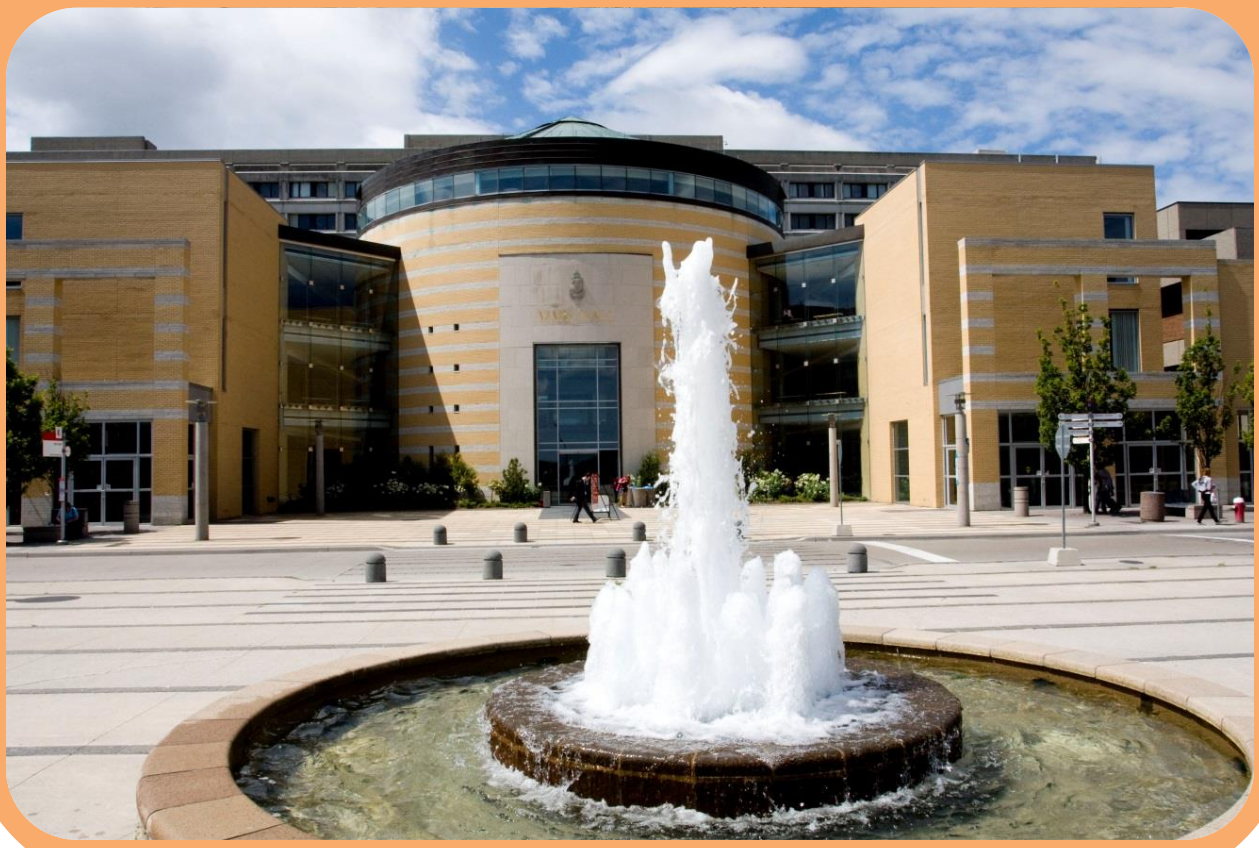
6th Annual
Muscle Health Awareness Day
May 22, 2015

Program and Abstracts



Muscle Health & Research Centre
Adaptation • Development • Metabolism • Disease

TORONTO 2015 Pan Am/Parapan Am

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Date: May 22, 2015

To: All Participants

From: David A. Hood, MHRC Director

Welcome to the 6th Annual Muscle Health Awareness Day

The Muscle Health Research Centre at York University welcomes you to **MHAD6**, our 6th annual "***Muscle Health Awareness Day***", designed to bring together scientists, faculty members, graduate students and post-doctoral fellows to discuss issues related to muscle physiology, metabolism, adaptation, development and disease.

We have 9 great speakers for **MHAD6**. The focus this year is on 1) metabolism, adiposity and exercise, 2) cardiac stem cells, disease and cell signaling, and 3) mechanisms of skeletal muscle wasting and disease.

Our goal is to give graduate students an opportunity to network and present their work in an informal, yet educational manner. We also want to highlight the research of both junior and senior faculty members. Every year we try to improve the format of this event, so any feedback or suggestions that you might have 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 and volunteers for their participation, and for helping to continue to make this a successful event. Please enjoy **MHAD6**!

Sincerely,

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

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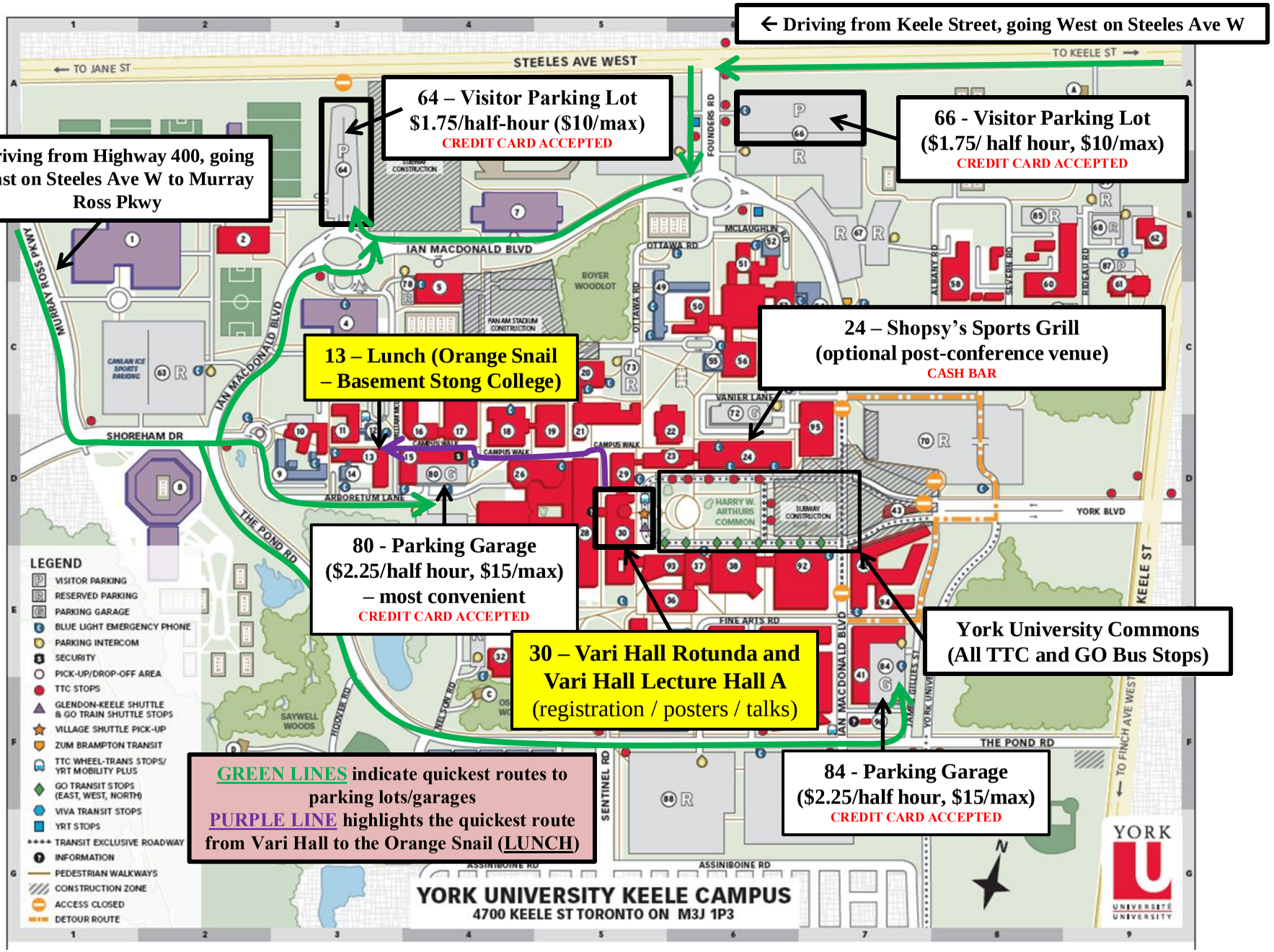
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(registration / posters / talks)
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6th Annual Muscle Health Awareness Day Program
May 22, 2015
Vari Hall Rotunda and Vari Hall A, York University

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

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

Session Chair: Dr. Rolando Ceddia, York University

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

9:05-9:35 – Dr. Julie St-Pierre, *McGill University*
Regulation of metabolic adaptation by the PGC-1 transcriptional coactivators

9:35-10:05 – Dr. Paul LeBlanc, *Brock University*
The membrane structure-protein function relationship: challenges with biological membranes

10:05-10:35 – Dr. Robert Ross, *Queen's University*
Exercise, adiposity and skeletal muscle: towards precision in exercise prescription

10:35 – 11:30 Break (Poster Presenting and Viewing)

Session 2: Cardiac Muscle (11:30-12:30)

Session Chair : Dr. Heather Edgell, York University

11:30-12:00 – Dr. Sara Nunes Vasconcelos, *Toronto General Research Institute/UHN*
Human stem cell-derived cardiomyocyte maturation by biomimetic topographical and electrical cues

12:00-12:30 –Dr. Robert Tsushima, *York University*
Targeting cell signaling pathways to protect the heart from ischemic injury

12:30 – 2:30 Lunch (Orange Snail – Stong College);
2:00-2:30 Poster Presentations and Viewing

Session 3: Muscle diseases and mechanisms of atrophy (2:30-4:40)

Session Chair: Dr. Christopher Perry, York University

2:30-3:00 – Dr. Simon Wing, *McGill University*
A role for deubiquitination in muscle wasting and differentiation

3:00-3:30 – Dr. John Dawson, *University of Guelph*
Cardiac actin mutations: from molecules to organisms

3:30-4:00 – Dr. Jean-Marc Renaud, *University of Ottawa*
Mechanisms making the Hyperkalemic Periodic Paralysis diaphragm asymptomatic: can it give answers to develop a treatment?

4:00-4:30 – Dr. Stuart Phillips, *McMaster University*
What is the predominant process causing muscle disuse-induced atrophy? A tale of mice and men!

4:30-4:40 – Poster Awards, Concluding Remarks

6th Annual Muscle Health Awareness Day Speaker Profiles

Dr. John Dawson, University of Guelph

Dr. Dawson is a Professor in the Department of Molecular and Cellular Biology at the University of Guelph. His current work focuses on the control actin polymerization and elucidating the biochemical links between mutations in actin and the development of cardiac disease.

Dr. Paul LeBlanc, Brock University

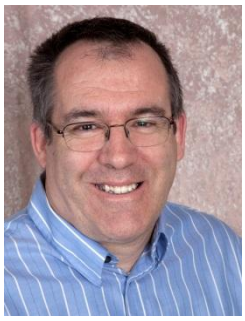
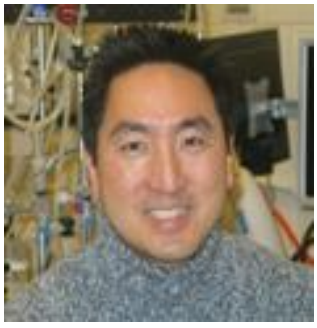
Dr. LeBlanc is an Associate Professor in the Department of Health Sciences, and the Director of the Centre for Bone and Muscle Health at Brock University. His research interests lie in examining the adaptation associated with diet and exercise, and how these impact the composition of skeletal muscle membranes (fatty acids and cholesterols) and the function of proteins contained within them.

Dr. Sara Nunes Vasconcelos, TGRI/UHN and University of Toronto

Dr. Nunes is an Assistant Scientist in the Division of Experimental Therapeutics at the Toronto General Research Institute, and an Assistant Professor at the Institute of Biomaterials and Biomedical Engineering at the University of Toronto. Her current research involves the development of vascularization strategies for regenerative medicine, as well as the nature and mechanistic basis of vessel maturation during adult neovascularization.

Dr. Stuart Phillips, McMaster University

Dr. Phillips is a Professor in the Department of Kinesiology, and the Director of the McMaster Centre for Nutrition, Exercise and Health Research at McMaster University. His work focuses on the mechanisms regulating skeletal muscle mass, quality and metabolic activity, in the context of resistance and/or aerobic training, disuse and aging. He is also interested in the relationship between feeding different protein compositions and meal timing, and how this modulates the response to exercise.

	**Dr. Jean-Marc Renaud**, University of Ottawa Dr. Renaud is a Professor in the Department of Cellular and Molecular Medicine and a member of the Neuromuscular Research Centre at the University of Ottawa. He is interested in the regulation of muscle contractility during exercise and fatigue, with specific focus on muscle membrane excitability and the regulation/activity of the membrane channels which govern this.
	**Dr. Robert Ross**, Queen's University Dr. Ross is a Professor in the School of Kinesiology and Health Studies at Queen's University. His research focuses on the characterization and management of obesity and related co-morbidities in adults, and the effectiveness of lifestyle-based interventions designed to manage these disease states.
	**Dr. Julie St-Pierre**, McGill University Dr. St-Pierre is an Associate Professor in the Department of Biochemistry at the Rosalind and Morris Goodman Cancer Centre located at McGill University. The aims of her research are to understand the regulation of mitochondrial metabolism under physiological and pathological conditions using metabolic profiling and global gene expression analyses.
	**Dr. Robert Tsushima**, York University Dr. Tsushima is an Associate Professor and the Associate Dean of Research and Partnerships in the Faculty of Science and Engineering at York University. His research examines the properties and regulation of ion channels and the role of SNARE proteins in cardiac and pancreatic tissues, as well as the mechanisms which modulate myocardial ischemic preconditioning protection, in the context of heart disease and diabetes.
	**Dr. Simon Wing**, McGill University Dr. Wing is a Professor in the Department of Medicine and the Program Director for the Research Institute of the McGill University Health Centre. His research is focused on the role of the ubiquitin-proteasome system in skeletal muscle protein degradation and the identification of key enzymes in this system that could be pharmacologically targeted to mitigate muscle wasting.

Poster Presentation Abstract List

Poster number	First Author (Surname)	Abstract Title	University Affiliation
1	Al-Sajee	Lack of Xin results in altered metabolic and mitochondrial profile in skeletal muscle	McMaster University
2	Altalhi	Diabetes impairs vessel arterio-venous specification in pre-vascularized engineered implants that support the transplantation and robust survival of stem cell-derived cardiomyocytes	University of Toronto/Toronto General Research Institute
3	Ammar	Diaphragm of Hyperkalemic periodic paralysis mouse has no contractility abnormality compared to the robust abnormalities in EDL and soleus	University of Ottawa
4	Bott	Skeletal-site specific effects of endurance running on bone structure in growing rats	Brock University
5	Brownlee	TFEB expression and activation in contracting skeletal muscle myotubes	York University
6	Ciccone	Angio-adaptive allies: Examining the relationship between human primary endothelial cells and human skeletal muscle myofibroblasts	York University
7	Coleman	Myostatin inhibition: A novel adjuvant therapy in the treatment of type one diabetes	McMaster University
8	Crilly	The influence of the Nrf2-Keap1 pathway on skeletal muscle and mitochondrial function	York University
9	D'Cruz	PD-LIM7 is a novel target of the ubiquitin ligase Nedd4-1 in skeletal muscle	St. Michael's Hospital/University of Toronto
10	Dhanani	Branched-chain amino acid catabolism is required for skeletal muscle cell differentiation	York University
11	D'souza	Satellite cell myogenic capacity is impaired in type 1 diabetic skeletal muscle	McMaster University
12	Ehyai	A p38 MAPK regulated MEF2:β-catenin interaction enhances canonical Wnt signalling	York University
13	Erlich	Effect of aging, PGC-1α, and exercise on levels of inflammatory proteins in muscle	York University
14	Haikalis	The effect of irisin on rescuing high-fat diet-induced obesity and diabetes	McMaster University
15	Hassanpour	Skeletal muscle protease activity: Dependence on exercise intensity and total energy expenditure	York University
16	Hughes	The effect of acute and chronic high intensity interval exercise on mitochondrial respiratory sensitivity to ADP	York University
17	Lamb	Integrated capillary responses to multiple vasodilators: implications for redundancy in active hyperaemia	University of Guelph

18	Liu	How do a-cardiac actin mutations lead to cardiomyopathy?	University of Guelph
19	Loustau	Angio-adaptation of adipose tissue by physical exercise in a context of obesity induced by a high fat diet	Université d' Avignon
20	Macpherson	Reduced cortical BACE1 content with one bout of exercise is accompanied by declines in AMPK, AKT, and MAPK signaling in obese, glucose intolerant mice	University of Guelph
21	Mandel	Prazosin prevents skeletal muscle capillary rarefaction in vivo	York University
22	Memme	Time course of signaling events associated with skeletal muscle adaptations to exercise	York University
23	Moghei	The effect of leucine and its metabolites on insulin signaling and glucose transport in L6 myotubes	York University
24	Moghimi	Vitamin D_3 supplementation at non-toxic doses attenuates disease pathophysiology in the spinal cord of the G93A mouse model of amyotrophic lateral sclerosis	York University
25	Morris	The effect of β-adrenergic stimulation of post-tetanic potentiation of concentric force in fast skeletal muscle	Brock University
26	Morton	The effect of high and low-load resistance training on body composition in young, resistance-trained men	McMaster University
27	Murphy	Hypoenergetic diet-induced reductions in myofibrillar protein synthesis are rescued by resistance training and balanced daily protein ingestion in older men	McMaster University
28	Nejatbakhsh	In-gel detection of a maleimide-based infrared-fluorescent dye as a simplified approach for the detection of protein redox state in cardiac muscle homogenate	York University
29	Nwadozi	Endothelial FoxO proteins regulate obesity associated skeletal muscle capillary rarefaction	York University
30	Oh	Genome-wide analysis of endurance exercise-induced atrial/ventricular remodelling	University of Toronto
31	Oikawa	Resistance training intensity determines neither strength nor muscular hypertrophic gains in young resistance trained men	McMaster University
32	Ojehomon	Developing zebrafish as an in vivo model of cardiomyopathy	University of Guelph

33	Polidovitch	Phosphodiesterase 3 (PDE3) inhibition ameliorates pressure overload-induced cardiac hypertrophy and dysfunction by antagonizing the calcineurin-NFAT signaling pathway	University of Toronto
34	Raiber	Are the discrepancies between self-report and objectively measured physical activity in individuals with obesity improved after accounting for differences in body weight and exercise energy expenditure?	York University
35	Ramos	The effects of chemotherapeutic microtubule stabilizing and destabilizing drugs on skeletal muscle mitochondrial H2O2 emission and respiration	York University
36	Rebalka	Fluvastatin causes hallmark myopathic characteristics and impaired lipid transport in diabetic skeletal muscle.	McMaster University
37	Saleem	METRNL and IL-15 – effective therapeutic interventions for high fat diet-induced obesity and glucose intolerance?	McMaster University
38	Skelly	Similar expression of mitochondrial genes following sprint interval exercise in men and women	McMaster University
39	Sun	The role of AP-1 in skeletal muscle regeneration	York University
40	Sun	Innovative prevascularization model for improving transplantation of human embryonic stem cell-derived cardiomyocytes	University Health Network/Toronto General Research Institute
41	Swarbeck	Moderate running exercise protects against sepsis-induced inflammatory response in skeletal muscle, lung and liver in aged mice	University of Western Ontario
42	Tian	Developing a method for measuring length-dependent activation in isolated cardiomyocytes	University of Toronto
43	Wales	Regulation of Hspb7 by MEF2 and AP-1 in muscle atrophy	York University
44	Wauchop	Mechanisms of atrial fibrillation induced by endurance exercise	University of Toronto
45	Zhou	Mild exercise training improves endurance capacity, but not measures of skeletal muscle health in T1DM mice	McMaster University

Lack of Xin results in altered metabolic and mitochondrial profile in skeletal muscle

Dhuha Al-Sajee[1], Meghan Hughes[2], Christopher Perry[2], John Provias[1], Sarah Zhou[1], Gary Mangan[1], Thomas Hawke[1].
[1] *Pathology and Molecular Medicine, McMaster University. Hamilton ON, Canada.*
[2] *Muscle Health Research Centre, York University, Toronto ON, Canada.*

Background/Objectives: Xin has been characterized as a cytoskeletal adaptor protein capable of binding numerous other cytoskeletal proteins (e.g. F-actin, Filamin-C). We recently demonstrated that Xin-deficient mice display a mild myopathy however the mechanisms leading to this phenotype are unknown. Preliminary investigations led us to suspect mitochondrial dysfunction in these mice leading us to hypothesize that Xin-/- mice have impaired mitochondrial form and function resulting in the myopathic features observed. **Methods:** Immunohistochemical stains, immunoblotting, transmission electron microscopy (TEM) and high-resolution respirometry (mitochondrial respiration kinetics) were used to study the mitochondrial/metabolic effects of the lack of Xin in skeletal muscle. **Results:** Xin-/- muscles show an altered metabolic profile demonstrated as an increase in total muscle lipid content and increased in oxidative enzymes in muscle sections [succinate dehydrogenase (SDH), cytochrome c oxidase (Cox)]. SDH and Cox data suggest mitochondrial proliferation, which was further confirmed by ultrastructural examination of muscles. Significant increases in mitochondrial areas and altered mitochondrial shapes in Xin -/- compared to controls were observed by TEM. Furthermore, Xin-/- muscles display elevated oxidative phosphorylation proteins. Despite these changes, total oxidative phosphorylation capacity in Xin-/- muscles was not different from controls. **Conclusions:** Xin-deficient mitochondria display significant abnormalities at the ultrastructural level and this dysfunction has led to their compensatory proliferation without a concomitant increase in oxidative capacity. Further investigations to uncover the causes of these mitochondrial defects are being undertaken.

Diabetes impairs vessel arterio-venous specification in pre-vascularized engineered implants that support the transplantation and robust survival of stem cell-derived cardiomyocytes

Wafa Altalhi[1,2,3], Mansoor Husain[1,2,3,4], Sara Nunes[3,4]
[1]*Laboratory Medicine and Pathology, University of Toronto,* [2]*McEwen Center for Regenerative Medicine,* [3]*Division of Experimental Therapeutics Cardiovascular, Toronto General Hospital Research Institute, and* [4]*Heart and Stroke Richard Lewar Centre for Excellence.*

Regenerative medicine of damaged cardiac tissue, such as myocardial infarction, consists of two approaches: 1) cell replacement and 2) vascularization of the new tissue. These revascularization approaches have the potential to regenerate ischemic tissues, holding promise for translational therapies. We have successfully used pre-vascularization approach to transplant hESC-cardiomyocytes in vivo. In case of diabetes, however, microvasculature is susceptible to dysfunction due to hyperglycemia and glycosylation end product. Therefore, revascularization may fail due to the lack of mature vasculature. Maturation is determined by the formation of hierarchical vascular network, consisting of specific arterio-venous (AV) types. This AV specification is essential for sustaining vascular function. We define factors that control AV specification in pre-vascularized engineered implants in health and diabetes by using the only pre-vascularized engineered implant described to yield vessels with specific AV identities.

Hypothesis: AV specification is dependent on perivascular cell (PVC) recruitment and is impaired in diabetes. **Results:** we find 50% reduction in PVC coverage in microvascular fragments from type I diabets model (streptozotocin-injected) compared to control non diabetic models. For further investigation, using microvessel fragments isolated from EphrinB2-GFP (arterial-reporter) mice and implanted into engineered constructs, we show for the first time that preventing PVC recruitment by blocking PDGFRß resulted in lack of proper AV identity (ubiquitous EphrinB2 expression) and absence of vessel network hierarchy (immature, narrow vessels). Controls exhibited mature networks with arterial and venous vessels. Analysis of endothelial cells co-cultured with PVCs of arterial or venous origin point to endothelial-PVC cell-cell Notch signaling involvement in arterial specification. No differences in the percentage of vessel perfusion or shear stress between microvessels in control or PDGFRß-blocked implants were observed. Lack of proper AV identity in the absence of PVCs (PDGFRß-blocked) was comparable to microvessels in constructs implanted into the diabetic mice. With regards to underlying mechanisms, qPCR data from diabetic microvascular fragments and high glucose preconditioned endothelial-PVC showed significantly reduced expression levels of genes involved in the Notch signaling pathway. **Conclusion:** our data are suggesting that an inability of vessels to acquire proper AV identity contributes to microvascular dysfunction in diabetes.

Diaphragm of Hyperkalemic periodic paralysis mouse has no contractility abnormality compared to the robust abnormalities in EDL and soleus

Tarek Ammar and Jean-Marc Renaud
Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada

Hyperkalemic periodic paralysis (HyperKPP) is an autosomal dominant disease that is caused by mutations in the SCN4A gene that encodes for the Nav1.4 channel, the skeletal muscle isoform of Na+ channels. HyperKPP patients suffer from both myotonia and limb paralysis, they rarely suffer respiratory distress. Despite the fact that Na+ influxes through mutated NaV1.4 channels in diaphragm, soleus and EDL muscles from a HyperKPP mouse model are all significantly greater than in their WT counterparts, the HyperKPP diaphragm is the only muscles being spared from the K+-induced paralysis. Measurements of resting membrane potential (resting Em) and force demonstrated that the lower force generation in HyperKPP EDL and soleus compared to wild type muscles at different [K+]e was in part related to greater membrane depolarization when [K+]e was increased. However, for a given a depolarized resting Em, HyperKPP diaphragm generated more force than WT diaphragm. The lack of paralysis in diaphragm is not related to any significant increases in Na+ K+ ATPase (NKA) α-1 and α-2 protein content, which actually only increased in HyperKPP EDL. However, NKA electrogenic contribution to resting Em increased only in HyperKPP diaphragm but not in soleus and EDL. Measurement of action potential (AP) amplitude showed that at a given depolarized resting Em HyperKPP diaphragm generated higher APs than WT muscles. We concluded that HyperKPP diaphragm is spared from symptoms because of because of higher NKA contribution to resting Em and higher AP amplitude at depolarized resting Em.

Skeletal-site specific effects of endurance running on bone structure in growing rats

Kirsten N. Bott, Sandra M. Sacco, Patrick C. Turnbull, Amanda B. Longo, Wendy E. Ward, Sandy J. Peters
Brock University

High impact activities, such as running, induce positive effects on bone mass and bone strength. However, there are discrepancies in the magnitude of changes due to exercise, mainly as a result of varying training protocols (i.e. duration, intensity, and mode), analysis techniques, and bone sites being measured (weight bearing vs. non-weight bearing sites within a bone). **Purpose:** To determine the effect of endurance running on structure and strength of weight bearing (tibia) and non-weight bearing (lumbar vertebra and mandible) bones in growing male rats. **Methods:** 8-week-old male Sprague-Dawley rats were randomly assigned to either the trained (n = 9) or control group (n = 10). Rats in the trained group underwent a progressive treadmill running program for 8 weeks (25 m/min for 1 hour, incline of 10%). Microcomputed tomography was used to measure bone structure and strength of tibia, lumbar vertebra and mandible at a resolution of 9 um. Outcomes included bone volume fraction, BV/TV; trabecular number, Tb.N; trabecular thickness, Tb.Th; trabecular separation, Tb.Sp. **Results:** At the proximal tibia, training resulted in higher BV/TV (23.77 ± 1.06 vs. 20.95 ± 0.91%, p = .03), Tb.N (2.53 ± 0.11 vs. 2.18 ± 0.09 mm^{-1}, p = .01) and lower Tb.Sp (0.26 ± 0.015 vs. 0.32 ± 0.02 mm, p = .05) compared to control rats. No significant differences at the tibia midpoint or lumbar vertebra were found. At the mandible, trained rats had lower BV/TV (63.00 ± 1.57 vs. 69.13 ± 1.45%, p = .01) and Tb.Th (0.22 ± 0.01 vs. 0.26 ± 0.01 mm, p = .0002), compared to control rats. This effect may be due to a lower amount of mechanical force due to chewing, as trained rats had a significantly lower weekly food intake (471.20 ± 2.82g vs. 384.30 ± 3.53 g/cage/week, p < .0001). **Conclusion:** Endurance running in growing male rats results in site-specific effects to the skeleton. Bone structure is improved by direct loading (tibia) while lumbar vertebra, a bone that is not loaded is unchanged, while mandible structure was compromised due to less loading resulting from a lower food intake induced by the endurance training protocol.

TFEB expression and activation in contracting skeletal muscle myotubes

Diane M. Brownlee and David A. Hood
Muscle Health Research Centre, York University, Toronto, Ontario, Canada.

Skeletal muscle adaptations during exercise depend on a functional mitochondrial pool. Optimal mitochondria are regulated by two opposing processes, termed mitochondrial biogenesis and mitochondrial autophagy (mitophagy). Though much is known about mitochondrial biogenesis, an understanding of the process of mitophagy activated by exercise is more elusive. Mitophagy provides the essential service of removing damaged or dysfunctional mitochondria from the cells, and completing a recycling process to allow the components and amino acids to be reused. A key mechanism in mitophagy is lysosomal biogenesis, this process is under the control of transcription factor EB (TFEB). TFEB is known to be activated following starvation, however, exercise-mediated TFEB activity in skeletal muscle has not been determined. Furthermore, the role of TFEB in mitophagy in skeletal muscle is unknown. To understand this we employed acute and chronic contractile activity, in vitro models of exercise using a C2C12 cell culture model. Following 2 and 5 hours of acute stimulation TFEB was activated and translocated to the nucleus. However, TFEB promoter activity was unaffected, suggesting that TFEB transcription

was not influenced by acute stimulation. To evaluate the effect of TFEB on muscle, adenoviral overexpression of TFEB was performed in myotubes prior to acute stimulation. Autophagy markers LC3, Cathepsin D, p62 and LAMP2 mRNA levels were slightly increased compared to control cells at basal levels following the adenoviral overexpression. Additionally, these autophagy markers demonstrated modest increases following 5 hours of stimulation. Interestingly, overexpression of TFEB markedly increased the mRNA levels of PGC1α, but significantly reduced TFEB mRNA in a contractile activity-dependent manner. Our data illustrate that TFEB activity in skeletal muscle is increased following acute stimulation, but that TFEB transcription and mRNA expression are unaffected by contractile activity. The relationship between in TFEB expression and PGC1α mRNA levels provides useful insight into the connections of the two transcriptional regulators. A decrease in TFEB mRNA was observed following a bout of exercise, which may indicate that TFEB is regulated through a negative feedback loop. Future work will include investigating the effects of chronic contractile activity on lysosomal markers and to investigate markers of mitophagy following acute and chronic stimulation.

Angio-adaptive allies: Examining the relationship between human primary endothelial cells and human skeletal muscle myofibroblasts

Joseph Ciccone[1], Emilie Roudier[1], Yasaman Alinejad[2], Julian Aiken[1], Marc-Antoine Despatis[3,4], Frederic Balg[2,4], Guillaume Grenier[2,4], Olivier Birot[1]

[1]York University, Faculty of Health, School of Kinesiology and Health Science, Angiogenesis Research Group, Toronto, ON, Canada,[2]Centre de Recherche du Centre Hospitalier de l'Université de Sherbrooke (CRCHUS), [3]Department of Vascular Surgery, [4]Department of Orthopedic Surgery, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, QC, Canada.

Angioadaptation is the process in which capillaries can regress, stabilize, or grow in order to adapt to physiological changes. Such process is tightly regulated by a balance between pro- and anti-angiogenic signals, which influence the microenvironment surrounding the capillaries. This microenvironment is also strongly under the influence of paracrine and physical interactions between endothelial cells and supporting cells such as myofibroblasts. **Objective:** Here we examined a possible pro-angiogenic paracrine interaction between a population of newly identified myofibroblast progenitors in human skeletal muscle and primary endothelial cells, and how this interaction may be altered under hyperglycemic conditions. **Methods:** Flow cytometry was used to sort myofibroblast progenitor cells (CD90+) from human skeletal muscle. The latter were differentiated using TGF-β (10 ng/ml) in medium containing 5 or 25 mM glucose representing normo- or hyperglycemic conditions, respectively. Conditioned media (secretome) was collected from CD90+ cells and myofibroblasts. The expression level of 55 proteins involved in regulating angio-adaption was measured in these secretomes using a proteome profiler array. Human dermal microvascular endothelial cells (HDMVECs) were treated with these secretomes, or cultured under normo- or hyperglycemic conditions. Their expression level for vascular endothelial growth factor-A (VEGF-A) and thrombospondin-1 (TSP-1) mRNA and protein, two major angio-adaptive factors, was determined by real-time qPCR and western blot. The functional angiogenic activity of HDMVECs was evaluated by measuring cell migration in the Boyden Chamber assay. **Results:** The secretome from differentiated MF was enriched in pro-angiogenic factors compared to CD90+ cells. VEGF-A mRNA and protein expression was increased in EC when treated with MF secretome and their migration was stimulated. In contrast,

CD90+ cell differentiation under HG resulted in decreased pro-angiogenic factor secretion in MF secretome and reduced EC migration. Finally, HDMVEC cultured under HG conditions also had reduced migratory activity. **Conclusion:** MF exert some pro-angiogenic stimulation on primary EC compared to progenitor cells. This pro-angiogenic affect is attenuated when cell differentiation occurs under HG. Funding: CIHR/NSERC.

Myostatin inhibition: A novel adjuvant therapy in the treatment of type one diabetes

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Background/Objectives: Type one diabetes (T1D) is characterized by hyperglycemia and hypoinsulinemia resulting in long term complications. Myostatin, a negative regulator of muscle mass, is elevated in T1D. Myostatin-deficient (MyoLn/Ln) mice exhibit muscle masses double that of controls (WT). We hypothesize that diabetic MyoLn/Ln mice will display an increase in healthy muscle mass, providing a greater "sink" for glucose disposal. This reduction in blood glucose will reduce time spent in hyperglycemia and thereby attenuate long-term complications. **Methods:** From 6-12 weeks of age (2-8 weeks of T1D) blood glucose levels and body mass of MyoLn/Ln diabetic mice were measured. Intraperitoneal insulin tolerance test and metabolic cage analysis was also performed. At 8 weeks of T1D, mice were euthanized, tissues collected and analyses performed. **Results:** MyoLn/Ln diabetic mice demonstrated greater body mass ($p<0.0001$), decreased polydipsia ($p=0.0008$) and hyperphagia ($p=0.0111$) and a greater reliance on lipid metabolism. Muscle masses and insulin sensitivity ($p<0.0001$; $p=0.006$ respectively) were also greater in the MyoLn/Ln vs. WT diabetic animals. Notably, the MyoLn/Ln diabetic mice recorded significant reductions in fed and fasted blood glucose levels ($p<0.05$). Western blot analysis revealed increased GLUT1 ($p=0.02$) and GLUT4 ($p=0.0785$) transporter expression in MyoLn/Ln diabetic muscle compared to WT diabetics. Immunohistochemical analysis of inguinal white adipose tissue revealed increased expression in UCP1 indicating a "beiging" of white adipocytes. **Conclusions:** Taken together, myostatin inhibition has improved the health of the diabetic mice. These findings show promise for targeting myostatin as an adjuvant therapy in the treatment of T1D.

The influence of the Nrf2-Keap1 pathway on skeletal muscle and mitochondrial function

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Nuclear erythroid 2 p45-related factor 2 (Nrf2) is regarded as a master regulator of endogenous antioxidant enzyme expression through the transcriptional activation of the antioxidant response element (ARE) located within the promoter region of its target genes. The Nrf2 signaling pathway is negatively regulated by Keap1. While it is well-established that the Keap1-Nrf2 pathway serves as a molecular switch that is highly responsive to the redox status of the cell, its expression and function within skeletal muscle remain to be elucidated. Thus, the purpose of our study was to examine the role of the Keap1-Nrf2 pathway in mediating changes in skeletal muscle phenotype using Nrf2 wild-type (WT) and knockout (KO) mice at 3 months of age. Although a 29% increase in body weight was observed in the Nrf2 KO mice, there were no observed differences in TA mass between the two genotypes. Since Nrf2 has also been implicated in mitochondrial function, we subjected these mice to an endurance exercise capacity

test. Interestingly, the Nrf2 KO mice exhibited a similar running capacity since their distance (1110m ±65) and time to exhaustion (62.6 min ±2.0) were the same as their WT counterparts. This may partially be explained by the lack of observed differences in mitochondrial content as measured by COX activity, or lactate production during exercise between the WT and KO mice. Additionally, PGC1α protein content did not differ between genotypes. These findings suggest that Nrf2 may be required for normal muscle growth, but that it is not essential to maintain mitochondrial content or endurance exercise performance in young animals.

PD-LIM7 is a novel target of the ubiquitin ligase Nedd4-1 in skeletal muscle

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Introduction and Objectives: Skeletal muscle atrophy is a condition associated with increased morbidity, impaired quality of life and increased health resource utilization and costs. It is characterized by a loss of muscle mass, which is known to result from increased proteolysis mediated by the ubiquitin proteasome system (UPS), but the specific cellular signalling networks responsible, remain unknown. We identified Nedd4-1, a UPS regulatory protein, as a positive mediator of muscle degradation in mouse models of disuse atrophy and demonstrated its up-regulation in the atrophied quadriceps of individuals with severe COPD. In this study we set out to determine the mechanism by which Nedd4-1 induces muscle wasting. **Methods:** We used mouse disuse models of muscle atrophy (tenotomy, denervation) in Nedd4-1 skeletal muscle specific knockout mice to identify Nedd4-1 muscle substrate(s) and study their potential involvement in the induction of muscle wasting. Mass spectrometry identified potential Nedd4-1 muscle interactor proteins/substrates. Confirmation of Nedd4-1 and interactor/substrate binding was undertaken with co-immunoprecpitation experiments, generation of fusion proteins and in vitro binding experiments, and assessing for co-localization of endogenous proteins in C2C12 myoblasts and differentiated myotubes. Confirmation that the interacting protein(s) were bona fide Nedd4-1 substrate(s) was undertaken with ubiquitination assays. **Results:** Using MUD PIT mass spectrometry, we identified PDLIM7, a member of the PDZ-LIM family of proteins, as a novel interactor of Nedd4-1 in skeletal muscle. The PDZ-LIM family of proteins is known to regulate muscle development and function. We found PDLIM7 expression was decreased in gastrocnemius muscle atrophied by denervation, concomitant with an increase in Nedd4-1 expression, and PDLIM7 protein levels were stabilized in denervated gastrocnemius muscle of Nedd4-1 skeletal muscle specific knockout mice. PDLIM7 and Nedd4-1 transfected into 293 cells co-immunoprecipitated and binding was mediated in a canonical fashion between the PY motif in PDLIM7 and the 2nd and 3rd WW domains of Nedd4-1. Endogenous Nedd4-1 and PDLIM7 co-localized in the cytoplasm of fully differentiated C2C12 myotubes, but did not demonstrate an interaction in undifferentiated myoblasts. Binding by Nedd4-1 resulted in PDLIM7 ubiquitination. **Conclusions:** We have identified PDLIM7 as a bona fide skeletal muscle substrate of Nedd4-1. This novel interaction likely underlies the biological process of Nedd4-1 mediated muscle breakdown and provides a potential target for therapeutic

manipulation in muscle wasting. Supported by: Neuromuscular Research Partnership Grant (CIHR/ALS Society Can/Muscular Dystrophy Canada) to JB.

Branched-chain amino acid catabolism is required for skeletal muscle cell differentiation
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The importance of branched-chain amino acids (BCAAs) in promoting skeletal muscle anabolism has been well studied. BCAAs isoleucine, leucine, and valine have been shown to have a profound effect on activating anabolic signaling pathways in skeletal muscle that result in increased protein synthesis. This occurs in part by the upregulation in activity of the mammalian target of rapamycin complex-1 protein (mTORC1). However, the regulation and importance of branched-chain amino acid metabolism in developing muscle has not been as well elucidated. Here, we studied BCAA metabolism during a 5-day differentiation of L6 rat myoblasts. When we attempted to differentiate myoblasts in leucine-free medium, we observed that myoblasts were not able to differentiate. However, adding the leucine metabolite α-ketoisocaproate (KIC) to the leucine-free medium was able to rescue myoblast differentiation, suggesting that metabolites of BCAA metabolism can regulate muscle cell differentiation. In skeletal muscle, two enzymes which regulate BCAA metabolism and produce such metabolites are the branched-chain amino transferase-2 enzyme (BCAT2) and the branched chain α-keto acid dehydrogenase complex (BCKDC). BCAT2 is the first enzyme in the BCAA catabolic pathway which catabolizes BCAAs to corresponding α-keto acids, which are then irreversibly decarboxylated by the BCKDC complex. We found that the abundance of BCAT2 nonsignificantly increased ~1.3-fold after five days of differentiation, whereas levels of the E1α subunit of BCKDC increased ~7-fold ($p<0.05$). Finally, blunting the expression of either BCAT2 or BCKDE1α severely impaired the ability of myoblasts to differentiate. Based on these findings, we suggest that BCAA catabolism is a critical process facilitating skeletal muscle cell differentiation.

Satellite cell myogenic capacity is impaired in type 1 diabetic skeletal muscle
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Skeletal muscle health is adversely affected upon Type 1 Diabetes (T1D) development. An integral component to skeletal muscle growth and repair is the satellite cell (SC). As such, the current study investigated the effects of the T1D environment on SCs in a genetic T1D mouse model (Akita) to elucidate changes to SC functionality with T1D exposure. Single myofibers and associated SCs, isolated from wildtype (WT) and Akita (T1D model) mice, were stained for markers of myogenesis (Pax7, MyoD, Myogenin), as well as components of the Notch signaling system. The number of Pax7+ SCs was reduced at rest and in response to an activating stimulus in T1D muscle vs. WT ($p<0.05$). Consistent with an impaired SC activation, SCs from Akita mice displayed reductions in MyoD and Myogenin expression ($p<0.05$). To elucidate a potential mechanism involved, quantification of components of the Notch signaling pathway was completed. The Notch signaling pathway was specifically investigated as previous work has shown that up-regulation of specific Notch targets can impair MyoD expression, and subsequently, SC activation. Relative to WT, T1D SCs demonstrated enhanced active Notch

(NICD) and Hes1 expression ($p<0.05$). Accordingly, these results suggest that hyper-activation of the Notch signaling pathway in T1D SCs, as evident by increased NICD and Hes1 expression, likely contributes to the observed impaired activation. To ascertain whether altered SC function would have a lasting impact on the SC population in T1D skeletal muscle, we examined SC content (i.e. Pax7+ cells) in muscle sections from aged (44 week) Akita and WT mice. As predicted, SC content was lower in aged Akita mice ($n=3$, $p<0.05$). This data is also supported by analyses of human skeletal muscle section that show a trend for reduced SC content in T1D skeletal muscle (n=2-3). Given these results, it is apparent that alterations in SC functionality in the T1D muscle detrimentally affect the maintenance of the SC population, and likely contributes to a decline in skeletal muscle health observed over the course of disease progression.

A p38 MAPK regulated MEF2:β-catenin interaction enhances canonical Wnt signalling

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Canonical Wnt/β-catenin signalling plays a major role in various biological contexts such as embryonic development, cell proliferation, and cancer progression, therefore characterization of nuclear β-catenin function and retention is critically important. Previously, a connection between p38 MAPK signalling and Wnt-mediated activation of β-catenin has been alluded to but poorly understood, and in this study, we investigate potential cross talk between p38 MAPK and Wnt/β-catenin signalling. Here, we show that loss of p38 MAPK function reduces β-catenin nuclear accumulation in Wnt3a stimulated primary vascular smooth muscle cells (VSMCs). Conversely, active p38 MAPK signalling increases β-catenin nuclear localization and target gene activity in multiple cell types. Furthermore, the effect of p38 MAPK on β-catenin activity is mediated through phosphorylation of a key p38 MAPK target, myocyte enhancer factor 2 (MEF2). We report a novel p38 MAPK mediated phosphorylation-dependent interaction between MEF2 and β-catenin in multiple cell types and primary VSMCs, resulting in: (a) increased β-catenin nuclear retention, which is reversed by siRNA mediated MEF2 gene silencing; (b) increased activation of MEF2 and Wnt/β-catenin target genes, and; (c) increased cell proliferation. These observations provide mechanistic insight into a fundamental level of cross talk between p38 MAPK/MEF2 signalling and canonical Wnt signalling.

Effect of aging, PGC-1α, and exercise on levels of inflammatory proteins in muscle

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The aging process involves a progressive decline in cellular and organismic function. Low-grade inflammation, apoptosis, and a decrease in autophagy are prevalent during aging, which could be elicited by mitochondrial dysfunction and oxidative stress. This dysfunction may be related to the level of PGC-1α, a transcriptional coactivator. Lack of PGC-1α with age, or in knockout (KO) animals, is associated with mitochondrial dysfunction and increasing inflammation. There

has been extensive work conducted on inflammation and aging, however limited research exists on the role of skeletal muscle in the activation of the inflammasome. The purpose of this study was to examine inflammatory proteins within aging muscle, and the role of PGC-1α in determining their expression. In addition, the potential effects of acute exercise and recovery on protein expression were evaluated. In 34 month old Fischer Brown Norway rats, PGC-1α is reduced by 40-60% in fast- and slow-twitch muscles compared to young, 6 month old animals. Caspase-1 was increased 30-fold. Similarly, Bax, Bcl-2, and Beclin-1 were increased 10-, 25-, and 5-fold respectively, in aged muscle. Interestingly, these proteins were unaffected by the lack of PGC-1α, since protein levels in young PGC-1α KO animals were not different from their WT counterparts. In addition, acute treadmill exercise (to exhaustion) followed by recovery (2 hours) had no impact on the levels of these proteins. Our findings suggest that an increase in inflammatory proteins during aging could be due to dysfunctional mitochondria, but may be only indirectly related to PGC-1α levels. Future work will evaluate the effect of chronic exercise on the expression of muscle inflammatory proteins, and their relationship to ROS production and mitochondrial function.

The effect of irisin on rescuing high-fat diet-induced obesity and diabetes

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Physical inactivity is a primary modifiable risk factor for obesity and type 2 diabetes (T2D) – metabolic diseases that are rampant in pediatric and adult population. Endurance exercise has been shown to prevent and/or attenuate the onset and progression of obesity and T2D. The myokine 'irisin' (cleaved product of Fndc5 in response to endurance exercise) has been touted to play a role in this process but the data available thus far is controversial with respect to the efficacy of irisin. Thus, we sought to determine if irisin mediates the effect of exercise training on improving high fat diet-induced obesity and diabetes. C57Bl/6 mice were fed high-fat diet (HFD; 60% kcal from fat) for 6 months until the animals were hyperglycemic and glucose intolerant. Subsequently, the animals were divided into HFD control, endurance exercise (15 m/min X 60 mins X 5 days/week, END), or given intravenous injections of recombinant irisin (50 ng/kg/day, 3x/week, IR). Animals were treated for 8 weeks. END mice had lower body and inguinal fat weight, higher muscle mass, and improved glucose tolerance profile ($P < 0.05$). Treatment with IR had no effect on fasting glucose levels or bodyweight, but improved glucose tolerance ($P < 0.05$). IR mice also had a significant increase in brown fat mass, just like mice in the END group. There was a marked improvement Ucp1 expression, and COX activity in inguinal fat and quadriceps muscle in the END animals respectively ($P < 0.05$), which was absent in the IR group. Our data clearly reflect that while irisin plays a role in improving glucose tolerance, its effectiveness in rescuing symptoms of obesity and diabetes at the whole body level and browning of inguinal fat is limited. Supported by NSERC and CIHR.

Skeletal muscle protease activity: Dependence on exercise intensity and total energy expenditure

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The aim of this study was to investigate whether skeletal muscle protease (calpain) activation is muscle type specific and responds in a dose-dependent manner to exercise intensity. **Methods:** Rats were assigned to groups – control and treadmill run exercise until exhaustion (at 15m/min, 25m/min and 35m/min and two energy expenditures. Soleus, plantaris and vastus lateralis muscles were excised and assayed for calpain-like activity and myeloperoxidase activities. Blood creatine kinase activities were also assessed for each group. **Results:** Blood CK and muscle protease activities increased in a dose-dependent manner following exercise (r = 0.66). Calpain-like activity was tissue specific - dependent on muscle fiber composition and location. PL and VL muscles increased their calpain-like activities as a result of varying loads. PL calpain-like activities increased ($p<0.05$) from 14.1 4.2 to 22.5 5.8 U/g wet wt for the 15 m/min group, while the 25 m/min group went from 17.6 1.9 to 33.8 8.0 U/g wet wt ($p<0.05$). Comparable results were found with the vastus lateralis muscles. Interestingly, varying exercise intensity at a constant energy expenditure resulted in greater changes (~33%) at the higher energy expenditure for the plantaris, compared to the vastus lateralis, which had a more pronounced exercise intensity influence (~50%) at the lower energy expenditure condition. In contrast, SOL did not respond to exercise conditions in any systematic fashion ($p>0.05$) and had larger inter-animal variability. Contrary to calpain, myeloperoxidase activities of all muscle fibers, a marker of muscle injury, increased with increasing intensities only at the lower energy expenditure condition. Furthermore, there was a strong correlation between myeloperoxidase and calpain at varying intensities ($r=0.98$) in both lower and higher energy expenditure conditions. **Conclusion:** Calpain activation with exercise is intensity and fibre type dependent, such that muscles with >FT respond at higher exercise intensities. Moreover, the strong correlations between calpain and myeoloperoxidase could indicate at least a partial interdependence of MPO on calpain activity. *Supported by NSERC*

The effect of acute and chronic high intensity interval exercise on mitochondrial respiratory sensitivity to ADP

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Background: ADP/ATP is believed to either diffuse slowly between mitochondria and the cytosol or be substituted with fast diffusing creatine (Cr) and phosphocreatine (PCr) ‘phosphate shuttling’ catalyzed by mitochondrial creatine kinase. Previous studies assessing Cr-dependent (-Cr) and independent (+Cr) ADP-stimulated respiratory kinetics showed acute exercise had no effect on or impairment on ADP/ATP diffusion but promoted phosphate shuttling. Conversely, chronic exercise impaired both models of energy exchange. To date, physiological concentrations of PCr/Cr have not been employed *in vitro* when assessing the effect of exercise on the regulation of energy exchange. In an attempt to reconcile the seemingly diverse findings to date, we employed a longitudinal study design to assess the acute and chronic effects of

exercise on energy exchange in human skeletal muscle when modeling *in vivo* PCr/Cr conditions *in vitro*. **Methods:** 11 healthy un-trained men (age, body weight and VO_2peak were 24+/- 1 yr, 74.6 +/- 3.5 kg, 52.1 +/- 1.9 ml/kg/min respectively) completed 9 exercise sessions over 3 weeks. Mitochondrial respiratory sensitivity to ADP (K_{mapp}ADP) was assessed -Cr, +Cr (20mM) and + PCr/Cr (2.4mM and 20mM) at 37°C in permeabilized vastus lateralis fibres from biopsies sampled Pre, immediately Post and 3hr-Post exercise (cycling ~1hr, 10 x 4 min intervals at 91% maximum heart rate separated by 2min rest). Biopsy trials were repeated on 5th and 9th exercise sessions. **Results**: Chronic effects of exercise: By the 9th session, resting K_{mapp}ADP (Pre) was more sensitive for –Cr (422µM, $p<0.05$ vs T5 Pre, 678µM and T1 Pre, 735µM), suggesting improved ADP/ATP diffusion, but showed opposing responses in phosphate shuttling conditions with increased sensitivity in +Cr (Pre, 63µM $p<0.05$ vs T5 Pre, 88µM and T1 Pre, 89µM) yet decreased in +PCr/Cr (1305µM, $p<0.05$ vs T5 Pre,1071µM and T1 Pre, 879µM). Acute effects of exercise: The 1st session increased sensitivity in -Cr K_{mapp}ADP Post (512 µM) and 3hr post (469uM) vs Pre (735µM, $p<0.05$) but had no effect on +Cr K_{mapp}ADP or + PCr/Cr K_{mapp}ADP. The 5th session decreased sensitivity in +PCr/Cr immediately Post (1356µM vs Pre 1071µM, $p<0.05$) and in +Cr by 3h Post (185µM vs Pre 88µM, $p<0.05$). The 9th session had no acute effect in any condition. **Conclusion:** Acute high-intensity exercise improves –Cr but not +PCr/Cr or +Cr energy exchange, contrary to previous findings.. 9 sessions improved -Cr, suggesting improved ADP/ATP diffusion, however, an impairment in sensitivity was observed when modeling *in vivo* concentrations of PCr and Cr despite an improvement with +Cr, challenging previous conclusions that phosphate shuttling is improved with training.

Integrated capillary responses to multiple vasodilators: implications for redundancy in active hyperaemia

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Introduction: Increase in skeletal muscle blood flow during heightened muscle activity is known as active hyperaemia and functions to ensure O_2 supply is matched to tissue metabolism. Hyperaemia is critical to the maintenance of skeletal muscle function, as a mismatch between flow and metabolism is detrimental to muscle health. Increases in flow are mediated by the release of multiple vasoactive substances, metabolically linked to the 'working' muscle. However, no vasodilator(s) have been shown to be obligatory in mediating the hyperaemic response. Thus, methodological approaches have failed at elucidating a uniting control mechanism. Given the critical nature of hyperaemia current thinking has led to the idea of a more integrated, redundant control system. Redundancy is the design of a system whereby one component has the same function as another, existing to engage if the first component fails, preventing entire system failure. This redundant design would require inhibitory interactions between vasodilators, whereby one vasodilator attenuates the effects of another. Therefore, when the first vasodilator is inhibited, its inhibition on the second dilator is removed and the effects of the second dilator will be expressed. Vasoactive substances trigger capillaries to send a dilatory signal (known as a conducted response) to specific upstream arteriolar sites, allowing for increased flow to stimulated capillaries. Coupled with their proximity to skeletal muscle, places capillaries in the ideal position to detect changes in skeletal muscle activity. Therefore, we tested whether capillaries integrate multiple vasodilator signals in a redundant manner. **Methods:** Using intravital microscopy of the hamster cremaster *in situ*, we stimulated the capillaries using

a combination of 10mM KCl, 10^{-6}M ADO and 10^{-6}M NO. **Results:** When applied to capillaries, alone, we found KCl, NO, and ADO, produced a vasodilation that propagated up the vascular tree causing dilation in upstream arterioles (conducted response). However, when ADO and NO were applied in the presence of KCl, we observed that the upstream dilation elicited by NO and ADO was significantly blunted. Furthermore, we found ADO also blunted the conducted response elicited by KCl, while NO had no effect on KCl's dilation. NO or ADO did not seem to effect one another's conducted dilation. **Discussion:** We have demonstrated that metabolic vasodilators interact to influence one another's vasodilatory ability. Thus, changes in flow during skeletal muscle activity are likely a product of vasodilator interactions rather than the summation of their individual's effects. **Conclusion:** Vasodilators relevant to skeletal muscle contraction interact and may comprise a complex integrated redundant system to ensure that blood flow is always matched to skeletal muscle metabolic demand.

How do a-cardiac actin mutations lead to cardiomyopathy?

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Cardiovascular diseases (CVD) are the leading cause of death worldwide. To develop treatments, we must understand the molecular cause(s) of these diseases. To date, 16 a-cardiac actin (ACTC) mutations have been identified in patients with either hypertrophic or dilated cardiomyopathies (HCM or DCM). The prevailing hypothesis in the field is that increased contractility leads to HCM. Changes in contractility may result from altered myosin motor duty ratios, particularly for four HCM-related ACTC mutations in the actomyosin binding site: H88Y, F90Delta, R95C, and E99K. We found the myosin duty ratio with the E99K ACTC variant was 2.26 times higher than WT. The other ACTC variants result in 20%-45% reductions in in vitro motility (IVM) velocities. Loaded myosin bed driving forces (Fd) were 7-10-fold higher with the ACTC variants than WT. Taken together, our data suggest that HCM-related ACTC mutations in the actomyosin binding site manifest higher myosin duty ratios and higher force generation, supporting the prevailing hypothesis. We are now investigating the impact of troponin-tropomyosin regulated ACTC variant proteins on myosin ATPase and IVM assays. With these levels of research, we hope to understand how altered protein properties lead to heart disease.

Angio-adaptation of adipose tissue by physical exercise in a context of obesity induced by a high fat diet

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Adipose tissue (AT) homeostasis and growth are dependent on microvasculature. This adipose capillary bed has a remarkable plasticity, a process called tissue angio-adaptation. In response to metabolic alterations linked to obesity, signaling pathways involved in the maintenance of vascular homeostasis of AT appear to be affected. We demonstrated that Mdm-2/ FoxO1 axis played a key role in skeletal muscle angio-adaptive response to exercise. But regulation of this process in adipose tissue occurring during obesity as well as physical exercise remains unknown. In this context, we studied the temporal effects of voluntary exercise in C57/Bl6 mice fed with a high fat diet (HFD) on the capillarisation of the epididymal (eWAT) and subcutaneous adipose

tissues (sWAT), and angiogenic factors: Murine-double-minute 2 (MDM-2), Forkhead box 1 (FoxO1) and their downstream effectors: Vascular endothelial growth factor (VEGF-A) and Thrombospondin (TSP-1). Morphometric analysis of mice showed a significant reduction of 30% in the ratio of fat/total mass in HFD trained mice after 7 weeks of training. In these mice, the mass of the eWAT and sWAT was also significantly reduced by 33 and 39%, respectively. Histological study demonstrated a significant decrease in adipocyte size by 26% in eWAT and 30% in sWAT compared to sedentary HFD mice. Furthermore, 7 weeks of exercise training significantly increased VEGF-A/TSP-1 expression ratio and Mdm2 protein expression while FoxO1 protein was decreased in AT of control and HFD mice. After training, a significant increase in the capillary density (capillaries/mm²) was observed in AT of control mice (55 and 36% respectively in eWAT and sWAT) and a capillaries/adipocyte ratio significantly increased in control mice (respectively by 41 and 17% in eWAT and sWAT) and in HFD mice (by 16 and 18% in eWAT and sWAT respectively), as measured by histological marking of CD31. These results showed for the first time that physical exercise acts as a pro-angiogenic stimulus in AT in favor of capillary growth, thru activation of MDM2, downregulation of FoxO1 and rise of VEGF-A/TSP-1 ratio.

Reduced cortical BACE1 content with one bout of exercise is accompanied by declines in AMPK, AKT, and MAPK signaling in obese, glucose intolerant mice

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Obesity and type 2 diabetes are significant risk factors in the development of neurodegenerative diseases, such as Alzheimer's disease. A variety of cellular mechanisms, such as altered AKT, AMPK, and increased inflammatory signaling contribute to neurodegeneration. Exercise training can improve markers of neurodegeneration but the underlying mechanisms remain unknown. The purpose of this study was to determine the effects of a single bout of exercise on markers of neurodegeneration and inflammation in brains from mice fed a high fat diet. Male C57BL/6 mice were fed a low (LFD, 10% kcals from lard) or a high fat diet (HFD, 60% kcals from lard) for 7wks. HFD mice underwent an acute bout of exercise (treadmill running: 15m/min, 5% incline, 120min) followed by a recovery period of 2h. The HFD diet resulted in increased body mass and glucose intolerance (both $p<0.05$). This was accompanied by an ~2 fold increase in the phosphorylation of AKT, ERK, and GSK in the cortex ($p<0.05$). Following exercise, there was a decrease in beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) ($p<0.05$). This was accompanied by a reduction in AMPK phosphorylation, indicative of a decline in cellular stress ($p<0.05$). AKT, ERK, and GSK phosphorylation were decreased following exercise in HFD mice to a level similar to that of the LFD mice ($p<0.05$). This study demonstrates that a single bout of exercise can reduce BACE1 content independent of changes in adiposity. This effect may be mediated through reduced AKT, ERK, GSK, and AMPK signaling in the cortex.

Prazosin prevents skeletal muscle capillary rarefaction in vivo

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Prolonged exposure to elevated glucocorticoids (GC) results in skeletal muscle capillary rarefaction, which may promote insulin resistance and limb ischemia. Conversely, treatment with the α1-adrenergic receptor inhibitor prazosin increases muscle blood flow and stimulates angiogenesis. We hypothesized that prazosin treatment would prevent GC- induced capillary rarefaction and improve markers of insulin resistance. Corticosterone (Cort) or placebo (wax) pellets (400mg/rat) were implanted subcutaneously in young male Sprague-Dawley rats. Two days after, prazosin was administered in the animal's drinking water (50mg/L). Plasma levels of Cort correlated significantly with fasting plasma insulin levels (r=0.76), suggesting that sustained elevations in Cort resulted in insulin resistance; this relationship was unaffected by prazosin (r=0.78). Skeletal muscle capillary-to-fiber ratio (C:F) correlated inversely with fasted plasma insulin levels (r=-0.77) and with plasma Cort levels (r=-0.62). In 16-day Cort-treated animals, prazosin significantly increased mRNA levels of pro-angiogenic VEGF-A but not levels of anti-angiogenic thrombospondin-1 (TSP1), thus resulting in an elevated ratio of VEGF-A to TSP1. Most notably, the Cort- induced capillary rarefaction (2.0+0.08 vs. 1.6+0.08) was prevented with prazosin treatment (1.6+0.08 vs. 2.0+0.11). Fasting plasma insulin increased dramatically with Cort (0.51+0.02 vs. 4.1+0.5ng/ml, $P<0.05$), and improved slightly with prazosin treatment (4.1+0.5 vs. 3.7+0.3ng/ml, $P<0.05$). This study demonstrates the potential benefits of prazosin in preventing the GC-induced loss of capillaries and lowering fasting plasma insulin levels (Funding: NSERC and HSF Canada).

Time course of signaling events associated with skeletal muscle adaptations to exercise

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Exercise is a well-established trigger for the synthesis of mitochondrial proteins via organelle biogenesis, regulated by the transcriptional coactivator PGC-1α. During stimulus-induced increases in protein synthesis, as with exercise, the unfolded protein responses of both the mitochondria (UPR^{mt}) and the endoplasmic reticulum (UPR^{ER}) play integral roles in maintaining cellular homeostasis. However, little work has focused on the role that the UPR^{mt} and the UPR^{ER} play in exercise-induced adaptations, specifically the time-course of UPR signaling relative to mitochondrial adaptations. To investigate this, we subjected rats to 3 hrs of contractile activity (CCA) for 1, 2, 3, 5 or 7 days, followed by 3 hrs recovery, and examined the gene and protein expression responses involved in early-onset adaptations to exercise. PGC-1α mRNA expression increased 12-fold as early as 1 day of CCA, while the UPR^{ER} factors XBP1s, ATF4 and CHOP displayed a 1.5-4-fold elevation in mRNA expression from 1-3 days. Similarly, chaperones BIP and HSP70 increased in mRNA and protein from1-7 days. Expression of upstream UPR^{mt} sensors (LonP, ERα and SirT3) remained unchanged over 7 days of CCA, but the mRNAs encoding the mitochondrial chaperones CPN10, mtHSP70 and HSP60 were elevated by 40-100% between 2-7 days of CCA. These changes preceded increases in mitochondrial content marked by COX enzyme activity. Thus, these findings indicate a potential role of the UPR^{mt} and the UPR^{ER} in early exercise-induced mitochondrial adaptations in skeletal muscle.

The effect of leucine and its metabolites on insulin signaling and glucose transport in L6 myotubes

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Branched-chain Amino Acids (BCAAs) are known to have positive effects in metabolic health through weight management and muscle protein synthesis. However, elevated levels of BCAAs (particularly leucine) and their metabolites have also been implicated in insulin resistance and type 2 diabetes mellitus (T2DM). One of such metabolites is α-ketoisocaproic acid (KIC). High levels of leucine result in hyperactivation of the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) and its substrate ribosomal protein S6 kinase1 (S6K1). This overactivation leads to a negative feedback loop that inhibits insulin receptor substrate 1(IRS-1) action and subsequently leads to insulin resistance. Yet, current findings are inconclusive as some studies also indicate benefits of high BCAA levels in improving insulin sensitivity. Here, we examine the effect of various concentrations of leucine (0 to 1600μM) in the presence or absence of other amino acids on glucose transport in L6 rat myotubes. Leucine alone at 200μM suppresses insulin-induced glucose uptake ($p < 0.05$). This occurs in parallel with increased phosphorylation of S6K1T389 and IRS-1Ser612, suggesting a link between increased mTORC1 activity and insulin resistance. Interestingly, the effect of leucine on glucose uptake disappears in the presence of other amino acids. We also show that KIC treatment results in significant suppression of insulin-stimulated glucose uptake at 200 μM ($p < 0.05$), concurrent with increased activation of mTORC1 pathway. These effects are abolished when mTORC1 is inhibited by rapamycin. Our findings suggest that specific metabolites of BCAAs may contribute to the development of insulin resistance and its comorbidities.

Vitamin D_3 supplementation at non-toxic doses attenuates disease pathophysiology in the spinal cord of the G93A mouse model of amyotrophic lateral sclerosis

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Background: Vitamin D_3 (D_3) at 50x the adequate intake (AI) improves paw grip endurance in G93A mice. However, *quadriceps* apoptosis increases in females, indicating a threshold of toxicity. ALS is a neuromuscular disease characterized by progressive degeneration of upper and lower motor neurons. **Objective:** To determine the influence of dietary D_3 supplementation at 50x the AI on oxidative damage, antioxidant enzymes, inflammation, apoptosis, neurotrophic factor, and neuron count. **Methods:** Beginning at age 25 d, 41 G93A mice were provided food *ad libitum* with either adequate (AI; 1 IU D_3/g feed; 12 M, 11 F) or high (HiD; 50 IU D_3/g feed; 10 M, 8 F) D_3. At age 113 d, the spinal cords were analyzed for protein content via western blot for oxidative damage (4-HNE, 3-NY), antioxidant enzymes (SOD2, catalase, GPx1), inflammation (TNF-α, IL-6, IL-10), apoptosis (bax/bcl-2 ratio, CASP3 cleaved/pro ratio), neurotrophic factor (GDNF), and neuron count (ChAT, SMI36/32 ratio). Differences were considered significant at $P \leq 0.10$, since this was a pilot study. **Results:** HiD females had 14% higher 3-NY ($P = 0.065$), 21% lower catalase ($P < 0.0001$), 21% higher TNF- α ($P = 0.003$), 13% lower IL-10 ($P = 0.042$), 13% higher CASP3 ($P = 0.010$) and 18% lower ChAT ($P = 0.024$) vs. AI. HiD males had 16% lower 3-NY ($P = 0.073$), 24% lower IL-6 ($P = 0.085$), 26% lower CASP3 ($P = 0.009$) and 19% lower SMI36/32 ($P = 0.098$) vs. AI. **Conclusion:** In G93A mice,

dietary D_3 at 50x AI is toxic in the spinal cord of females but attenuates disease pathophysiology in males. This is in accord with results in the *quadriceps*, as well as functional and disease severity outcomes. Future studies need to identify the sex-specific therapeutic dose of D_3 for ALS. ***Grant Funding Source**: NSERC, Faculty of Health-York U.*

The effect of β-adrenergic stimulation of post-tetanic potentiation of concentric force in fast skeletal muscle

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The purpose of this study was to examine the influence of β-adrenergic stimulation on the amplitude and time-course of force potentiation, in fast twitch mouse muscle. Stimulation induced elevations in myosin RLC phosphorylation, as catalyzed by the skeletal isoform of myosin light chain kinase (skMLCK) is known as the primary mechanism for isometric twitch force potentiation in mammalian skeletal muscle. β-adrenergic stimulation may interact with this process by inhibiting the dephosphorylation of the RLC via myosin light chain phosphatase (skMLCP), thus augmenting the "potentiated state". To test the hypothesis that concentric twitch force potentiation is subject to an influence of β-adrenergic stimulation, we assessed the time course of concentric force potentiation in the absence and presence of epinephrine (1 μM). To this end, extensor digitorum longus (EDL) muscles from wildtype (WT) mice were isolated and incubated for 30-minutes in normal (CON) or epinephrine containing (EPI) Tyrode's solution (in vitro, 25°C) and underwent a twitch timeline over 480 seconds. Concentric force potentiation was assessed by eliciting twitches (30% Vmax) before and after a standard conditioning stimulus (CS) to determine a relative twitch force increase (post/pre). Pilot data showed that this protocol induced concentric twitch potentiation in both conditions as well as no difference between 1μM, 2μM, or 3μM incubations (n = 3). The CS potentiated mean concentric force of the CON and EPI condition to 1.29 ± 0.79 and 1.39 ± 0.77 of unpotentiated, pre-CS values, respectively (n = 8, $P < 0.05$). An analysis between groups showed that after 30 s EPI had significantly increased mean concentric force (n = 8, $P<0.05$) which was partially maintained (1.19 ± 0.80) until 480s, however in the CON group this value dissipated to pre-CS values. In addition, no significant differences in twitch kinetics accompanied epinephrine incubation, as rate of force development (dF/dt) and ½ relaxation values were only slightly elevated. These results indicate interactions between epinephrine and RLC phosphorylation in fast muscle that modulates potentiation as part of the sympathetic fight-or-flight response.

The effect of high and low-load resistance training on body composition in young, resistance-trained men

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Background: Body composition is an important predictor of metabolic health and athletic performance. Performing resistance exercise training (RET) results in a change in body composition where lean body muscle mass increases and fat mass decreases. To date, how various RET protocols impact body composition in a resistance-trained population remains largely unknown. **Methods:** Forty-nine resistance-trained men (mean±SEM, 23.6±0.4 years,

85.9±2.2 kg, 180.5±0.9 cm) were randomly allocated to either a high repetition group (HRG, n=24) at 20-25 repetitions/set or a low repetition group (LRG, n=25) at 8-12 repetitions/set, all for 3 sets to volitional failure. All participants completed a 12-week progressive RET program that included 4 training sessions per week. Changes in total body mass (TBM), total fat mass (TFM), bone mineral content (BMC), total lean mass (TLM), appendicular lean mass (ALM), leg lean mass (LLM), and body fat percentage (BF%) were all measured via dual-energy x-ray absorptiometry. **Results:** In response to RET, LBM (1.58±0.29 kg; P<0.001), ALM (1.12±0.24 kg; P<0.001) and LLM (0.62±0.19 kg; P<0.001) increased while BF% (-0.69±0.27 %; P<0.05) and TFM (-0.57±0.26 kg; P<0.05) decreased. TBM and BMC did not change as a result of the training. There were no significant differences between groups however the change in TBM (HRG; 0.08±0.47 and LRG; 1.19±0.33 kg; P=0.06) and TLM (HRG; 1.01±0.18 and LRG; 1.62±0.28 kg; P=0.07) showed a trend to be greater in the LRG. **Conclusion:** These results indicate that RET performed to failure at either a high or low repetition range induces similar changes in body composition.

Hypoenergetic diet-induced reductions in myofibrillar protein synthesis are rescued by resistance training and balanced daily protein ingestion in older men

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In overweight/obese older adults weight loss has numerous clinical benefits but may accelerate sarcopenic muscle loss; thus, strategies to enhance weight loss with a high fat-to-lean ratio are paramount. In overweight and obese older men, we aimed to determine how the distribution of per-meal dietary protein affected the synthesis of muscle proteins during: energy balance (EB); energy restriction (ER); and ER plus resistance training (ER+RT). Participants were provided with a 4-wk ER diet in which total protein intake was distributed across four daily meals in the proportions 25:25:25:25% in participants randomized to the balanced group (BAL) and 7:17:72:4% in the skewed group (SKEW; n = 10/group). In Phase 1 of ER (wk 0-2) participants continued their habitual physical activity and in Phase 2 (ER+RT; wk 3-4) participants performed RT (3 d/wk). Acute rates of muscle protein synthesis (%/h; myofibrillar and sarcoplasmic) were measured during a 13-h primed continuous infusion of L-[ring-13C6] phenylalanine in response to a BAL or SKEW pattern of protein intake while participants were in EB, after 2-wk ER, and after 2-wk ER+RT. Additionally, ingested D2O was used to quantify chronic (%/d) myofibrillar protein synthesis (MPS) and the synthesis of individual skeletal muscle proteins during Phase 1:ER (wk 0-2) and Phase 2:ER+RT (wk 3-4). Acute MPS was lower in ER than EB in both groups ($p < 0.001$), but was 19% higher in BAL than SKEW ($p < 0.05$). In ER+RT, MPS increased above ER in both groups and BAL was not different from EB ($p = 0.9$). In SKEW MPS remained 14% lower than EB ($p < 0.01$) and 16% lower than BAL ($p < 0.01$). The D2O-determined rates of MPS showed an increase in synthesis of 26 (of 69 measured) individual muscle proteins during Phase 2:ER+RT vs. Phase 1:ER ($p < 0.05$), with no group differences. During ER in overweight/obese older men, a BAL consumption of protein

acutely stimulated the synthesis of muscle contractile proteins more effectively than a traditional, SKEW distribution. Combining RT with a BAL protein distribution 'rescued' the lower rates of MPS during ER. We also report, in addition to changes in MPS, that individual protein synthetic rates were increased with RT showing the potency of this stimulus. These data suggest that the combination of a BAL pattern of daily protein intake, with even protein distribution at each meal, and RT represents an effective strategy to allow for fat mass loss during ER without exacerbating sarcopenic muscle loss.

In-gel detection of a maleimide-based infrared-fluorescent dye as a simplified approach for the detection of protein redox state in cardiac muscle homogenate

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Introduction: Allosteric and covalent modifications of rate-limiting enzymes are thought to be the primary mechanism by which muscle metabolism is regulated. However, reactive oxygen species (ROS) may also regulate muscle metabolism considering 1) nutritional and exercise challenges generate ROS concurrent with altered metabolism, and 2) several enzymes contain ROS-sensitive cysteines. Mass spectrometry detects protein redox state but is expensive and is not easily accessible. IRdye800CW-maleimide (IRD), a highly sensitive maleimide-based infrared dye, has been reported to detect the redox state of immunoprecipitated proteins in cardiac muscle using modified western blot procedures, but has yet to be validated as a novel tool to detect redox conditions throughout the proteome using common in-gel and in-well assays. Therefore, our purpose was to establish the efficacy of IRD in detecting redox conditions across a wide range of protein masses in cardiac muscle lysate separated by SDS-PAGE or detected through an in-well approach. **Methods**: Sprague Dawley rat heart was treated with reducing agents dithiothreitol (DTT) and tris(2-carboxyethyl) phosphine (TCEP) or hydrogen peroxide containing sodium iodide (H_2O_2+NaI) then incubated with IRD (Licor Biosciences) in either CHAPS buffer (pH 7.1) or Tris-HCl (pH7.1). Samples were separated by SDS-PAGE and fluorescent densitometry quantified (Odyssey, Licor Bioscience). **Results**: Compared to Tris-HCl, samples treated with IRD in CHAPS buffer showed greater variability when treated with DTT, TCEP and H_2O_2. With Tris-HCl, TCEP significantly increased fluorescence vs untreated (untreated mean: 29.9 a.u, TCEP mean: 51.4 a.u, $p<0.05$) indicating reduced cysteines. H_2O_2+NaI decreased fluorescence compared to untreated (H_2O_2+NaI mean: 2.7 a.u, $p<0.05$) suggesting oxidation of protein thiols. Contrary to previous findings, DTT showed no significant increase in fluorescence vs untreated sample (DTT mean: 23.9 a.u) possibly through inactivation by protonation at the pH required for dye reactivity with cysteines. Similar, albeit non-significant trends were observed using in-well assays with Tris buffer. Using CHAPS buffer showed no differences among DTT, TCEP and H_2O_2+NaI samples when compared to untreated using both in-gel and in-well assays. **Conclusion**: The greater TCEP fluorescence and lower H_2O_2fluorescence suggests IRD accurately detects protein redox state across much of the cardiac proteome using an in-gel assay with a strong potential for in-well detection of protein redox state. Given the current findings, IRD appears to be a new and simple approach for detecting cellular redox conditions and may be of use to researchers requiring an assessment of tissue redox states.

Endothelial FoxO proteins regulate obesity associated skeletal muscle capillary rarefaction

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Obesity is associated with capillary loss (rarefaction) in skeletal muscle, which can affect muscle function and whole body metabolism. Endothelial forkhead box O (FoxO) transcription factors are potent repressors of capillary growth (angiogenesis) in skeletal muscle and we have shown that its deletion enhances angiogenesis during chronic endurance exercise and ischemic recovery. We hypothesized that FoxO proteins contribute to obesity-related capillary rarefaction. Three PolyI:C injections were administered to induce an endothelial directed FoxO1/3a/4 deletion in Mx1-Cre+;FoxO1/3a/4L/L mice (FoxOΔ) but not in control Mx1-Cre-;FoxO1/3a/4L/L littermates (FoxOL/L). After 16 weeks on a high-fat (HF) or normal chow (NC) control diet, protein levels of FoxO1 (NC: 0.8±0.1 vs. HF: 1.2±0.1) but not FoxO3a (NC: 0.6±0.1 vs. HF: 0.8±0.1) were elevated in the gastrocnemius muscle. This correlated with the occurrence of angiogenesis in the skeletal muscle of FoxOΔ mice (NC: 1.8±0.06 vs. HF: 2.2±0.07) but not FoxO L/L mice (NC: 1.8±0.04 vs. HF: 1.9±0.08). Metabolic characterization revealed reduced weight gain (11.7±1.0 vs. 17.2±1.2 g, respectively) and improved insulin sensitivity (plasma glucose AUC during insulin tolerance test: 23.6±2.1 vs. 37.5±5.8, respectively) in the HF-FoxOΔ group compared to HF-FoxOL/L mice. Analysis of existing GEO datasets from NC and HF mouse muscle established ontology enrichment of angiogenesis-related pathways among genes that correlated inversely with FoxO1 expression. These factors, putatively repressed by FoxO1, are postulated to be differentially expressed in FoxOΔ and FoxOL/L mice. These results provide novel evidence that endothelial FoxO proteins contribute to obesity related impairments to skeletal muscle angiogenesis, weight gain and insulin sensitivity.

Genome-wide analysis of endurance exercise-induced atrial/ventricular remodelling

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Cardiac remodeling is a term used to describe the changes in cardiac biochemistry, gene expression, structure and function of the heart in response to stress, which themselves can be categorized into physiologic (i.e. exercise) and pathophysiologic (i.e. disease). Our lab has identified distinct remodeling patterns between the atria and ventricles of the mouse heart in response to endurance exercise. In particular, the atria developed fibrosis, inflammation and atrial fibrillation (AF), whereas the ventricles showed improved contractile function with reduced vulnerability to induced arrhythmia. Also, our microarray studies showed significant increase in the activation of the TNFa-NFκB pathway, a central regulator of cardiac inflammation and cardiomyocyte survival, in exercise-associated atrial remodeling. In addition to the differential electrical remodeling, we have also' shown differential levels of NFκB activity in exercised ventricle vs. atria, with an elevation of NFκB activity in the atria, while no significant difference in the ventricles. We have also found the involvement of NFκB in regulation of Ito channels, which are responsible for the transient outward K+ current. Dynamic transcriptional regulation of NFκB activity in response to a range of stimuli in each chamber could cause these differential exercise-induced effects. This suggests that the pro-arrhythmic effects of endurance

exercised atria are mediated by altered NFκB-associated gene expression (i.e. ion channel expression) and structural changes (i.e. fibrosis). In addition to further network analysis of our microarray studies, I will also perform deep RNA-sequencing to obtain full atrial/ventricular transcriptomes for 4 mice groups: exercised and sedentary CD1s and TNFa knockout mice. Using RNA-Seq results, I will identify additional genes or pathways with modified expressions using gene ontology and pathway analysis. In conclusion, together with preliminary data from our lab, my study will allow in-depth examination of mRNA and protein expression levels of different ion channels as well as genes related to cardiac inflammation and fibrosis, to examine the underlying mechanism of differential exercise-induced electrical and structural cardiac remodeling in the atria and ventricle.

Resistance training intensity determines neither strength nor muscular hypertrophic gains in young resistance trained men

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Background: Resistance exercise training (RET)-induced skeletal muscle hypertrophy contributes to strength gains. Increased skeletal muscle mass can also lead to numerous health benefits. Previously, we reported that exercise repetition intensity (load as a percent of maximal strength – %1RM) was not a determinant of RET-induced gains in muscle strength and size in an untrained population as long the loads were lifted to volitional muscular failure; however, whether the same is true in trained persons remains to be experimentally confirmed. The aim of this study was to determine the effects of 12 weeks of RET on muscle strength and hypertrophy in a previously-trained population. **Methods:** Forty-nine resistance-trained men (mean ± SEM, 23 ± 1 years, 85.9 ± 2.2 kg, 180 ± 1 cm) were randomly allocated into a high-repetition group (HIGH, n=24: 20-25 repetitions per set to failure) or a low-repetition group (LOW, n=25: 8-12 repetitions per set to failure). Strength (1RM) was assessed throughout the intervention for leg press, bench press, shoulder press, and leg extension. Changes in lean body mass (LBM) and leg lean mass (LLM) were assessed using a dual-energy x-ray absorptiometry (DXA). **Results:** In response to chronic RE there were significant increases in strength in all exercise protocols with no differences between groups ($p>0.05$). Similarly, LBM and LLM increased significantly following training in the HIGH group (1.01 ± 0.87kg, $p<.001$, 0.66 ± 0.92 kg, $p<0.01$ respectively) and the LOW group (1.62 ± 1.4 kg, $p<.001$; 0.66 ± 0.99 kg, $p<0.01$ respectively) with no significant differences between groups ($p<0.05$). **Conclusions:** These data show that RET performed to volitional failure at either a high load or a low load induces similar adaptations strength and lean mass accrual in a resistance-trained young men.

Developing zebrafish as an in vivo model of cardiomyopathy

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Cardiomyopathy is a major cause of heart failure and it is defined as the disease of the heart muscle, with hypertrophic and dilated cardiomyopathy (HCM and DCM) being the two most prevalent types. The development of HCM and DCM has been linked to mutations in sarcomere proteins, including the cardiac actin gene, *ACTC.* To date, sixteen mutations in *ACTC* have been

found in patients with HCM or DCM. Our goal is to determine the molecular mechanisms of HCM and DCM development resulting from mutations in the *ACTC* gene. To understand how these mutations affect the physiology of whole organisms, we are developing zebrafish as an *in vivo* model due to its optical transparency, rapid cardiovascular development and low maintenance costs. First, to overcome the toxic phenotype of systemic and transient *ACTC* misexpression by mRNA injection, we are developing transgenic zebrafish lines that stably express *ACTC* mutants under the control of a heart-specific promoter. Second, we found that knocking down zebrafish cardiac actin genes with morpholinos showed reduced heart rates, edema, and stunted tails. To counter the transient nature of morpholinos, we are using CRISPRs to stably truncate all cardiac actin genes in the zebrafish genome. We hope our physiological and morphological characterizations of the resulting zebrafish lines will provide more insight into how *ACTC* mutations develop into cardiomyopathies and a system for future development of cardiomyopathy-specific therapeutics.

Phosphodiesterase 3 (PDE3) inhibition ameliorates pressure overload-induced cardiac hypertrophy and dysfunction by antagonizing the calcineurin-NFAT signaling pathway

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Cyclic nucleotide phosphodiesterases (PDEs) hydrolyze cAMP and/or cGMP, and thus determine the spatiotemporal kinetics of intracellular cyclic nucleotide signalling. PDE3 family variants (PDE3A, PDE3B) are highly expressed in the heart and vasculature. Family specific PDE3 inhibitors, which do not discriminate between PDE3A and PDE3B, have been shown to increase myocardial contractility, and enhance arterial vascular relaxation. Chronic use of these agents for treatment of heart failure (HF) was shown to worsen outcomes in patients with HF stemming from ischemic-heart disease, and improve outcomes in patients with HF stemming from non-ischemic-heart disease. Based on the evidence we hypothesized that selective modulation of PDE3 family variant activities influences the progression of HF of non-ischemic etiology. To test our hypothesis we used a mouse model of pressure-overload induced HF. We found PDE3A, and not PDE3B protein expression to be elevated, and PDE3-mediated cAMP hydrolyzing activity to be enhanced in failing hearts subjected pressure-overload. Interestingly, chronic ablation of PDE3 variant activities with a family-specific inhibitor (milrinone) was found to suppress the development of cardiac contractile dysfunction, hypertrophic cardiac remodeling, and activation of the calcineurin-NFAT signaling pathway in hearts subjected to pressure-overload. These cardioprotective effects of PDE3 ablation were attributed to inhibition of PDE3A and not PDE3B activity, as PDE3A knock-out (PDE3A-/-) and not PDE3B knock-out (PDE3B-/-) mice exhibited attenuated cardiac contractile dysfunction and hypertrophic cardiac remodelling in response to pressure-overload. These data suggest that modulation of PDE3A and not PDE3B activity by family specific PDE3 inhibitors influences the progression of HF of non-ischemic etiology.

Are the discrepancies between self-report and objectively measured physical activity in individuals with obesity improved after accounting for differences in body weight and exercise energy expenditure?

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Accelerometers can objectively measure the duration and intensity of physical activity (PA). However, existing accelerometer intensity thresholds do not account for body weight, thus individuals who are overweight or obese will have greater energy expenditure (EE) at the same intensity thresholds. This may negatively bias objective PA measures in individuals with obesity. Using NHANES, established intensity thresholds for accelerometers were adjusted to account for differences in body weight, to produce a similar EE rate as lean individuals at moderate and vigorous (MV) intensity thresholds. Using existing thresholds, 78% of overweight and obese over-reported MVPA by 43.6+ 75.0min/day. New thresholds increased the mean duration of objectively measured MVPA by 6.0+12.2 in overweight and 16.2+24.8min/day in obese, while decreasing the frequency of over-reported MVPA by 5% and 16%, respectively. This study suggests even with intensity thresholds that account for body weight, individuals still tend to over-report PA.

The effects of chemotherapeutic microtubule stabilizing and destabilizing drugs on skeletal muscle mitochondrial H2O2 emission and respiration

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Certain chemotherapy drugs trap cancer cells in the G2 mitotic phase of the cell cycle, inhibiting proliferation by either stabilizing or destabilizing microtubules. This action can lead to increases in reactive oxygen species ultimately inducing cell death in tumours. Reports of muscle pain and weakness following in vivo administration of these drugs suggest there may be direct effects on skeletal muscle. Based on an emerging model demonstrating microtubules directly impair outer mitochondrial membrane to ADP/ATP permeability, the purpose of this study was to determine whether these agents directly alter skeletal muscle mitochondrial bioenergetics. We hypothesize that a microtubule-stabilizing drug (Taxol) will restrict ADP/ATP transport and limit the ability of ADP to lower mitochondrial H2O2 emission, and also decrease ADP stimulated respiration. We also hypothesize that microtubule-destabilizing drugs (nocodazole, vinblastine, colchicine, SB225002) will result in greater ADP/ATP permeability and increase the sensitivity of ADP to lower H2O2 emission, while increasing ADP stimulated respiration. Permeabilized muscle fibre bundles prepared from female mouse soleus and white gastrocnemius were incubated for 2 hours in stabilizing or destabilizing drugs at 4°C and were used to determine H2O2 emission +/- ADP (high-resolution spectrofluorometry) and ADP-stimulated respiration (high-resolution respirometery). None of the drugs affected ADP-stimulated respiration in either muscle, although taxol tended to decrease respiration (control; 155.4± 30.5, taxol; 91.2± 6.8, p= 0.07). In soleus, all drugs improved the ability of ADP to lower succinate-induced H2O2 emission albeit at different ADP concentrations (50μM ADP: control 233.7 ± 37.6, cholchicine 128.5 ± 27.7; 100μM ADP: control 204± 28.2, taxol 75.3 ± 17.8, vinblastine 95.0 ± 23.2, SB225002 94.8 ± 23.8; $p < 0.05$ in all comparisons). In white gastrocnemius, taxol and vinblastine induced a surprising increase in succinate induced H2O2 emission (control 111.0 ± 13.8, taxol 160.0 ±

10.9, vinblastine 132.8 ± 13.5; p=0.001 in both comparisons) yet still improved the ability of ADP to lower emission, as did colchicine and vinblastine (50μM ADP: control 51.5 ± 1.7, taxol 39.6 ± 8.3, colchicine 25.3 ± 7.6; 100μM ADP: control 58.6 ± 14.2, vinblastine 29.2 ± 2.7, nocodazole 22.6 ± 3.3; p<0.05 in all comparisons). In conclusion, microtubule stabilizing and destabilizing agents had no effect on ADP-stimulated mitochondrial respiration in soleus or white gastrocnemius contrary to our hypothesis. However, the ability of ADP to suppress H2O2 emission was improved by all drugs in soleus and most drugs in white gastrocnemius, with 2 drugs increasing H2O2 emission in the absence of ADP in white gastrocnemius. Future work will determine whether these responses are due to microtubule-dependent increased ADP permeability in the outer mitochondrial membrane and whether this leads to altered exercise capacity in mice.

Fluvastatin causes hallmark myopathic characteristics and impaired lipid transport in diabetic skeletal muscle

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Background: With 27% of the Canadian population and 47% of the American population currently prescribed statins, this class of lipid-lowering agents is the most widely prescribed pharmaceutical in North America. Upwards of 87 million individuals taking statins complain of myalgia. In the diabetic state, muscle growth, development, and metabolism are already negatively affected. Because of the existing muscular complications in diabetes, it is imperative to assess the effects of statins in the diabetic muscle. **Methods:** Four weeks after diabetic onset, male streptozotocin-induced diabetic (STZ) and WT C57Bl6/J mice were randomly assigned to receive control chow or diet enriched with 600 mg/kg Fluvastatin. Twenty-four days after the commencement of diet administration, muscles were harvested and analyzed. **Results:** WT and STZ muscles from Fluvastatin-consuming mice exhibit increased macrophages, necrotic and centrally-nucleated myofibers, as well as decreased fiber areas. In STZ-Fluvastatin mice, an abundance of ectopic lipid droplets was observed within the muscles, whereas the intramyocellular lipid content was not different from WT mice. Fluvastatin had no effect on lipid content in WT muscle. **Conclusions:** Consistent with the literature, by observing several of the hallmark characteristics of myopathy in this investigation, it is apparent that this short-term administration of Fluvastatin is causing myopathy in both WT and STZ animals. Additionally, the absence of an effect of Fluvastatin on WT lipid content, coupled with the increased external and decreased internal lipid content observed in STZ-Fluvastatin tissue may indicate problems in lipid transport into the muscle fibers, indicating a negative effect of Fluvastatin on the shuttling of lipids in diabetic skeletal muscle.

METRNL and IL-15 – effective therapeutic interventions for high fat diet-induced obesity and glucose intolerance?

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Physical inactivity is a primary modifiable risk factor for obesity and type 2 diabetes (T2D), metabolic diseases that are rampant in both pediatric/adult populations. Endurance exercise has been shown to prevent/attenuate the onset and progression of obesity/T2D. We postulated that exercise mediated these pro-metabolic effects on distal fat depots and other organs via secretory myokines. Our lab has identified two such myokines: interleukin-15 (IL-15) and meteorin-like protein METRNL. Here we administered these two myokines, singularly and in combination, to deduce the respective potency in treating obesity by utilizing a diet-induced model of obesity. C57Bl/6 mice were fed high-fat diet (HFD; 60% kcal from fat) for 6 months until the animals were hyperglycemic and glucose intolerant. Subsequently the animals were divided into HFD control, endurance exercise (15 m/min for 60 min, 5 days/week, END), or given intravenous injections of recombinant METRNL (0.4 ng/kg/day, 3x/week, MET), IL-15 (25 ng/kg/day, 3x/week), or a combination group of both IL-15 and METRNL (COMBO) for 8 weeks. Treatment with METRNL or COMBO normalized serum glucose levels, improved glucose tolerance, and reduced body and fat weight, increased pancreas weight as effectively as the mice in END group (all $P < 0.05$). Inguinal fat analyses showed a marked improvement in gene networks involved in mitochondrial biogenesis and browning of the fat (increased ucp1, prdm16, and cidea expression), and COX activity in END, MET, and COMBO groups in tandem. IL-15 treatment alone had no effect on these indices. Our data clearly establishes treatment with METRNL as an effective therapeutic approach to counteract diet-induced obesity. Funded by NSERC and CIHR.

Similar expression of mitochondrial genes following sprint interval exercise in men and women

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Sprint interval training (SIT), or brief bursts of "all out" exercise with short recovery periods, induces physiologic remodelling similar to moderate-intensity continuous training despite large differences in total exercise volume. A few recent studies have suggested there may be sex-based differences in the adaptive response to SIT. Specifically, relative to women, men have been shown to experience greater increases in muscle protein synthesis, mitochondrial biogenesis and improved markers of glycemic control after several weeks of SIT. The potential impact of sex on the acute metabolic response to SIT has not been previously examined and may explain the observed differences in training adaptations. **Purpose/hypothesis**: To examine the acute response of genes involved in mitochondrial biogenesis to SIT in men and women matched for initial fitness. We hypothesized that in response to a session of SIT, women would have an attenuated increase in mRNA expression for proteins involved in mitochondrial biogenesis. **Methods**: Sedentary men and women were recruited [men/women: n = 8/8, age = 23±4/22±3 yr,

peak aerobic capacity (VO2peak) = 45±6/45±10 ml/kg fat free mass/min]. Women were tested in the mid-follicular phase of their menstrual cycles (day 9±2). Subjects completed an exercise session consisting of 3 x 20 sec "all-out" cycling efforts at a resistance equal to 5.0% of body weight, interspersed with 2 min of recovery. Skeletal muscle biopsies from the vastus lateralis were obtained before, immediately after, and 3 h following exercise. Gene expression of peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PGC-1α), PGC-1α-related coactivator (PRC), PPARδ, and Sirtuin 1 (SIRT1) was determined using real-time quantitative polymerase chain reactions. **Results**: Exercise increased the mRNA expression for proteins involved in mitochondrial biogenesis at 3 h vs. rest, but there were no differences between men and women (men/women: PGC-1α = ~7.3/~7.0-fold increase, PRC = ~1.5/~1.5-fold increase, PPARδ = ~1.2/~1.4-fold increase, SIRT1 = ~1.3/~1.3-fold increase, $p<0.05$ for the effect of exercise). **Conclusion:** A single session of SIT involving 1 min of "all-out" exercise increases the expression of genes involved in mitochondrial biogenesis, with no differences between men and women. This research suggests that men and women respond similarly to SIT with respect to the expression of mitochondrial genes and that the metabolic basis for the observed sex-based differences in training adaptations remains to be elucidated. Supported by NSERC.

The role of AP-1 in skeletal muscle regeneration

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Skeletal muscle makes up around 40% of adult human body weight. Development and regeneration of skeletal muscle is a tightly orchestrated and regulated process. Failure of skeletal muscle to develop pre-natally leads to embryonic lethality, while inability to maintain or repair skeletal muscle post-natally affects quality of life, sometimes even causes death. So it is of great importance to study skeletal muscle development and regeneration in order to overcome skeletal muscle diseases, such as skeletal muscle wasting, which is a common feature of diabetes, duchenne muscular dystrophy, sarcopenia and cachexia. Activator protein-1 (AP-1) is a ubiquitously expressed transcription factor which also has tissue-specific functions. Previous study in our lab showed that the AP-1 subunit, Fra-2, is expressed and co-localized with Pax7 (satellite cell marker) in adult resident stem cells, also termed as satellite cells, and restricted expression in undifferentiated 'reserve' cell population, the analog of resident stem cell, in myogenic cell culture. Inhibition of Fra-2 enhances myogenic differentiation, indicating a positive role of Fra-2 in maintaining myogenic progenitor cells and reserve cells quiescence. To better understand the role of AP-1 in skeletal muscle regeneration, I went on and observed restricted expression of all the AP-1 components in 'reserve' cell population, which makes AP-1 a very promising candidate in skeletal muscle regeneration, which is majorly mediated by satellite cells in adult. Single fiber culture was employed to study the role of AP-1 in satellite cells. ChIP-seq analysis and mass spectrometry was performed to identify target genes and interacting partners respectively.

Innovative prevascularization model for improving transplantation of human embryonic stem cell-derived cardiomyocytes

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Heart failure is the leading cause of death globally. Because the adult heart lacks regenerative capacity, loss of cardiomyocytes, or heart cells, always results from myocardial infarction (MI) or other diseases. The compensatory response from damaged heart ultimately develops into a failure to suffice blood supply to meet metabolic needs of the body, when heart failure occurs. Existing clinical therapies aiming at slowing the rate of cardiac compensatory response cannot stop the progress of the disease. Cell transplantation provides such a possibility of cardiac regeneration. Human embryonic stem cell-derived cardiomyocytes (hESC-CM) represent a potential novel unlimited autologous cell source to facilitate repair and regeneration of injured, diseased or aged heart. However, this approach has been limited by poor survival of hESC-CMs after transplantation, which has been attributed to ischemia (due to insufficient blood supply). This is particular the case for infarcted heart because of the ischemic environment in the infarct area. Though a number of strategies have been pursued in order to promote vascularization and to improve graft survival, including the use of angiogenic growth factors and the co-implantation of endothelial cells with cardiomyocytes, the time required for new blood vessels to form (1-2 weeks) in these approaches is too long compared to the rapid death of transplanted cells (1-3 days). This necessitates other approach such as to prevascularize implants by incorporating pre-made vasculatures to promote early blood perfusion of transplanted hESC-CMs. We have developed methods to obtain microvessel fragments (MFs) from easily accessible and dispensable adipose tissue and pre-form an adaptive microvascular system capable of rapidly progressing into a mature, stable and efficient perfusion circuit upon implantation. Our preliminary data demonstrated that co-implantation of hESC-CMs and MFs lead to the survival of implanted cells after subcutaneous implantation into immunodeficient Rag1 mice. Our ongoing study is focusing upon the generation of pre-vascularized cardiac tissues composed of adipose-derived MFs and hESC-CMs to improve the survival and retention of transplanted hESC-CMs to regenerate the heart tissue and improve cardiac function post-MI.

Moderate running exercise protects against sepsis-induced inflammatory response in skeletal muscle, lung and liver in aged mice

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Background: Sepsis is a systemic inflammatory response to local infection. It is associated with 40-50% mortality in Intensive Care Units. Despite many animal studies and more than 70 clinical trials, there is no definitive treatment of sepsis. The lack of treatment could be due to inappropriate models of sepsis using young animals, as the majority of septic patients are elderly (average 64 yr). Since little is known about the effect of age on the inflammatory response in

sepsis, we aimed to examine sepsis in mice aged to the equivalent of human 64 yr. Since running exercise offers protection across a variety of diseases, we also examined the effect of running on sepsis in aged mice. We hypothesized that aging worsens the outcome of sepsis and that exercise prevents this worsening. **Methods:** Male mice C57BL/6 were aged to 22 mo. During aging, mice were prevented from becoming obese, because obesity itself worsens the outcome of sepsis. Accordingly, mice were fed 70% of ad libitum food. At the target age of 22 mo, mice were subjected to control condition or to severe sepsis initiated by intraperitoneal injection of fecal slurry (3.75 g/kg). At 7 h of sepsis, we used (i) intravital microscopy to assess capillary plugging (a hallmark of sepsis) in hindlimb skeletal muscle, (ii) lung lavage to measure the surfactant function, pulmonary cytokines IL6 and KC, and protein leakage, (iii) lung and liver homogenates to measure myeloperoxidase (inflammatory neutrophil infiltration), and (iv) systemic blood to determine platelet count (low count signifies severe sepsis). Starting 2 mo prior to target age, a subgroup of mice ran voluntarily on wheels, in a one-day-on and one-day-off fashion to allow recovery between runs. These mice were fed 90% of ad libitum food to prevent obesity during their last 2 mo of aging. **Results:** When compared to our published data from septic young mice (2-3 mo), aging significantly increased sepsis-induced capillary plugging and lung myeloperoxidase level. Sepsis in aged mice also caused high levels of pulmonary IL6 and KC, and liver myeloperoxidase, and low levels of surfactant function and systemic platelet count. Exercise (i) reduced the high levels of capillary plugging, lung and liver myeloperoxidase, and pulmonary IL6 and KC, and (ii) restored the low levels of surfactant function and platelet count toward control. **Conclusion:** Moderate running exercise protects against the high level of inflammatory response in skeletal muscle, lung and liver in septic aged mice.

Developing a method for measuring length-dependent activation in isolated cardiomyocytes

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Rationale: It has been well-established for more than a century that systolic contractile force increases with the extent of ventricular filling in the heart, a phenomenon known as Frank-Starling's Law which is associated with increased contractile force with increasing sarcomere lengths, a phenomenon called length-dependent activation (LDA). In turn LDA is associated with, and largely determined by, length-dependent binding of Ca2+ to the contractile proteins with notable changes in Ca2+ transient amplitudes. We hypothesize that cardiac relaxation at the termination of the blood ejection during the cardiac cycle is strongly influenced by LDA activation. Consequently, we further hypothesize that the diastolic dysfunction seen in 50% of heart failure patients arises from changes in LDA. **Objective:** To measure length-dependent activation in Intact single isolated cardiomyocytes. **Methods and Results:** Ventricular myocytes were isolated from the endocardium of mouse hearts by enzmatic digestion. Individual isolated cells with clear striations were attached to glass rods using a bio-adhensive MyoTak (Ionoptix). We simultaneously measured sarcomere length, Ca2+ and force in single isolated cardiomyocytes following pacing at 0.5 Hz using external field stimulation (10V/cm, 2ms pulse width) with 1.2 mM extracellular Ca2+. The sarcomere length was estimated in real-time using fast-fourier transform of a portion of the cell image containing ~20 sarcomeres. Ca2+ was measured using Fura-2 loaded into the cell via incubation of the myocytes with the acetoxymethyl ester of Fura 2 (Fura2-AM). Force was measured via deflection of the glass rods arising from forces generated by both (or either) contraction or passive elements following

stretch above slack length. The deflections were calibrated into force units in separate experiments in which the glass was deflected with an Akers strain gauge (AE301) force transducer. Two important features of these recordings should be noted. First there is a large passive force that develops when cells are stretched from 1.8µm (slack length) to over 2.0µm. Second, there is a large amount of internal shortening during each contraction. As a result of these features, in order to estimate the active force-length relationship (and therefore LDA), it is necessary to accurately subtract the passive force generated by the cardiomyocytes from the active force generated during the contraction at the same sarcomere lengths. In order to determine the active force-sarcomere length relationship, the force-sarcomere length during passive stretch (no contractions) was plotted along with the relationship of the peak force and simultaneous sarcomere length observed during contractions. Finally the active force during muscle contraction was calculated by subtracting the passive force from the total force. The active force-calcium relations were also plotted and compared at short and long sarcomere lengths. **Significance and Conclusion**: We have developed a method for accurately measuring the LDA which will be useful for assessing the role of changes In LDA in heart disease.

Regulation of Hspb7 by MEF2 and AP-1 in muscle atrophy

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Although the individual roles of MEF2 and AP-1 in myogenesis have been explored, the relationship between these proteins in muscle development, disease and aging has not been clearly defined. Using MEF2A ChIP-exo data, c-Jun ChIP-seq data and predicted AP-1 consensus motifs, we identified common MEF2 and AP-1 target genes, several of which have a function in regulating the actin cytoskeleton. Since muscle atrophy often targets the actin cytoskeleton for degradation, we characterized the expression of five putative MEF2/AP-1 target genes (Dstn, Flnc, Hspb7, Lmod3 and Plekhh2) under glucocorticoid induced atrophy using Dexamethasone (Dex) in C2C12 myoblasts. Hspb7, a small heat shock protein with no characterized role in skeletal muscle to date, showed strong upregulation in Dex treated cells. Further investigation into the regulation of Hspb7 expression revealed a common role for MEF2 and AP-1 in regulating this gene under atrophic conditions. Loss of MEF2A, Fra-2 or c-Jun using siRNA-mediated gene silencing prevented Dex-regulated induction of Hspb7, however, only MEF2A could co-operate with Dex to induce Hspb7 expression in myoblasts. RNA isolated from the gastrocnemius of 3-, 8- and 20-week old mice showed that Hspb7, MEF2A, Fra-2 and c-Jun expression decrease with age. In C2C12, Hspb7 expression was detected late in myogenesis and was localized in the cytoplasm. Exogenous expression of Hspb7 led to the accumulation of pro-LC3B, the form of LC3B expressed prior to cleavage into LC3B-I. Lastly, Hspb7 was able to interact with pro-LC3B but not LC3B-I or –II. Together this data indicates that MEF2 and AP-1 are necessary but not sufficient to regulate Hspb7 expression, and may rely on additional regulation by the glucocorticoid receptor. Additionally, we provide evidence of a role for Hspb7 in autophagy which has implication in muscle atrophy and aging.

Mechanisms of atrial fibrillation induced by endurance exercise

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Atrial fibrillation (AF) is the most common supraventricular arrhythmia. Long-term endurance exercise causes a 2-10 fold increase in the risk of developing AF. The molecular mechanisms underlying endurance exercise induced AF remain largely unknown. Our laboratory has developed two 6-week endurance exercise mouse models (swim and voluntary running on free wheels), which revealed increased vulnerability to AF *in vivo* and *ex vivo*. Parasympathetic nerve activity (PNA) acts on the SA node to induce sinus bradycardia and acts on myocytes across the atria to induce repolarizing I_{KAch} currents which results in PNA mediated spatially heterogeneous shortening of action potential durations (APDs). Sinus bradycardia and shortened APDs increase the probability of propagation of ectopic re-entry circuits, which underlie AF. Both the swim and free wheel models demonstrate *in vivo* exercise mediated sinus bradycardia with no bradycardia in *ex vivo* denervated atria. Heart rate variability (HRV) studies demonstrate exercise-mediated elevation of HRV high frequency power in both models. Together, reversal of *in vivo* sinus bradycardia in denervated atria and elevated HRV high frequency power indicate that free wheel and swim models have increased parasympathetic nerve activity (PNA). Results from intracellular action potential (AP) recordings from myocytes in *ex vivo* denervated atria indicate that swim exercise induces action potential duration (APD) prolongation whereas free wheel exercise induces arrhythmogenic APD shortening. Perfusion with a muscarinic receptor agonist in sedentary controls induces spatially heterogeneous APD shortening across the atria. Work is currently underway to characterize this relationship in the exercise models, however PNA induced APD heterogeneity in sedentary controls and elevated PNA in exercise models indicates that in exercise models, PNA may induce in increased spatially heterogeneous shortening of APDs resulting in temporally divided areas of the atria and disruption of a coordinated wave of depolarization. These results in combination with previous work within our laboratory demonstrating fibrosis within the atria of swim exercised mice indicates that swim and free wheel exercise induce increased vulnerability to AF mediated by distinct mechanisms involving structural and electrical remodeling respectively.

Mild exercise training improves endurance capacity, but not measures of skeletal muscle health in T1DM mice

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Type 1 diabetes mellitus (T1DM) is a disease that causes the body to secrete little to no insulin to the rest of the body. This leads to the diminished ability to control blood glucose, and in the long-term, may potentially lead to neuropathy, nephropathy, myopathy, retinopathy and more. Currently, exercise is prescribed as a treatment for type 2 diabetes, as exercise has been shown to attenuate the onset of the disease and improve glycemic control. This may be due to the ability of exercise to stimulate GLUT4 receptors in skeletal muscle, allowing for glucose metabolism and glycogen storage in the muscle. Several studies in T1DM patients have suggested that exercise

has the potential to decrease blood glucose and improve insulin sensitivity as well. The current study aims to elucidate the effects of a mild intensity exercise protocol on measures of skeletal muscle health, and how this may translate into attenuating the long-term complications of diabetes. Wild-type and C57BL/6-Ins2Akita/J (Akita) mice, a model for T1DM, underwent a low-intensity treadmill running regime three times a week, for 16 weeks total. It was determined that although an improved endurance capacity was observed ($P<0.05$), there were no corresponding changes in the measures of skeletal muscle health and glycemic control. This may suggest that the improvements in endurance capacity after exercise did not stem from improvements in muscle health, and further research is required to provide insight as to whether exercise is an effective treatment for T1DM, and the mechanisms behind it.

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