to determine the influence of photoperiod on fat accumulation in wild mice that are responsive to photoperiod. Adult white-footed mice (Peromyscus leucopus) were housed for 4 weeks, in a long-day (16 hours light) or short-day (8 hours light) environment at thermoneutrality. Energy balance was monitored throughout the study and body composition assessed at the end of the 4-week

study. Photoperiod did not alter weekly food intake or body weight gain. However, circadian feeding behavior was altered in that mice housed in the short-day environment fed throughout their 16-hour dark phase, whereas long day mice fed only during their 8-hour dark phase. Total cumulative food intake over 24 hours however was not altered by photoperiod. Photoperiod altered epididymal fat mass but not inguinal or intrascapular fat mass, as long-day mice had larger epidydimal fat pads than short-day. Thus, the altered circadian feeding behavior that is induced by photoperiod may be an important signal for adipose tissue accumulation.

Neuromuscular biology of skeletal muscle-specific AMPK knockout mice

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The neuromuscular junction (NMJ) is the synapse between the motoneuron and muscle and is essential for the transmission of electrochemical signaling to physical muscle contraction. Recent work demonstrates that adenosine monophosphate-activated protein kinase (AMPK) regulates synaptic morphology, integrity, and function. However, the precise role of AMPK at the NMJ has yet to be investigated. Thus, the purpose of this study was to determine whether skeletal muscle AMPK is necessary for the maintenance of NMJ morphology and gene expression. Wild-type (WT) mice and animals deficient in skeletal muscle AMPK β1 and β2 subunits (mKO) were utilized. The soleus (SOL) and extensor digitorum longus (EDL) muscles were whole-mounted and immunolabelled for pre- and postsynaptic components of the NMJ. Images were captured with confocal microscopy and analyzed with a systematized workflow, "NMJ morph". Twenty NMJs were analyzed per muscle. Real-time, quantitative polymerase chain reaction was also conducted on tibialis anterior (TA) muscles to analyze NMJ mRNA transcripts. Numerous NMJ morphology metrics were modestly altered in mKO muscles relative to their WT counterparts, such as number of presynaptic branch points, nerve terminal area, postsynaptic compactness and AChR perimeter. Area of synaptic contact was ~25% higher ($p < 0.05$) in mKO versus WT SOL and EDL muscles. TA muscle mRNA transcripts involved in NMJ maintenance and plasticity, such as AChR subunits, Dok7, LRP4, rapsyn, and MuSK, were 2-4-fold higher in mKO animals relative to WT mice, which suggests a dynamic remodeling of the synapse. Our results suggest that in the absence of skeletal muscle AMPK, the structure of the NMJ is largely maintained, which may be due in part, to concurrent, robust alterations in synaptic gene expression.

Age-related changes in human single muscle fibre passive elastic properties are sarcomere length dependent

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Introduction:

Physiological studies of single muscle fibres have revealed an age-related decrease in the ability of single fibres to produce force, independent of changes in fibre size [1, 2], as well as an increase in the active elastic properties of the fibre [3]. Together, this suggests that the structures responsible

for active force production within the muscle cell are altered with aging. However, whether or not the passive mechanical properties of single muscle fibres in humans are altered with age is unknown. The aim of this study was to characterize single muscle fibre passive elastic modulus and stress in young and old men.

Methods:

A total of 20 healthy males participated in the study (young males [YM]: N=10, mean age: 25.4 years; old males [OM]: N=10, mean age: 68.9 years). Needle biopsies using suction were obtained from the vastus lateralis (VL) in line with the muscle fascicles and immediately placed in physiological storage solution. Single muscle fibres were dissected and placed in a chamber filled with relaxing solution and attached on one end to a motor to stretch the fibres and on the other end to a force transducer. SLs were measured via laser diffraction. Each fibre was stretched in 0.25 μm/sarcomere increments and allowed to stress relax for 120s before force and length measurements were recorded; this ensured only the elastic properties of the fibre were tested. A minimum of 7 stretches were conducted on each fibre. The elastic modulus-SL relationship was assumed to follow a logistic function; passive mechanical characteristics of each fibre were obtained by fitting the integral of the logistic function to experimental stress-SL data. Fitted equations were evaluated across the physiologic range of SLs and 'beyond thick and thin filament overlap' (1.8 to 4.5 μm) and averaged across all fibres.

Results and Discussion:

A total of 182 single fibres were tested and analyzed (90 YM and 92 OM).

Muscle fibres from older individuals had a larger mean passive elastic modulus from SLs between 1.9 μm to 2.65 μm. This resulted in a larger average passive stress from SLs between 2.1 μm to 3.55 μm. These were found to be significantly different ($p<0.05$). However, at longer SLs (i.e. > 3.55 μm) the fibres from young individuals had a higher (but not statistically significant) passive modulus than those from old, resulting in similar passive stresses at longer SLs.

Conclusions:

The passive elastic modulus in muscle fibres from older individuals was larger at short SLs but not different at long SLs as compared to young. The mechanism behind the SL dependant changes to the elastic modulus between young and old fibres is currently unknown. One possible explanation is that the structures responsible for passive stiffness, such as titin and the basement membrane are altered with age. Current work is aiming to measure differences in titin isoform expression between young and old human muscle samples.

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Investigating Sex-Specific Mechanisms of Diastolic Dysfunction in a New Model of Heart Failure with Preserved Ejection Fraction

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INTRODUCTION: Extensive epidemiological evidence of heart failure with reduced ejection fraction (HFrEF) shows that prolonged neurohormonal activation leading to systolic dysfunction can be effectively targeted with blockades such as Beta Blockers, ACE Inhibitors and Angiotensin Receptor Blockers. In contrast to HFrEF, heart failure with preserved ejection fraction (HFpEF), characterized by diastolic dysfunction and left ventricle ejection fraction > 45%, is poorly understood and completely lacks pharmacological treatment options for patients. This is largely due to a lack of animal models that truly recapitulate the clinical HFpEF phenotype. To this end, we have developed the first model of HFpEF that encompasses both the cardiac pathology and the co-morbidity crisis characteristic of this challenging clinical population. Thus, the primary objective was to investigate a mechanistic cause for diastolic dysfunction in this new model of HFpEF.
METHODS: In male and female splenectomized and sham rats (fed a chow diet), cardiac function was assessed at 9 and 52-weeks post-surgery using M-Mode echocardiography of the left ventricle (LV) and invasive hemodynamics of the left and right ventricles (RV), respectively. We also evaluated whole-body physiology (e.g. body weight, glucose sensitivity, hematocrit). Histological analysis of the LV and RV assessed interstitial fibrosis using Picrosirius Red. In male hearts, CD 206+ staining quantified alternatively activated (M2-like) macrophage populations in both ventricles.
RESULTS: At both 9 and 52-weeks post-splenectomy, male and female rats showed significant diastolic dysfunction (elevated EDP, -dP/dtmin) without any evidence of systolic decompensation (preserved EF) in either sex. Further, by 52 weeks, this dysfunction was associated with systemic and pulmonary hypertension (increased LV and RV pressures), obesity, reduced glucose sensitivity, and anemia. Elevations in interstitial fibrosis of the left and right ventricles were observed in splenectomized males compared to shams, however, there were no differences in fibrosis between female sham and splenectomy groups. CD 206+ staining revealed increases in alternatively activated (M2-like) macrophages in splenectomized males compared to shams.
CONCLUSIONS: Splenectomy, in otherwise healthy male and female rats, caused a HFpEF-like phenotype characterized by diastolic dysfunction with preserved systolic function, systemic and pulmonary hypertension, obesity, glucose intolerance, and anemia. Despite evidence that pro-fibrotic remodeling of the heart leads to impairments in diastole, only in males did excessive fibrosis correlate with diastolic dysfunction. Chronic activation of M2-like macrophages promotes cardiac fibroblast polarization which increases the deposition of fibrillar collagens in the extracellular matrix (ECM) causing a stiffer, more fibrotic ventricle that cannot relax properly. In females, however, fibrosis did not correlate with impairments in diastole, confirming that ECM quantity is not the underlying mechanism for diastolic dysfunction in female rats. In this HFpEF model, sterile inflammation correlates with diastolic dysfunction suggesting that while HFrEF is characterized by neurohormonal dysfunction, HFpEF is a disease of inflammatory dysregulation.

Investigating the Role of ACTC Genes in Zebrafish Development
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Cardiomyopathy is an inherited heart disease caused by mutations in sarcomere proteins. Cardiac actin (ACTC) is a sarcomere protein that can have mutations leading to cardiomyopathy. Creating an in vivo model for cardiomyopathy is of upmost importance as this can give a clear picture as to what is happening in the human body. Zebrafish has become an advantageous model for studying cardiovascular disease due to its optical transparency and the fact that they can survive early development with a fatal heart defect. We have shown that zebrafish have three ACTC genes, and knowing the specific roles each zebrafish ACTC (zfACTC) gene plays, would inform decisions regarding which gene to edit for model development. To understand the roles zfACTC genes play during the stages of heart development, we performed in situ hybridization and quantitative PCR, determining the stage and tissue specific expression of each zfACTC gene. We demonstrate that although all three zfACTC genes are expressed during heart development, there is tissue specific expression during the early stages of heart morphogenesis. These results support a gene switch hypothesis where certain sarcomere proteins are replaced during development based on the physiological demand of the organism. With these data, we can now target a specific zfACTC gene to express changes found in human cardiac ACTC mutations, thereby developing a model for cardiomyopathy, observing how the disease develops and lastly, performing drug therapeutics to reduce the symptom or progress of development.

Mitochondrial Maintenance in Aged Human Cardiac Muscle

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One the hallmarks of aging is the structural changes in the heart that accompany biochemical and metabolic alterations. Since cardiovascular disease is the leading cause of death in the developed world and is highly associated with aging, the maintenance of cardiac health with age is of upmost importance. Within cardiomyocytes, mitochondria contribute 95% of all ATP required, demonstrating the reliance of the myocardium on the functionality of these organelles. Many of the age-dependent changes that occur at the molecular level in the heart converge on the mitochondrion. Previous data have demonstrated decrements in the synthesis of mitochondria in aged cardiac tissue, however little is known about the maintenance and the clearance of these organelles. Thus, we hypothesized that mitochondrial quality control mechanisms such as mitochondrial autophagy, mitophagy, would be impaired in the aged human heart. Right atrial tissues were collected from young (≤50 years) and aged (≥70 years) patients undergoing coronary artery bypass surgery (CABG). Samples were obtained both immediately pre- and post-cardiopulmonary bypass as a model of ischemia-reperfusion injury. Patients were matched for hypertension and dyslipidemia, and for medications including statin and ACE inhibitors. Exclusion criteria included a prior stroke or myocardial infarction, a history of smoking, or other comorbidities (e.g. diabetes, cancer). Mitochondrial markers COX I and VDAC were significantly reduced by 50-60% in the aged samples, however no differences were observed for Citrate Synthase or UQCRC2. This suggests that atrial mitochondrial composition changes took place with age. The upstream markers of autophagy Beclin-1 and transcriptional regulators TFEB and TFE3 did not change with age, suggesting that aging does not affect the drive for autophagy. Despite this, constituents of the autophagosome p62 and LC3-II were reduced by 50 and 30%, respectively, in the aged samples, indicative of increased autophagy flux. The mitophagy marker Parkin, displayed a trend to decrease with age, suggesting a decline in the signaling for mitophagy. The lysosomal marker Cathepsin D was unaffected by age in cardiac muscle, but Mucolipin increased by 2-fold in aged atrial muscle. Analysis of post-CPB samples indicated marked elevations in HSP70 and Caspase-3 protein compared to pre-CABG values. This was only evident in the atria of aged individuals. Our data suggest that increased mitophagy flux could account for the reductions in mitochondrial markers in aged atria, and that ischemia-reperfusion injury triggered an apoptotic stress response that is more prominent with age.

Sodium nitrate supplementation prevents cardiac dysfunction and attenuates left ventricular mitochondrial ROS emission in high-fat diet fed mice

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Introduction: Heart disease and diabetic cardiomyopathy are multifactorial conditions involving structural and functional changes within the left ventricle (LV), which in part have been attributed to mitochondrial dysfunction. Therefore, nutritional interventions that improve mitochondrial bioenergetics may be particularly beneficial in preventing pathological changes associated with obesity and high fat diets. One possible intervention is the consumption of sodium nitrate, as oral supplementation has been shown to improve cardiac contractility and influence LV mitochondrial bioenergetics in healthy rodents. However, little is known regarding the efficacy of nitrate to prevent diabetic cardiomyopathy, or the underlying mechanism-of-action. Therefore, we examined if dietary nitrate supplementation was capable of preventing high-fat diet induced cardiac abnormalities, in association with improved mitochondrial bioenergetics.
Methods: Male C57Bl/6N mice (n=30) were fed a control diet (10% fat) or a high-fat diet (HFD, 60% fat) in the absence (HFD) or presence (HFD+NaNO3) of 4mM sodium nitrate via drinking water for 8 weeks. Following the intervention, echocardiography was performed, and 3 days later the LV was excised for histological analysis and preparation of permeabilized muscle fibers for mitochondrial respiration and reactive oxygen species (ROS) emission experiments. Results: Compared to lean controls, HFD mice presented with ~25% reduction ($p<0.05$) in end-diastolic volume, stroke volume, and ultimately cardiac output, in association with a ~3-fold increase in fibrosis. In stark contrast, NaNO3 supplementation completely normalized these detrimental effects. The improvements in cardiac function following NaNO3 supplementation cannot be attributed to mitochondrial respiratory function, as NaNO3 consumption did not improve maximal mitochondrial respiratory capacity, sensitivity to ADP, or mitochondrial sensitivity to L-carnitine. In contrast, while HFD increased maximal (succinate-supported), and submaximal (presence of 100μM ADP) mitochondrial ROS emission rates ~60% and ~3-fold, respectively, NaNO3 consumption fully prevented these responses.
Conclusion: Dietary nitrate supplementation prevented the detrimental effects of HFD feeding on LV fibrosis and mitochondrial H2O2 emission rates, mechanisms which may be implicated in the NaNO3-mediated improvements in cardiac function. Altogether, these data implicate oral NaNO3 consumption as a promising therapeutic strategy to preserve cardiac function in diabetic or insulin resistant states.

Reproducibility in the cardiometabolic responses to high-intensity interval exercise in adults with type 1 diabetes

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Aims: Patients with type 1 diabetes (T1D) often report a rise in their blood glucose level following brief, intense exercise. We sought to determine the reproducibility of the cardiometabolic responses to high-intensity interval training (HIIT).
Methods: Sixteen adults with T1D, using an optimized multiple daily injection with basal insulin glargine 300 U/mL (Gla-300), performed four fasted HIIT sessions over a 4–6-week period. Exercise consisted of high-intensity interval cycling and multimodal training over 25 min.

Results: Heart rate and rating of perceived exertion rose similarly in all sessions, as did lactate, catecholamine and growth hormone levels. Plasma glucose increased in response to HIIT in 62 of 64 visits (97%), with an overall increase of 3.7 ± 1.6 mmol/L (Mean ± SD) ($P<0.001$). In within-patient comparisons, the change in plasma glucose among the four HIIT sessions was significantly correlated with a composite correlation of 0.58 ([$r2 = 0.34$]; 95% CI 0.35–0.80; $P<0.01$).
Conclusions: Intersession observations of four separate HIIT sessions showed high intrasubject reproducibility in the cardiometabolic responses to exercise, including the rise in plasma glucose, when adults with T1D perform the activity in a fasted state.

Microtubule disorganization is associated with mitochondrial dysfunction in Duchenne muscular dystrophy

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In Duchenne muscular dystrophy (DMD), a genetic mutation results in a loss of dystrophin which leads to microtubular disorganization, cellular degeneration and muscle weakness. However, a mechanism by which microtubule disorganization contributes to muscle weakness in DMD has not been established. We considered a model that proposes tubulin, the structural component of microtubules, may directly bind VDAC2 on the outer mitochondrial membrane and alter its permeability to ADP import. Given ADP stimulates oxidative phosphorylation and attenuates H2O2 emission through modulation of membrane potential, the model predicts that tubulin-VDAC interactions may be a novel regulator of cellular energy and redox homeostasis. Furthermore, as VDAC is also thought to be involved in formation of the mitochondrial permeability transition pore (mPTP) in response to calcium stress – a phenomenon that occurs in DMD – tubulin-VDAC interactions may also influence the maximal mitochondrial calcium retention capacity (CRC) required to trigger cell death. Considering these relationships between VDAC and bioenergetic control, we hypothesized that the disorganized microtubule network in DMD would be linked to impairments in ADP's control of bioenergetics and mPTP formation through altered tubulin-VDAC binding. Methods: Permeabilized fibre bundles and single fibers from 4-week old wildtype (WT) and D2.mdx (DMD mouse) were prepared from extensor digitorum longus (EDL) muscles and used for mitochondrial bioenergetic and histological assessments. Results: D2.mdx EDL demonstrated impaired ADP-stimulated respiration at a range of ADP concentrations (-37-43% at 25mM, 100mM, 500mM and 5mM ADP, $p<0.003$) and an impaired ability of ADP to attenuate H2O2 emission (71% increase in H2O2 at 500mM ADP, $p=0.04$) despite similar CRC. There were no differences in the protein content of electron transport chain complex subunits (Complexes I to V) or VDAC2 despite significantly decreased adenine nucleotide translocase (ANT) ($p=0.03$). a-tubulin–VDAC2 interactions were unchanged despite alterations to a-tubulin network in D2.mdx fibers. Conclusions and Discussion: Disorganized microtubules in EDL from D2.mdx mice are related to mitochondrial dysfunction, but this is not due to altered a-tubulin–VDAC2 interactions. It remains to be determined if interactions between other tubulin or VDAC isoforms are altered in DMD and contribute to impaired VDAC-dependent bioenergetics. In addition, reduced ANT content may partially explain impaired mitochondrial function in dystrophic EDL.

Progressive Suppression of Autophagy with Denervation

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Skeletal muscle is a highly malleable tissue that is responsible primarily for locomotion and metabolic control. Lack of contractile activity (i.e. disuse) promotes muscle atrophy. In recent years, various models have been employed to investigate the effects that disuse have on the breakdown of muscle protein. One of the processes in which intracellular components are degraded is termed autophagy. Autophagy is a selective degradation process in which damaged proteins are selected for, engulfed, and subsequently degraded at the lysosomes. This process is required to maintain the health of the cell by degrading dysfunctional proteins and organelles. Aberrant autophagy has been found to contribute to muscle atrophy. Yet, little work has been conducted to examine the time-dependent changes in the regulation of autophagy following muscle disuse. Thus, the objective of this work was to better understand the process of autophagy in the context of muscle disuse. We employed unilateral hindlimb denervation of the peroneal nerve in Sprague-Dawley rats (n=7) to study atrophy of the tibialis anterior (TA) and the extensor digitorum longus (EDL) muscles. The contralateral hindlimb served as a control where the nerve remained intact. The animals were denervated for either 1, 3 or 7 days. TA muscles were subsequently removed, weighed and protein lysates were created. We observed significant reductions of 25-30% ($p<0.05$) in EDL and TA muscle mass by 7 days post-denervation. Using western blotting techniques, significant elevations in Tfeb, Beclin1 and ATG-7 were measured by 7 days post-denervation. Interestingly, both the precursor protein LC3-I and the lipidated mature protein LC3-II were upregulated 2-fold by 7 days of denervation, corresponding to no change in the LC3-II/LC3-I ratio. We also observed 1.5-fold elevations in both LC3-II and p62 protein at 7 days. To assess changes in autophagy flux through our time-course, a subset of animals was injected with colchicine (0.4mg/kg/day) for 2 days prior to sacrifice, and TA muscles were subsequently extracted. p62 flux remained unchanged at 1 day of denervation, while LC3-II flux increased. After 3 days, p62 flux decreased while LC3-II remained increased compared to control, but to a lesser degree than after 1 day. Both p62 and LC3-II flux were reduced by 7 days of denervation, suggesting that autophagy flux is reduced at the later time-point of denervation. Since the LC3II/LC3-I ratio was unchanged, this implies that the suppression of flux was potentially due to an impairment in downstream lysosomal machinery, as compared to the upstream proteins involved in the synthesis of the autophagosomes. Our data suggest that denervated muscle appears to progress toward an impairment in protein breakdown mechanisms via autophagy that are essential for the maintenance of homeostasis in skeletal muscle.

The Effect of a High-Fat Diet on Glucose Tolerance in Mice Lacking Endothelial-Derived Erythropoietin

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I. Erythropoietin Biology in the Endothelium

Erythropoietin (Epo), a cytokine produced by the kidney in response to hypoxia, is classically known for stimulating erythropoiesis (i.e. red blood cell production). Emerging evidence demonstrates the existence of non-renal sources of Epo and that Epo has roles beyond promoting erythropoiesis. Interestingly, in recent studies, the administration of recombinant (i.e. artificially glycosylated) Epo in rodents was shown to have anti-obesity effects and improve glucose tolerance, suggesting Epo may be implicated in regulating energy metabolism. However, the physiological relevance of non-renal sources of endogenous Epo has yet to be determined. Recent work in our lab has identified the endothelium as an important source of Epo. Thus, we created a novel rodent model by knocking out Epo expression in the endothelium (EpoKO-Endo) to assess whole-body metabolism. We hypothesized that glucose regulation would become dysregulated in EpoKO-Endo mice, as compared to the wild-type (WT) littermates, without affecting erythropoiesis. At 8-10 weeks of age EpoKO-Endo and WT male mice were placed on a regular chow or high-fat diet (HFD) for 6 weeks. Upon completion of the feeding intervention, an oral glucose tolerance test was performed, and mice were placed in a comprehensive lab animal monitoring system (CLAMS) for 48 hours to evaluate energy intake and utilization. No differences in glucose tolerance or metabolic parameters measured via CLAMS were observed between genotypes for mice fed a regular chow diet. While the HFD increased body weight similarly in the WT and EpoKO-Endo mice, resulting in significantly greater weight gain than the chow-fed mice, these results were not paralleled in the oral glucose tolerance test. Surprisingly, compared to the WT mice, EpoKO-Endo males appeared to be protected against glucose intolerance in response to the HFD, and were comparable to chow-fed mice. Our data contrasts the current research published on Epo in the literature which suggests Epo regulates metabolic homeostasis by improving glucose tolerance. Evidently, exogenous human-recombinant Epo (as investigated in current literature) does not mimic the effects of endogenous Epo, suggesting that further research investigating the endogenous functions of Epo is necessary. Thus, altered Epo function may have important implications in metabolic health and disease, such as obesity and type 2 diabetes, prompting the need to understand the underlying mechanisms of how endogenous Epo regulates glucose utilization.

II. Consideration of Sex

While the majority of Epo biology has been investigated in males, a few studies have identified that estrogen significantly affects Epo expression. In addition, females are more susceptible to anemia than males. Thus, we were interested in investigating sex as a variable in our study to determine whether there would be differences in males and females in response to the HFD

intervention. Male and female mice responded comparably to the HFD with significantly increased body weights. Unexpectedly, female WT mice did not develop glucose intolerance, as observed with the WT males. Evidently, Epo biology between males and females is divergent. This finding opens exciting new avenues in Epo biology for improving our understanding of how this cytokine regulates energy metabolism between males and females and encourages future research to continue investigating Epo-related sex differences.

Acute olanzapine-induced hyperglycemia is exacerbated in female AMPK beta 1 mice.

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Olanzapine is a second-generation antipsychotic drug used widely in the treatment of schizophrenia. Though effective in reducing psychoses, acute olanzapine treatment causes acute increases in blood glucose and chronically leads to weight gain. A primary contributor to acute olanzapine-induced hyperglycemia is glucagon mediated-increases in liver glucose production. Prior work by our lab demonstrated that exhaustive exercise or treatment with 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), approaches which activates the energy sensing enzyme 5'AMP activated protein kinase (AMPK), protect against olanzapine-induced hyperglycemia. The purpose of this study was to determine if 1) olanzapine-induced hyperglycemia would be exacerbated in AMPK beta 1 deficient (KO) mice and 2) if A769662, a specific allosteric activator of AMPK beta1 containing complexes, could mitigate the effects of olanzapine on glucose homeostasis. We hypothesized that the absence of AMPK beta 1, a subunit which is primarily found in the liver, would worsen the acute metabolic effects of olanzapine and that A769662 would prevent olanzapine-induced hyperglycemia in control mice. Female AMPK beta1 KO or wild type (WT) mice were treated with olanzapine (5 mg/ kg) and blood glucose was taken at baseline and 30, 60 and 90 minutes post-injection. As anticipated, the protein content and phosphorylation of AMPK alpha was significantly reduced in liver from AMPK beta 1 KO mice and this coincided with a greater olanzapine-induced increase in blood glucose compared to WT mice. Serum glucagon concentration was not significantly different between genotypes after OLZ treatment nor were there any differences in the protein content of the gluconeogenic enzymes PEPCK and G6Pase. Interestingly, when challenged with glucagon (1.0 mg/kg IP) or epinephrine (0.5 mg/kg IP) the rise in blood glucose was greater in KO compared to WT mice, suggesting that an increase in the responsiveness to hormonal gluconeogenic signals could explain the potentiated effect of olanzapine in AMPK beta1 KO. Similarly, pyruvate-induced increases in blood glucose following olanzapine treatment were more pronounced in beta1 KO compared to WT mice. Olanzapine reduced physical activity, RER and carbohydrate oxidation while increasing fat oxidation in both genotypes. Surprisingly, co-treatment with A769662 (30 mg/kg) did not attenuate olanzapine-induced increases in blood glucose in female C57BL6/J mice. Our findings provide evidence that reductions in AMPK activity potentiate the effects of acute olanzapine treatment on blood glucose, whereas specifically targeting AMPK beta1 containing complexes is not sufficient to protect against olanzapine-induced hyperglycemia.

ADPr-actin trimer: A short-F-actin complex for determining atomic structures of F-ABP complexes

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Actin is one of the most abundant and conserved proteins involved in a myriad of biological functions critical for maintaining eukaryotic life. Actin forms filaments in the cytoskeleton and is involved in cellular processes ranging from intracellular trafficking to force generation. The functions of actin rely on the ability of monomeric actin to assemble into filaments that can organize into dynamically reorganizing cytoskeletal architectures. The process of dynamic reorganization of actin filaments is modulated by several regulatory proteins called Actin Binding Proteins (ABPs). Understanding the interaction of actin with ABPs will enhance our understanding of eukaryotic cellular processes. Despit the significance of these interactions, the field lacks atomic details of interactions of F-actin with ABPs. With recent advances in cryo-Electron Microscopy (cryo-EM), structural models of F-actin alone and bound to ABPs have been proposed. However, the resolution from EM studies lack important atomic details, such as those at high radius. Consequently, traditional X-ray crystallography remains the most widespread technique to obtain high resolution structures. However, the ability of actin to self-assemble into filaments of varying lengths limits the application of X-ray crystallography. To overcome the challenge posed by the inherent property of actin to self-assemble, a short, non-polymerizable F-actin complex called ADPr-trimer was generated. The aim of my project is to generate heterocomplexes of ADPr-trimer with F-ABPs for structural work. A candidate-based approach was applied to determine interactions of ADPr-trimer with purified ABPs (myosin subfragment1, cofilin and gelsolin). Cofilin bound to ADPr-trimer, overcoming polymerisation inhibition properties of ADPr-trimer and forming filaments, and myosin did not bind ADPr-trimer. Gelsolin bound to ADPr-trimer and the resulting complex (GS:3mr) was purified for crystal trials; however, the complex failed to yield crystals. Applying recent advances in EM imaging, the GS:3mr is being screened for homogeneity by negative stained microscopy for Single particle analysis. In addition, an unbiased approach which involves pull-down of ABPs from cell lysates using ADPr-trimer affinity columns coupled with mass spectrometry was employed to identify ADPr-trimer binding proteins. An identified potential interactor, MLC6B, was produced and is being validated for future structural work. Understanding atomic interactions of actin with ABPs with proteomics and structural determination will advance our knowledge of the mechanisms of actin filament organization and force generation - processes critical for maintaining eukaryotic life.

Guided Active Play Tracks Children's Physical Activity Participation

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Background: Cardiometabolic risk factors and changes in body composition established during childhood show poor-to-moderate relationships with physical activity (PA) participation and

fitness. It was been debated that the parameters surrounding the quantification of physical activity (laboratory vs field settings; assessment periods of 1-d vs 3-d vs 7-d) contribute to the poor and inconsistent relationships reported for children. It has been suggested that the poor tracking of year-over-year physical activity participation underlies the poor-to-moderate relationships between PA and risk factors reported in the literature. This study aims to assess the tracking of children's physical activity participation during a self- paced guided active play program using cooperative games. Methods: Children (n=14; age 8.8[)1.5yrs) were recruited from a community centre camp program and assessed over two consecutive years for height, weight, BMI, waist circumference, total grip strength, leg power, systolic blood pressure, diastolic blood pressure and estimates of aerobic power. PA participation was assessed during a self-paced guided active play program (lhr/d on 4d/wk for 5-wks). PA was measured daily with accelerometry (ACC) (ActiGraph GT3X+) with vector outputs (10sec epochs) used to estimate energy expenditure (kcal/session; kcal/min), metabolic equivalents (MET) and intensity levels using laboratory derived equations. Tracking of the variables between year 1 and 2 was analyzed using Spearman rank order correlations and Kappa statistics. Paired t-tests were used to assess differences in performance and physical activity between year | and 2. Results: Children's developmental changes from year 1 to year 2 were typical for age, growth and physiological variables. PA (kcal/session; and Ical/min) was greater at year 2 vs year 1 ($p<0.001$) and showed strong tracking (1=0.90, p=0.001, k=0.68). %Moderate-to- Vigorous (MV) PA was also greater at year 2 ($p<0.001$) and exhibited moderate tracking ($r=0.74$, $p<0.01$, $k=0.47$). For physiological variables only, children's muscle strength increased at year 2 ($p<0.001$) and exhibited strong tracking ($r=0.81$, $p<0.001$, $k=0.47$). Minimal changes in BMI, waist circumference, muscle power, systolic blood pressure, aerobic power was evident at year 2 ($p>0.05$). Conclusion: Evidence suggests that children are more likely to participate in physical activity with an unstructured play environment, which aligns with the self-paced, cooperative, noncompetitive nature of this program. This guided active play approach to track children's PA participation may promote stronger relationships between cardiometabolic risk factors during development.

Enabling skeletal muscle repair and functional recovery following denervation-induced injury using ultrasound mediated gene delivery (UMGD)

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Rationale/Significance: Skeletal muscle is essential for mobility and its health relies on innervation. Accidental limb trauma with peripheral nerve injury results in immediate loss of muscle function and muscle atrophy. This is reversible if re-innervation occurs in a timely manner, due to muscle's robust capacity for regeneration and self-repair. However, due to peripheral nerves' limited rate of regeneration (approximately 1mm/day), proximal limb trauma (e.g. above the elbow) and/or delayed re-innervation can result in permanent, irreversible damage to distal targets (e.g. hand and fingers) by the time of nerve regrowth: muscle architecture is destroyed, restorative potential is lost, and it is non-receptive to re-innervation. Permanent physical disability results, negatively impacting quality of life and inducing significant societal costs, such as

increased health care burdens, loss of productivity, and significant career restructuring. There are currently no practical and preventative therapies to sustain denervated muscle awaiting re-innervation.
Objective: Use the novel approach of ultrasound mediated gene delivery (UMGD) to promote the repair and regeneration of denervated muscle and sustain its receptivity to reinnervation.
Methods: Using the rat Tibial Nerve Transection model, we denervate the gastrocnemius and soleus muscles in one hindlimb; the contralateral leg serves as unoperated control. UMGD is then administered as follows: For each gene, DNA minicircle plasmids or empty control plasmids are charge-coupled to cationic lipid microbubbles and are introduced via jugular vein infusion in the anesthetized rat following nerve transection. The ultrasound imaging transducer is then swept over the denervated muscles during, and for 10 minutes, after infusion, causing microbubble destruction and release of minicircle into the target tissue. Gene expression is retained for 6-8 weeks. Muscle is harvested at serial time points, from 2 weeks to 3 months following nerve transection. Muscle weights, myofiber type-specific cross sectional area, vascular density, satellite cell count, fibro-adipogenic progenitor (FAP) content, and extent of fibro-fatty degeneration are determined with morphometry, histology, immunohistochemistry, flow cytometry and Western blotting.
Results: We have established the baseline time course of key denervation-mediated events in the gastrocnemius after tibial nerve transection including myofiber type-specific atrophy, alterations in satellite cell content, and progression of fibrofatty infiltration, to determine the optimum timeline for intervention with UMGD therapy. In regards to the progression of fibrofatty infiltration, we have developed and optimized an experimental method for identifying fibro-adipogenic progenitor (FAP) cells, a population of resident interstitial cells that have been recently characterized in acute and chronic muscle injury models, but whose role is unclear in denervation injury. We have shown that FAPs increase their numbers throughout the first 5 weeks following denervation. We have conducted a pilot UMGD trial, delivering Insulin Growth Factor 1 (IGF-1) to counteract denervation-induced muscle proteolysis and myofiber wasting. We will present our preliminary results.
Conclusions: We have characterized the long-term post-denervation changes in rat gastrocnemius muscle, and demonstrate the influx of FAPs in the context of other post-denervation pathologic features. UMGD provides a novel tool to transiently and precisely introduce tissue-specific gene therapy to sustain muscle regenerative/repair mechanisms while awaiting reinnervation."

Characterizing alpha-cardiac actin variants associated with cardiomyopathy
Zi Teng and John F. Dawson

Heart disease is the number one cause of death worldwide, costing the Canadian economy 21.2 billion dollars annually. The most commonly inherited heart disease is cardiomyopathy and is the result of abnormal cardiac muscle. The two main types of cardiomyopathy are hypertrophic cardiomyopathy (HCM), where the walls of the ventricles are thick and stiff, and dilated cardiomyopathy (DCM), where the walls of the ventricles are thin due to weakened cardiac muscle. The basic contractile unit of the heart muscle is the sarcomere, where α-cardiac actin forms filaments and β-myosin is the motor protein moving along the filamentous actin (F-actin), resulting in contraction. At the molecular level, muscle contraction is fine-tuned by two other proteins,

troponin and tropomyosin. These proteins bind to F-actin and open myosin binding sites on F-actin in respond to calcium resulting in heart muscle contraction. Many genetic mutations, including mutations in ACTC1 encoding α-cardiac actin (ACTC), have been linked to the pathogenesis of DCM and HCM. Currently, there are 16 ACTC variants found independently in patients with cardiomyopathy. Of these, the HCM-linked S271F ACTC variant and the DCM-linked T126I ACTC variant have yet to be characterized biochemically. According to widely-held model, HCM-linked ACTC variants and DCM-linked ACTC variants should display hypercontractility or hypocontractility, respectively. Therefore, I hypothesized that the S271F ACTC variant will exhibit increased contractility and the T126I ACTC variant will exhibit diseased contractility. T126I ACTC variant exhibits no changes in its intrinsic properties. However, when regulated with troponin and tropomyosin, this variant requires more calcium to result in half maximal myosin activity compared to WT ACTC, aligning with my hypothesis. Further experiments with S271F ACTC variant will shed more light on how amino acid substitution in ACTC will impact its biochemical properties and regulation by troponin and tropomyosin leading to different forms of cardiomyopathy.

Regulation of myogenic gene transcription by Smad7: b-catenin complex.

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Smad7 promotes skeletal muscle differentiation and growth, previous studies from our lab documented a non-canonical role of nuclear Smad7 during myogenesis, independent of its role in TGF-β signaling. In addition, Wnt signaling pathway plays critical roles in various aspects of developmental and regenerative myogenesis. Further characterization of the myogenic function of Smad7 revealed β-catenin, effector of Wnt signaling as a Smad7 interacting protein. Reporter gene analysis and chromatin immunoprecipitation demonstrated that Smad7 and β-catenin are co-operatively recruited to the extensively characterized ckm promoter proximal region to facilitate its muscle restricted transcriptional activation in myogenic cells. Biochemical analysis utilizing GST pull down assay identified a Smad7 interaction domain (SID) between aa575-683 of β-catenin. Depletion of endogenous Smad7 and β-catenin in muscle cells reduced ckm promoter activity indicating their role during myogenesis. Deletion of the β-catenin SID substantially reduced the effect of Smad7 on the ckm promoter and exogenous expression of SID abolished β-catenin function, indicating that SID functions as a transdominant negative regulator of β-catenin activity. β-catenin interaction with the Mediator kinase complex through Med12 subunit led us to identify MED13 as an additional Smad7 binding partner. Collectively, these studies document a novel function of a Smad7-MED12/13-β-catenin complex at the ckm locus, indicating a key role of this complex in the program of myogenic gene expression underlying skeletal muscle development and regeneration.

Fatty Acid-Induced Hepatocellular Carcinoma Growth is Mediated by Decreasing Mitochondrial H2O2 Emission Coupled to Increased Glutathione Levels

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Rationale and Hypothesis: High fat diets are associated with increased hepatocellular carcinoma (HCC) risk, as well as increased HCC growth rates. Accelerated HCC growth in response to fatty acid challenges has been attributed to a variety of signalling pathways, but the role of metabolic and redox flexibility in mediating a pro-growth environment in this context has not been examined. Here we examined the direct effect of fatty acid challenges on HCC growth in relation to altered metabolic and redox homeostasis. Experimental approach: The HCC cell line HepG2 was incubated with 0μM, 50μM and 100μM palmitoylcarnitine (PCarn) for up to 48 hrs. We measured clonogenic survival, mitochondrial H2O2 emission and glutathione following PCarn incubations with and without the glutathione depleting agent buthionine sulfoximine (BSO) and inhibition of uncoupling protein-2 (UCP2) activity by genipin. Results: 100μM PCarn increased clonogenic survival in HepG2 by 8% more than control ($p<0.05$) at 48 hrs which represents a marked early effect considering the population doubling time of HepG2 cells is ~48 hrs. This was associated with an increase in both reduced and oxidized glutathione at both 24 and 48 hrs ($p<0.05$). Depleting glutathione with BSO prevented PCarn-stimulated growth ($p<0.05$). In a separate experiment, acute incubations of 100μM PCarn increased H2O2 emission within the first 10 minutes ($p<0.05$) followed by a decrease in H2O2 at 1 hr that remained lower at 24 hrs ($p<0.05$). The acute increase in H2O2 followed by a more chronic depression in H2O2 suggested PCarn might have triggered a compensatory mechanism. In support of this notion, inhibition of UCP2 with genipin sensitized HepG2 cells to PCarn-induced decreases in clonogenic survival ($p<0.05$) without a change in UCP2 protein content. Conclusion: Collectively, this data suggests that PCarn-induced HCC growth is in part attributed to elevated glutathione. Increases in glutathione may be a result of UCP2 rapidly attenuating PCarn-induced H2O2 emission which may permit greater glutathione synthetic rates and creation of a pro-growth redox environment.

The Impact of Acute Exercise on Skeletal Muscle-Specific Coactivator-Associated Arginine Methyltransferase 1 Knockout Mice

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Coactivator-associated arginine methyltransferase 1 [CARM1, also known as protein arginine methyltransferase 4 (PRMT4)] catalyzes the methylation of arginine residues on target proteins, which drives numerous gene expression programs. Skeletal muscle CARM1 appears to have important functions during myogenesis, as well as in response to exercise and during disuse-induced phenotypic plasticity. However, a mechanistic understanding of the role(s) of CARM1 in skeletal muscle biology is lacking. In this study, we test the hypothesis that CARM1 is involved in the molecular response to acute physical activity in skeletal muscle. Male and female 12-week-old skeletal muscle-specific CARM1 knockout (mKO) mice and wild type (WT) littermates were randomly assigned to one of three experimental groups: sedentary (SED), acute exercise (0AE), or acute exercise followed by 3 hours of recovery (3AE). RT-qPCR, Western blotting, immunofluorescence, and immunoprecipitation assays will be performed to assess the effects of

CARM1 on multiple aspects of gene expression in skeletal muscle. Functional tests demonstrated decreased ($P < 0.05$) exercise capacity and maximum grip strength in mKO compared to WT mice. In TA muscles, PRMT1 and PRMT5 levels were similar between genotypes, indicating that compensatory PRMT induction did not occur in mKO animals. Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) protein levels were significantly higher in the mKO mice, as compared to WT animals. The basal activation status of AMP-activated protein kinase was similar between WT and mKO mice. A significant increase in muscle AMPK activation was observed in both WT and mKO animals at 0AE followed by a return to resting levels at 3AE. This ongoing study aims to increase our understanding of the functions of CARM1 in maintaining and remodelling skeletal muscle phenotype.

DCA Can be used to Prevent Olanzapine-Induced Weight Gain

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University of Guelph

Olanzapine is an atypical antipsychotic drug primarily used to treat disorders such as schizophrenia and bipolar disorder. Unfortunately, olanzapine has severe metabolic side effects, including weight gain and hyperglycemia, at least partially attributable to increased hepatic glucose production. Inhibiting pyruvate dehydrogenase kinase 4 (PDK4), and subsequently activating pyruvate dehydrogenase (PDH), can reduce hepatic glucose production by favoring glucose oxidation. Moreover, genetic ablation of PDK4 has been shown to reduce HFD-induced body weight gain. With this in mind, we sought to determine whether chronic inhibition of PDK4 can limit olanzapine-induced hyperglycemia. We predicted that olanzapine will induce hyperglycemia and glucose intolerance, while co-treatment of olanzapine with dichloroacetate (DCA), a PDK4 inhibitor, will prevent chronic olanzapine-induced weight gain and hyperglycemia. Methods: Female mice were fed a high fat diet (45% fat) supplemented with or without OLZ (50mg/kg diet). Half of the OLZ treated mice were given DCA (2g/L, dissolved in their water). Body weight, food intake, and water consumption were tracked throughout the experiment. Fed blood glucose measurements were taken from a tail vein and a glucose tolerance test were performed in the 5th week of HFD. Results: DCA decreased OLZ-induced weight gain and feed efficiency. DCA consumption and mouse weight gain were negatively correlated. There was no correlation between olanzapine consumption and weight gain in the DCA-treated group, suggesting that the reduced weight gain is not due to decreased olanzapine consumption. Fed blood glucose measurements were lower ($p=0.079$) in the group co-treated with DCA, although glucose tolerance was not different in the OLZ or DCA groups. Conclusion: Co-treatment of olanzapine with DCA reduces olanzapine-induced weight gain and lowers fed blood glucose.

Low dose lithium supplementation reduces muscle inflammation and serum creatine kinase in mdx mice

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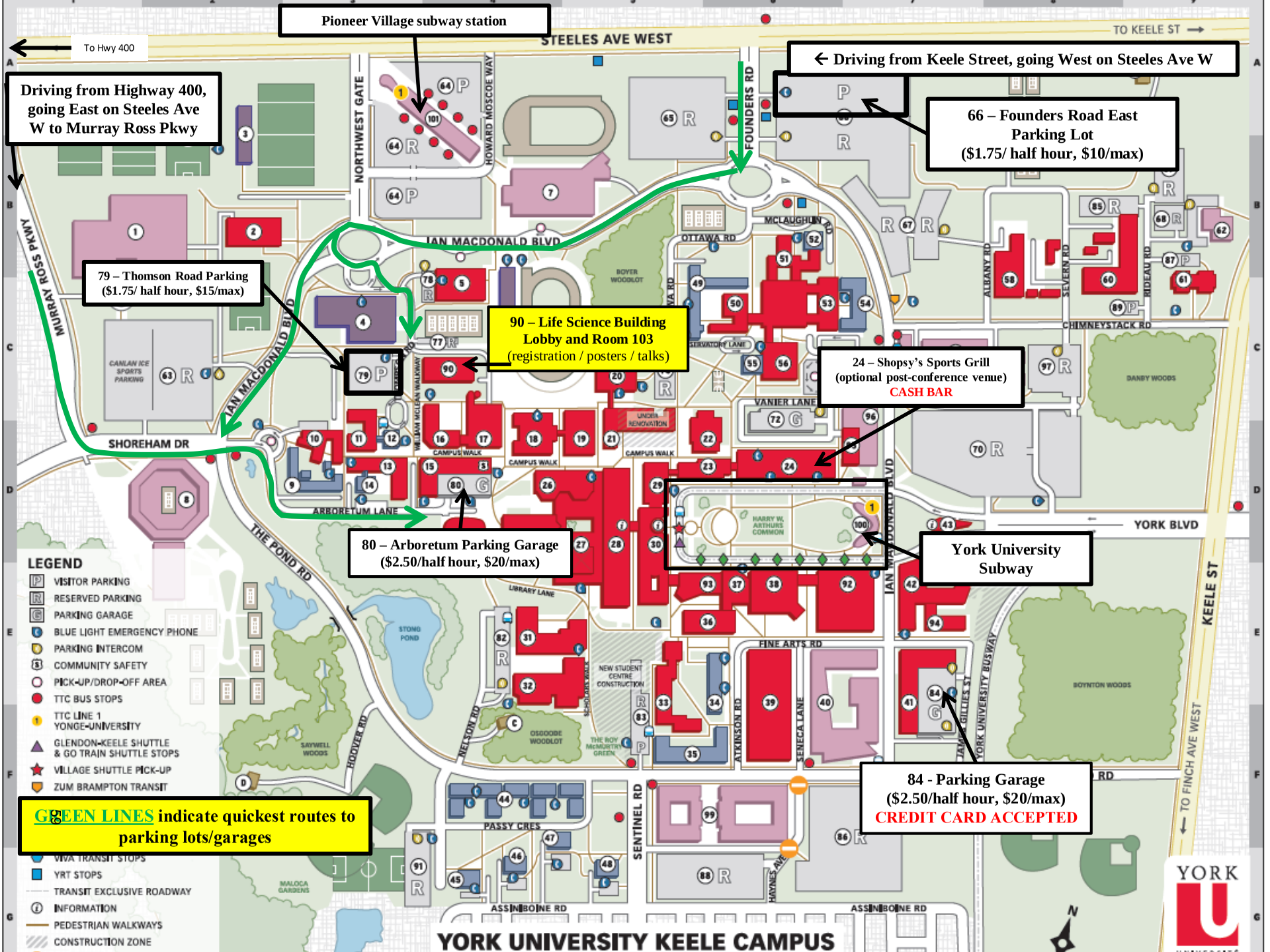
Duchenne's muscular dystrophy (DMD) is an X-linked disorder causing severe muscle degeneration and premature death due to cardiorespiratory failure. DMD patients are characterized by an absence of dystrophin, causing muscle fibres to be susceptible to contraction-induced damage leading to eventual muscle wasting. In addition, inflammation is chronically elevated in dystrophic muscles and is thought to have a significant role in the pathology. Glycogen synthase kinase 3 (GSK3) can promote inflammation and has been shown to have increased activity in animal models of DMD. Lithium is a natural GSK3 inhibitor, and previous studies have shown that lithium can exert anti-inflammatory effects. This study aimed to target GSK3 with low dose lithium supplementation to determine the effects on muscle inflammation as well as serum creatine kinase activity in DBA/2J mdx mice. We included 3 groups in our study: 1) wild-type (WT) healthy control; 2) mdx control; and 3) mdx treatment, the latter of which was fed 10mg/kg/day of lithium chloride via their drinking water for 6 weeks. The red gastrocnemius, white gastrocnemius and diaphragm muscles were collected and compared using mRNA analyses for IL-6 and TNF-a. Serum was collected to measure creatine kinase activity using a commercially available kit that was fitted onto a 96-well plate.

NOTES


Pioneer Village subway station
STEELES AVE WEST
TO KEELE ST
To Hwy 400
Driving from Highway 400, going East on Steeles Ave W to Murray Ross Pkwy
← Driving from Keele Street, going West on Steeles Ave W
66 – Founders Road East Parking Lot ($1.75/ half hour, $10/max)
79 – Thomson Road Parking ($1.75/ half hour, $15/max)
90 – Life Science Building Lobby and Room 103 (registration / posters / talks)
24 – Shopsy's Sports Grill (optional post-conference venue) CASH BAR
80 – Arboretum Parking Garage ($2.50/half hour, $20/max)
York University Subway
84 - Parking Garage ($2.50/half hour, $20/max) CREDIT CARD ACCEPTED
GREEN LINES indicate quickest routes to parking lots/garages
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ARBORETUM LANE
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KEELE ST
TO FINCH AVE WEST
FINE ARTS RD
SENTINEL RD
PASSY CRES
ASSINIBOINE RD
OTTAWA RD
MCLAUGHLIN RD
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VANIER LANE
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LIBRARY LANE
NELSON RD
HOOVER RD
ATKINSON RD
SENECA LANE
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LEGEND
VISITOR PARKING
RESERVED PARKING
PARKING GARAGE
BLUE LIGHT EMERGENCY PHONE
PARKING INTERCOM
COMMUNITY SAFETY
PICK-UP/DROP-OFF AREA
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TTC LINE 1 YONGE-UNIVERSITY
GLENDON-KEELE SHUTTLE & GO TRAIN SHUTTLE STOPS
VILLAGE SHUTTLE PICK-UP
ZUM BRAMPTON TRANSIT
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