7th Annual
Muscle Health Awareness Day
May 27, 2016

Program and Abstracts

Muscle Health & Research Centre

Adaptation • Development • Metabolism • Disease

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Date: May 27, 2016

To: All Participants

From: David A. Hood, MHRC Director

Welcome to the 7th Annual Muscle Health Awareness Day

The Muscle Health Research Centre at York University welcomes you to **MHAD7**, our 7th annual "***Muscle Health Awareness Day***", designed to bring together scientists, faculty members, graduate students and post-doctoral fellows to discuss issues related to skeletal and cardiac muscle physiology, metabolism, adaptation, development and disease.

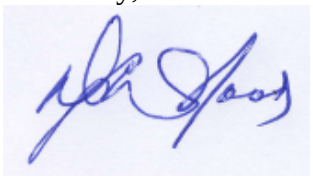
MHAD7 is dedicated to the memory of our colleague and former MHRC Faculty member, **Dr. Enzo Cafarelli**, who passed away earlier this year of cancer. He was a wonderful mentor and supporter of MHAD who will be greatly missed within the exercise physiology community in Canada.

We have 9 great speakers for **MHAD7**. The focus this year is on 1) glucose and fat metabolism, adiposity and exercise, 2) cardiac stem cells and mitochondria, 3) muscle vascularity, and 4) skeletal muscle regeneration, disease and weakness.

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, volunteers and sponsors for their participation, and for helping to continue to make this a successful event. Please enjoy **MHAD7**!

Sincerely,



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

MHAD7 is dedicated to the memory of

Dr. Enzo Cafarelli

1942-2016

Professor, School of Kinesiology and Health Science

MHRC Faculty Member,

Colleague, Mentor and Friend

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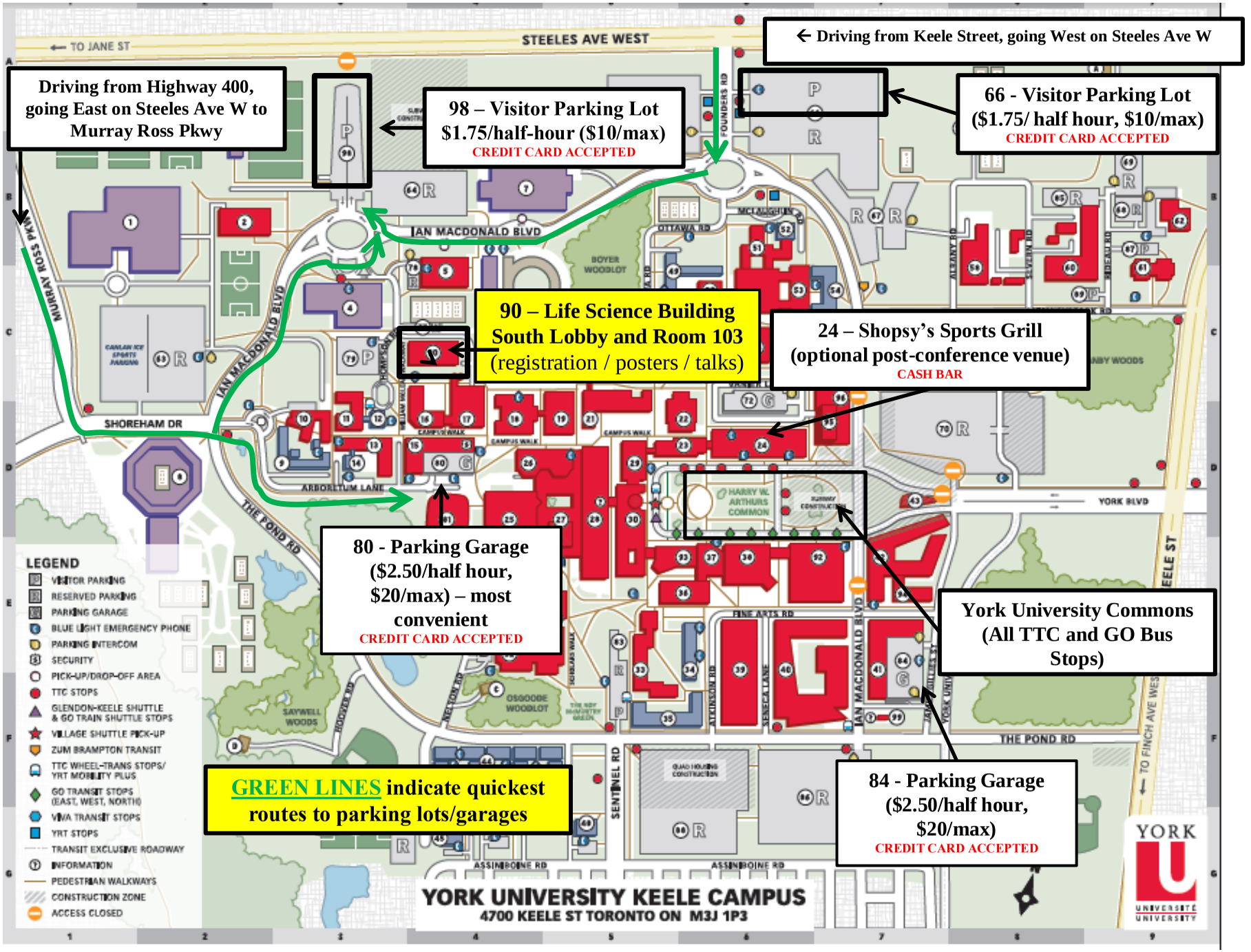
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7th Annual Muscle Health Awareness Day Program
Friday May 27, 2016
Life Science Building South Lobby and Room 103, York University

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

Session 1:Muscle, metabolism and exercise (9:00-10:35)
Session Chair: Dr. Ola Adegoke, *York University*

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

9:05-9:35 – Dr. Amira Klip, *The Hospital for Sick Children/University of Toronto*
Regulation of GLUT4 in muscle cells by insulin and contraction

9:35-10:05 – Dr. Erin Kershaw, *University of Pittsburgh*
Role of adipocyte and skeletal muscle lipolysis in muscle metabolism and function

10:05-10:35 – Dr. Martin Gibala, *McMaster University*
Physiological and health adaptations to low-volume interval training

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

Session 2: Cardiac muscle and circulation (11:30-12:30)
Session Chair: Dr. Tara Haas, *York University*

11:30-12:00 – Dr. Ren-Ke Li, *UHN/Toronto General Research Institute (TGRI)*
Cardiac cell therapy: From repair to rejuvenation

12:00-12:30 –Dr. Graham Fraser, *University of Western Ontario*
Microvascular supply and demand: How adequate muscle oxygenation is achieved

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

Session 3: Muscle, growth and repair (2:00-4:00)
Session Chair: Dr. John McDermott, *York University*

2:00-2:30 – Dr. Jeff Dilworth, *University of Ottawa*
Epigenetic regulation of cell fate transitions during muscle regeneration

2:30-3:00 – Dr. Jim Dowling, *The Hospital for Sick Children/University of Toronto*
Fish and mice and patients, oh my! Drug discovery for myotubular myopathy.

3:00-3:30 – Dr. Jane Batt, *St. Michael's Hospital/University of Toronto*
ICU Acquired Weakness (ICUAW)- A devastating complication of critical illness

3:30-4:00 – Dr. Yan Burelle, *University of Montreal*
Integrating mitochondrial quality control in our understanding of muscle plasticity: Lessons learned from the cardiac system

4:00-4:10 – Poster Awards, Concluding Remarks

7th Annual Muscle Health Awareness Day Speaker Profiles

	**Dr. Jane Batt**, Keenan Research Centre for Biomedical Science, St. Michael's Hospital Dr. Batt is a Respirologist and Scientist at the Keenan Research Centre for Biomedical Science at St. Michael's Hospital. Her research is focused on defining the molecular mechanisms underlying skeletal muscle atrophy, and identifying novel mediators of muscle mass loss.
	**Dr. Yan Burelle**, Université de Montréal Dr. Burelle is an Associate Professor in the Department of Biomedical Sciences in the Faculty of Medicine at the University of Montreal. His research examines mitochondrial function and mitochondrial quality control mechanisms, and their roles in cardiac and skeletal muscle disease.
	**Dr. Jeff Dilworth**, University of Ottawa Dr. Dilworth is a Senior Scientist in the Regenerative Medicine Program at the Ottawa Hospital Research Institute, and an Associate Professor in the Department of Cellular and Molecular Medicine, as well as the Department of Medicine. The aims of his research are to better understand the role of epigenetic regulation of stem cells, and how this influences stem cell health and function.
	**Dr. James Dowling,** The Hospital for Sick Children / University of Toronto Dr. Dowling is a Physician in Neurology and a Senior Scientist in Genetics and Genome Biology at the Hospital for Sick Children, as well as an Assistant Professor in Molecular Genetics at the University of Toronto. His research focuses on the development of gene- and drug-based therapies for childhood neuromuscular diseases, such as congenital myopathies and muscular dystrophies.

	**Dr. Graham Fraser**, University of Western Ontario Dr. Fraser is an Adjunct Assistant Professor in the Department of Medical Biophysics at the Schulich School of Medicine and Dentistry at the University of Western Ontario. He is interested in the study of oxygen transport and the regulation of microvascular blood flow.
	**Dr. Martin Gibala**, McMaster University Dr. Gibala is a Professor and Chair in the Department of Kinesiology, and a member of the Exercise Metabolism Research Group, both at McMaster University. His current work focuses on the study of the metabolic adaptations to high-intensity interval training (HIIT), and the role of nutrient availability to influence adaptations to exercise training.
	**Dr. Erin Kershaw,** University of Pittsburgh Dr. Kershaw is an Associate Professor of Medicine in the Division of Endocrinology at the University of Pittsburgh. Her current research examines the roles of glucocorticoid and lipid metabolism in the development of obesity and the metabolic syndrome.
	**Dr. Amira Klip,** The Hospital for Sick Children / University of Toronto Dr. Klip is a Senior Scientist in Cell Biology at the Hospital for Sick Children, and a Professor in Biochemistry, Paediatrics and Physiology at the University of Toronto. Her research interests lie in unraveling the mechanisms by which muscle contraction and insulin regulate glucose uptake.
	**Dr. Ren-Ke Li,** Toronto General Research Institute (TGRI)/ University Health Network Dr. Li is a Senior Scientist at the TGRI, and a Professor in the Division of Cardiovascular Surgery at the University of Toronto. His work focuses cell transplantation into damaged myocardial tissue for the restoration of cardiac function and tissue engineering for the repair of cardiac defects.

Poster Presentation Abstract List

Poster number	First Author (Surname)	Abstract Title	University Affiliation
1	Abbaszadeh	Glycolytic control of stem cell fate decision	York University
2	Allison	Stair Climbing: A practical form of sprint-interval training	McMaster University
3	Beatty	The role of mRNA translation inhibitor programmed cell death 4 (PDCD4) regulates apoptosis essential for muscle differentiation	York University
4	Bonafiglia	Acute regulation of PGC-1α mRNA predicts the response in SDH activity following training	Queen's University
5	Calic	Influence of Children's Aerobic Power and Muscle Function on Guided Sport Specific Active Play during Early Childhood	York University
6	Castellani	Acute Exhaustive Exercise Protects Against Antipsychotic-Induced Hyperglycemia In Male C57BL/6J Mice	University of Guelph
7	Cervone	Acylated and deacylated ghrelin do not directly alter insulin-dependent or independent skeletal muscle glucose uptake in rats	University of Guelph
8	D'Aquila	Teneurin C-terminal Associated Peptide (TCAP), a novel regulator of glucose uptake and metabolism in skeletal muscle: A potential therapeutic for neuromuscular dysfunction	University of Toronto
9	Dial	Expression of the Dystrophin-Associated Protein Complex in the Absence of Skeletal Muscle AMPK	McMaster University
10	Edgett	The effect of acute and chronic sprint-interval training on LRP130, SIRT3, and PGC-1α expression in human skeletal muscle	Queen's University
11	Erlich	Regulation of TFEB transcriptional activity and translocation during exercise by PGC-1α	York University
12	Farquharson	Endothelial-Derived Erythropoietin: A Novel Role In Regulating Skeletal Muscle Glycogen Content	University of Guelph
13	Hekmat	Cardiac autonomic responses to structured vs. unstructured physical activity in children	York University
14	Henein	Characterizing The Role of Actin Variants in HCM	University of Guelph
15	Hughes	Early onset of muscle-specific alterations in mitochondrial bioenergetics in the D2.B10-DMD^{mdx}/2J mouse model of Duchenne Muscular Dystrophy	York University
16	Klein	Novel paracrine requirement for cardiac-derived erythropoietin in cardiogenesis	University of Guelph
17	Knuth	Brown adipocyte recruitment following chronic cold exposure and short photoperiodism in the white-footed mouse, *Peromyscus leucopus*	Trent University
18	Kolahdouzan	Vitamin D_3 deficiency negatively impacts disease pathophysiology in the spinal cord of the G93A mouse model of amyotrophic lateral sclerosis	York University
19	Lakin	Exercise-induced right ventricular dysfunction and increased arrhythmia susceptibility in mice	University of Toronto
20	Liu	Analysis of Regulated Thin Filaments Variants	University of Guelph

21	Miotto	In the absence of phosphate shuttling, exercise reveals the in vivo importance of creatine-independent regulation on mitochondrial ADP transport	University of Guelph
22	Moosavi	Modulation of the UPR in relation to mitochondrial biogenesis adaptations in muscle cells	York University
23	Motamed	PDCD4 Depletion Stabilizes Myofibrillar Protein Abundance in L6 Myotubes	York University
24	Nguyen	Chronic cold exposure alters gut microbiota that promotes browning of white adipose tissue	McMaster University
25	Nissar	'Forcing' changes in the muscle stem cell transcriptome	University of Toronto
26	Oh	Differential Atrial and Ventricular Remodeling in Response to Endurance Exercise	University of Toronto
27	Ojehomon	Developing Zebrafish as a Model Organism to Study Cardiomyopathy	University of Guelph
28	Peppler	Physical activity protects against lipopolysaccharide-induced inflammation in tricep muscle and adipose tissue	University of Guelph
29	Rebalka	Fluvastatin causes myopathic characteristics and altered lipid compartmentalization in diabetic muscle, but may facilitate repair following skeletal muscle damage	McMaster University
30	Rusiecki	Chronic endurance training in mice alters gut microbiome and enhances overall endurance capacity in their sedentary co-habitants	McMaster University
31	Saleem	Using exosomes to 'cure' Duchenne Muscular Dystrophy (DMD) – a non-immunogenic genetic therapy to restore dystrophin expression in mdx mouse model of DMD	McMaster University
32	Salerno	Does Estrogen Protect Against Skeletal Muscle Damage and the Cellular Stress Response?	University of Toronto
33	Sidhu	Exploring actomyosin molecular interactions using short F-actin oligmers	University of Guelph
34	Sové	Characterizing insulin resistance using a spatial mathematical model of glucose transport in skeletal muscle interstitium	Western University
35	Stouth	Protein Arginine Methyltransferase Expression During Denervation-induced Skeletal Muscle Plasticity	McMaster University
36	Triolo	Cellular remodelling of C2C12 myoblasts during recovery in response to electrical stimulation	York University
37	Turnbull	Nutritional targeting of mitochondrial bioenergetics in cancer: lipid incubation increases Caspase 3/7 activity and H2O2 emission in MCF7 cancer but not HT29 and non-cancer epithelial cells	York University
38	Zarrin-Khat	The Role of Mitophagy in Aging Cardiac Muscle	York University

Glycolytic control of stem cell fate decision

Maryam Abbaszadeh[1,2], Debasmita Bhattacharya[1,2], Deanna P. Porras[1,2], Anthony Scimè[1,2]
[1]Stem Cell Research Group, [2]Molecular, Cellular and Integrated Physiology, Faculty of Health, York University, Toronto, Ontario, Canada, M3J 1P3

Stem and progenitor cell fates play a crucial role in health and disease progression. While white adipocytes store energy in the form of lipid, brown-type adipocytes metabolize fatty acid and glucose, thus maintaining energy homeostasis. Interestingly, we found that the metabolic state of quiescent stem and progenitor cells regulates their adipocyte lineage fates. Our lab has previously shown that the transcriptional co-repressor p107 acts as an adipocyte lineage determinant factor. Its absence leads to the differentiation of brown-type over white adipocytes of non-committed stem and progenitor cells. Our new data brings to light how p107 accomplishes this, by altering the glycolytic state of non-committed stem cells. Notably, at quiescence, p107-depleted stem and progenitor cells undergo anaerobic glycolysis. Indeed, we found that oxamate, which inhibits LDHa, a necessary enzyme of the glycolytic pathway, blocks brown adipocyte differentiation of non-committed stem cells. Importantly, we found a significant decrease in the expression of pro-thermogenic factors in differentiated knockdown p107 cell lines and knockout primary adipogenic stromal vascular cells with oxamate treatment. On the other hand, the addition of lactate, a by-product of enhanced glycolysis, to non-committed stem cells, resulted in brown versus white adipocyte differentiation. This data is the first to couple metabolic reprogramming to the control of stem cell and progenitor adipocyte lineage fates.

Stair Climbing: A practical form of sprint-interval training

Mary K. Allison[1], Brian J. Martin[1], Martin J. MacInnis[1], Brendon Gurd[2], and Martin J. Gibala[1]
[1]McMaster University, Hamilton, ON; [2]Queens University, Kingston, ON

Sprint Interval Training (SIT), involving brief intermittent bursts of intense exercise, has been touted as a time-efficient alternative to traditional endurance training for improving cardiorespiratory fitness (CRF) and clinical markers of health. Most SIT protocols, such as Wingate-based cycling, have been studied in a laboratory setting and require specialized equipment, which is impractical for many individuals. Stair climbing may be a more suitable and accessible alternative to laboratory-based SIT. While established as an effective form of exercise to enhance CRF, the minimum effective "dose" of stair climbing remains unknown. PURPOSE: To determine whether brief and intermittent bouts of intense stair climbing improves CRF. METHODS: Twelve sedentary but otherwise healthy women (age = 26±11 y; BMI = 23.6±3.0 kg/m2) trained 3 d/wk for 6 wk. Each 10-min training session involved a 2-min warm up, 3x20-s bouts of intense stair climbing interspersed with 2 min of recovery, and a 3-min cool-down. Training was performed using the stairwell of a 6-storey campus building. Subjects were instructed to climb stairs as fast as safely possible. Recovery periods involved descending the stairs slowly and walking on flat ground. RESULTS: Participants climbed 59±4 stairs (height climbed = 11.4±0.8 m) during each bout. Mean power output was 365±40, 354±38 and 337±35 W, with corresponding ratings of perceived exertion scores of 12±1, 14±2, and 16±2, respectively. Training elicited 81±4% of maximum heart rate (HRmax) on average over the 10-min session, 86±3% HRmax during the 3x20-s bouts, and peak HR was 94±3% of HRmax. Peak oxygen uptake (VO2 peak) increased by 12% from 28.9±3.7 to 32.4±3.6 mL•kg-1•min-1 after training ($p<0.01$). Absolute VO2 peak similarly increased from 1.8±0.2 to 2.0±0.3 mL•min-1

($p<0.01$) as body mass was unchanged after training (62.2±9.5 vs. 62.6±9.6 kg; $p>0.05$). CONCLUSION: Brief and intermittent bouts of intense stair climbing, involving only 3 min of "all-out" exercise within a 30-min time commitment per week increased CRF by ~1 metabolic equivalent over 6 wk. This change is similar to that previously reported after laboratory-based SIT protocols of similar duration, and traditional endurance training involving a much higher exercise volume and time commitment.

The role of mRNA translation inhibitor programmed cell death 4 (PDCD4) regulates apoptosis essential for muscle differentiation

Brendan Beatty, Naomi Maeda, Olasunkanmi Adegoke
Muscle Health Research Centre, York University

Skeletal muscle wasting is an important indicator of disease progression in a variety of diseases including cancer, HIV/AIDS, and diabetes. Preventing muscle wasting has the potential to improve patient outcomes. Understanding the process of muscle development is essential to preserving muscle mass. Myogenesis is the process of muscle formation. It is a major part of embryonic development, but also required for repair/regeneration of adult muscle tissue. Muscle differentiation, a part of myogenesis, is a process in which myoblasts fuse and form elongated, multi-nucleated myotubes, synonymous with myofibrils. Programmed Cell Death 4 (PDCD4), a substrate of the mechanistic target of rapamycin complex 1 (mTORC1), has classically been studied in cancer models as a tumour suppressor. Because it inhibits mRNA translation initiation and is pro-apoptotic, it may regulate cell differentiation. We studied this in L6 skeletal muscle cells. PDCD4 expression increased at the onset of differentiation (day 1 and day 2) by 50%. Consistent with its role as an inhibitor of mRNA translation, we observed a two-fold increase in the association of PDCD4 with eukaryotic initiation factor-4A(eIF4A) on day 2 of differentiation compared to day 0. This change in PDCD4 expression was not non-specific because the level of S6, another substrate of ribosomal protein S6 kinase (S6K1), did not change. S6K1 and AKT are involved in regulating PDCD4 abundance and intracellular location. The levels of phosphorylated S6K1 and AKT tended to rise during differentiation. Finally, apoptosis has recently been shown to be needed for muscle cell differentiation. The increase in processed caspase-3 during differentiation coincided with the rise in PDCD4. Indeed, cells depleted of PDCD4 are unable to process caspase-3. Taken together PDCD4's role in muscle differentiation represents a possible therapeutic target for muscle wasting conditions.

Acute regulation of PGC-1α mRNA predicts the response in SDH activity following training

J.T. Bonafiglia[1], B. A. Edgett[1], B. L. Baechler[2], J. Quadrilatero[2], and Brendon Gurd[1]
[1]School of Kinesiology and Health Studies, Queen's University, Kingston, ON K7L 3N6, Canada; [2]Department of Kinesiology, University of Waterloo, Waterloo, ON N2L 3G1, Canada

Elevations in mitochondrial protein content following repeated exercise bouts (i.e. training) are preceded by transient increases in mRNA expression of the transcriptional coactivator peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1α). However, whether this transcriptional regulatory response to acute exercise predicts skeletal muscle adaptation to training is unknown. The purpose of the present study was to determine whether a relationship exists between acute changes in PGC-1α mRNA expression and skeletal muscle

adaptation following training. Skeletal muscle biopsies were obtained from fourteen healthy men (22.4 ± 2.4 years) at rest (PRE) and 3 hours after (3HR) one session of sprint interval training (SIT; 8 x 20-second intervals at ~170% of VO2peak work rate separated by 10 seconds of load-less cycling). Participants then completed 6 weeks of SIT (4 days per week) and additional biopsies were collected ~72 hours after the 8th (MID) and last training session (POST). To examine skeletal muscle adaptations to SIT, succinate dehydrogenase (SDH) activity was measured in type I and type IIA fibres via histochemical analysis. PGC-1α mRNA was elevated 3 hours following one bout of SIT (+284%, $p < 0.001$) and 6 weeks of SIT had a main effect on SDH activity (type I: +31%; type IIA: +37%; $p < 0.001$). The acute response in PGC-1α mRNA was strongly related to the changes in SDH activity following training (type I: $r = 0.67$, $p < 0.05$; type IIA: $r = 0.81$, $p < 0.001$). This study shows that the response in PGC-1α mRNA expression following acute exercise predicts the subsequent training response in SDH activity. Furthermore, these results suggest that the individual sensitivity to exercise training may be detected by the magnitude of change in PGC-1α mRNA following acute exercise.

Influence of Children's Aerobic Power and Muscle Function on Guided Sport Specific Active Play during Early Childhood

Dusan Calic, Moghaddaszadeh, A., Hynes, L. and Belcastro, A.N.
Pediatric Exercise Physiology Laboratory, School of Kinesiology and Health Science, York University, Toronto, ON, M3J 1P3.

INTRODUCTION: There is a lack of research on the relationships between fitness parameters (i.e. grip strength) and physical activity (i.e. %MVPA) during the early childhood period (specifically 5-7years). The purposes of this study were to quantify: a) the participation (time and intensity of PA) of children in guided active play format using sport specific game skills by measuring a range of physical activity characteristics b) the relationships among muscle strength, leg power, and aerobic power (CRF) with physical activity characteristics (kcal/10sec, kcal/session, METs, %Sedentary time, %MVPA time) during early childhood. **METHODS:** Fourty-two children (ages 6.3±0.8 years; weight 28.8±7.56 kg) registered in a summer camp program at an elementary school in northwest Toronto, were recruited to participate in a sport-specific (guided active play) program for 5 weeks (5x/week; 1hr/day). Estimates of energy expenditure and %moderate-vigorous PA (%MVPA) were used to assess PA participation from regression equations derived from calibrated motion sensors (ActiGraph GT3X+). Muscle function and CRF were recorded before and after the 5-wk sports program using procedures such as the 20m shuttle run, standing vertical jump, and grip strength using a hand grip dynamometer (coefficient of variability <3%). Statistical procedures were performed using ANOVA and Pearson Correlation 'r' (SPSS 23.0). **RESULTS:** Children's PA participation averaged 182±53 kcal/session, 0.55±0.04 kcal/10sec, and 3.60 ±0.6MET across the 5wks. The proportion of time children spent in MVPA was 39±10% ($p<0.05$). Minimal differences in PA participation were observed for age (5-7years) ($p>0.05$). Leg power was positively correlated with %MVPA for 4 out of the 5 sports (handball, football, track/field, and basketball ($p<0.05$), while strength was positively correlated with %MVPA for 3 out of the 5 sports (soccer, football, and handball) ($p<0.05$). Age and weight were only associated with soccer ($p<0.05$) but no other sport. **CONCLUSION:** There are definite advantages behind using a sport-specific guided active play program for improving children's PA participation during the early childhood period. Significant relationships do exist for muscle function and CRF with PA participation. Improving these

fitness parameters with involvement in MVPA from an early age could prove beneficial in middle-late childhood where motor and fitness competence drives PA participation.

Acute Exhaustive Exercise Protects Against Antipsychotic-Induced Hyperglycemia In Male C57BL/6J Mice

Laura N. Castellani, Willem T. Peppler, Rebecca E. MacPherson, David C. Wright
Department of Human Health and Nutritional Sciences, University of Guelph, Guelph Ontario Canada, N1G 2W1

Second generation antipsychotics (SGAs) such as olanzapine are efficacious in treating symptoms of schizophrenia and bipolar disorders. However, use of SGAs is linked to robust metabolic disturbances. Notably, acute olanzapine (OLZ) exposure has been shown to result in rapid impairments in glucose homeostasis. Given the reputed ability of exercise to promote insulin-independent glucose uptake as well as to improve insulin sensitivity and potentiate glucose stimulated insulin secretion, the purpose of the present study was to assess the ability of exercise to protect against acute OLZ-induced hyperglycemia. Male C57BL/6J mice were exercised to exhaustion (20% incline, approximately 75 minutes) or remained sedentary immediately prior to olanzapine treatment (5mg/kg; intraperitoneal injection). Notably, previously exercised mice were protected against OLZ-induced increases in blood glucose and this was accompanied by increases in serum interleukin-6 (IL-6) and insulin. Interestingly, IL-6 alone was not sufficient to mimic the protective effects of exercise. Similarly, the protective effective of exercise was maintained in IL-6 -/- mice suggesting that IL-6 is not necessary for exercise-induced glycemic control. Exhaustive exercise did however enhance whole body insulin tolerance and hepatic insulin action following olanzapine treatment. In conclusion, the current study suggests a protective effect of exhaustive exercise against acute OLZ-induced elevations in blood glucose, perhaps as a result of exercise-induced improvements in peripheral insulin action.

Acylated and deacylated ghrelin do not directly alter insulin-dependent or independent skeletal muscle glucose uptake in rats

Daniel T. Cervone and David J. Dyck
University of Guelph

Ghrelin is an appetite-stimulating (orexigenic), gut-derived hormone that regulates feeding, energy balance and peripheral tissue metabolism. It is a 28-amino acid peptide that can be acylated post-translationally which governs its binding to the growth-hormone secretagogue receptor subtype 1a (GHS-R1a). While acylated ghrelin (AG) is largely considered to be the active form, given its capacity to stimulate growth hormone (GH) release and food-seeking behaviour, recent evidence suggests that deacylated ghrelin (DAG) can also have distinct or even antagonist effects to its counterpart, both centrally (eg. DAG blunts the orexigenic effect of AG, when administered intracerebroventricularly) as well as peripherally. Overall, metabolic *in vitro* and *in vivo* studies with ghrelin have been controversial. Thus, ghrelin's direct action on peripheral tissues such as skeletal muscle needs to be elucidated, independent of its modification to DAG (abundant circulating form) and in particular, in the absence of secondary GH release. Skeletal muscle is responsible for the clearance of as much as 80% of an ingested oral glucose load, and ghrelin's peak prior to a meal could mediate this. As such, we sought to determine the direct *ex vivo* effects of various concentrations of AG and DAG on isolated rat oxidative (soleus)

and glycolytic (extensor digitorum longus - EDL) skeletal muscle glucose uptake and insulin sensitivity. This was assessed using a validated radioactive glucose tracer assay within our lab, as well as Western blots to analyze the activation of proteins involved in insulin and glucose transporter signalling pathways. Ghrelin, at both physiological and supraphysiological concentrations, does not influence submaximal (0.5mU/mL) or maximal (10mU/mL) insulin signalling or insulin's ability to stimulate glucose transport in soleus or EDL skeletal muscle. In line with this, various ghrelin concentrations did not alter glucose uptake independent of insulin. Phosphorylation of the signal transducer and activator of transcription 5 (STAT5) was used as a positive control to ensure that growth hormone was exerting an effect cellularly, at the level of the muscle. Further, we show for the first time that AG and DAG can act independent of GHS-R1a (as negligible mRNA expression of GHS-R1a is found within peripheral tissues like skeletal muscle) and increase the phosphorylation of calmodulin-dependent protein kinase 2 (CAMK2), though this may be fibre type-dependent as it was only apparent in glycolytic EDL. We observed no changes from ghrelin or growth hormone treatment on markers of insulin signalling, while insulin, expectedly, increased the activation of phosphorylated residues of Akt (Ser473). We conclude that both forms of ghrelin have no significant or direct influence on skeletal muscle's ability to uptake glucose, in rats. Further molecular investigation is required for ghrelin's direct action on key metabolic tissues.

Teneurin C-terminal Associated Peptide (TCAP), a novel regulator of glucose uptake and metabolism in skeletal muscle: A potential therapeutic for neuromuscular dysfunction

Andrea L. D'Aquila[1], Yani Chen[1], Leanne Wybenga-Groot[2], Marius Locke[3], and David A. Lovejoy[1,4]

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Skeletal muscle health is essential for the well-being and active lifestyles required for a fit population. Glucose is a necessary source of energy where aberrant glucose regulation can lead to endemic pathologies such as diabetes, obesity and associated muscular dysfunction. Skeletal muscle accounts for 40% of glucose-associated energy requirements, yet despite this, our understanding of energy metabolism in muscle is not well understood. Recent studies indicate that TCAP-1, a bioactive peptide on the C-terminus of teneurin protein, enhances neuronal metabolism via up-regulation of glucose transporters in tissues. This peptide hormone has been established as one of the most ancient peptide hormones that regulate glucose transport, predating the evolution of insulin. Recently, we have established that TCAP-1 significantly increases insulin-independent glucose uptake by examining radioactive glucose uptake into rodent skeletal muscle in vitro and in vivo using functional positron emission tomography (fPET) data. These studies led me to hypothesize that TCAP-1 may be critical to skeletal muscle viability. To assess if this increase in glucose uptake is translated to increased muscle function, I next assessed muscle function following TCAP-1 administration by performing an in vivo muscle stimulation test. TCAP-1 treatment was found to significantly increase muscle contraction force, and prolong contraction speed and relaxation rate during a fatigue protocol, indicating enhanced muscle function. Histological analyses of these muscles demonstrate that TCAP-1 treatment increases oxidative capacity as seen by increases in NADH production, however, this effect was only seen in a subset of muscle fiber types. Importantly, ADGRL1, the

receptor of TCAP, had an expression pattern consistent with the fibers affected by TCAP-1 actions, thus suggesting a fiber-specific mechanism of action. Moreover, preliminary studies done on mitochondrial respiration of skeletal muscle cells demonstrate TCAP-1 has dose-dependent effects upon the mitochondria and its activation. Thus, TCAP-1 may regulate glucose metabolism to produce a higher oxidative capacity of the cell, providing additional energy for muscle and protecting it from damaging situations. This may provide a potential therapeutic for muscular dysfunction.

Expression of the Dystrophin-Associated Protein Complex in the Absence of Skeletal Muscle AMPK

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The dystrophin-associated protein complex (DAPC) is composed of proteins that are highly expressed along the sarcolemma. The DAPC provides a mechanical link between the intracellular cytoskeleton and extracellular matrix, as well as a signal transduction apparatus from the periphery to the interior of muscle fibers. The signalling molecule AMP-activated protein kinase (AMPK) is a powerful regulator of phenotypic plasticity. Recent evidence has shown that chronic AMPK activation alters the expression of DAPC components. However, a more comprehensive understanding of the influence of AMPK on the DAPC is lacking. Therefore, the purpose of this study was to investigate the role of AMPK in the expression of the DAPC. Extensor digitorum longus (EDL) and soleus (SOL) muscles representing fast glycolytic and slow oxidative tissues, respectively, were obtained from wild-type (WT) mice, as well as from mice deficient in both isoforms of the AMPK-β subunit in skeletal muscle (AMPK-MKO). RT-PCR and Western blotting measured mRNA and protein content of DAPC components, respectively. In WT animals, utrophin and laminin mRNA expression, as well as laminin and β-dystroglycan protein content were ~25-60% higher ($p < 0.05$) in the SOL versus EDL muscle. In contrast, neuronal nitric oxide synthase (nNOS) mRNA was 60% lower in SOL. The AMPK-MKO animals displayed a different pattern of fiber-type specific expression of DAPC components. In these mice, laminin, γ-sarcoglycan (SG), dystrophin, and utrophin transcripts, as well as dystrophin and utrophin protein expression were 40-55% greater ($p < 0.05$) in the SOL versus the EDL. β-SG and nNOS mRNA content were lower in the EDL muscle of AMPK-MKO mice, as compared to their WT counterparts. We also assessed the expression of peroxisome proliferator activated receptor coactivator-1α (PGC-1α) and Ca2+/calmodulin dependent protein kinase II (CAMKII), factors that contribute to the upstream regulation of the DAPC. PGC-1α mRNA content was higher in SOL relative to EDL muscles of both genotypes. CAMKIIα protein expression was significantly lower in SOL muscles versus EDL muscles of WT and AMPK-MKO, while CAMKIIβ exhibited the opposite pattern. PGC-1α mRNA tended to be higher in the muscles from AMPK-MKO mice compared to WT animals. Conversely, CAMKII mRNA content tended to be reduced in the AMPK-MKO muscles, as compared to WT. These data indicate a fiber-type specificity to DAPC expression in skeletal muscle. Furthermore, AMPK deficiency results in a differential profile of DAPC components, as well as in alternations in the expression of alternative upstream DAPC regulators. Our results suggest that AMPK contributes to the expression profile of the DAPC.

The effect of acute and chronic sprint-interval training on LRP130, SIRT3, and PGC-1α expression in human skeletal muscle

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The present study examined changes in LRP130 gene and protein expression in response to an acute bout of sprint-interval training (SIT) and 6 weeks of SIT in human skeletal muscle. In addition, we investigated the relationships between changes in LRP130, SIRT3 and PGC-1α gene or protein expression. Fourteen recreationally active men (age: 22.0 ± 2.4 years) performed a single bout of SIT (eight, 20-second intervals at ~170% of VO_2peak work rate, separated by 10 seconds of rest). Muscle biopsies were obtained at rest (PRE) and 3 hours post-exercise. The same participants then underwent a 6 week SIT program with biopsies after 2 (MID) and 6 (POST) weeks of training. In response to an acute bout of SIT, PGC-1α mRNA expression increased (284%, $p < 0.001$); however, LRP130 and SIRT3 remained unchanged. VO_2peak and fibre-specific SDH activity increased in response to training ($p < 0.01$). LRP130, SIRT3, and PGC-1α protein expression were also unaltered following 2 and 6 weeks of SIT. There were no significant correlations between LRP130, SIRT3, or PGC-1α mRNA expression in response to acute SIT. However, changes in protein expression of LRP130, SIRT3, and PGC-1α were positively correlated at several time points with large effect sizes, which suggests that the regulation of these proteins may be coordinated in human skeletal muscle. Future studies should investigate other types of exercise known to increase PGC-1α and SIRT3 protein to identify if LRP130 expression is altered in response to other exercise protocols.

Regulation of TFEB transcriptional activity and translocation during exercise by PGC-1α

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Muscle health is strongly dependent on the maintenance of functional mitochondria for the provision of ATP. The mitochondrial pool is governed by two processes: mitochondrial biogenesis and autophagy (mitophagy). Mitochondrial biogenesis is coordinated by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), while transcription factor EB (TFEB) regulates lysosomal biogenesis and is therefore required for autophagy. It has been established that exercise is able to activate PGC-1α and therefore mitochondrial biogenesis. However, little is known about the activation of TFEB during exercise in muscle. Since the two processes are strongly linked, the purpose of this study was to further elucidate the relationship between TFEB and PGC-1α under exercise conditions in wild type (WT) and PGC-1α knockout (KO) mice to determine whether PGC-1α is required for TFEB promoter activity and translocation. Protein levels of TFEB were reduced by 70% in whole body PGC-1α KO mice compared to their WT counterparts. In contrast, muscle-specific overexpression of PGC-1α resulted in a 50% increase in TFEB protein expression. Since TFEB is involved in lysosomal biogenesis, markers of chaperone-mediated autophagy were measured to determine if they were affected by PGC-1α-mediated TFEB downregulation. However, no changes were observed in

Hsc70 or Lamp-2A between the two genotypes. TFEB promoter activity was increased with acute exercise to a greater extent in WT compared to KO animals, but translocation of TFEB into the nucleus following exercise was increased in both WT and KO mice. Our findings confirm the positive correlation between the two master regulators of biogenesis and autophagy, PGC-1α and TFEB. PGC-1α does not seem to have an effect on markers of chaperone-mediated autophagy, despite its regulation of TFEB expression. Thus, it is probable that this correlation is working to promote other forms of autophagy, as well as mitochondrial biogenesis, in order regulate the turnover of the mitochondrial network.

Endothelial-Derived Erythropoietin: A Novel Role In Regulating Skeletal Muscle Glycogen Content

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The master regulator of hematopoiesis, erythropoietin (EPO), is no longer recognized as just a hematopoietic cytokine. EPO is important for neurogenesis, is an inotropic agent, induces cell proliferation and confers profound cytoprotection. Interestingly, EPO expression increases in whole muscle following both local hypoxia and acute exercise. However, the cell type responsible (e.g., endothelium, myocyte, fibroblast) for production and the physiological relevance remains to be elucidated. Thus, our objective was to understand EPO signaling in whole muscle in health and following exercise. We hypothesize that the endothelial is the source of EPO in muscle following exercise; furthermore, loss of endothelium-derived EPO impairs endurance exercise capacity. To investigate endothelial-derived EPO, we generated constitutively active endothelial-specific (using Tie2-Cre, with expression beginning E8) EPO knock-out mice using the cre-lox system (EPOfl/fl:Tie2-cre+/-; EPOEndo-Δ). Generation of EPOEndo-Δ pups followed Mendelian genetics with adult mice showing normal hematocrit, body weight and cardiac function as compared to littermates (wt). EPO expression was decreased in only glycolytic, but not oxidative, muscles of healthy adult EPOEndo-Δ mice as compared to wt. In response to exercise, EPOEndo-Δ showed reduced running distance. To assess the cause of exercise intolerance, we investigated in vitro force and fatigability. While force was normal, EPOEndo-Δ displayed greater fatigability of the extensor digitorum longus (glycolytic muscle) but not the soleus (oxidative muscle). We next examined glycogen content in healthy adult muscles from wt and EPOEndo-Δ mice, which showed reduced glycogen content only in glycolytic muscles as compared to wt. Here we show that the endothelium of only glycolytic muscles produces EPO and that loss of endothelial-derived EPO causes exercise intolerance along with reduced glycogen content in only (fast-twitch) glycolytic muscles. These data suggests the first physiological role of non-renal derived EPO, as it plays a critical role in regulating glycogen content in fast-twitch glycolytic muscles.

Cardiac autonomic responses to structured vs. unstructured physical activity in children

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INTRODUCTION: The cardiac autonomic nervous system influences a number of cardiovascular parameters such as cardiac output through its direct effect on heart rate. During physical activity the sympathetic (SNS) and parasympathetic nervous systems (PNS) regulate the cardiovascular responses to increased physiological demand as a result of the activity. However, the precise nature of the SNS and PNS response is uncertain following different types of physical activity types. The purpose of this study was to determine the contribution of SNS and PNS to different types and intensity of physical activity. **METHODS:** Children (n=6) (age 9.4±1.1 yrs, BMI 21.1±2.3 kg/m2) were randomly assigned to one of two-structured treadmill (TM) exercise protocols (either continuous or random) with speeds ranging from 4-10km/hr. Children (n=9) registered to participate in an unstructured active play program were recruited from a local community centre. Adults (n=?)(age 21.6± 2.1 yrs) were also assessed during TM and active play. The oxygen consumption (VO2) responses were determined using a CosMed2 portable oxygen analyzer. Physical activity (PA) (accelerometer Actigraph GT3X+) and heart rate (HR) (Polar Corp) were assessed every 10sec during TM and active play. HRV (ActiLife Software) was determined for each participant and the R-R intervals analyzed using Fast Fourier-Transformation (Kubios Software) to measure low (LF) and high (HF) frequencies. These variables were expressed as percentages of the total frequencies (%LF – SNS and %HF – PNS) and as a ratio of LF/HF. The standard deviation of the R-R intervals (SDRR) was also obtained. Statistical analysis was performed on standardized workloads of 4 and 6 METs for each condition by using ANOVA and/or Pearson correlation 'r' with an alpha level of p=0.05. **RESULTS:** In children, SDRR was higher for active play than the random and continuous TM exercise (195.8+/-272.7 ms vs 58.6+/-20.2 ms and 33.1 +/- 16.2 ms, respectively) ($p>0.05$). %LF for active play in the children group was 22.4% lower than the random TM exercise and 15.7% lower than the continuous TM exercise ($p<0.05$). On the other hand, HF% for active play was 15.6% higher than continuous and 22.3% higher than random TM exercise ($p<0.05$). LF/HF ratio for children was lower for active play in comparison to the other two conditions ($p>0.05$). Compared to the adult group no significant differences were observed. **CONCLUSION:** Compared to treadmill exercise, active play, an unstructured type of PA, results in lower HRV through lower cardiac sympathetic stimulation and higher parasympathetic contribution. As a result greater health benefits can be achieved through unstructured physical activity participation for a given intensity of physical activity.

Characterizing the Role of Actin Variants in HCM

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Cardiovascular disease (CD) is the leading cause of death worldwide. Hypertrophic cardiomyopathy (HCM) is an inherited CD that affects 1 in 500 people. HCM causes an overgrowth in the left ventricle, which results in a smaller ventricle and less oxygenated blood transported throughout the body. For these reasons, HCM is the leading cause of sudden cardiac death in people under 30 years old. Myocardium contraction is increased in hearts with HCM,

suggesting an increase in the force (ensemble force). The myocardium consists of myosin and actin, two motor proteins that form a cross bridge to shorten the sarcomere in the presence of ATP. The duty ratio (*r*) of the muscle is the fraction of time that myosin spends bound to actin during the cross bridge cycle. Human alpha-cardiac actin (ACTC) variants with single amino acid substitutions to subdomain 1 have been linked to HCM. This subdomain is the binding interface for myosin, suggesting there is an alteration to the r. I hypothesized that the r for these ACTC variants are increased. To test my hypothesis I used an ACTC activated myosin ATPase assay. The results show that the *r* for H88Y was similar to wild type, F90Δ and R95C have a smaller *r* than wild type, and E99K has a larger *r*. The amino acid properties at these residues are likely causes for this result. To further analyze these variants in HCM, animal models with gene modifications will be useful.

Early onset of muscle-specific alterations in mitochondrial bioenergetics in the D2.B10-DMD^{mdx}/2J mouse model of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a progressive muscle wasting disease resulting from mutations in the X-linked gene dystrophin. The loss of dystrophin in the dystroglycan complex causes severe muscle pathology yet the specific signaling mechanisms leading to this muscle wasting remain unclear. The mitochondrion has increasingly been considered a key contributor to the pathology seen in DMD yet the characterization of specific changes occurring within the mitochondria have been limited. We hypothesized that young D2.B10-DMD^{mdx}/2J would exhibit elevated levels of mitochondrial H_2O_2 emission and concurrent decreases in mitochondrial respiration. At 4 weeks of age, male D2.B10-DMD^{mdx}/2J and DBA.2J healthy controls were sacrificed and the left ventricle, diaphragm and quadriceps muscles were removed to allow for the preparation of permeabilized muscle fibre bundles. Mitochondrial H_2O_2 emission was detected using Amplex UltraRed in a high resolution spectrofluorometer. In the left ventricle, complex I-derived mitochondrial H_2O_2 (5mM Pyruvate/2mM Malate) was 57% higher in D2.B10-DMD^{mdx}/2J relative to healthy controls (306.2+/- 33.3 vs 194.7 +/- 26.3 pmol/sec/mg dry wt). However, there were no differences in complex I-derived H_2O_2 in the diaphragm or quadriceps relative to control. Oroboros Oxygraph-2k high resolution respirometers were used to study mitochondrial respiration. Pyruvate and malate (5mM/2mM) were again used as complex I substrates followed by the addition of physiological (25µM) and supraphysiological (5mM) concentrations of ADP to stimulate state III respiration. No differences in respiration were observed in the left ventricle or diaphragm. However, quadriceps muscle from the DMD mice showed significantly lower rates of respiration at both 25µM (6.6 +/- 1.4 vs 16.5 +/- 3.6 pmol/sec/mg dry wt) and 5mM ADP (18.7 +/- 2.7 vs 53.9 +/- 15.9 pmol/sec/mg dry wt). These findings at an early age highlight the need for further characterization of the alterations in mitochondrial function associated with the progression of DMD.

Novel paracrine requirement for cardiac-derived erythropoietin in cardiogenesis

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Erythropoietin (EPO) is more than just a hematopoietic cytokine. It is involved in cell proliferation, cytoprotection, cardiac inotropy and embryonic development. Extrarenal production has been identified in multiple tissues however the significance of these sources is unknown. EPO signalling is required for normal cardiac development and there is evidence to suggest the heart is a source of EPO production. Our objective was to identify the physiological significance of cardiac-derived EPO during development. We hypothesized that paracrine production of EPO in the heart is required for cardiogenesis. To investigate the developmental effects of cardiac-derived EPO, we generated constitutive, cardiomyocyte-specific EPO knockout mice ($EPO^{fl/fl}$:Mlc2v-$cre^{+/-}$; $EPO^{\Delta/\Delta\text{-CM}}$) using the Cre-lox system. Adult $EPO^{\Delta/\Delta\text{-CM}}$ hearts showed cellular hypertrophy, that was absent at the whole heart level, with increased ratio of capillaries per cardiomyocyte, compared to wild-type littermates. $EPO^{\Delta/\Delta\text{-CM}}$ mice displayed increased heart rate, cardiac contractility and decreased stroke volume. In response to exercise, $EPO^{\Delta/\Delta\text{-CM}}$ showed increased running distance, compared to wild-type. Unexpectedly, left ventricular EPO expression was elevated in $EPO^{\Delta/\Delta\text{-CM}}$ mice with increased serum EPO levels and higher hematocrits. This suggests over-compensation from another cell type in the heart. Here we show that the heart is a source of EPO and loss of cardiomyocyte-specific EPO production causes reciprocal up-regulation in another cell-type within the heart, resulting in morphological and functional alterations. These findings highlight previously unrealized importance of cardiac-derived EPO production during development; identifying physiological significance of a non-renal source of EPO.

Brown adipocyte recruitment following chronic cold exposure and short photoperiodism in the white-footed mouse, *Peromyscus leucopus*

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The global increase in the prevalence of obesity and related metabolic diseases has ignited a spark amongst researchers to determine the most effective, inexpensive, and least invasive treatment to tackle this epidemic. Brown adipose tissue (BAT) is responsible for generating heat to maintain stable body temperature under cold environmental conditions. The recent discovery of brown adipocytes in typically white adipose tissue (WAT) depots upon β_3-adrenergic stimulation has attracted the attention of researchers, since these cells have the potential to burn energy in these fat depots. Many studies using inbred, laboratory mouse models, have observed the development of brown adipocytes in subcutaneous WAT depots more so than in visceral WAT. Our study examines whether development of brown adipocytes in WAT depots occurs following exposure to 12 weeks of cold (10°C) in *Peromyscus leucopus*. This mouse is abundant and widely distributed in North America, is not inbred, and has a closer phylogenetic relationship to humans than its laboratory counterpart, *Mus musculus*. We studied the gross anatomical features and performed Haematoxylin & Eosin histology on the interscapular BAT (iBAT), inguinal subcutaneous WAT (iWAT), and the visceral epididymal WAT (eWAT) depots. Following cold exposure, the mass of iBAT increased, demonstrating a possible increase in

activation of non-shivering thermogenesis in this depot. In contrast, eWAT depot mass decreased and iWAT did not change. Histological examination of the fat depots suggests a decrease in size of adipocytes in the iBAT and eWAT depots, but not in the iWAT depot. Furthermore, we observed many clusters of multilocular adipocytes and increased vascular tissue, consistent with the appearance of browning of adipocytes in the eWAT depot. This suggests that in non-inbred *P. leucopus*, brown adipocytes may be induced in the visceral more so than the subcutaneous WAT depot. This may have important implications both for interpreting studies of BAT biology in lab mice, and for developing an appropriate model to study BAT biology for application towards human health.

Vitamin D_3 deficiency negatively impacts disease pathophysiology in the spinal cord of the G93A mouse model of amyotrophic lateral sclerosis

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BACKGROUND: Dietary vitamin D_3 (D_3) restriction reduces paw grip endurance and motor performance in G93A mice, and increases inflammation and apoptosis in the quadriceps of females. ALS, a neuromuscular disease, causes progressive degeneration of motor neurons in the brain and spinal cord. **OBJECTIVE:** We analyzed the spinal cords of G93A mice following dietary D_3 restriction at 2.5% the adequate intake (AI) for oxidative damage (4-HNE, 3-NY), antioxidant enzymes (SOD2, catalase, GPx1), inflammation (TNF-α, IL-6, IL-10), apoptosis (bax/bcl-2 ratio, cleaved/pro-caspase 3 ratio), neurotrophic factor (GDNF) and neuron count (ChAT, SMI-36/SMI-32 ratio). **METHODS:** Beginning at age 25 d, 42 G93A mice were provided food *ad libitum* with either adequate (AI; 1 IU D_3/g feed; 12 M, 11 F) or deficient (DEF; 0.025 IU D_3/g feed; 10 M, 9 F) D_3. At age 113 d, the spinal cords were analyzed for protein content. Differences were considered significant at $P \leq 0.10$, since this was a pilot study. **RESULTS:** DEF mice had 16% higher 4-HNE ($P = 0.056$), 12% higher GPx1 ($P = 0.057$) and 23% higher Bax/Bcl2 ratio ($P = 0.076$) vs. AI. DEF females had 29% higher GPx1 ($P = 0.001$) and 22% higher IL-6 ($P = 0.077$) vs. AI females. DEF males had 23% higher 4-HNE ($P = 0.066$) and 18% lower SOD2 ($P = 0.034$) vs. AI males. DEF males had 27% lower SOD2 ($P = 0.004$), 17% lower GPx1 ($P = 0.070$), 29% lower IL-6 ($P = 0.023$) and 22% lower ChAT ($P = 0.082$) vs. DEF females. **CONCLUSION:** D_3 deficiency exacerbates disease pathophysiology in the spinal cord of G93A mice, the exact mechanisms are sex-specific. This is in accord with our previous results in the quadriceps, as well as functional and disease outcomes.

Exercise-induced right ventricular dysfunction and increased arrhythmia susceptibility in mice

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BACKGROUND: The effect of prolonged intensive endurance exercise (PE) on right ventricular (RV) function is controversial, with evidence of acute RV systolic and diastolic impairment observed in both elite and recreational endurance athletes. In addition, repetitive

bouts of intensive PE have been linked to exaggerated RV remodeling and a propensity to arrhythmias arising from the RV. However, the mechanisms linking these responses are unclear. **METHODS:** In vivo admittance catheterization and pressure-volume analysis with vena cava occlusion was performed acutely (within 30 min) and following 6 week intensive training with and without dobutamine infusion (1.5mg/kg) to characterize RV functional reserve. Electrical properties and RV arrhythmia vulnerability was assessed using an octapolar recording/stimulation electrophysiology catheter. **RESULTS:** Acutely, RV volumes were dilated following intensive exercise (n=18) relative to non-exercised controls (n=16) and 30 minute exercised mice (n=18), with concurrent reductions in RV contractility ($P<0.05$) and functional reserve ($P<0.05$). Myocardial relaxation (tau) was delayed with elevated filling pressures ($P<0.05$). Following 6 weeks of intensive training, RV systolic and diastolic impairment persisted (n=18), with a reduced ability to augment ventricular function following dobutamine infusion. While ventricular effective refractory periods (VERPs) were prolonged, RV arrhythmia inducibility was enhanced ($P<0.05$) in 6-week intensively trained mice (n=50) compared to moderately trained mice (n = 24) and non-exercise controls (n=24). Dobutamine infusion did not increase arrhythmia susceptibility in all groups (n=15) ($P>0.05$). **CONCLUSION**: Acute prolonged intensive endurance exercise is associated with RV functional impairment, which may be linked to adverse structural, electrical and functional remodeling and increased RV arrhythmia susceptibility in the long-term.

Analysis of Regulated Thin Filaments Variants

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Cardiovascular disease (CVD) is the leading cause of death worldwide, and in Canada, someone dies from the disease every 7 minutes. Due to rising mortality and economic burden, it is crucial that we understand the molecular cause(s) of these diseases so treatments can be developed.To date, 16 alpha 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 decreased contractility leads to DCM while increased contractility leads to HCM. Part of that change in contractility may result from altered myosin motor duty ratios, particularly for four HCM-related ACTC mutations in subdomain 1 of actin resulting in changes at the actomyosin interface: H88Y, F90Δ, R95C, and E99K. We sucessfully extracted the key regulatory proteins Troponin and Tropomyosin from bovine hearts through ammonium sulfate cuts and myosin II from rabbit hind legs. Myosin full length was cleaved to become heavy meromyosin (HMM) using chymotryptic digestion. We also optimized our protocol to purify ACTC variants from Sf21 Insect Cells using affinity chromatography based on his-tagged gelsolin 4-6, a calcium-dependent actin binding protein. Manual analysis of in vitro motility (IVM) velocity shows decrease with the ACTC variants H88Y and E99K, while R95C and F90Δ exhibit the same or even higher velocity than WT recombinant. Tests of an automatic motility measurement program FAST produce the same velocities as manual analysis. We will be using automatic analysis in the future. My IVM analysis supports the idea that different mutations in subdomain 1 have different effects on actomyosin interactions.To explore the possibility that cardiac dysregulation occurs at the level of the regulation of variant ACTC proteins, the impact of these variants on troponin-tropomyosin regulated ACTC was investigated. First, myosin ATPase and in vitro motility (IVM) assays were done by comparing tissue purified cardiac actin

from bovine hearts and WT recombinant cardiac actin, which results in similar pCa50. With these data in hand, I can now investigate the effects of the subdomain 1 mutations on regulated thin filament interactions with myosin. The results of my research will help identify the molecular causes of hypertrophic cardiomyopathy and provide a starting point for the development of treatements designed to correct specific dysregulation leading to disease.

In the absence of phosphate shuttling, exercise reveals the in vivo importance of creatine-independent regulation on mitochondrial ADP transport

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The transport of cytosolic ADP into the mitochondria is a major control point in metabolic homeostasis, as ADP concentrations directly affect glycolytic flux and oxidative phosphorylation rates within mitochondria. A large contributor to the efficiency of this process is thought to involve phosphocreatine (PCr)/Creatine (Cr) shuttling through mitochondrial creatine kinase (Mi-CK), whereby PCr is regenerated and ADP is concentrated near adenine nucleotide translocase (ANT). However, the biological importance of alterations in Cr-independent ADP transport during exercise remains unknown. Therefore, we utilized a Mi-CK knockout (KO) model to determine whether in vivo Cr-independent mechanisms are biologically important for sustaining energy homeostasis during exercise. Ablating Mi-CK did not alter protein contents of ADP transporters (voltage-dependent anion channel (VDAC); ANT), cytosolic CK, or mitochondrial markers (complexes I-V of the electron transport chain; carnitine palmitoyl transferase-1; pyruvate dehydrogenase). Exercise time to volitional fatigue was also similar between wildtype and KO mice at moderate and high exercise intensities. Moreover, skeletal muscle metabolic profiles after exercise, including glycogen, PCr/Cr ratios, free ADP/AMP and lactate were similar between genotypes. While these data suggest the absence of PCr/Cr shuttling is not detrimental to maintaining energy homeostasis during exercise, KO mice displayed a dramatic increase in Cr-independent mitochondrial ADP sensitivity after exercise in permeabilized muscle fibres. Specifically, while mitochondrial ADP sensitivity decreased with exercise in wildtype mice, in stark contrast, exercise increased mitochondrial Cr-independent ADP sensitivity in KO mice. As a result, the apparent ADP Km was 50% lower in KO mice after exercise, suggesting in vivo activation of VDAC/ANT can regulate mitochondrial ADP transport to maintain exercise tolerance and fuel utilization as opposed to simple diffusion. This was further supported using a mitochondrial isolation preparation, which demonstrated a lack of compensatory re-location of the cytosolic CK to the mitochondrial intermembrane space, as well as similarities in ADP sensitivity in the presence or absence of Cr in KO mice. Altogether, we provide novel insight that Cr-independent ADP transport mechanisms are biologically important for regulating ADP sensitivity during elevated metabolic requirements, while highlighting complex regulation and the plasticity of the VDAC/ANT axis to support ATP demand during exercise.

Modulation of the UPR in relation to mitochondrial biogenesis adaptations in muscle cells

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Skeletal muscle is the largest organ of our body and it plays a major role in metabolic homeostasis. Basally, skeletal muscle has a low mitochondrial content. However, with exercise, there is an increased abundance via mitochondrial biogenesis. This process exerts high protein synthesis demands upon the cell, placing it under stress. In order to maintain proteostasis, the unfolded protein response (UPR) is activated to induce a series of transduction pathways, targeting the increase of chaperones and proteases to attenuate the accumulation of potentially toxic misfolded proteins, and if necessary, to trigger apoptosis of the cell. The UPR can be activated both in the endoplasmic reticulum (ER), as well as in the mitochondria. Whether the UPR is necessary for mitochondrial adaptations is still unknown. Thus, the purpose of this study is to investigate the modulation, as well as the necessity, of the UPR in differentiation- and chronic contractile activity (CCA)- induced models of mitochondrial adaptations. For the differentiation-induced mitochondrial biogenesis model, C2C12 muscle cells are treated with anti-UPR chemical chaperone mimetic Tauroursodeoxycholate acid (TUDCA) prior to differentiation for 24 h. After day 0 or day 4 of differentiation, cells were found to have increased mitochondrial content marekrs despite decreased myogenesis: COX-I and COX-IV by 1.5 fold, and PGC-1α by 1.3 fold ($p<0.05$). With TUDCA treatment, the effect of TUDCA in the CCA-induced model of mitochondrial biogenesis seems to potentially increase mitochondrial content, as well as increase the activation of the mitochondrial UPR. Interestingly, there was an interaction effect of TUDCA and CCA for COX-IV levels which increased relative to vehicle by 2.6 fold, and 1.7 fold for mitochondrial UPR marker CPN10 ($p<0.05$). Further work is required to specifically target UPR pathways to better investigate their role in mitochondrial adaptations. Such findings could have therapeutic benefits related to skeletal muscle health.

PDCD4 Depletion Stabilizes Myofibrillar Protein Abundance in L6 Myotubes

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Skeletal muscle is the largest organ in the human body constituting 40-45% of one's total body mass. As a highly active metabolic tissue, skeletal muscle is vital for maintaining substrate homeostasis and is a major contributor to whole-body metabolism. Skeletal muscle mass is regulated by protein turnover, which is the balance between the rates of protein synthesis and protein degradation. The mechanistic target of rapamycin complex 1/ribosomal protein S6 kinase 1 (mTORC1/S6K1) pathway is critical in integrating both intracellular and extracellular signals to regulate protein turnover. A recently characterized downstream target of the mTORC1/S6K1 pathway, programmed cell death protein 4 (PDCD4), has been shown to regulate protein synthesis. PDCD4 is a pro-apoptotic protein that binds to eukaryotic mRNA translation initiation factor 4A (eIF4A) to inhibit mRNA translation and decrease protein synthesis. However, upon p70S6K1-mediated phosphorylation, PDCD4 is targeted for proteolysis via the ubiquitin–proteasome system. Surprisingly, PDCD4 depletion decreases myofibrillar protein synthesis in myotubes. Therefore, the aim of this study was to examine PDCD4's role in muscle myofibrillar protein content to determine its overall effect on protein turnover. We hypothesized that PDCD4

depletion would increase myofibrillar protein content in L6 myotubes. Myotubes treated with dexamethasone on day 5 of differentiation exhibited a decrease in ph-p70S6K1 ($P < 0.05$), which corresponded with a significant increase in PDCD4 expression ($P < 0.001$). In PDCD4-depleted myotubes, MHC-1 and troponin expression was significantly elevated compared to control by 163% and 136%, respectively ($P < 0.001$). Therefore, PDCD4 may serve as a therapeutic target in skeletal muscle, which may reduce the loss of myofibrillar protein content associated with disease and aging.

Chronic cold exposure alters gut microbiota that promotes browning of white adipose tissue

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The interactions between the microbiota and host have been shaped by their co-evolution, resulting in highly interdependent metabolic functions. The gut microbiota plays vital role in maintaining host energy homeostasis by regulating energy harvest, storage, and expenditure. In developed countries, high-fat diets and energy-rich foods have resulted in a disequilibrium of host energy, where the energy retained from food intake grossly exceeds energy expenditure, which, in turn, contributes to the high prevalence of obesity. Browning of the white adipose tissue has been touted as next generation therapy for obesity and type 2 diabetes. We hypothesize that cold-exposure, a well-described stimulus of browning of white adipose tissue, enforces its pro-metabolic effects through alteration in gut microbiota. Through differential bacteria culturing of fecal matter, we observed that exposing mice to cold (n = 10 C57Bl/6J, 3 month old male mice kept at kept at 4 °C for 2 weeks) leads to a dramatic depletion of gut microbiota vs. mice kept at thermoneutral conditions (n = 10 C57Bl/6J, 3 month old male mice kept at kept at 30 °C for 2 weeks). Moreover, exposure to cold not only reduces bacterial load, but results in key changes in the composition gut microbiota, which results in induction of browning gene program in inguinal white adipose tissue. This alteration in cold-induced gut microbiota induces mitochondrial activity and promoted thermogenic gene expression in inguinal white adipose tissue. These findings help to solidify the microbiota's role in maintaining homeostatic energy balance, which presents a potential therapeutic means for metabolic disorders and obesity.

‘**Forcing’ changes in the muscle stem cell transcriptome**

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KASH and SUN are integral membrane protein domains that localize to the outer- and inner nuclear membranes, respectively. The domains interact to form a bridge across the nuclear membranes that link the cytoskeleton to the nuclear lamina. Nesprins 1 and 2 possess KASH domains that bind cytoplasmic f-actin. Observations indicating that the Nesprin/Sun bridge can move chromosomes by transferring forces initiated outside the nucleus led to speculations that

the complex might physically open inaccessible regions of the genome to modify gene expression. We hypothesize that the extracellular niche plays a role in regulating muscle stem cell self-renewal and specification through mechanotransduction signals propagated by the Nesprin/Sun complex. We find that Nesprins, but not SUN proteins, are expressed in quiescent skeletal muscle stem cells (MuSCs) using immunofluorescence. SUN expression is induced shortly (2 hrs) after culture suggesting that the Nesprin/Sun bridge is assembled during MuSC activation. Knock down of Nesprin expression in MuSC using siRNA showed a decrease in cells committed to the myogenic lineage. To uncover novel genes influenced by the transmission of extracellular mechanical stimuli, we performed RNAseq on primary myoblasts cultured upon soft or stiff substrates and expressing GFP or a Nesprin dominant-negative mutant that cannot bind f-actin. Upon LINC complex disruption on stiff substrate, Lim-domain-only-7 (Lmo7) was significantly downregulated, which is a transcription activator of myogenic regulatory genes such as MyoD. Interestingly, Lmo7-null mice were reported to exhibit Emery-Dreifuss muscular dystrophy (EDMD) phenotypes. Our study hopes to provide new insight into the pathogenesis of EDMD, and elucidate mechanisms that regulate muscle stem cell fate.

Differential Atrial and Ventricular Remodeling in Response to Endurance Exercise

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Cardiac remodeling refers to biochemical, genetic, structural and functional changes that occur in the heart in response to stress, classified as physiological (i.e. exercise) or pathophysiological (i.e. heart disease). Our recent studies have identified distinct remodeling patterns in mouse atria and ventricles in response to endurance swim exercise: exercise induced fibrosis, inflammation and atrial fibrillation (AF) in atria, while improving contractility and reducing arrhythmia vulnerability in ventricles. This exercise-induced atrial remodeling was prevented by genetic (TNFαKO) or pharmacological blockade (etanercept) of TNFα-NFκB signaling, a key regulator of cardiac inflammation and cardiomyocyte survival. Etanercept treatment starting at 3-weeks after exercise commencement did not reverse the pathological atrial remodeling, indicating that remodeling occurs at an early stage during exercise training. To evaluate the early TNFα-mediated atrial remodeling with exercise, WT and TNFαKO mice swam for 90minutes/session, 2sessions/day for 2-weeks. I performed deep-RNA-sequencing (RNA-seq) on isolated 2-week exercised mouse atria and ventricles. RNA-Seq reads were mapped and aligned to the mouse reference genome (mm10) using the Tophat-Cuffdiff pipeline. Enriched gene sets were identified using Gene Set Enrichment Analysis (GSEA) with curated(c2cp) and mouse specific gene set database (Mouse_GO_AllPathways). Gene sets were ranked and only those with $p<0.05$ and FDR<0.25 were considered. Although the inflammation-associated gene sets, such as IL1, IL6, TOLL-like receptors, and NFκB, which is downstream of TNFα, were enriched in the sedentary atria and ventricle groups instead of the exercised groups, there was high consistency between clusters of enriched gene sets in the atria and ventricles. However, the number of significantly enriched gene sets was greatly reduced in exercised ventricles (34 gene sets) compared to exercised atria (76 gene sets), suggesting that ventricles are less susceptible to exercise-induced transcriptomic remodeling. To examine how TNFα is involved in the distinct remodeling and to refine gene sets that are specifically mediated by TNFα during exercise, the (Swim-Sedentary)

WT-(Swim-Sedentary) TNFαKO bioinformatics analysis was performed. Enrichment maps suggested TNFα's involvement in Wnt, Hedgehog signaling, cell-cell adhesion and mechanotransduction-associated pathways, such as the focal adhesion and integrin pathways, in the atria, but not in the ventricles, implying that TNFα-mediated atrial(but not ventricular) remodeling in exercise is driven via mechanotransduction. This can be a potential mechanism underlying the pathological remodeling observed in the atria versus the beneficial remodeling in the ventricles in response to swim endurance exercise. This study is consistent to human studies where intense endurance is linked to AF, despite significant ventricular benefits.

Developing Zebrafish as a Model Organism to Study Cardiomyopathy

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Determining the molecular mechanisms that lead to cardiomyopathy will help in future development of cardiomyopathy-specific therapeutics. To understand how actin protein mutations contribute to the development of cardiomyopathy, we are developing zebrafish as an *in vivo* model to study the physiological effect this disease has on humans. Due to its optical transparency, rapid cardiovascular development and easy gene manipulation, zebrafish is a great *in vivo* model for genetic studies of vertebrate development and cardiovascular physiology. Our goals are to knock out zebrafish cardiac actin (*zfACTC*) genes using CRISPRs, and express human *ACTC* in zebrafish using Tol2 transposons. Although the human genome contains a single ACTC gene, zebrafish have a partly duplicated genome. Presently, we are working on two genes that have been shown to be expressed in the heart of the zebrafish: *zfACTA1b* and *zfACTC1a*. Knocking down the *zfACTA1b* gene resulted in a reduction in cardiac function before 72 hours post-fertilization, while knocking down *zfACTC1a* reduced cardiac function after 72 hours post-fertilization. These data suggest that zebrafish ACTC genes are active at different stages of development; a fact we can exploit in our *ACTC* mutation characterization in zebrafish. We hope our physiological characterization of the resulting zebrafish lines will provide more insight into how ACTC mutations develop into cardiomyopathy.

Physical activity protects against lipopolysaccharide-induced inflammation in tricep muscle and adipose tissue

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Sepsis is a systemic inflammatory response to infection, and can progress to organ dysfunction, shock, and even death. The prevalence of sepsis is increasing, however there are currently no preventative strategies to protect against the deleterious effects. The purpose of this study was to determine if habitual physical activity, in the form of voluntary wheel running (VWR), could protect against sepsis-induced inflammation in tricep muscle, inguinal adipose tissue (iWAT), and epididymal adipose tissue (eWAT) of mice. Male C57BL/6J mice (n=40, ~8 weeks of age) were housed in individual cages with access to a running wheel (VWR), or as sedentary controls (SED), for 10 weeks. Sepsis was induced using an intraperitoneal injection of 2 mg/kg lipopolysaccharide (LPS), or an equivalent volume of saline (SAL) as a control. Mice were euthanized after 6 hours of LPS exposure, and tissues were removed. First, we characterized the

effects of VWR on overall metabolic health. VWR led to attenuation in body mass gain, improved glucose tolerance, and induced iWAT browning as shown by increases in PGC1α and UCP1 protein content (main effect of VWR, $p<0.001$ and $p<0.0001$ respectively). Second, we measured the mRNA expression of inflammatory markers TNF-α and IL-1β. In iWAT, there was a protective effect of VWR on attenuating LPS induced increases in TNFα ($p<0.001$) and a main effect of VWR for reducing IL-1β ($p<0.05$), however there was no effect of VWR in tricep muscle or eWAT. Third, we assessed indices of IL6 signaling using IL6 and SOCS3 mRNA expression along with the phosphorylation of STAT3 (pSTAT3). In tricep muscle, there was a protective effect of VWR on LPS induced increases in IL6 and SOCS3 mRNA expression ($p=0.11$ and $p<0.001$, respectively) along with a main effect of VWR for reducing pSTAT3 protein content ($p<0.05$). In iWAT, there was a main effect of VWR for reducing IL6 and SOCS3 mRNA expression ($p<0.001$ and $p<0.05$, respectively), but no effect on pSTAT3 protein content. In eWAT, however, the protective effect of VWR against LPS induced inflammation was less pronounced, as only a main effect for reducing IL6 mRNA expression was observed ($p<0.05$). Overall, the results from this study demonstrate that habitual physical activity, in the form of VWR, can modulate the response to LPS-induced inflammation in multiple tissues.

Fluvastatin causes myopathic characteristics and altered lipid compartmentalization in diabetic muscle, but may facilitate repair following skeletal muscle damage

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With 47% of the American population currently prescribed statins, this class of lipid-lowering agents is among the most widely prescribed pharmaceuticals in North America. Furthermore, recent guidelines released by the American College of Cardiology and the American Heart Association recommend all diabetic individuals be prescribed statins to reduce their atherosclerotic cardiovascular disease risk. While effective in reducing the incidence of cardiovascular disease, the most common clinical complaint surrounding statin therapy is myopathy. The purpose of this study is two-fold: (i) investigate whether statin myopathy is exacerbated in a diabetic environment, and (ii) investigate the impact of Fluvastatin on muscle repair. Six weeks after diabetes onset, male streptozotocin-induced diabetic (STZ) and WT C57Bl6/J mice were assigned to receive control chow or a diet enriched with 600 mg/kg Fluvastatin. The tibialis anterior muscle of one hindlimb was injured via cardiotoxin injection, and twenty-four days after the commencement of diet administration (ten days after cardiotoxin injury), muscles were harvested and analyzed. WT and STZ muscles from Fluvastatin-treated mice displayed myopathy (increased presence of macrophages, necrotic, split and centrally-nucleated myofibers and decreased fiber area). No difference in severity of myopathy was noted between WT and STZ muscle. In STZ-Fluvastatin mice, a significant increase in extracellular (ectopic) lipids was observed; the result of significant decreases in the skeletal muscle expression of fatty acid transporters (FAT/CD36, FABPpm). Fluvastatin had no effect on lipid content in WT muscle, indicating impaired lipid compartmentalization as a strictly diabetic complication of Fluvastatin administration. Following muscle damage, regenerating Fluvastatin-treated WT and STZ muscles contained less macrophages and a greater capillary density than their control treated counterparts. Although statin administration is detrimental to overall muscle health, our findings do suggest that Fluvastatin may facilitate repair of both healthy and diabetic skeletal muscle.

Chronic endurance training in mice alters gut microbiome and enhances overall endurance capacity in their sedentary co-habitants

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Regular aerobic exercise has numerous health benefits, including combating type 2 diabetes, metabolic syndrome, atherosclerosis, and colorectal cancer, amongst many others. A factor that plays a common etiological role in these metabolic pathologies is gut microbiome dysbiosis. The gut microbiome represents an estimated 100 trillion commensal microorganisms essential for normal host physiology. We believe that the systemic benefits of endurance exercise are, in part, mediated by changes in the gut microbiota. To test this hypothesis, we have either singly housed, or group housed sedentary mice with mice subjected to 12 weeks of endurance exercise (forced-treadmill run, 3x/week for 20 weeks; n = 10 C57Bl/6J, male mice for all groups). Here we assessed if co-housing sedentary mice with endurance exercised mice transfers beneficial changes to their gut microbiota and improves their overall endurance performance compared to single housed sedentary mice. Fecal samples were collected for differential plating and colony assessment, and monthly endurance stress test was administered to assess overall endurance capacity. Twenty weeks of group housing resulted in increased number of colony forming units in differential plating, and enhanced endurance capacity in sedentary group housed mice compared to their single housed co-hanbitants. Group-housed sedentary mice displayed an improved glucose tolerance than single-housed sedentary. These results indicate that beneficial effects of exercise may occur due to a shift in gut microbiota in endurance exercised mice, which is then transferred into sedentary mice through group housing.

Using exosomes to 'cure' Duchenne Muscular Dystrophy (DMD) – a non-immunogenic genetic therapy to restore dystrophin expression in mdx mouse model of DMD

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Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder caused by a recessive X-linked genetic mutation in the gene encoding dystrophin. Currently, there is no cure for DMD, and recent efforts have focused on viral-based modes of gene delivery to express truncated forms of dystrophin, or rely on exon skipping strategies to skip the locus of mutation. Here we utilized bioengineered exosomes, 40-120 nm sized extracellular vesicles, to deliver full-length dystrophin mRNA (DMD) to mdx mice allogenically. C57BL/10ScSn-Dmdmdx (mdx) mice (~14-15 weeks old) were randomly divided into prednisolone (PRED, 2mg/kg BW/day), vehicle (VEH, i.v. injections of 0.9% sterile saline), exosomes only (EXO, i.v. injections of empty exosomes), and exosomes + DMD mRNA (EXO+mRNA, i.v. injections of exosomes containing 150 ng of DMD mRNA) groups. C57BL/10ScSn wild type (WT) mice were also included as control animals. Treatment of mdx mice with exosomes + DMD mRNA rescued the absence of dystrophin protein expression in skeletal muscle (EDL, SOL, TA, diaphragm), and heart as assessed by Western blotting and immunohistochemistry. This occurred in tandem with a complete attenuation in elevated serum creatine kinase levels, muscle hypertrophy, and grip

strength deficits. Interestingly, the improved grip strength in EXO+mRNA group vs. the other groups was still significantly lower than maximum grip strength measured in WT mice. Moreover we treated dermal fibroblasts isolated from control and DMD patients with DMD mRNA only, exosomes only, and exosomes + DMD mRNA. Similar to our in vivo observations, we restored dystrophin protein expression in DMD patients treated with our bioengineered exosomes. Our data clearly establish treatment with non-immunogenic bioengineered exosomes as efficient delivery vehicles for treating diseases of genetic origin. Funded by NSERC, CIHR and Exerkine Corporation.

Does Estrogen Protect Against Skeletal Muscle Damage and the Cellular Stress Response?
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Estrogen may modulate the cellular stress response (CSR) and thus play a role in protecting skeletal muscle from damage. To clarify the relationship between estrogen and the CSR, the expression of Hsps was examined in estrogen-void skeletal muscle following controlled lengthening contractions (LC) of the tibialis anterior (TA) muscle. TA muscles from ovary-intact (E+; n=12) and ovariectomized (OVX; n=12) female Sprague-Dawley rats were subjected to either 20 or 40 LCs. Contractile measures of twitch, tetanus, ½ relaxation time were measured prior to, directly after, and 10 minutes after LCs, while torque production was measured throughout. No differences were observed in contractile measures between E+ and OVX rodents. However, 24 hours after stimulation measures of muscle damage showed significant differences in fibre area, ($P<0.0001$) and circularity ($P<0.05$), between E+ and OVX rats following both 20 and 40 LCs. Hsp72 content was increased in TA muscles from OVX rats compared to E+ following 40 LCs ($P <0.05$), but not after 20 LCs. Taken together, low estrogen does not appear to affect muscle contractile properties but may render muscles more susceptible to muscle damage.

Exploring actomyosin molecular interactions using short F-actin oligmers
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Movement is a fundamental property of all life-forms. Actin, a cytoskeletal protein, interacts with myosin in an actomyosin complex to form the basis of cellular movement. We want an atomic resolution understanding of the force generating actomyosin complex; however, the property of actin to self-assemble into filaments (F-actin) makes it unsuitable for X-ray crystallography. Therefore, we propose that short, stable non-polymerisable F-actin oligomers of defined length can provide a platform to study protein-protein interactions of the force generating actomyosin complex. We have developed the ADPr-trimer as such a short F-actin complex. The results from native gels, DLS and mass spectrometry indicate that ADPr-trimer does not bind myosin S1 fragment. Nevertheless, ADPr-trimer has been shown to interact with other ABPs, such as gelsolin, and thus ADPr-trimer might be used to elucidate molecular interactions between F-actin and ABPs. An alternative short actin dimer was shown to interact with myosin S1 in preliminary experiments. Therefore, we will pursue this dimer as an alternative candidate for exploring molecular interactions in actomyosin complex.

Characterizing insulin resistance using a spatial mathematical model of glucose transport in skeletal muscle interstitium

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Impaired glucose uptake in type II diabetes is a result of decreased insulin sensitivity and leads to high blood glucose concentrations. Insulin sensitivity is typically measured using serial blood samples during a euglycemic clamp and while these measurements give a global index of insulin resistance, they fail to account for the spatial complexity of vascular geometry that affect glucose uptake in the muscle. To explore how skeletal muscle capillary geometry impacts glucose transport we used a spatial mathematical model to quantify the effects insulin resistance on glucose uptake in the muscle. The mathematical model describes the steady-state diffusive transport of both insulin and glucose between capillaries and muscle fibers in skeletal muscle interstitium. Blood insulin and glucose concentrations were used as boundary conditions at the capillary wall. Uptake of insulin into the fiber was calculated using insulin flux at the muscle fiber surface according to Michaelis-Menton kinetics. Glucose uptake by the muscle fiber was specified to be linearly proportional to glucose concentration and insulin flux. In this work, the governing equations were solved on a two-dimensional cross-section of skeletal muscle orthogonal to capillary blood flow. The geometry was discretized using triangular elements and the governing equations were solved using a Galerkin finite element method. Convergence was verified by successively refining the mesh to ensure the solution was independent of the choice of discretization. Blood insulin and blood glucose concentrations were altered to simulate fasting and post-prandial conditions. We simulated insulin resistance by decreasing the rate constant describing the kinetics of glucose uptake by a percentage of the severity of insulin resistance. Glucose uptake rate with no insulin resistance was 165% higher in post-prandial conditions when compared to fasting conditions. The rate of glucose uptake decreases approximately linearly with insulin resistance in both fasting and post prandial conditions. The slope for the post prandial condition is 10 times larger than that of the fasting condition.

Protein Arginine Methyltransferase Expression During Denervation-induced Skeletal Muscle Plasticity

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Protein arginine methyltransferase 1 (PRMT1), PRMT4 (also known as co-activator-associated arginine methyltransferase 1; CARM1) and PRMT5 are critical components of a diverse set of intracellular functions including cell signaling and transcriptional regulation. Despite the limited number of studies investigating PRMT biology in muscle, evidence strongly suggests that PRMT1, CARM1 and PRMT5 are important players in the regulation of skeletal muscle plasticity. However, their role in disuse-induced muscle remodeling is unknown. Thus, our study objective was to determine whether denervation-induced muscle disuse alters PRMT expression and activity in skeletal muscle and to contextualize PRMT biology within the early disuse-evoked signaling events that precede muscle atrophy. After unilateral sectioning of the sciatic nerve, mice were subjected to 6, 12, 24, 72, or 168 hours of denervation (n= 8-9/group). The contralateral limb served as an internal control. Western blot analyses were employed to determine protein expression levels in the denervated tibialis anterior (TA) muscle, relative to the

contralateral, non-denervated, control TA muscle across the experimental time course. A ~32% reduction ($p < 0.05$) in TA muscle mass was observed in the denervated hind limb after 168 hours. PRMT1, CARM1 and PRMT5 protein expression were significantly increased by 2.7-, 1.3-, 1.8-fold, respectively, after 72 hours and by 3.4-, 1.3-, 2.2-fold, respectively after 168 hours of inactivity in the denervated hind limb, as compared to the control limb. This differential response to denervation-induced muscle disuse suggests a unique sensitivity to, or regulation by, potential upstream signaling and transcriptional pathways. These denervation-induced increases in PRMT expression were accompanied by a ~40% elevation in cellular mono-methyl arginine content at 72 and 168 hours, a marker of global PRMT methyltransferase activity. Muscle RING-finger protein-1 (MuRF1) protein expression was also significantly elevated by 4.5- and 3.4-fold, respectively, after 72 and 168 hours of denervation, suggesting that PRMT expression may be mediated by factors governing the muscle atrophy program. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) protein expression was diminished by ~51% ($p < 0.05$) in the denervated limb after 6 hours and remained depressed throughout the time course. In contrast, p38 mitogen-activated protein kinase (p38) phosphorylation status (phosphorylated/total) was elevated by ~43-52% ($p < 0.05$) in the denervated muscle following 6 and 12 hours. AMP-activated protein kinase (AMPK) phosphorylation status was lowered by ~40% ($p < 0.05$) after 6 and 12 hours of denervation. By 168 hours, AMPK phosphorylation status was 65% greater in the denervated limb than in the control limb. Our data suggest that alterations in AMPK, p38, and PGC-1α signaling are among the earliest signals that precede the induction of the atrophy program in response to neurogenic muscle disuse. Furthermore, PRMT1, CARM1 and PRMT5 may contribute to the remodeling of muscle during denervation-induced atrophy.

Cellular remodelling of C2C12 myoblasts during recovery in response to electrical stimulation

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Muscle regeneration following muscle damage requires quiescent satellite cells to enter the cell cycle, proliferate to increase cell number, exit the cell cycle and subsequently fuse and mature into multinucleated muscle fibres. Regulation of this process involves the ordered expression of myogenic regulatory factors and cell cycle proteins. Muscle cell cycle arrest can be regulated by AMPK and AKT. The interplay of these two proteins is important in the maintenance of healthy muscle tissue, and any stimulus that alters the activity of these proteins may alter this process. Electrical stimulation (ES) is a method commonly employed to elicit adaptations in muscle cells. Recently, ES has been shown to cause effects within proliferating myoblasts. Our lab has previously focused on the long term effects of ES on proliferating myoblasts, but no research to date has focused on the response of these cells in the recovery phase following stimulation. Thus, we have employed a model whereby proliferating myoblasts are subjected to ES for 4hr/day followed by a recovery period. Myogenic specific protein, cell cycle inhibitor protein as well as intracellular signalling protein levels were measured. Increases in pAKT were found following stimulation, with no changes in the myogenic targets of AKT. Additionally, no changes in the activation of AMPK were found, and cell cycle inhibitor p27 protein content decreased following stimulation. Stimulation induced an increase in p42 and p44 MAPK phosphorylation, and a

decrease in Mef2A protein content. Interestingly, there was an upregulation of autophagy marker LC3II, which was subsequently diminished, potentially indicating cellular remodelling. Our results suggest important events occur in the post-ES recovery phase.

Nutritional targeting of mitochondrial bioenergetics in cancer: lipid incubation increases Caspase 3/7 activity and H2O2 emission in MCF7 cancer but not HT29 and non-cancer epithelial cells

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A benchmark of cancer cells is the reliance on glycolysis characterized by decreased mitochondrial oxidative phosphorylation (the Warburg effect). Recent evidence suggests that fatty acids (palmitoylcarnitine) may force a shift to mitochondrial oxidative phosphorylation in HT29 cancer cells causing cell death, yet non-cancer cells appear to be resistant. These results present the intriguing possibility that 'lipid-therapy' might be well tolerated by healthy cells while retaining potency in cancer. However, the mechanism of lipid-induced cancer cell death is not known. Our investigation hypothesized that fatty acid treatment in cancer cells will increase NADH generation leading to H2O2 emission and greater apoptosis-related caspase 3/7 activity while non-cancerous cells will be resistant to this fat exposure despite enhanced NADH generation. Two adenocarcinomas (HT29 colon and MCF7 breast cancer) and a non-cancer colonocyte CCD-841 were incubated for 3 and 24hr with intralipid containing emulsified polyunsaturated fatty acids. Using in-well spectrofluorometry, we measured H2O2 emission in living adherent cells (Amplex Ultrared, Life Technologies), NADH production using a tetrazolium salt (XTT) and activation of caspase 3/7 using a caspase-specific activated fluorophore (CellEvent, Life Technologies). After 24hr at 0.625% intralipid (~22mM), both MCF7 and CCD-841 demonstrated greater NADH production compared to HT29 (23%), however, H2O2 emission in MCF7 was greater than CCD-841 (45%) and HT29 (55%) suggesting MCF7 are more sensitive to NADH 'overload' from lipids. This occurred concomitantly with MCF7 demonstrating a greater caspase 3/7 activity vs CCD-841 (30%). Of note, caspase 3/7 activity also increased in HT29 despite no increase in H2O2 rates suggesting a non-H2O2 mechanism related to lipid exposure. These results suggest that lipid stimulates caspase 3/7 activity in MCF7 through H2O2 generation stemming from increased NADH production but this does not occur in normal CCD-841 despite equally elevated NADH. However, increased caspase 3/7 in HT29 occurred without any increase in NADH or H2O2 suggesting an alternative mechanism explains HT29 susceptibility to fatty acid treatment. These findings raise the intriguing possibility that fatty acid stress might be tolerated by healthy cells while possibly initiating cell death in breast and colon cancers, although the mechanisms may be diverse.

The Role of Mitophagy in Aging Cardiac Muscle

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Mitochondrial autophagy (mitophagy), a highly conserved cellular housekeeping process by which damaged mitochondria are digested and recycled via lysosomes, occurs continuously in the heart to maintain tissue homeostasis. Unfortunately, the efficiency of this process has been reported to decline with age and has been considered a major mechanism underlying cardiac senescence. Still, mitophagic activity remains ill-defined in aging cardiac muscle, as a result of steady state measurements made with aims to elucidate a dynamic process. In this study we evaluated *in vivo* levels of autophagic and mitophagic flux in order to elucidate age-related changes in pathway mechanics. This was accomplished by comparison of 6-7-month (young) and 35-36-month old (aged) male Fischer 344 Brown Norway F1 rats, which were subjected to successive intraperitoneal injections of the microtubule destabilizer, colchicine. We report a significant, though modest 6% age-associated cardiac hypertrophy in the senescent animals, as indicated by mean heart weight per body weight values (HW/BW) of 2.3 ± 0.02 mg/g, and 2.5 ± 0.05 g/mg, in young and aged animals, respectively. Immuoblot analyses of whole cardiac muscle and isolated mitochondrial proteins revealed trends for increased flux of the autophagosomal marker, LC3 II, as well as a 3-fold increase in the mitochondrial localization of the mitophagy marker, Nix, with age. Together with aged-related increases in the levels of lysosomal proteins: LAMP2, LAMP2a, as well as Cathepsin D, by 72%, 50%, and 57%, respectively, our findings suggest a cardioprotective increase in autophagic/mitophagic activity with age. Furthermore, this notion was supported by comparable levels of oxygen consumption as well as mitochondrial content, as measured by cytochrome C oxidase (COX) activity, between young and aged animals. In addition to these findings, future functional and morphological assessments of aged mitochondria will aid in the determination of the required levels of autophagic activity to maintain a relatively healthy aged myocardium.

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