Program and Proceedings of the 11th Annual Muscle Health Awareness Day

York University, May 22, 2020

Muscle Health & Research Centre

Adaptation • Development • Metabolism • Disease

health | YORK UNIVERSITÉ UNIVERSITY

David A. Hood, PhD

Professor,
Canada Research Chair
in Cell Physiology,
School of Kinesiology &
Health Science

Director,
Muscle Health Research
Centre

302 Farquharson Bldg.,
York University
4700 Keele St.
Toronto ON
Canada M3J 1P3

Email: dhood@yorku.ca
Web: yorku.ca/dhood/

Date: May 22, 2020

To: All Participants

From: David A. Hood, MHRC Director

Welcome to the 11th Annual Muscle Health Awareness Day Via Zoom

The Muscle Health Research Centre at York University welcomes you to **MHAD11**, our 11th annual "***Muscle Health Awareness Day***", designed to bring faculty members and trainees together to discuss issues related to skeletal and cardiac muscle physiology, metabolism, adaptation, development and disease.

There is a first time for everything, and clearly the format of this meeting is different from the face-to-face interactions that we have enjoyed in previous years. However, we are determined to carry on, and are pleased to present 8 great speakers who have agreed to deliver their "Muscle Health" research via Zoom. The focus this year is on skeletal muscle and cardiovascular physiology.

We have also adopted a different "poster" presentation style, reminiscent of the "3-min thesis" format. Our level of sponsorship this year, along with reduced expenses, have allowed our judges to select 8 Abstracts from the MSc and PhD student submissions, and fund them with abstract awards valued at $200. Two of these presentations will also be selected for AJP (Cell Physiology) Oral Presentation Awards. It should be noted that a total of 9 faculty members were asked to be involved in the selection of these awards, not only from York, but from other institutions as well.

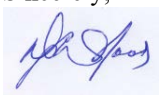
Read through the Program: If you submitted an abstract, you will find it published in these MHAD Proceedings. Sixty (60) abstracts were submitted for the meeting, and while awards can't be given to all of them, reading through these gives you a chance to see the breadth of muscle health research that we are disseminating this year.

Provide feedback: Please send any ideas for alternative abstract delivery methods, or other feedback on the format of the meeting, to mhrc@yorku.ca so that we can improve MHAD in coming years.

Next year, MHAD12 will change date, as it will be the "pre-conference day" for the **International Biochemistry of Exercise Conference (IBEC)** to be held at the Marriott Eaton Centre, June 14-17, 2021, and organized by York University. Much more information on this exciting meeting will be sent out soon.

We thank all of our presenters and sponsors for their participation, and for helping to make this a successful event.

Sincerely,



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

11th Annual Muscle Health Awareness Day Program

Friday May 22, 2020

From York University via ZOOM

Session 1: Skeletal Muscle

Session Chair: Dr. David A. Hood

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

9:05-9:35 – Dr. Imed Gallouzi, *McGill University*
The role of the RNA Binding Protein HuR in muscle function and integrity: new avenue to treat disease-induced muscle wasting.

9:35-10:05 – Dr. Jacob Haus, *University of Michigan*
Enzymes for Dicarbonyl Detoxification in Skeletal Muscle are Attenuated with Obesity and Diabetes by Mechanisms of Acetylation.

10:05-10:35 – Dr. Scot Kimball, *Pennsylvania State University*
Regulation of the Mechanistic Target of Rapamycin (mTOR) as a Mechanism for Modulating Protein Synthesis in Skeletal Muscle.

10:35 – 10:45 Break

Session Chair: Dr. Arthur Cheng

10:45 – 11:05 - Four (4) Abstract Presentations, 5 mins per Abstract
(3 min presentations + 2 mins questions)

11:05-11:20 – Dr. Sally A Miller, *Advanced Biosystems Specialist, Nikon Instruments Inc.*
Live Cell Imaging; Nikon's approach to Gentle, Fast, and Flexible Confocal Systems

Session 2: Muscle Physiology

Session Chair: Ms. Heather Johnston

11:20-11:50 – Dr. Sunita Mathur, *University of Toronto*
Sarcopenia in chronic lung disease: evaluation and relationship to health outcomes

11:50-12:20 – Dr. Sherry Grace, *York University*
Knowledge Translation and Implementation Science in Cardiac Research

12:20 – 12:30 Break

Session Chair: Dr. Ali Abdul-Sater

12:30-12:50 - Four (4) Abstracts presentations, 5 mins per Abstract
(3 min presentations + 2 mins questions)

12:50-1:05 – Dr. Natalia Fedianina, *Flow Cytometry Consultant, Beckman Coulter Life Sciences*
Applications of Flow Cytometry in Muscle Health Research

Session 3: Cardiovascular Physiology

Session Chair: Dr. Heather Edgell

1:05-1:35 – Dr. Philip J. Millar, *University of Guelph*
Regulation of muscle sympathetic nerve activity during exercise.

1:35-2:05 – Dr. Kimberly Dunham-Snary, *Queen's University*
Mitochondrial-nuclear genetic interaction in health and disease.

2:05-2:35 – Dr. Richard L. Hughson, *University of Waterloo*
Effect of spaceflight on astronaut vascular and cardiometabolic health. Results from the Vascular Series Experiments

2:35-2:40 – Acknowledgment of Student Abstract Awards and Adjournment

11th Annual Muscle Health Awareness Day Speaker Profiles

Dr. Imed Gallouzi, *McGill University*

Dr. Imed Gallouzi is a Professor and Associate Chair in the Department of Biochemistry at McGill. His research area is mRNA metabolism during the cell cycle and cell differentiation. He uses the tools of molecular and cell biology to study problems in this field. Dr. Gallouzi's long-term research goals focus on understanding the cellular mechanisms involved in the regulation of mRNA turnover and how they affect cell growth and differentiation.

Dr. Jacob Haus, *University of Michigan*

Dr. Jacob Haus is an Associate Professor of Movement Science and Director of the Human Bioenergetics Laboratory at the University of Michigan School of Kinesiology. The focus of the Haus Lab is to identify strategies for the prevention and treatment of diabetic complications. Using aerobic exercise and caloric restriction, they have identified functionally redundant mechanisms, mediated by cellular bioenergetics, that attenuate inflammation and oxidative stress. Their rationale is that once these mechanisms are fully elucidated, progress towards the prevention and treatment of diabetic complications may be possible.

Dr. Scot Kimball, *Pennsylvania State University*

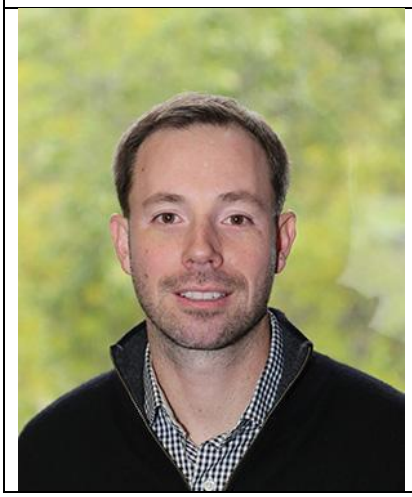
Dr. Kimball is a Professor in the Department of Cellular and Molecular Physiology in the College of Medicine. He is a physiologist with an interest in macronutrients as signaling molecules that regulate mRNA translation. His focus is on pre-clinical investigations of the signaling pathways and mechanisms through which amino acids, carbohydrates, and fatty acids act to modulate the initiation and elongation phases of mRNA translation, with an emphasis on anabolic resistance that manifests in skeletal muscle in response to disuse.

Dr. Sherry Grace, *York University*

Dr. Sherry Grace is a Professor in the School of Kinesiology and Health Science in the Faculty of Health at York University. Professor Grace's research centers on optimizing post-acute cardiovascular care globally, as well as outcomes (including mental health). She has published ~250 papers which have been cited ~11,000 times and authored clinical practice guidelines internationally.

Dr. Sunita Mathur, *University of Toronto*

Dr. Mathur is a physiotherapist and Assistant Professor in the Dept of Physical Therapy. She is the leader of the Muscle Function and Performance Lab, and her research is aimed at improving the health of skeletal muscle in people suffering from profound muscle atrophy and weakness in order to help them regain function and independence. Her research crosses multiple patient populations including people with advanced lung disease, solid organ transplantation and critical illness.



Dr. Philip J. Millar, *University of Guelph*

Dr. Millar is an Associate Professor in the Department of Human Health and Nutritional Sciences at the University of Guelph. He is also an Affiliate Scientist with the Toronto General Research Institute. His work is focused on understanding the mechanisms that regulate sympathetic outflow to skeletal muscle in humans, and the neural contributions to regulating blood pressure at rest and during exercise. Dr. Millar's research program is supported by NSERC, CFI, Parkinson Canada, and an Ontario Early Research Award.

Dr. Kimberly Dunham-Snary, *Queen's University*

Dr. Dunham-Snary studies mitochondrial biology, specifically how genetic and structural changes to mitochondria alter cell function in both physiology and pathology. Her research focuses on cardiopulmonary pathophysiology and the complex etiology of cardiometabolic diseases. She earned her MS in Forensic Science from Penn State University and her PhD from the University of Alabama at Birmingham prior to joining the Department of Medicine at Queen's University, where she is currently a CIHR-funded, senior postdoctoral fellow in the laboratory of Dr. Stephen Archer.

Richard L. Hughson, *University of Waterloo*

Dr.Hughson is the Schlegel Research Chair in Vascular Aging and Brain Health, and the Senior Director of Research at the Schlegel-University of Waterloo Research Institute for Aging. He is a Fellow of the Canadian Academy of Health Sciences, and the recipient of the CSEP Honour Award and the NASA Exceptional Scientific Achievement Medal. His research focuses on cardiovascular adaptations to exercise, inactivity and aging to explore the impact of increased arterial stiffness on brain blood flow and why older persons experience dizziness and increased risk of falling. He is principal investigator on six studies of astronauts on the International Space Station.

Conference Sponsors

Next year: MHAD12 is the IBEC pre-Conference day

health | YORK UNIVERSITÉ UNIVERSITY

ATP ADP

18th International Biochemistry of Exercise Conference (IBEC)
Sponsored by York University, Toronto, Canada

Welcome | Program Overview | Speakers & Topics | Exhibitors & Sponsors | FAQs | Travel & Accommodation | Conference Registration | Abstract Submission

Theme: "Exercise for Health, Adaptation and Rejuvenation".
The research highlighted in this Conference will provide the foundation for our understanding of the **molecular basis of improvements in tissue health resulting from exercise.**

Featured Keynote Speakers:

- Dr. Bruce Spiegelman
- Dr. Laurie Goodyear
- Dr. Håkan Westerblad

+ 14 Symposia and workshops

Dates: Mon June 14 – Thurs 17, 2021

Marriott Hotel Downtown at the Eaton Centre

Abstract Presentations

Muscle Health Awareness Day (MHAD11, May 22, 2020)

Abstract Number	First Author (Surname)	Abstract Title	University Affiliation
1.	**Abou Sawan**	Translocation and colocalization of the mtor-rheb-lysosomal protein complex to the sarcolemma may be associated with reduced reliance on dietary amino acids to support post-exercise myofibrillar remodeling after resistance training in young men	*University of Toronto*
2.	**Al Amrani**	Scoliosis in spinal muscular atrophy type i in the nusinersen era	*University of Toronto*
3.	**Baker**	Muscle stem cell fate and identity is directed by the mitochondrial fusion protein opa1	*University of Ottawa*
4.	**Bellissimo**	Does the mitochondrial-enhancing drug olesoxime prevent cell death and improve muscle size in duchenne muscular dystrophy	*York University*
5.	**Bhattacharya**	Muscle stem cell proliferative fates are directed by p107 regulation ofmitochondrial atp generation	*York University*
6.	**Braun**	Neuronatin is expressed in murine skeletal muscle and negatively regulates serca activity	*Brock University*
7.	**Cheema**	Rapamycin treatment in fibroblasts from healthy and melas individuals	*York University*
8.	**Da Silva Rosa**	Therapeutic targeting of skeletal muscle nix in early-onset insulin resistance	*University of Manitoba*
9.	**Davari**	Effect of pdcd4 depletion on myofibrillar protein abundance, mtorc-1 signaling and autophagy	*York University*
10.	**Delfinis**	Exploring the relationship between mitochondrial dysfunciton and skeletal muscle during cancer	*York University*
11.	**Eadie**	The regulation of heme metabolism in myocytes by hypoxia and ischemia: substrate-enzyme dyssynchrony	*Dalhousie University*
12.	**Estafanos**	Practical ‘activity snacks’ reduce postprandial insulinemia in healthy men and women.	*University of Toronto*
13.	**Finch**	The effects of low dose lithium supplementation on the genetic markers of adipose browning	*Brock University*
14.	**Fletcher**	Mechanisms behind local control of skeletal muscle blood flow	*University of Guelph*
15.	**Fredo-Cumbo**	Deficiency of the autophagy gene atg16l1 induces insulin resistance through irs1 degradation	*Hospital for Sick Children*
16.	**Geromella**	Gsk3 inhibition uncouples serca ca2+ transport efficiency and augments serca’s energy expenditure in c2c12 cells	*Brock University*
17.	**Ghahramani Seno**	Valproic acid improves survival rate and skeletal muscle phenotype in mtm1 knockout mice	*Hospital for Sick Children*
18.	**Grafham**	Evidence for unaltered skeletal muscle repair in young adults with well-controlled type 1 diabetes mellitus	*McMaster University*
19.	**Habib**	Flow-mediate dilation is greater in upright posture in young healthy adults	*York University*
20.	**Huber**	The cardio-splenic axis: a new player in acute regulation of cardiac function	*University of Guelph*
21.	**Hoffman**	Somatostatin receptor type 2 antagonism restores counterregulatory function and confers hypoglycemic resistance in a rodent model of advanced type 2 diabetes mellitus	*McMaster University*

22.	**Holjak**	Is there genetic drift in the spontaneously hypertensive rat colony?	*University of Geulph*
23.	**Hutchinson**	Dietary n-3 vs n-6 pufa differentially modulate macrophage-myocyte inflammatory cross-talk	*University of Guelph*
24.	**Jones**	Same codon, different diseases? A look into the reason for disease onset	*York University*
25.	**Kim**	Role of heme regulated inhibitory kinase control of hypertrophy and sex differences in the diastolic dysfunction	*Dalhousie University*
26.	**Kustermann**	A novel in-frame deletion of 52-55 dmd mouse model preserves muscle function	*Hospital for Sick Children*
27.	**Li**	Calpain activation mediates myocardial abnormalities in tail-suspended mice by promoting nadph oxidase activation via p38 and erk1/2 mapk pathways	*Soochow University*
28.	**Maani**	Mirnas as clinical biomarkers in myotubular myopathy	*University of Toronto*
29.	**Madu**	Depletion of e1α subunit of branched-chain ketoacid dehydrogenase complex improves myofibrillar protein abundance and anabolic signaling in myotubes	*York University*
30.	**Mann**	Effect of regulating branched-chain amino acid metabolism on insulin sensitivity	*York University*
31.	**Marrow**	A novel radio-telemetry method for the measurement of pleural pressure and physiological rhythms in freely behaving mice	*University of Guelph*
32.	**Martin**	Comparing quadriceps strength and size between lung transplant candidate diagnostic groups before and after transplantation	*University of Toronto*
33.	**Mazo**	The influence of acute aerobic and resistance exercise on mtor signaling and autophagy in untrained human skeletal muscle	*University of Michigan*
34.	**McFee**	Adapting a novel muscle endogenous repair assay for industry and academic adoption	*University of Toronto*
35.	**Memme**	A role for atf4 in mitochondrial regulation within skeletal muscle	*York University*
36.	**Messner**	Heterozygous sod2 deletion impairs serca function in soleus muscles from female mice	*Brock University*
37.	**Mofford**	Investigating candesartan for the prevention of pulmonary hypertension in rats with diastolic dysfunction	*University of Guelph*
38.	**Mora**	The effect of a chemotherapy drug cocktail on branched- chain amino acid metabolism	*York University*
39.	**Murugathasan**	Investigating the effect of exercise on inflammatory responses	*York University*
40.	**Ng, A**	Hemin therapy – a novel treatment for diastolic dysfunction in rats	University of Guelph
41.	**Ng, S**	Next generation ampk activation elicits adaptive gene expression in the skeletal muscle of dystrophic animals	*McMaster University*
42.	**Northrup**	Intron retention: what is it and how do we evaluate it?	*Dalhousie University*
43.	**Oliveira**	Mitochondrial maintenance in aged human right atrial tissue following ischemia-reperfusion injury	*York University*
44.	**Ouellette**	Impaired skeletal muscle regeneration in type 1 diabetes mellitus is characterized by a dysfunctional sphingolipid pathway response	*University of Windsor*
45.	**Pierdoná**	Using extracellular vesicles to predict individual response to exercise in obese youth	*University of Manitoba*
46.	**Pooni**	Accuracy of energy expenditure measurements by activity monitors in type 1 diabetes and control subjects	*York University*

47.	**Rahman**	Persistent necrosis and impaired regeneration related to elevated pai-1 in aged skeletal muscle	*University of Waterloo*
48.	**Richards**	The role of pgc-1α in the expression of cardiolipin synthesis enzymes in skeletal muscle	*York University*
49.			
50.	**Ryan**	Creatine supplementation exhibits sex differences in white adipose tissue and increases mitochondrial markers in female rats	*Brock University*
51.	**Sadek**	Inflammation-mediated activation of inos triggers energy crisis in skeletal muscle to promote atrophy	*McGill University*
52.	**Saumur**	Associating muscle function with phases of reactive stepping phases young, healthy adults	*University of Toronto*
53.	**Savinova**	Making the most of the covid crisis – mining online cardiac databases	*University of Guelph*
54.	**Slavin**	The role of atf5 in skeletal muscle uprmt regulation following endurance exercise	*York University*
55.	**Steffensen**	Understanding the role of actin's c-terminus in actomyosin force generation	*University of Guelph*
56.	**Stokes**	Molecular transducers of human skeletal muscle remodeling under different loading states	*McMaster University*
57.	**Te**	Enabling skeletal muscle repair and functional recovery following denervation-induced injury using ultrasound mediated gene delivery (umgd)	*St. Michael's Hospital*
58.	**Tokarz**	Insulin-stimulated glut4 translocation is reduced by palmitate and linked to defective rac1-induced actin remodelling	*The Hospital for Sick Children*
59.	**Triolo**	Regulation of the autophagy-lysosome system in response to hindlimb denervation	*York University*
60.	**Vithy**	Multi-exon skipping as a potential therapy for nemaline myopathy	*University of Toronto*
61.	**Wells**	Clamping skeletal muscle po2 eliminates hyperinsulinemic microvascular blood flow response	*Memorial University of Newfoundland*
62.	**Whitley**	Gsk3 inhibition with low dose lihtium supplementation augments fatigue resistance in soleus muscles	*Brock University*
63.	**Williamson**	Interruption of sedentary time with intermittent walking or body weight squats improves skeletal muscle dietary amino acid utilization with minimal impact on anabolic-related intramuscular signaling	*University of Toronto*
64.	**Yu**	The effects of exercise training on resting metabolic rate in youth with overweight or obesity	*York University*

MHAD11 Abstract Winners

A panel of 9 faculty member judges from the University of Guelph, the University of Toronto, York University and McMaster University was asked to provide feedback to select the winners of this year's MHAD11 Abstract awards.

Name	Affiliation
PhD students	
Amber Hutchinson	University of Guelph
Sean Ng	McMaster University
Matthew Triolo	York University
Eric Williamson	University of Toronto
Simone Da Silva Rosa	University of Manitoba
MSc Students	
Nicole Baker	University of Ottawa
Emily Hoffman	York University
Jaryd Te	St. Michael's Hospital

All 8 Abstract Awardees are in the running for the American Journal of Physiology (Cell Physiology) Oral Presentation Award, to be given out at the end of the meeting.

Proceedings of the Muscle Health Awareness Day (MHAD11, May 22, 2020)

Abstracts

TRANSLOCATION AND COLOCALIZATION OF THE MTOR-RHEB-LYSOSOMAL PROTEIN COMPLEX TO THE SARCOLEMMA MAY BE ASSOCIATED WITH REDUCED RELIANCE ON DIETARY AMINO ACIDS TO SUPPORT POST-EXERCISE MYOFIBRILLAR REMODELING AFTER RESISTANCE TRAINING IN YOUNG MEN

Sidney Abou Sawan[1], Nathan Hodson[1], Julia M. Malowany[1], Cassidy Tinline-Goodfellow[1], Daniel W. D. West[1,2], Dinesh Kumbhare[2], Daniel R. Moore[1]

[1]University of Toronto, Toronto, Canada, [2]Toronto Rehabilitation Institute, Toronto, Canada.

INTRODUCTION: The translocation and colocalization of mechanistic target of rapamycin (mTOR) with regulatory proteins is purported to be critical for translation initiation after resistance exercise (RE). Acute RE enhances the incorporation of dietary amino acids into skeletal muscle proteins for up to 24h in controlled lab-based settings. Resistance training (RT) can alter the magnitude and duration of the RE-induced elevation of muscle protein synthesis. The present study examined how training status and mTOR regulation modulates the RE-induced incorporation of dietary protein into muscle tissue protein over 24h in a free-living setting. METHODS: Ten recreationally active men (age: 22±3yr; BMI: 23.4±2.8kg/m2; body fat: 19.8±7.5%; means±SD) underwent 8 weeks of whole-body resistance exercise 3x/week (4 sets × 10-12 repetitions at 75% 1-repetition maximum). Muscle biopsies were obtained immediately before- (REST) and 24h after- an acute bout of RE in the untrained (UT) and trained (T) state while consuming crystalline amino acid, mixed macronutrient diets containing the equivalent of 1.6g protein/kg/d (modelled after egg). Dietary amino acid incorporation into the contractile myofibrillar fraction (LC/MS/MS) was determined by enriching the diet to 15% with [2H5]-phenylalanine or [13C6]-phenylalanine. Regulation of mTOR was determined by immunofluorescence microscopy. RESULTS: RT increased mTOR-WGA (sarcolemma marker) by ~23% and mTOR-UEA1 colocalization (capillary marker) by ~14% irrespective of RE (training effect; $P<0.05$). RT increased mTOR-LAMP2 (lysosomal marker) and mTOR-Rheb colocalization by ~10% (training effect; $P<0.05$). Resting LAMP2-WGA colocalization was increased by 10% in T compared to UT ($P<0.05$) and Rheb-WGA colocalization increased by ~10% (training effect; $P<0.05$). RE increased dietary phenylalanine incorporation above REST (0.062±0.023 MPE) in UT by ~34% (0.084±0.021 MPE, $P=0.016$) but not in T (0.072±0.022 MPE; $P=0.22$). CONCLUSION: An enhanced proximity of the mTOR-Rheb-lysosomal protein complex to the sarcolemma (and ostensibly near capillaries and ribosomes) after training could suggest an enhanced capacity for protein synthesis that is independent of dietary amino acid-induced stimulation of muscle protein synthesis. Our data demonstrates that resistance exercise enhances dietary amino acid incorporation into myofibrillar protein primarily in the untrained state, suggesting myofibrillar remodeling in the trained state may be less dependent on exogenous amino acids

SCOLIOSIS IN SPINAL MUSCULAR ATROPHY TYPE I IN THE NUSINERSEN ERA

Fatema Al Amrani[1], Reshma Amin[2], Jackie Chiang[2], Jennifer Boyd[1], Lauren Weinstock[3], Jiri Vajsar[1], James Dowling[1], Hernán Gonorazky[1]

[1]Department of Paediatrics, Division of Neurology, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. [2]Department of Paediatrics, Division of Respirology, Hospital for Sick Children, University of Toronto, Toronto, Ontario, [3]Department of physiotherapy, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Background: Spinal muscular atrophy (SMA) (5q) is an autosomal recessive neurodegenerative neuromuscular condition with a high degree of mortality and morbidity. A progressive scoliosis due to a rapid deteriorating axial muscle tone is a key factor with a major impact on the respiratory and motor function in 100% of SMA type 1 and 2. However different efforts and all the interventions that have been tried in the past have little or no effect on the progression of scoliosis. Currently, with the introduction of new therapies, such antisense oligo therapy, the natural history of the SMA population has changed. However, most of the reports have focused in the motor scores and respiratory function. Little is known on the impact of this novel therapy in the progression of the scoliosis. Objectives: To document the development and progression of scoliosis in SMA-type I treated with nusinersen. Methods: Retrospective study in patients with SMA-type I treated with nusinersen and followed at our institution were enrolled in this study. Data about patient's diagnosis, age at the first nusinersen dose, number of nusinersen doses, motor functional assessment at baseline and follow-up, X-ray spine and respiratory parameters were obtained from the charts. Results: Twenty five percent (9/34) were found to have SMA-type I from all patients followed at the neuromuscular clinic at our institution. Sixty six percent (7/9) met inclusion criteria and 1/7 was excluded due to extubation for redirection of care. All patients (6/6) showed improvement in their CHOP-INTEND scores with subsequent doses of nusinersen. All patients (6/6) has spine X-ray and follow up X-ray. Fifty percent (3/6) had x-ray done in the first year of life and showed 17 degrees at 6 month, 19 degrees at 9 month and 12 degrees at 11 month respectively. Fifty percent (3/5) had X-ray done after the first year of life and showed 34 degrees at 17 month, 32 at 29 month and 40 at 35 months. Conclusion: Despite the significant improvement in motor functional assessment in patients with SMA-type I, there was significant progression of scoliosis. Scoliosis developed in the first year in patients with SMA-type I treated with nusinersen. Although it is hard to estimate with this small sample but the progression of scoliosis is estimated to be > 15 degrees per year.

MUSCLE STEM CELL FATE AND IDENTITY IS DIRECTED BY THE MITOCHONDRIAL FUSION PROTEIN OPA1

Nicole Baker[1], John Girgis[1], Damian Chwastek[1], Peter Feige[4], Ryo Fujita[2], Colin Crist[2], Yan Burelle[3], Michael Rudnicki[4], Mireille Khacho[1].

[1]Department of Biochemistry, Microbiology and Immunology, Center for Neuromuscular Disease (CNMD), Ottawa Institute of Systems Biology (OISB), Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada2Lady Davis Institute for Medical Research, Jewish General Hospital, Department of Human Genetics, McGill University, Montreal, Quebec, Canada [3]Interdisciplinary School of Health Sciences, University of Ottawa, Ottawa, ON, Canada[4]Sprott Center For Stem Cell Research, Ottawa Hospital Research Institute, Regenerative Medicine Program, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

During aging and muscle degenerative diseases, there is a decline in muscle stem cells (MuSCs) and muscle regeneration, though the underlying reason is unknown. Interestingly, mitochondrial fragmentation is a common feature in aging and degenerative diseases, however, how this impacts MuSC function and maintenance has not been investigated. To address the effect of mitochondrial fragmentation in MuSCs, we generated a knockout mouse model using the Pax7CreERT2 inducible system to target deletion of the

mitochondrial fusion protein Opa1 specifically within MuSCs (Opa1-MKO). Analysis of MuSC function following in vivo muscle injury revealed a defect in the regenerative potential of Opa1-MKO MuSCs. Moreover, following injury there was a substantial decrease in the number of MuSC in Opa1-MKO animals with a concomitant increase in the number of committing (MyoD+/MyoG+) cells, illustrating that loss of Opa1 drives MuSC towards commitment at the expense of self-renewal. Furthermore, loss of Opa1 in MuSCs alters the quiescence state, priming MuSCs for rapid activation, as indicated by a reduction in quiescence-related genes (Pax7, CD34), increased EdU incorporation, and enhanced in vitro cell cycle kinetics upon activation. These data are the first to demonstrate a novel role for mitochondrial structure in the regulation of MuSC maintenance and regenerative capacity.

DOES THE MITOCHONDRIAL-ENHANCING DRUG OLESOXIME PREVENT CELL DEATH AND IMPROVE MUSCLE SIZE IN DUCHENNE MUSCULAR DYSTROPHY

Catherine Bellissimo, Luca Delfinis, Meghan Hughes, Peyman Tadi, Christina Amaral, Ali Dehghani and Christopher Perry

Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Canada, M3J 1P3

Mitochondrial dysfunction has been proposed as a secondary contributor to muscle weakness in Duchenne muscular dystrophy (DMD) – a severe muscle-wasting disease affecting ~1:3500 males. Olesoxime, (TRO19622) a mitochondrial-targeting compound, improved motor function in a model of amyotrophic lateral sclerosis (ALS) and increased life span in both models of ALS and spinal muscle atrophy. We have previously demonstrated mitochondrial dysfunction occurs in diaphragm and quadriceps in a mouse model of DMD (D2.mdx) and we hypothesized that treatment with olesoxime would improve mitochondrial bioenergetics, prevent cell death and improve mass in these muscles. Male D2.mdx mice received a daily oral gavage of corn oil supplemented with olesoxime (DRUG) (30mg/kg body weight) or without (VEH, corn oil vehicle) from age 10 to 28 days. Age-matched wildtype (WT) animals were used as healthy controls. A sample size of N=11-17 per group was used in this study. To elucidate if muscle degradation and cell death impacted muscle volume, serum creatine kinase (CK) and caspase activities (a marker of apoptosis) were examined. Complex I-supported ADP-stimulated mitochondrial respiration was significantly improved in both the quadriceps (+50.4%) and diaphragm (+34.7%) in the DRUG group compared to VEH. This improvement was related to greater whole body lean volume (+3.0%) and hindlimb muscle volume (+6.5%) assessed by microCT imaging in DRUG vs VEH while total trunk fat (-2.4%) and subcutaneous fat (-10.5%) trended lower ($p=0.067$ and $p=0.13$, respectively). Olesoxime significantly lowered serum CK levels compared to the VEH group (-57.7%), but caspase-3, -8 and -9 activities were not altered. In summary, olesoxime significantly increased Complex I-supported mitochondrial respiration which was related to improved muscle volume and reduced serum CK levels. These positive responses to olesoxime will be compared to histological measures of muscle cross-sectional area, necrosis and muscle force production.

MUSCLE STEM CELL PROLIFERATIVE FATES ARE DIRECTED BY p107 REGULATION OFMITOCHONDRIAL ATP GENERATION

Debasmita Bhattacharya and Anthony Scimè

Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Canada, M3J 1P3

Deficiencies in myogenic stem cell self-renewal and/or the number of their proliferating committed progenitors (MPCs) are associated with muscle wasting diseases and complications, such as muscular

dystrophy and sarcopenia. Stem cell fates including the decisions of their progenitors to continue proliferating or to differentiate are controlled by their metabolic states. In this regard, the metabolic profile of MPCs is due to control mechanisms that utilize ATP generated by glycolysis in the cytoplasm and oxidative phosphorylation in the mitochondria. Importantly, we have uncovered an intriguing mechanism that regulates MPC proliferative capacity through ATP generation, by the transcriptional co-repressor p107. Using primary cells and cell lines, we found that during MPC proliferation, p107 is found in the mitochondria interacting at the mitochondria DNA promoter. Here it represses mitochondrial encoded genes that are integral subunits of electron transport chain complexes leading to a decrease in the ATP generation rate. The consequence of ATP generation regulated by p107 is linked to MPC cell cycle control. In vivo, regenerating tibialis anterior muscle of mice genetically deleted for p107 had more proliferating MPCs compared to wildtype controls, indicative of a faster MPC proliferative capacity. This was corroborated in vitro, where an improved facility to generate more ATP in genetically deleted p107 MPCs was concomitant with increased cell cycle rate. Whereas, over expression of p107 specifically in the mitochondria resulted in cell cycle arrest. Understanding this control mechanism will provide novel approaches to manipulate muscle stem cells for regenerative medicine applications in muscle wasting diseases and complications.

NEURONATIN IS EXPRESSED IN MURINE SKELETAL MUSCLE AND NEGATIVELY REGULATES SERCA ACTIVITY

Jessica L. Braun and Val A. Fajardo

Department of Kinesiology, Brock University, St. Catharines, ON; Centre for Bone and Muscle Health, Brock University, St. Catharines, ON

The sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) pump catalyzes the active transport of Ca2+ into the sarco(endo)plasmic reticulum (SR/ER). In muscle, SERCA is imperative for relaxation and ensuring a sufficient SR Ca2+ load for subsequent contractions. Phospholamban (PLN) and sarcolipin (SLN) are both well-known SERCA regulators that bind to the pump and reduce its affinity for Ca2+. Neuronatin (NNAT) is a vital protein for healthy brain development that shares sequence homology with both PLN and SLN. Thus, NNAT is thought to have a role in regulating SERCA in the brain, though there is limited research directly showing NNAT as a SERCA regulator. Here we investigated NNAT protein expression in murine skeletal muscle as well as its role as a potential SERCA regulator. Endogenous expression of NNAT was found in a greater extent in the soleus, compared with the extensor digitorum longus (EDL) ($p = 0.01$). Co-immunoprecipitation experiments showed NNAT interaction with both SERCA1a and SERCA2a in the soleus. Finally, blocking NNAT-SERCA interactions via NNAT antibody incubation significantly improved maximal SERCA activity in the soleus ($p = 0.04$), but had no effect in the EDL ($p = 0.46$). This result is likely explained by differences in NNAT and SERCA expression, since the soleus muscle has significantly higher NNAT content and lower SERCA pump density. In conclusion, these findings suggest that NNAT is, in fact, a negative regulator of the SERCA pump in muscle; however, further investigation into its biological role in muscle is necessary.

RAPAMYCIN TREATMENT IN FIBROBLASTS FROM HEALTHY AND MELAS INDIVIDUALS

Nashwa Cheema and David A. Hood

Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Ontario, M3J 1P3, Canada

Mitochondrial diseases are a challenge to clinicians as patients are multi-symptomatic and exhibit a wide range of manifestation. Patients exhibit severe myopathy where accumulation of damaged mitochondria results in muscle deterioration and dysfunction. One of the most common myopathy is mitochodrial encephalopathy lactic acidosis stroke-like episodes (MELAS). There are several potential therapeutics, however, no current treatment has been a consistent intervention. Recently, MELAS patients treated with rapamycin had improved clinical outcomes. However, the cellular mechanism of rapamycin effects in MELAS patients is currently unknown. In this study, we first characterized extent of mitochondrial dysfunction in three fibroblast cell lines from MELAS patients and healthy individuals. We observed that mitochondria were fragmented, had a 3-fold decline in the average speed of mitochondria, 2-fold reduced mitochondrial membrane potential and 1.5 fold decline in basal respiration. MELAS fibroblasts had high levels of ROS and increased lysosomal function when compared to healthy controls. In MELAS fibroblasts, treatment with 24hr rapamycin resulted in an increase in lysosomal number, protein expression of the lysosomal marker vacuolar ATPase and protease function. Besides the lysosomal changes, we also observed that rapamycin treated MEALS fibroblasts had reduced ROS emission, higher levels of antioxidant enzyme glutathione peroxidase and a rescue of declining mitochondrial respiration. Our studies suggest that rapamycin has the potential to benefit MELAS patients through the activation of autophagic pathways mediated via lysosomes.

THERAPEUTIC TARGETING OF SKELETAL MUSCLE NIX IN EARLY-ONSET INSULIN RESISTANCE

Simone C. da Silva Rosa*[1,5,6], Matthew Martens[1,5,6], Jared Field1,[5,6], Donald Chapman[6], Christof Rampitsch[4], Vernon Dolinsky[2,5,6] and Joseph Gordon[1],[3,5,6]

Departments of Human Anatomy and Cell Science[1], Pharmacology and Therapeutics[2], College of Nursing[3], Plant Science[4], and The Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme[5] of the Children's Hospital Research Institute of Manitoba[6], University of Manitoba, Winnipeg, Canada.

Lipotoxicity is a form of cellular stress caused by the accumulation of lipids resulting in mitochondrial dysfunction and insulin resistance in muscle. Previously, we demonstrated that Nix, a lipotoxicity-responsive gene, accumulates in response to diacylglycerols induced by high-fat and sucrose (HFS) feeding and exacerbated by exposure to gestational diabetes (GDM) during fetal development. Here we identify a novel phosphorylation residue, activated by cilomilast treatment that can prevent Nix-induced mitochondrial dysfunction in muscle cells. In a series of gain- and loss-of-function experiments in rodent and human myotubes, we demonstrate that Nix accumulation triggers mitochondrial depolarization, fragmentation, calcium-dependent activation of DRP-1, and mitophagy. In addition, Nix-induced mitophagy leads to myotube insulin resistance through activation of mTORS6K inhibition of IRS-1. Through detailed phospho-peptide mapping of Nix, we identified a novel phosphorylation residue within the transmembrane domain, modulated by PKA activating agents, such as adrenergic agonist clenbuterol and the phosphodiesterase-4 inhibitor cilomilast. Treatment of myotubes with these agents serves to prevent Nix-induced mitochondrial dysfunction and restore insulin sensitivity. Furthermore, Nix knock-down or clenbuterol/cilomilast treatment

rescued palmitate-induced phosphorylation of Ser1101 on the insulin receptor substrate-1 (IRS-1) and prevented insulin resistance. These findings provide insight into the role of Nix-induced mitophagy and muscle insulin resistance during an overfed state. Finally, our data supports the hypothesis that Nix regulates mitochondrial metabolism and insulin signaling in myotubes and suggests a mechanism by which pharmacological activation of PKA may circumvent the mitochondrial dysfunction characteristic of insulin resistance.

EFFECT OF PDCD4 DEPLETION ON MYOFIBRILLAR PROTEIN ABUNDANCE, MTORC-1 SIGNALING AND AUTOPHAGY

Logan Davari, Olasunkanmi Adegoke

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

Skeletal muscle comprises of approximately 40% of the total body mass of a healthy individual and is responsible for functions such as locomotion and glucose homeostasis. The loss of muscle mass and strength have been associated with many metabolic diseases, cancer cachexia, and age-related sarcopenia. Within skeletal muscle, the mechanistic/mammalian target of rapamycin complex 1 (mTORC1) signaling is crucial for the maintenance of muscle mass and function, by playing a central role in protein synthesis. Previous research has highlighted that programmed cell death protein 4 (PDCD4), a substrate of mTORC1/S6K1, is important for the differentiation of myoblast into myotubes. However, the long-term effect of PDCD4 depletion on fully differentiated myotubes has not yet been investigated. In this study, we investigated the effect of PDCD4 knockdown on L6 myotubes differentiated for 4 days. Knockdown of PDCD4 led to a significant increase in myofibrillar protein content, shown through myosin-heavy chain 1 (MHC) and troponin ($p<0.05$). In addition, PDCD4 knockdown led to a significant increase in protein kinase B (AKT) and ribosomal protein S6 kinase (S6K1), both markers of mTORC1 signaling ($p<0.05$). Lastly, we found no changes in markers of autophagy following the knockdown of PDCD4. Our data demonstrates that PDCD4 depletion in L6 myotubes may have the potential to be a possible therapeutic target for increasing muscle mass, as many diseases such as cancer cachexia or age-related sarcopenia occur with the loss of skeletal muscle.

EXPLORING THE RELATIONSHIP BETWEEN MITOCHONDRIAL DYSFUNCITON AND SKELETAL MUSCLE DURING CANCER

LJ Delfinis and CA Bellissimo, SN DiBenedetto, S Gandhi and CGR Perry.

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

25-80% of cancer patients exhibit cachexia depending on the type and stage of cancer. Cancer cachexia is an ongoing loss of skeletal muscle mass that cannot be fully reversed by nutritional support. Mitochondria are organelles within muscle cells that regulate three vital cellular processes: ATP production, reactive oxygen species (ROS) generation and calcium retention capacity (CRC) in relation to triggering of the permeability transition pore prior to apoptosis. It was recently identified that cancer can induce mitochondrial dysfunction in skeletal muscle. Mitochondria can facilitate energy exchange from the matrix to the cytosol through two different phosphate shuttling mechanisms. We aimed to investigate these two pathways and the potential role of mitochondrial creatine kinase (mtCK) in cancer cachexia. 8-week-old

CD2F1 male mice (n=12) were injected subcutaneously with 5x10^5 colorectal 26 adenocarcinoma (C26) cancer cells or phosphate-buffered saline (PBS control, n=10) per flank. Tumours developed for 27-32 days. Tumour volume and grip strength were measured daily and weekly respectively. After tumour development, mice were sacrificed. Both the quadriceps and diaphragm muscles were separated into permeabilized muscle fibers and used for measurements of mitochondrial respiration. After 27-32 days of tumour bearing, there were reductions in body weight (-19.6%; $P < 0.05$) and muscle mass (-15%-28%; $P < 0.05$) in the plantaris, gastrocnemius, tibialis anterior, extensor digitorum longus and quadriceps muscles compared to PBS. Surprisingly, C26 cancer increased complex I-supported ADP-stimulated mitochondrial respiration in diaphragm and quadriceps by 26.9%-50.7% ($P <0.05$). A similar increase was seen in the presence of creatine to support mitochondrial-cytoplasmic creatine-based phosphate shuttling, but only in the diaphragm (33.7%; $P <0.05$). In conclusion, C26 cancer cells successfully induce muscle atrophy. However, a mitochondrial dysfunction was not seen. Instead, when the creatine-independent mechanism of energy exchange was utilized, mitochondrial respiration was upregulated in tumour-bearing mice. Moreover, both muscles seemed to respond differently to cancer, ultimately suggesting that cancer may have unique effect on different muscles.

THE REGULATION OF HEME METABOLISM IN MYOCYTES BY HYPOXIA AND ISCHEMIA: SUBSTRATE-ENZYME DYSSYNCHRONY

Ashley L. Eadie, Mathew J. Platt, Jade P. Marrow, Jason S. Huber, Allison J. Titus, Jeremy A. Simpson, and Keith R. Brunt

Department of Pharmacology, Dalhousie University, New Brunswick, Canada; Department of Human Health and Nutritional Sciences, University of Guelph, Ontario, Canada; IMPART investigator team Canada

Current strategies for the treatment of acute myocardial infarction (AMI) are limited and better pharmacological interventions are needed to improve weakened cardiac muscle function. Overexpression of the enzyme heme oxygenase-1 (HMOX1) has been shown to exert cytoprotective effects in models of AMI and is a highly attractive therapeutic target. HMOX1 is the stress-inducible enzyme responsible for the catabolism of heme, the functional backbone by which oxygen is carried in proteins like hemoglobin and cytochromes in the electron transport chain. As a potent inducer of HMOX1 and an FDA-approved heme-surrogate for the treatment of porphyria, hemin is a novel strategy for AMI treatment. To date, studies investigating hemin pharmacology and its use in models of ischemic muscle injury are limited. The objective of the present study was to provide a temporal and pharmacokinetic characterization of the changes in cardiac heme regulatory enzyme expression in response to hemin and AMI. Echocardiographic, hemodynamic and protein quantification analyses were performed in mice following a single intraperitoneal hemin injection, permanent ligation AMI, or daily hemin treatment pre- or post-AMI. Interestingly, HMOX1 — a heme and stress-inducible enzyme — was induced at only 3 days post-AMI and did not increase concomitantly with heme content or heme-synthesizing enzymes. Echocardiographic analyses showed preserved left ventricular morphology and cardiac output with hemin administration both pre- and post-AMI. Invasive hemodynamics revealed preserved left ventricular function in mice administered daily hemin when initiated 3h pre-AMI but not when initiated 2h post-AMI. Similarly, hemin administration significantly increased H9C2 cardiomyotubule viability when administered prior to H2O2-mediated injury but not with simultaneous or 2h post-injury hemin administration. This suggests that time-dependent mechanisms are involved in conferring hemin-mediated cardioprotection and could suggest differential mechanisms related to myocyte survival and cardiac remodeling.

PRACTICAL 'ACTIVITY SNACKS' REDUCE POSTPRANDIAL INSULINEMIA IN HEALTHY MEN AND WOMEN.

Stephanie Estafanos, Eric Williamson, Julia Malowany, Daniel R. Moore, Dinesh Kumbhare, Jenna B. Gillen.

Faculty of Kinesiology & Physical Education, University of Toronto, Toronto, ON, Canada.

Uninterrupted sedentary time is an independent risk factor for the development of metabolic diseases. Interrupting prolonged sitting with brief, intermittent walks can improve postprandial glucose metabolism; however, the efficacy of other types of exercise that do not require equipment nor space beyond one's immediate sedentary area remain to be investigated. PURPOSE: To determine the impact of interrupting prolonged sitting with practical 'activity snacks' on postprandial glycemia and insulinemia in healthy adults. METHODS: Fourteen participants (7 males,7 females; 23±5yr; 24±5kg/m2; 40±8ml/kg/min; 7032±2287steps/d) completed three 7.5hr trials in a randomized order consisting of uninterrupted sitting (SIT), sitting with intermittent (every 30 min) walking (WLK; 2min at 3.1mph) or sitting with intermittent squats (SQT; 15 'chair stands with calf raise'). Mixed-macronutrient liquid meals (~55:30:15% carbohydrate:fat:protein) provided 20% ('breakfast'; 406±87kcal) and 30% ('lunch'; 609±130kcal) of daily energy needs to mimic traditional Western meal patterns. Blood was obtained every 30min and analyzed for plasma glucose and insulin concentration. Positive incremental area under the curve (iAUC) for glucose, insulin and insulin:glucose ratio were calculated 1 and 3h postprandially using the trapezoidal rule. RESULTS: Postprandial glucose and insulin did not differ across conditions following breakfast. After lunch, peak insulin concentration was lower in SQT (51.6±26.7, $p<0.001$) and WLK (62.2±34.9, $p<0.05$) compared to SIT (78.9±43.0μIU/ml). The insulin:glucose iAUC 3h following lunch was also reduced by the activity snacks (SQT: 489±300; WLK: 541±401) compared to SIT (700±398, $p<0.05$). Insulin iAUC 1h following lunch was lower in SQT (1412±902, $p<0.01$) and WLK (1575±1145, $p<0.05$) relative to SIT (2231±1540μIU/ml x 1h, $p<0.01$), however 3h insulin iAUC was only reduced in SQT (SQT: 2992±1735 vs. SIT: 3954±2261μIU/ml x 3h; $p<0.05$). CONCLUSION: Interrupting prolonged sitting with short walks or repeated chair stands reduces postprandial insulinemia following lunch in healthy adults. Our results add to the evidence suggesting that short 'activity snacks' can help mitigate cardiometabolic risk factors associated with prolonged sitting.

THE EFFECTS OF LOW DOSE LITHIUM SUPPLEMENTATION ON THE GENETIC MARKERS OF ADIPOSE BROWNING

Michael S. Finch, Chantal Ryan, Rachel Fenech, Val A. Fajardo, Rebecca E.K. MacPherson

Faculty of Applied Health Sciences, Brock University, St. Catharines ONT, L2S 3A1

Introduction: Mammals possess two types of adipose tissue, white (WAT) and brown (BAT). Traditionally, WAT is known for playing a role in energy storage while BAT plays a role in non-shivering thermogenesis. Recent work has demonstrated that WAT's thermogenic capacity is inhibited through a glycogen synthase kinase 3 beta (GKS3β)-dependent pathway. Lithium, a natural inhibitor of GSK3ß, could have the potential to induce WAT browning. Objective: To determine if low-dose lithium supplementation can induce WAT browning and reduce/blunt the detrimental effects of an obesogenic diet. Methods: C57BL/6J mice (N=36)

were divided into three study arms: 1) chow, 2) high fat diet (HFD), and 3) HFD with lithium supplementation (HFD+Li) (10mg/kg/day lithium chloride via their drinking water). After 12 weeks, mice were anesthetized and inguinal white adipose (iWAT), epididymal white adipose (eWAT) and brown adipose tissues (BAT) were collected. Samples were analyzed for gene expression (Ucp1, Fgf21, Prdm16 and Pparg1), and will be tested for GSK and mitochondrial protein content via western blotting and GSK activity assay. Additionally, tissue will be fixed in formalin and prepared for histological analysis of adipocyte size and morphology. Results: As expected, HFD reduced genomic expression of important browning-related genes which was significantly elevated with the addition of lithium. Conclusions: The activation of the genes of interest suggests that lithium has an effect on BAT-related genetic programs. Lithium's ability to induce adipose browning will be further elucidated after protein concentration analysis.

MECHANISMS BEHIND LOCAL CONTROL OF SKELETAL MUSCLE BLOOD FLOW

Nicole M. Fletcher and Coral L. Murrant

Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, Canada, N1G 2W1

Matching perfusion to metabolic demand is essential for tissues, such as skeletal muscle, to remain healthy and viable. In order for this relationship to occur, active muscle fibres must communicate their metabolic needs to the surrounding vasculature. This is achieved by the release of local vasodilatory substances during muscle contraction that diffuse to the surrounding vasculature and initiate a vasodilatory signal. The conduction of the vasodilatory signal along the length of the vessel through gap junctions to distant vasculature promotes increases in tissue blood flow. Acetylcholine (ACh) classically elicits conducted responses (CR) through gap junction dependent mechanisms, however, recent evidence has demonstrated that certain vasodilators (i.e. pinacidil, PIN) utilize a pannexin/purinergic dependent signaling pathway at the capillaries. Therefore, our first study aimed to determine if all vasodilators utilized gap junction dependent signaling mechanisms within the arteriolar microvascular network. Using intravital microscopy of the hamster cremaster muscle in situ, we stimulated transverse arterioles locally via micropipette application of ACh and PIN in the presence and absence of the gap junction uncoupler halothane (HAL). Changes in vessel diameter were measured locally and upstream of the local stimulation site. ACh elicited a CR upstream (2.7+/-0.3 um) that was significantly inhibited in the presence of HAL (ACh/HAL: 1.2+/-0.7 um, $p=0.03$). PIN induced a CR (2.3+/-0.7 um) however, it was not significantly inhibited by HAL (PIN/HAL: 2.6+/-0.5 um, $p=0.6$). HAL did not significantly affect the local site's vasodilatory ability when stimulated with ACh or PIN ($p>0.05$). These data demonstrate that vasodilatory signals elicited by ACh conduct to the upstream vasculature via gap junctions whereas PIN induced CR are not gap junction mediated. Thus, another intervascular signaling pathway may be involved in coordinating blood flow to active muscle fibres, which is required for optimal tissue perfusion.

DEFICIENCY OF THE AUTOPHAGY GENE ATG16L1 INDUCES INSULIN RESISTANCE THROUGH IRS1 DEGRADATION

Scott Frendo-Cumbo [1,2], Javier R. Jaldin-Fincati1, Etienne Coyaud[3], Estelle M. N. Laurent[3], Logan Townsend[4], Ramnik J. Xavier[5], Nicolas J. Pillon[6], Brian Raught [2,3], David C. Wright[4], John H. Brumell1,[2] and Amira Klip1,[2]

[1] Hospital for Sick Children, Toronto, ON; [2] University of Toronto, Toronto, ON; [3] Princess Margaret Cancer Centre, Toronto, ON; [4] University of Guelph, Guelph, ON; [5] Massachusetts General Hospital, Harvard Medical School, Boston, MA; [6] Karolinska Institutet, Stockholm, Sweden

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

GSK3 INHIBITION UNCOUPLES SERCA Ca2+ TRANSPORT EFFICIENCY AND AUGMENTS SERCA'S ENERGY EXPENDITURE IN C2C12 CELLS

Mia S. Geromella[1], Sophie I. Hamstra[1], Fereshteh Moradi[2], Lucas A. Maddalena[2], Brian D. Roy[1], Rebecca E.K. MacPherson[3], Jeffrey A. Stuart[2], Val A. Fajardo1

[1]Department of Kinesiology, Brock University, St. Catharines, ON, L2S 3A1
[2]Department of Biological Sciences, Brock University, St. Catharines, ON, L2S 3A1
[3]Department of Health Sciences, Brock University, St. Catharines, ON, L2S 3A1

Adaptive thermogenesis is a cellular process that accelerates energy expenditure while increasing heat production in response to prolonged cold exposure or caloric excess. Enhancing adaptive thermogenesis may be relevant in combatting diet-induced obesity and glucose intolerance. Skeletal muscle via sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) uncoupling and brown/beige adipose via mitochondrial uncoupling are the two primary sites for adaptive thermogenesis in mammals. Recent evidence has shown that glycogen synthase kinase 3 (GSK3) negatively regulates adipose-based thermogenesis by repressing uncoupling protein-1 expression in brown adipocytes. However, to our

knowledge, there have been no studies examining whether GSK3 also negatively regulates muscle-based thermogenesis via SERCA uncoupling. To this end, C2C12 cells were treated with 0.5 mM lithium chloride – a well-known GSK3 inhibitor – at the onset of differentiation for 7 days. SERCA coupling ratios were obtained by measuring the rates of Ca2+ uptake using a fluorophore-based assay and the rates of ATP hydrolysis using a spectrophotometric assay at physiological Ca2+ concentrations ranging from a pCa of 5-7. Basal respiration was measured in absence and presence of 10 mM MgCl2 to estimate SERCA's contribution to resting energy expenditure. Our results show that when treated with lithium, C2C12 cells had significantly reduced Ca2+ uptake (-59%, $p = 0.03$) with no change in SERCA activity. This led to a reduction in SERCA's coupling ratio, though only trending towards significance ($p = 0.07$). Corresponding with these results, we found that lithium treatment significantly increased C2C12 basal respiration (1.6-fold, $p = 0.04$). Moreover, incubating C2C12 cells with 10 mM MgCl2 showed that SERCA's contribution to basal respiration was significantly elevated with lithium treatment (1.9-fold, $p = 0.03$). Altogether, these data suggest that GSK3 inhibition via lithium treatment augments SERCA energy expenditure likely through uncoupling Ca2+ transport from ATP hydrolysis and future studies will investigate the underlying cellular mechanisms.

VALPROIC ACID IMPROVES SURVIVAL RATE AND SKELETAL MUSCLE PHENOTYPE IN MTM1 KNOCKOUT MICE

Ghahramani Seno M. M[1]., Volpatti J.[1], Sabha N.[1], Alper Celik[2], Sarikaya E.[1] and Dowling J.J.[1]

[1]Program in Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; [2]The Center for Computational Medicine Hospital for Sick Children, Toronto, ON, Canada

X-linked myotubular myopathy (XLMTM) is a rare congenital neuromuscular disorder affecting boys at a rate of 1 in 50,000 live births. The majority (~80%) of the affected children are born with serious and diffuse skeletal muscle weakness and hypotonia that necessitate immediate and sustained extensive care including tracheostomy and feeding by gastric intubation. Considerable proportions (~25%) of the cases succumb to death within their first year of life and the majority do so later due mainly to respiratory failure. The molecular pathology of X-MTM starts with pathogenic variants of MTM1 gene, however the precise downstream molecular events are still under investigation. Our initial work on Zebra fish model of the disorder showed possible benefits from histone deacetylase (HDAC) inhibitors such as valproic acid (VPA). To further verify this effect in mammalians, we used VPA to treat our mtm1 Knockout mice and showed that VPA, indeed, improves longevity and some aspect of the muscle phenotypes in this model. Considering the long run experience on using VPA in humans for neuropsychiatric disorders and its reasonable safety level this finding propose VPA as a therapeutic approach that would be worth of trying in human patients.

EVIDENCE FOR UNALTERED SKELETAL MUSCLE REPAIR IN YOUNG ADULTS WITH WELL-CONTROLLED TYPE 1 DIABETES MELLITUS

Grace K. Grafham[1], Cynthia M.F. Monaco[1], Linda A. Brandt[2], Mark A. Tarnopolsky [2] and Thomas J. Hawke [1]

[1]Department of Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada; [2] Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada

There is strong evidence that skeletal muscle health is compromised early in the course of type 1 diabetes mellitus (T1D). These impairments include mitochondrial dysfunction, decreased satellite cell (SC) content, and a progressive loss of muscle mass, power, and strength. Maintaining healthy muscle requires successful muscle repair. To date, the impact of T1D on human skeletal muscle repair has not been established; however, attenuated repair would account for the reduced functional capacity which often characterizes those with diabetes. The purpose of this study was to determine the impact of T1D on the recovery of skeletal muscle function, morphology, and ultrastructure after 300 unilateral eccentric contractions (90°/s) of the knee extensors. Eighteen men and women (18-30 years old) with (n=9) and without (n=9) T1D performed the eccentric damage protocol. Pre-damage, and at 48- and 96-hours post-damage, subjects gave a blood sample and vastus lateralis biopsy, and performed a maximal isometric knee extension. Given the sex-specific differences in muscle damage, control and T1D men and women were analyzed separately. Force production and recovery were comparable between control and T1D men and women. Exercise-related increases in creatine kinase activity and ultrastructural damage were also comparable between groups. There was a trend for T1D men to have greater type II muscle fibers than T1D women ($p=0.055$). Surprisingly, total SC content was not different between groups; however, SC proliferation was trending lower at 48-hours post-damage in T1D women relative to controls ($p=0.07$). In those with T1D, there was no correlation between muscle damage and HbA1c, but HbA1c was strongly correlated with self-reported vigorous physical activity levels ($r_s=0.881$, $p=0.002$). Contrary to preclinical studies, our data is the first to show that skeletal muscle repair is largely unaltered in otherwise healthy young adults with T1D. We attribute these differences to glycemic control and speculate that muscle repair is unaffected if individuals are optimally managing their diabetes. Considering the exercise-related dysglycemia seen in T1D, our results emphasize a need to define the dose of physical activity required for persons with T1D to maintain euglycemia and ultimately, maximize their skeletal muscle health.

FLOW-MEDIATE DILATION IS GREATER IN UPRIGHT POSTURE IN YOUNG HEALTHY ADULTS

KH Habib, KR Murray, H Edgell

School of Kinesiology & Health Sciences, York University, Toronto, Canada

Orthostatic intolerance is often associated with vascular dysregulation leading to feelings of lightheadedness during upright posture. The baroreflex is activated during upright posture; however, intolerance can occur when there is an inadequate increase of peripheral vascular resistance. Hence, the purpose of this study was to assess vasodilatory capacity in the upright posture via measurements of brachial flow-mediated dilation (FMD) at the level of the heart. We hypothesized that healthy participants in the upright posture would experience significantly lower shear rate and FMD compared to the supine posture likely due to known increases of sympathetic activity. We recruited 10 female and 8 males (20.6 ± 1.8years, 27.0 ± 6.04 Kg/m2) with no history of cardiovascular or respiratory diseases. Women were tested in the low hormone phase of the menstrual cycle (days 2-5) and were not taking hormonal contraceptives. All participants completed 2 randomized FMD protocols (2 minutes baseline, 5 minutes right forearm occlusion, and 3 minutes recovery; 30-minute interval) in supine posture and 70° head up tilt. FMD tests were both conducted with the brachial artery at the level of the heart to control for gravitational fluid shifts during upright posture. FMD was measured using duplex ultrasonography. Shear rate and percent FMD were calculated using automated software (Cardiovascular Suite). In the upright posture, participants exhibited a greater FMD response

compared to the supine posture (14.8 ± 3.82% vs 9.4 ± 5.10%, P=0.005). However, we observed no difference in shear rate between postures (940 ± 272s-1 vs 984 ± 322 s-1, P≥0.05). Furthermore, we observed no difference in the baseline diameter between postures (3.5 ± 0.79 mm vs 3.4 ± 0.59 mm, P≥0.05). There is a greater FMD response in the upright posture compared to the supine posture, suggesting greater vasodilatory capacity in the upright posture.

THE CARDIO-SPLENIC AXIS: A NEW PLAYER IN ACUTE REGULATION OF CARDIAC FUNCTION

Herrera S, Huber JS[1], Edgett BA, Brunt KR, Simpson JA[2]

[1]Department of Human Health and Nutritional Science, University of Guelph, Guelph, Ontario, Canada; [2]Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

Perspective: Emerging epidemiological studies suggest that splenectomy increases the risk of chronic cardiovascular disease but have yet to establish the mechanisms responsible for this relationship. The mammalian spleen has separate immune and haematological functions, along with bidirectional neural projections. While recent studies of splenic function have focused on immunological activity using organ ablation, little attention has been directed towards the neural-splenic axis. Our preliminary data demonstrates an acute link between in vivo splenic nerve stimulation and increases in left ventricle pressure and contractility. Interestingly, changes in cardiac function occurred without alteration in heart rate, suggesting the possibility of a splenic-specific hormone responsible for regulating cardiac function. Our overall objective is to understand how neural control of the spleen acutely affects heart function. Our hypothesis is that the spleen synthesizes a hormone under neural control that, when secreted, increases cardiac contractility.

Methods: To isolate the role of the spleen in regulating cardiac function, we will conduct an in vitro splenic perfusion model. In the experimental group, spleens will be harvested from Sprague Dawley rats (n=15) and the splenic artery will be cannulated and attached to an isolated perfusion system. The splenic nerve will be stimulated, and splenic effluent collected for 1 minute before and during stimulation. The control group will follow an identical protocol, minus the nerve stimulation. Collected effluent will be subjected to 1D SDS-PAGE, with protein bands of interest sent for punitive protein identification. Further, to assess the effect of splenic effluent on cardiac function, effluents will be injected intravenously into rats, with cardiac function assessment by invasive hemodynamics.

Expected Results and Significance: In contrast to non-stimulated splenic effluent, stimulated splenic effluent will increase cardiac contractile function in vivo and contain at least one unique protein as observed by protein electrophoresis. This work will reveal a novel, physiological importance of the spleen, and its role in regulating heart function. This could propose new treatment strategies to restore contractility in failing myocardium.

SOMATOSTATIN RECEPTOR TYPE 2 ANTAGONISM RESTORES COUNTERREGULATORY FUNCTION AND CONFERS HYPOGLYCEMIC RESISTANCE IN A RODENT MODEL OF ADVANCED TYPE 2 DIABETES MELLITUS

Emily G. Hoffman, Ninoschka C. D'souza, Mehran Nejad-Mansouri, Parinaz Zaree Bavani, Julian Aiken, Richard Liggins, and Michael Riddell

Introduction: Anti-diabetic medications aim to delay or prevent vascular complications that can lead to multiple organ failure in type 2 diabetes (T2D); however, their therapeutic potential is compromised by the threat of iatrogenic hypoglycemia. Somatostatin receptor type 2 antagonists (SSTR2a), which reverse SST-mediated α-cell inhibition, have been shown to delay or prevent the onset of hypoglycemia by augmenting glucagon counterregulation in various rodent models of type 1 diabetes. Objective: To investigate the efficacy of the SSTR2a, PRL-2903, as a prophylactic measure against insulin-induced hypoglycemia in a rodent model of late-stage, T2D. Methods: T2D was induced in male, Sprague Dawley rats (n=18) through 3-weeks of high-fat feeding (to establish insulin resistance) followed by a low-dose injection of the β-cell toxin, streptozotocin (35 mg/kg, IP). Two hypoglycemic challenges were conducted in a crossover deign, separated by a 1-week washout period. T2D rats received an injection of SSTR2a (10 mg/kg PRL-2903, SC) or vehicle (n=9/group/challenge; n=18/group combined) 1-hour prior to the induction of hypoglycemia (3 IU/kg NovoRapid, SC). Normal chow-fed (NCF, n=6) rats served as non-diabetic controls. Results: SSTR2a raised the blood glucose nadir from 3.4±0.17 to 4.2±0.26 mmol/L ($p=0.04$ vs. T2D-vehicle) and reduced the incidence of hypoglycemia (blood glucose <3.9 mmol/L) by 40%. Hypoglycemic onset was delayed by 20±6.95 minutes in treated rats ($p=0.01$ vs. T2D-vehicle). The glucagon response to insulin challenge, which deteriorated 7-fold in the T2D-vehicle group ($p=0.001$ vs. NCF), was fully restored with SSTR2a treatment (AUC analysis, $p=0.09$ vs. NCF). Immunohistochemical staining of islet tissue revealed a 70% increase in the expression of somatostatin type 2 receptors on T2D α-cells ($p=0.04$ vs. NCF). Glucagon content per islet was elevated by 40% in T2D rats ($p<0.00001$vs. NCF), while somatostatin content was not significantly different between groups. Discussion: We demonstrated, for the first time, that SSTR2a treatment reduced the frequency and severity of hypoglycemic exposure in a model of advanced T2D. The increased prevalence (and potential activation) of somatostatin type 2 receptors in the pancreatic islets may offer insight into the mechanism underlying SST-mediated, alpha-cell dysfunction in T2D. In conclusion, we propose somatostatin antagonism as an important therapeutic tool for restoring optical glycemic control in patients with T2D.

IS THERE GENETIC DRIFT IN THE SPONTANEOUSLY HYPERTENSIVE RAT COLONY?

Emma Holjak [1], Keith R. Brunt[2,3], and Jeremy A. Simpson [1,2]

[1] Department of Human Health and Nutritional Sciences, University of Guelph, ON, Canada [2]IMPART Team Canada Investigator Network, [3] Department of Pharmacology, Dalhousie Medicine New Brunswick, NB, Canada

The Spontaneously Hypertensive Rat (SHR) Colony was established in the early 1960's and is the most common model used to study heart failure. The SHRs are primarily characterized by the development of essential hypertension, which onsets at 8 weeks of age and fully develops by 14 weeks of age.

Cardiovascular fitness is an important component of sexual health. As heart failure in the SHR develops around the age animals are selected for breeding, there may be a natural selection bias towards healthier animals. To investigate if there was drift in the SHR colony over the past half century, we performed a

systematic review to explore indices of cardiovascular health. We hypothesized that because cardiovascular health impacts sexual fitness, hypertension-induced heart failure becomes less pronounced in the SHR colony with time. To this end, we searched PubMed to retrieve SHR-relevant articles published between 1964-1984 (early-SHR) and 2000-2020 (late-SHR). Studies reporting at least 3 cardiovascular/hemodynamic parameters were selected.

We first evaluated systolic blood pressure, the key defining feature of the SHR colony. As expected, SBP was similar between both groups. Although an elevated blood pressure can cause heart disease it is not indicative of the severity of heart failure. Thus, to evaluate cardiovascular fitness, we next evaluated indices of cardiac contraction (dP/dt max) and relaxation (dP/dt min). The late-SHR colony had significantly higher dP/dt max and dP/dt min values in comparison to the early-SHR colony ($p < 0.05$), indicating a shift towards improved cardiac function that appears to be independent of age, methods/techniques and anesthetics. Interestingly, LVEDP was elevated in the late-SHR colony ($p < 0.05$), in comparison to the early-SHR, suggesting cardiac compliance or end-diastolic volume has also changed with time. Here we show that the SHR colony, while has maintained stable hypertension over time, has significantly better cardiac function in recent years.

This historical perspective of the SHR should be taken into account when using this very common model of HF as natural selection may have altered how this model recapitulates hypertension-induced sequelae of heart failure in humans. However, the shift in this model could also provide important insights into natural adaptation to hypertension-induced heart failure, revealing therapeutic targets not previously known. Further, changing breeding strategies should be considered to better ensure genetic drift is minimized in animal colonies where cardiovascular fitness is compromised in animals that are used for breeding.

DIETARY N-3 VS N-6 PUFA DIFFERENTIALLY MODULATE MACROPHAGE-MYOCYTE INFLAMMATORY CROSS-TALK

Amber L. Hutchinson, Danyelle M. Liddle, Rufaida Ansari, Lindsay E. Robinson

Human Health and Nutritional Sciences, University of Guelph, Guelph ON, Canada, N1G 2W1

Skeletal muscle is the primary site for insulin-stimulated glucose uptake. In obesity, increased circulating inflammatory cytokines interfere with skeletal muscle insulin signaling, leading to local and whole-body insulin resistance (IR). Moreover, obese skeletal muscle is characterized by accumulation of infiltrated M1 macrophages and ensuing macrophage-myocyte paracrine interactions (cross-talk) contribute to local inflammation and IR. Such macrophage-myocyte inflammatory cross-talk provides a potential intervention target for anti-inflammatory nutrients, including dietary long-chain n-3 polyunsaturated fatty acids (PUFA). Using a co-culture model designed to mimic the degree of $CD11b^+$ cell accumulation in obese skeletal muscle (40% of immune cells), differentiated L6 myocytes were co-cultured with purified splenic $CD11b^+$ cells from male Sprague Dawley rats (7-wk old) consuming one of three isocaloric diets: i) high-fat (HF; 54% *kcal* lard, 6% *kcal* soybean oil), ii) high-fat with n-3 PUFA (HFn-3; 39% *kcal* lard + 15% *kcal* menhaden oil + 6% *kcal* soybean oil) or iii) high-fat with n-6 PUFA (HFn-6; 45% *kcal* lard + 15% *kcal* soybean oil) for 2, 8 or 12-wk (n= 8-12/diet). Co-cultures were stimulated for 24h with lipopolysaccharide (LPS, 10 ng/mL) to mimic *in vivo* obese endotoxin levels. $CD11b^+$ cells were also cultured alone for 24h in conditioned media collected from L6 myocytes stimulated with LPS for 24h (LCM). In co-cultures, HFn-6 increased mRNA expression of inflammatory markers compared to HF and HFn-3 at 8- (*iNos*; $P \leq 0.05$) and

12-wk (*Tnfa*, *Il-6*, *Il-1b*; $P \leq 0.05$). Similarly, at 8-wk $CD11b^+$ cells from HFn-6 rats that were treated with LCM, had increased mRNA expression of inflammatory cytokines (*Tnfa, Il-1b*) and M1 polarization markers (*iNos, Cd86*) compared to both HF and HFn-3, and the same effects were seen with *Il-6* and *Il-1b* at 12-wk ($P>0.05$). Lastly, HFn-3 reduced mRNA expression of *Tnf-a* compared to HF at 12-wk ($P>0.05$). Together, these data suggest that n-6 PUFA support macrophage-myocyte inflammatory cross-talk, in part by promoting M1 macrophage polarization. Further, these data provide mechanistic insight into the benefits of n-3 vs n-6 PUFA inclusion in a high-fat diet in mitigating skeletal muscle inflammation in obesity. (NSERC)

SAME CODON, DIFFERENT DISEASES? A LOOK INTO THE REASON FOR DISEASE ONSET.

Michael R. Jones, Dr. John F. Dawson

University of Guelph

Cardiovascular disease is the highest-ranking cause of death globally and a large percentage of these diseases are genetic. Cardiomyopathy is a family of cardiovascular diseases which include hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). Hypotheses for cardiomyopathy onset range from calcium sensitivity changes or higher contractile force generation. A residue in cardiac actin (actc1) contains a potential resolution to the mystery of cardiomyopathy onset and differentiation: point mutations in the codon encoding amino acid residue R312 of actin results in either HCM or DCM. Characterizing the differences between these mutations could clarify what causes different forms of cardiomyopathy. To test these actin variants, the intrinsic stability, actomyosin interactions and regulatory protein interactions were measured in vitro using a combination of assays. Intrinsic stability was measured with thermal shift assays and polymerization assays, while actomyosin interactions and regulatory protein interactions were measured by observing filament velocity over a bed of myosin with an in vitro motility assay. We found that these actin variants do not vary in calcium sensitivity or intrinsic stability compared to wild type protein. Rather, an increased filament velocity for both the HCM and DCM variants was observed when bound to regulatory proteins. Since the variants are similar in their characteristics, one conclusion is that these mutations first result in HCM, with one progressing to DCM. These findings provide an example of how understanding the molecular cause of a cardiomyopathy patient's disease is critical for providing optimal treatments in the future.

ROLE OF HEME REGULATED INHIBITORY KINASE CONTROL OF HYPERTROPHY AND SEX DIFFERENCES IN THE DIASTOLIC DYSFUNCTION

MinJi Kim1[3], Ashley Eadie [1,3], Victoria Northrup [1,3], Leslie Ogilvie [2,3], Jason Huber [2,3], Jeremy Simpson [2,3], and Keith Brunt [1,3]

[1]Department of Pharmacology, Dalhousie University, Halifax, Canada, B3H 4R2; [2]Department of Human Health & Nutrition, University of Guelph, Guelph, Canada, N1G 2W1; [3]IMPART investigator team Canada, Saint John, E2K 5E2

Cardiomyocytes changes it shapes and function to adapt to changing conditions, pressures or stress thus altering the cardiac extracellular matrix and heart function. Most scientific research has focused on the maintenance of systolic dysfunction, but not on the diastolic dysfunction. Diastolic function is regulated by complex mechanisms of passive compliance (e.g. fibrosis) and active relaxation (e.g. hypertrophy) with

specific sex-differences. Maladapted hypertrophy limits the diastolic function, and the extracellular signal-regulated protein kinases 1 and 2 (Erk1/2) is a key player regulating cardiac hypertrophy. Heme regulated inhibitory kinase (HRIK) also partly regulates protein synthesis in hypertrophy. It is important to recognize that females are more prone to diastolic dysfunction. Estrogen receptors regulate Erk1/2 to reduce hypertrophy in the heart. It is not known what affect estrogen has on heme regulated inhibitory kinase. I hypothesize that Erk1/2 signaling for hypertrophy is controlled by heme-regulated HRIK negative regulation of protein synthesis. Further, heme bioavailability accounts in part for the sex differences observed in cardiomyocyte hypertrophy. A recently developed model of isolated diastolic dysfunction caused by splenectomy (the most heme replete organ in the body) will be used in the study. I will test how cardiomyocyte stimuli affects protein synthesis in hypertrophy with special attention to HRIK that controls eif2alpha to regulate synthesis of protein from mRNA. I will use H9c2 cardiomyotubes as an in vitro model. I will be able to account for sex by carbon filtering media to remove sex hormones and conditionally adding estrogen as a controlled variable. I will induce hypertrophy by specific combinations of reagents: first, dobutamine (or Angiotensin-II) and hydrogen peroxide; second, lipopolysaccharide and high-glucose. This will model the hypertrophy mechanisms found distinctly in systolic dysfunction (sympathetic stress) and diastolic dysfunction (inflammation & metabolism stress) to identify common or distinct mechanisms. I will measure biomarkers that indicate the diastolic dysfunction over time compared with a control in both sexes. Based on these findings, I will add hemin to the animal model to determine if it has any impact on diastolic function and mechanisms of hypertrophy. By analyzing those in the animal model, it could help to find out which signals and which stress regulate diastolic function related to hypertrophy. Discovering whether we can model part of the mechanisms of diastolic dysfunction using an in vitro model will allow us to provide new information to the cell biology field. We can then more accurately determine how hormones like estrogen alter the response to these stimuli.

A NOVEL IN-FRAME DELETION OF 52-55 DMD MOUSE MODEL PRESERVES MUSCLE FUNCTION

Monika Kustermann, Tatianna W. Y. Wong, Evgueni A. Ivakine, Ronald D. Cohn

Program in Genetics and Genome Biology, The Hospital for Sick Children Research Institute, Toronto, Ontario, Canada

Duchenne muscular dystrophy (DMD) is a life-limiting X-linked recessive neuromuscular disorder, affecting approximately 1 in 5000 boys. DMD is caused by mutations in the *DMD* gene encoding dystrophin, a member of the dystrophin glycoprotein complex (DGC) which protects muscle fibers from contraction-induced injury. DMD is marked by absent dystrophin expression, often caused by frame-shift mutations. Among the dystrophinopathies, Becker muscular dystrophy (BMD) is a milder disorder typically caused by in-frame *DMD* mutations, resulting in shortened yet partly functional dystrophin formation. Both the clinical presentation and prognosis of BMD patients are improved compared to those with DMD. Therefore, conversion of out-of-frame *Dmd* mutations into in-frame mutations by means of exon-skipping, is appealing as a potential therapeutic option. This study first created a DMD mouse model mirroring a patient deletion of exons 52-54 (*Dmd Δ52-54*). Such a mutation may be amenable to exon skipping to create a deletion of exons 52-55, the latter being a known mutation causing BMD. We then generated a mouse model of the in-frame deletion mutation *Dmd Δ52-55*, recapitulating the result of exon skipping in our DMD mouse. To date, *Dmd Δ52-55* is the first BMD mouse model generated. Both mouse models were subsequently subjected to histopathological and functional analysis. *Dmd Δ52-54* mice lacked dystrophin expression, exhibited a muscular dystrophy phenotype, and had compromised motor and cardiac function. In contrast,

the *Dmd Δ52-54* mice showed expression of a truncated dystrophin which localized correctly to the sarcolemma and recruited DGC components, which were absent in *Dmd Δ52-54*. Furthermore, this shortened dystrophin maintained muscle integrity and protected from exercise-induced damage similar to wildtype dystrophin. Thus, our results indicate that therapies targeting the conversion of DMD-causing frameshift mutations into BMD-like in-frame mutations may restore shortened dystrophin expression and improve dystrophic phenotype.

CALPAIN ACTIVATION MEDIATES MYOCARDIAL ABNORMALITIES IN TAIL-SUSPENDED MICE BY PROMOTING NADPH OXIDASE ACTIVATION VIA P38 AND ERK1/2 MAPK PATHWAYS

Huili Li[1], Liwen Liang[1], Lina Qu[2], Lulu Zhang[1], Guo-Chang Fan[3], Peter A Greer [4,5], Jianmin Li[6], Douglas L Jones [7,8,9], Tianqing Peng [8,9]

[1]Institutes of Biology and Medical Sciences, Soochow University, Suzhou 215123, China; [2]State Key Laboratory of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, Beijing 100094, China; [3]Department of Pharmacology and Systems Physiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA; [4]Division of Cancer Biology and Genetics, Queen's University Cancer Research Institute, and [5]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6; [6]Department of Pathology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325003, China; 7Department of Physiology and Pharmacology, Western University, London, Ontario, Canada N6A 5C1; [8]Lawson Health Health Research Institute of London Health Sciences Centre, London, Ontario, Canada N6A 4G5; [9] Department of Medicine, Western University, London, Ontario, Canada N6A 5C1

Microgravity conditions cause myocardial abnormalities including atrophy and dysfunction. However, the underlying mechanisms are incompletely understood. This study investigated if and how calpain activation promotes myocardial abnormalities under microgravity. Simulated microgravity was induced by tail-suspension in mice with cardiomyocyte-specific deletion of Capns1 and their wild-type littermates. Tail-suspension time-dependently reduced cardiomyocyte size, heart weight and myocardial function in mice; and these changes were accompanied by calpain activation, NADPH oxidase activation and oxidative stress in heart tissues. These effects of tail-suspension were attenuated by deletion of Capns1. Notably, the protective effects of Capns1 deletion were associated with the prevention of phosphorylation of Ser345 on p47phox, and attenuation of ERK1/2 and p38 activation in hearts of tail-suspended mice. In cultured neonatal mouse cardiomyocytes, simulated microgravity induced calpain activation, phosphorylation of Ser345 on p47phox, and activation of ERK1/2 and p38, all of which were prevented by calpain inhibitor-III. Furthermore, inhibition of ERK1/2 or p38 attenuated phosphorylation of Ser345 on p47phox in cardiomyocytes under simulated microgravity. This study demonstrates for the first time that calpain promotes NADPH oxidase activation and myocardial abnormalities under microgravity by facilitating p47phox phosphorylation via ERK1/2 and p38 pathways. Thus, calpain inhibition may be an effective therapeutic approach to reduce microgravity-induced myocardial abnormalities.

MIRNAs AS CLINICAL BIOMARKERS IN MYOTUBULAR MYOPATHY

Nika Maani, Nesrin Sabha, Dakota Gustafson, Matthew Alexander, and James Dowling.

University of Toronto, Department of Molecular Genetics; University of Toronto, Department of Laboratory and Medicine and Pathology; Hospital for Sick Children, Department of Pediatrics; and University of Alabama at Birmingham, Department of Pediatrics.

Myotubular myopathy (MTM) is one of the most severe neuromuscular diseases reported in children, to date. MTM is associated with severe disabilities, early death and is without existing therapy. Maani et al (2018)., recently identified tamoxifen as a novel therapeutic candidate for MTM that improves muscle structure, strength and prolongs survival in MTM mice through modulation of dynamin-2 (DNM2), a known disease modifier. As clinical trials for tamoxifen in MTM are imminent, there remains a need for a reliable, non-invasive biomarker that faithfully reflects disease severity and treatment response, facilitating disease monitoring and therapy testing. Disease progression is currently monitored by clinical examination and invasive muscle biopsy, as no convincing biomarkers that correlate with changes in disease status have been identified. MicroRNAs (miRNAs) are emerging as viable candidates for disease biomarkers in childhood muscle disease, as well as novel therapeutic targets. In this study we seek to establish a novel platform for correlating disease severity and treatment response with miRNA levels in MTM, using the high throughput fluidigm platform. Doing so, we seek to identify and validate the first non-invasive biomarker for MTM. Moreover, we will investigate the potential of miRNAs to act as modulators of MTM pathogenesis through genetic modulation of miRNAs of interest in skeletal muscle. This study has immense potential to not only advance clinical practice for MTM patients, but also accelerate the clinical evaluation of novel therapies. Insights from this study will also broadly influence the study of miRNAs and methods of biomarker discovery in muscle disease. Supported by the National Institutes of Health (NIH) and the Myotubular Trust.

DEPLETION OF E1α SUBUNIT OF BRANCHED-CHAIN KETOACID DEHYDROGENASE COMPLEX IMPROVES MYOFIBRILLAR PROTEIN ABUNDANCE AND ANABOLIC SIGNALING IN MYOTUBES

Glory Madu and Olasunkanmi A. J. Adegoke

School of Kinesiology & Health Science, Muscle Health Research Centre, York University, Toronto, Canada

Introduction: Branched-chain amino acids (BCAAs) are essential amino acids that are crucial for skeletal muscle anabolism. Thus, alterations in their levels are associated with muscle atrophic diseases such as cancer, chronic inflammatory and neurological disorders. Others have linked impairments in BCAA metabolism to the development of insulin resistance and its sequelae. Compared to the effects of theses amino acids, much less is known on how impairment in BCAA catabolism affects skeletal muscle. BCAA catabolism starts with the reversible transamination by the mitochondrial enzyme branched-chain aminotransferase 2 (BCAT2). This is followed by the irreversible carboxylation, catalyzed by branched-chain ketoacid dehydrogenase (BCKD) complex. We have shown that BCAT2 and BCKD are essential for the differentiation of skeletal myoblasts into myotubes. Here, we investigated the effect of depletion of BCAT2 or of E1□ subunit of BCKD in differentiated myotubes.

Methods: On day 4 of differentiation, L6 myotubes were transfected with the following siRNA oligonucleotides: scrambled (control), BCAT2, or E1α subunit of BCKD.

Results: Forty-eight hours after transfection, compared to control or BCAT2 siRNA group, we observed improved myotube structure in BCKD-depleted cells. BCKD depletion augmented myofibrillar protein levels: myosin heavy chain (MHC, 2-fold) and tropomyosin (4-fold), $p<0.05$, $n=3$. To further analyze the increase in myofibrillar protein content, we examined signaling through mTORC1 (mechanistic target of rapamycin complex 1), a vital complex necessary for skeletal muscle anabolism. BCKD depletion increased the phosphorylation of mTORC1 upstream activator AKT (52%, $p<0.05$, $n=3$), and of mTORC1 downstream substrates by 25%-86%, consistent with the increase in myofibrillar proteins. Finally, in myotubes treated with the catabolic cytokine (tumor necrosis factor-α), BCKD depletion tended to increase the abundance of tropomyosin (a myofibrillar protein).

Conclusion: We showed that depletion of BCKD enhanced myofibrillar protein content and anabolic signaling. If these data are confirmed in vivo, development of dietary and other interventions that target BCKD abundance or functions may promote muscle protein anabolism in individuals with muscle wasting conditions.

EFFECT OF REGULATING BRANCHED-CHAIN AMINO ACID METABOLISM ON INSULIN SENSITIVITY

Gagandeep Mann

York University

Abstract text (no figures, no Bold): Branched-chain amino acids (BCAAs) have displayed metabolic benefits, and play a role in muscle protein synthesis. However, elevated levels of BCAAs and their metabolites have been linked to the pathogenesis of insulin resistance and type 2 diabetes mellitus. BCAA metabolic enzymes, branched-chain amino acid aminotransferase 2 (BCAT2) and downstream of BCAT2, branched-chain alpha-keto acid dehydrogenase complex (BCKD) are responsible for the metabolism of BCAAs. Both enzymes are downregulated in conditions like obesity, insulin resistance and type 2 diabetes, which could allude to the increase in BCAA levels in these conditions. This poses the question whether BCAAs are inhibiting insulin-stimulated glucose uptake, or if the dysregulations of BCAA metabolic enzymes are the reason for reduced insulin sensitivity and that the elevated levels of BCAAs are a consequence of this. This makes upregulating BCAA metabolism through their enzymes a therapeutic target against insulin resistance. It has been previously demonstrated in my lab that α-ketoisocaproic acid (KIC), a metabolite of leucine, inhibits insulin stimulated glucose uptake, but is converted back to leucine in order to do so. This further supports the potential benefit of upregulating BCAA metabolic flux to help increase insulin sensitivity. Thus, I analyzed the effect of manipulating BCAA metabolic enzymes on insulin sensitivity. Preliminary results support our hypothesis, as BCKD knockdown showed a decrease in insulin-stimulated glucose uptake in starved conditions ($p<0.05$). BCKD knockdown with KIC supplementation showed a further suppression of insulin-stimulated glucose uptake ($p<0.05$). Conversely, knockdown of BCKD's inhibitor, branched-chain alpha-keto acid dehydrogenase kinase (BDK) showed an increase in insulin-stimulated glucose uptake in starved conditions. These results emphasize that regulating BCAA metabolism and the metabolic enzymes involved can be a potential therapeutic target for managing insulin resistance.

A NOVEL RADIO-TELEMETRY METHOD FOR THE MEASUREMENT OF PLEURAL PRESSURE AND PHYSIOLOGICAL RHYTHMS IN FREELY BEHAVING MICE

Jade P. Marrow[1, 3,] Andrew J. Foster[1], Melissa A. Allwood[1], Keith R. Brunt[2,3], and Jeremy A. Simpson[1,3]

[1]Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada. [2]Department of Pharmacology, Dalhousie Medicine New Brunswick, Saint John, NB, Canada. [3]IMPART Team Canada Investigator Network.

Background: For effective gas exchange to occur, the respiratory system relies on the coordinated action of three major components: neural drive, proper lung compliance and mechanics, and respiratory muscle function. Techniques that evaluate respiratory drive and function are critical for understanding lung physiology in health and disease, however we are limited in our ability to chronically assess these factors in freely behaving animals. Accordingly, our aim was to develop a surgical approach for the placement of a telemetry device to effectively record pleural pressure over the long-term and detect differences in respiratory timing and drive, providing the first tool to assess these parameters for circadian influence. Methods: Adult male C57Bl/6 mice were implanted with radio-telemetry devices to record heart rate, temperature, activity, and pleural pressure during 24 h normoxia, 24 h moderate hypoxia (15% O2), and return to 48 h normoxia. To assess circadian rhythms, cosinor analysis was performed by applying the best-fit cosine wave and recording wave characteristics (e.g., mesor, amplitude, acrophase) over each 24 h period. Statistical analyses were completed on 12-hour averages during both sleep- and wake-phases. Differences were considered significant at $p<0.05$. Results: Compared to baseline normoxia, 24 h of hypoxia induced a significant increase in ventilation, owing to increases in respiratory frequency and pleural pressure. Respiratory frequency was modulated by a significant reduction in expiratory time without changes to the duration of inspiration. Further, ventilatory drive, as assessed by inspiratory pleural pressure divided by inspiratory time, was significantly elevated during the 24 h exposure period and remained elevated during 48 h of normoxic recovery. Application of cosinor analysis revealed that respiratory frequency has a clear circadian rhythm, which is disrupted by a hypoxic stress. Conclusion: For the first time, we have demonstrated that radio-telemetry is an effective tool for the continuous and chronic recording of pleural pressure to facilitate circadian rhythm analyses. Thus, radio-telemetry of pleural pressure can complement traditional methods for evaluating respiratory function in health and disease.

COMPARING QUADRICEPS STRENGTH AND SIZE BETWEEN LUNG TRANSPLANT CANDIDATE DIAGNOSTIC GROUPS BEFORE AND AFTER TRANSPLANTATION

Christian Martin, Manoela Ferreira, Nathalia Maia, Tyler Saumur, and Sunita Mathur

Muscle Function and Performance Laboratory, Department of Physical Therapy, University of Toronto, Canada, M5G 1V7

There are limited studies comparing muscle atrophy and dysfunction in pre-transplant lung diseases, including those with chronic obstructive pulmonary disease (COPD) and interstitial lung disease (ILD), before and after lung transplantation. The objective of this study was to compare quadriceps strength and size between patients with COPD and ILD before lung transplant, and changes from pre to 3 months post-transplant. Patients with COPD or ILD who were listed for lung transplant in the Toronto Lung Transplant Program between August 2018 and June 2019 were included in the study. B-mode ultrasound was used to measure quadriceps total thickness (thicknesses of the rectus femoris, vastus intermedius, and vastus lateralis, cm), and rectus femoris cross-sectional area (CSA, cm2). Quadriceps strength was measured using the Medup dynamometer (Nm). Between-group differences at pre-transplant were tested using one-way

ANOVA. Within-group differences from pre- to post-transplant were tested using paired samples t-tests (α=0.0167). Nine patients with COPD (65 + 4 years, 6males, 3 females) and 16 with ILD (61 + 10 years,10 males, 6 females) were included. A significant difference was seen between diagnostic groups pre-transplant in rectus femoris thickness ($p = 0.003$). ILD patients showed decreases across all measures from pre- to post-transplant: quadriceps thickness (-1.0 + 0.7cm, $p < 0.001$), rectus femoris CSA (-2.1 + 1.1 cm2, $p < 0.001$), and quadriceps strength (-24 + 35 Nm, $p = 0.015$). COPD patients showed a significant decrease only in quadriceps strength (-11 + 7 Nm, $p = 0.002$). Patients with ILD appear to have larger quadriceps size in comparison with the COPD group and demonstrated greater deficits pre- to post-transplant. The difference between these lung disease groups could be multifactorial, owing to impacts such as disuse, systemic inflammation, or malnutrition within each group. Future research should investigate the effects of rehabilitation within each group and its improvements on muscle function before and following lung transplantation.

THE INFLUENCE OF ACUTE AEROBIC AND RESISTANCE EXERCISE ON MTOR SIGNALING AND AUTOPHAGY IN UNTRAINED HUMAN SKELETAL MUSCLE

Corey E. Mazo[1], Kaylin R. Sweeney[2], Chad C. Carroll[3], Andrew C. D'Lugos[2], Jacob M. Haus[1], Jared M. Dickinson[4]

[1]School of Kinesiology, University of Michigan, [2]College of Health Solutions, Arizona State University, [3]College of Health and Human Sciences, Purdue University, [4]Department of Health Sciences, Central Washington University

Exercise serves as a powerful stimulus for skeletal muscle adaptation. In particular, exercise elicits a structural and contractile response specific to the type of exercise performed. However, the mechanisms regulating these specific adaptations have yet to be completely elucidated. The purpose of this investigation was to examine the molecular response of skeletal muscle to acute aerobic (AE) and resistance exercise (RE). In particular, we focused on two molecular processes: 1) mammalian/mechanistic target of rapamycin (mTOR) signaling, a known regulator of muscle protein synthesis, and 2) autophagy, the process of recycling damaged/dysfunctional cellular components. Six healthy, recreationally active young men (27□3y) completed the study. In a counter-balanced, crossover design, participants completed two trials: AE: 40 minutes of cycling at 70% HR max, and RE: 8 sets of 10 repetitions ay 65% 1 rep-max. Muscle biopsies were taken from the vastus lateralis at baseline, 1 and 4 hours after exercise. Total and phosphorylated protein content was measured via western blot. mTORSer2448 phosphorylation increased at 4h following both RE ($p<0.001$) and AE ($p=0.06$), however, the increase after RE was greater compared to AE ($p=0.026$). Additionally, p70S6K1Thr389 was also significantly increased at 4h following both AE and RE ($p<0.001$). No changes were observed for 4E-BP1Thr37/46 or eEF2Thr56 ($p>0.05$). In regard to markers of autophagy, a significant decrease in LC3BII was observed at 1h postexercise following both AE ($p=0.008$) and RE ($p=0.004$). No changes in the LC3BII/I ratio or p62 protein content were observed ($p>0.05$). Lastly, PGC1alpha protein content increased at 4h only after AE ($p=0.031$). These data indicate that in untrained individuals both acute AE and RE have similar influence on markers of autophagy and also stimulate, to some degree, mTOR signaling in skeletal muscle. Thus, examination of additional molecular pathways, perhaps those related to PGC1alpha, or an extended time course may be necessary to identify the molecular mechanisms mediating specific adaptions of skeletal muscle to AE and RE.

ADAPTING A NOVEL MUSCLE ENDOGENOUS REPAIR ASSAY FOR INDUSTRY AND ACADEMIC ADOPTION

Matthew McFee[1-3], Amir Meysami[1-3], Bella Xu[1-4], Sadegh Davoudi[1-3], Penney Gilbert[1-3,5], Alison McGuigan[1,2,4]

[1]University of Toronto, Toronto, ON, Canada [2]Institute of Biomaterials and Biomedical Engineering, Toronto, ON, Canada [3]Donnelly Centre for Cellular and Biomolecular Research, Toronto, ON, Canada [4]Department of Chemical Engineering and Applied Chemistry, Toronto, ON, Canada [5]Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

In healthy individuals, skeletal muscle injury can self-repair through the activity of muscle stem cells (MuSCs) residing within the tissue, a process known as 'endogenous repair'. In age and in disease, MuSC endogenous repair potency is lost, and it is widely believed that therapeutics designed to target and stimulate endogenous repair will serve to improve muscle health in these patient populations. However, validation of drugs with potential to stimulate muscle endogenous repair proves to be a continued bottleneck in drug discovery. To address this limitation and compress the drug discovery pipeline, we developed an in vitro stem cell mediated skeletal muscle endogenous repair platform (MEndR) that predicts in vivo outcomes of a gold-standard MuSC potency assay in mice. The assay makes use of primary myoblasts established from human biopsy tissue that are seeded into a tea bag paper scaffold and differentiated to form striated muscle fibre. Freshly isolated MuSCs are 'engrafted' into the tissues, which are then injured to induce and study the process of stem cell mediated repair in culture. Endpoint metrics such as donor fibre diameter, nuclear index, and surface coverage are used to evaluate the extent of regeneration in this predictive phenotypic assay. Three major roadblocks currently exist that hinder the MEndR assay from being widely adopted by industry and academia. First, access to human muscle biopsies is not feasible for many industry and academic labs, and primary myoblasts have a finite culture life, which limits screening efforts. Next, the assay makes use of MuSCs from mice, which introduces expense and species specificity limitations. Finally, the current scale of the MEndR assay is too large to conduct phenotypic drug screens. In this address, we provide an overview of the MEndR assay and share our progress made in addressing the first aforementioned hindrance to adoption.

A ROLE FOR ATF4 IN MITOCHONDRIAL REGULATION WITHIN SKELETAL MUSCLE

Jonathan M. Memme and David A. Hood

Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, ON, CANADA, M3J 1P3.

As mitochondria are responsible for the maintenance of metabolic health in muscle, they contribute to muscle dysfunction and systemic disease. The master regulator of mitochondrial biogenesis, PGC-1□, promotes the synthesis of mitochondrial proteins transcribed by both nDNA and mtDNA. However, the coordinated expression of these genomes is important in preserving proteostasis, as imbalances in nDNA:mtDNA expression can disrupt mitochondrial viability, triggering the mitochondrial unfolded protein response (UPRmt). The primary regulator of the UPRmt is ATF4, which improves organelle protein handling by augmenting the expression of mitochondrial chaperones to re-establish homeostasis. ATF4 has been suggested to regulate skeletal muscle health by mediating aging-related, and disuse-induced muscle atrophy and decline. However, whether ATF4 is necessary for mitochondrial biogenesis in skeletal muscle has yet to be determined. Our data indicate that ATF4 protein increased ~3-fold following 4 days of differentiation in C2C12 myotubes. This induction of ATF4 corresponded with 3-5-fold increases in mitochondrial content

measured by proteins COX I and IV, despite modest decreases in the notable UPRmt proteins mtHSP70, HSP60, and CPN10. Overexpression and knock-down of ATF4 in cultured myoblasts impaired their ability to form multinucleated myotubes, suggesting that ATF4 expression must be fine-tuned to facilitate myotube formation. Moreover, ATF4 mRNA and protein content were induced 1.5-1.8-fold following acute contractile activity in both rat tissue and cultured cells, respectively, preceding increases in mitochondrial content. We also observed a robust 90% increase in ATF4 protein expression in 7-day denervated mouse muscle, which corresponded with an approximately 20% reduction in mitochondrial content. These data implicate ATF4 as a potentially potent regulator of mitochondrial remodeling in muscle, that is capable of mounting appropriate, stress-specific signaling under various conditions of muscle development, contractile activity and chronic inactivity. Future studies using knockdown and overexpression of ATF4 will explore whether ATF4 is necessary for the regulation of mitochondrial content and function. This will help solidify whether ATF4 could be deemed a suitable target for mitochondrial-targeted therapies in order to promote overall metabolic health.

HETEROZYGOUS SOD2 DELETION IMPAIRS SERCA FUNCTION IN SOLEUS MUSCLES FROM FEMALE MICE

Holt N Messner[1], Dr. Jeffrey A Stuart[2], Dr. Val Andrew Fajardo[1]

[1]Faculty of Applied Health Sciences, Brock University, St. Catharines, ON, Canada

[2]Department of Biological Sciences, Brock University, St. Catharines, ON, Canada

Sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) pumps actively transport intracellular Ca2+ back into the sarcoplasmic reticulum and are the primary facilitators of muscle relaxation, restoring intracellular [Ca2+] to resting levels. Like many proteins, SERCA pumps are subject to oxidation which may affect their functional capacity. Superoxide dismutase 2 (SOD2) is a critical antioxidant enzyme localised in the mitochondrial matrix which converts superoxide radicals into less reactive H2O2. The present study sought to explore the structural and functional consequences of halved SOD2 expression, particularly to SERCA isoforms, in skeletal fast- and slow-twitch muscle. To this end, we obtained soleus and extensor digitorum longus (EDL) muscles from wild-type (WT) and SOD2 heterozygote (SOD2+/-) C57BL/6J mice (n=8 per genotype, aged 6-7 months). SERCA activity assays were performed along with immunoprecipitation experiments to examine SERCA2a (slow isoform) and SERCA1a (fast isoform) tyrosine nitration and glutathionylation. Relative to WT, soleus muscle showed significant impairments in SERCA function, with a reduction in SERCA's affinity for Ca2+, whereas no differences were observed between genotypes in the EDL. This corresponded well with Western blot data showing significantly elevated SERCA2a tyrosine nitration in the soleus, with no change in SERCA1a tyrosine nitration in the EDL. Glutathionylation of SERCA may act as an adaptive modality to preserve function during moderate oxidative stress. However, our data shows that glutathione-SERCA2a adducts were instead decreased in SOD2+/- soleus compared to WT. This was in contrast to the EDL, where no significant measurable differences in glutathione-SERCA1a adducts were found between genotypes. These results suggest less oxidation in EDL relative to soleus. Altogether, our data show that heterozygous deletion of SOD2 influences SERCA activity in slow-twitch, but not fast-twitch skeletal muscle. The differential response between muscles is likely due to differences in oxidative species production and SERCA isoform-specific susceptibility to tyrosine nitration.

INVESTIGATING CANDESARTAN FOR THE PREVENTION OF PULMONARY HYPERTENSION IN RATS WITH DIASTOLIC DYSFUNCTION

Jonathan D. Mofford [1], Leslie M. Ogilvie[1], Brittany A. Edgett [1,2], Anabelle M. Ng[1], Mathew J. Platt[2], Keith R. Brunt [2,3], Jeremy A. Simpson[1,2].

[1]University of Guelph, Department of Human Health and Nutritional Sciences, [2]IMPART team Canada Investigator Network, [3]Department of Pharmacology, Dalhousie Medicine

Introduction: Heart failure is a global pandemic, and a leading cause of death in developed countries. Clinically, approximately half of heart failure patients display preserved ejection fraction characterized by diastolic dysfunction. Group 2 pulmonary hypertension (PH-II) is a common comorbid condition in patients with heart failure, and currently lacks effective treatment strategies. Previous work from our lab identified Candesartan, an angiotensin receptor blocker, as a potential treatment for PH-II in a rodent model of pressure overload-induced heart failure with reduced ejection fraction, significantly reducing pulmonary blood pressure compared to other drugs of its class. Given the efficacy of Candesartan in cases of heart failure with reduced ejection fraction, we sought to evaluate Candesartan for the treatment of PH-II in a rat model of a heart failure with preserved ejection fraction (HFpEF)-like syndrome. We hypothesize that rats with a HFpEF-like syndrome will develop pulmonary hypertension associated with diastolic dysfunction by 9 weeks post-splenectomy, and that Candesartan will prevent the development of pulmonary hypertension, but not diastolic dysfunction, in these rats.

Methods: 8-week-old female Wistar rats were subjected to splenectomy to induce diastolic dysfunction consistent with a HFpEF-like syndrome or sham surgery. Rats were aged to 5 weeks post-surgery and randomized to Candesartan or vehicle treatment. Candesartan was administered orally at 15mg/kg body weight mixed with Nutella daily for 4 weeks; vehicle-treated rats received Nutella without Candesartan. At 9 weeks post-surgery, cardiac structure and function were evaluated by M-mode echocardiography and invasive catheterization of the left and right ventricles of the heart.

Preliminary Results: Candesartan treatment prevented the development of pulmonary hypertension compared to vehicle-treated rats, as evidenced by reduced right ventricle pressure. Interestingly, hemodynamic assessment and echocardiography revealed that Candesartan significantly reduces left ventricular end diastolic pressure and posterior wall thickness; effectively slowing the progression of diastolic dysfunction and concentric remodeling of the left ventricle by 9 weeks post-surgery.

Conclusion: Candesartan, an antihypertensive drug commonly prescribed to heart failure patients, demonstrates the capacity to treat comorbid PH-II in a rat model of HFpEF. Furthermore, early treatment with Candesartan may slow or arrest the development of diastolic dysfunction in this model. While further investigations in large animal models and patients are necessary, these preliminary data suggest that Candesartan could represent the first successful therapy for treating PH-II in patients with HFpEF.

THE EFFECT OF A CHEMOTHERAPY DRUG COCKTAIL ON BRANCHED- CHAIN AMINO ACID METABOLISM

Stephen Mora and Olasunkanmi A.J. Adegoke

York University

Cachexia, or muscle wasting is a common co-morbidity of many clinical diseases, including cancer, chronic kidney disease and heart failure. Although development of cachexia is associated with tumour burden and disease-related malnutrition, other studies have suggested a causative link between chemotherapy treatment and cachexia. Since previous studies have shown that chemotherapy-induced cachexia is not fully reversible by nutritional support, we investigated the effects of a common chemotherapy drug cocktail on branched-chain amino acid (BCAA) metabolism. On day 4 of differentiation, L6 myotubes were treated with vehicle (1.4μL/mL DMSO) or the common chemotherapy drug cocktail folfiri (a mixture of CPT-11 (20μg/mL), leucovorin (10μg/mL), and 5-fluorouracil (50μg/mL)) for 24-48h. Compared to myotubes treatment with vehicle, those treated with folfiri showed reductions in myofibrillar protein content, shown through myosin heavy chain-1 (MHC) and troponin (n=3). We next investigated a known regulator of BCAA metabolism, branched-chain alpha-ketoacid dehydrogenase complex (BCKD), an enzyme responsible for the irreversible decarboxylation of the keto-acids. Although protein content of BCKD was unchanged (n=3), the activity of this enzyme was significantly decreased 24 and 48h following treatment with folfiri (n=3). Interestingly, BCKD's negative regulator branched-chain alpha-ketoacid dehydrogenase complex regulator kinase (BDK) was increased 24h following treatment with folfiri compared to vehicle (n=3). Due to the decreased activity of BCKD, our data suggests deregulated metabolism of BCAAs following treatment with folfiri in myotubes.

INVESTIGATING THE EFFECT OF EXERCISE ON INFLAMMATORY RESPONSES

Mayoorey Murugathasan, Ardavan Jafari, Ali A. Abdul-Sater

School of Kinesiology and Health Science, Muscle Health Research Centre (MHRC), Faculty of Health, York University, Toronto, ON, Canada.

Inflammation is one of the earliest immune responses when the body encounters "danger" in the form of infection or tissue injury. It plays a crucial role in defending us from and removing the injurious agents and initiating the healing process. However, excessive or prolonged inflammation predisposes a number of adverse chronic diseases and health conditions, including cancer, cardiovascular disease, type II diabetes, obesity and autoimmune disorders. This study aims to investigate how structured exercise programs of various intensities modulate the inflammatory responses and associated signaling pathways as well as to elucidate the underlying mechanisms. C57/Bl6 mice were subjected to long-term moderate exercise training on a treadmill. Non-exercising sedentary mice served as controls. Splenocytes, peritoneal macrophages and bone marrow-derived macrophages (BMDMs) were isolated and stimulated with Lipopolysaccharide (LPS). Western blotting, qPCR, Seahorse and Flow Cytometric analyses were performed to evaluate how exercise affects the induction of key inflammatory transcription factors, pro- and anti-inflammatory cytokine expression, metabolic pathways that promote inflammation or tissue repair, and mitochondrial parameters such as oxidative stress and mitochondrial membrane potential. Long-term moderate exercise reduces the activation of pro-inflammatory transcription factors (NF-**κ**B, IRF3), thereby reducing expression of pro-inflammatory cytokines (IL-1β, TNF-α, IFN-β). In addition, it reduces the intracellular levels of inflammatory signaling proteins (iNOS, Hif-1α, p-P65, p-ERK, p-S6). However at the same time, it increases

the expression of anti-inflammatory cytokines (IL-10) and signaling proteins (Arginase-1, IκB-α). Furthermore, long-term moderate exercise reduces mitochondrial oxidative stress and sustains mitochondrial membrane potential in BMDMs. Mechanistically, these observations demonstrate that moderate exercise intensity drives macrophages away from the pro-inflammatory (M1) phenotype and towards the anti-inflammatory (M2) phenotype. Our results provide crucial mechanistic insights into how exercise regulates inflammation, which might be eventually used to optimize physical activity programs that provide clinically meaningful protection from excessive or unwanted prolonged inflammation.

This research was supported by a grant from Natural Sciences and Engineering Research Council (NSERC) grant number: RGPIN-2017-05703.

HEMIN THERAPY – A NOVEL TREATMENT FOR DIASTOLIC DYSFUNCTION IN RATS

Anabelle M. Ng[1], Jonathan D. Mofford[1], Leslie M. Ogilvie[1], Keith R. Brunt[1,2], Jeremy A. Simpson[1,3]

[1]University of Guelph, Department of Human Health and Nutritional Sciences

[2]Dalhousie University, Department of Pharmacology, [3]IMPART Investigator Team Canada

Introduction: Heart failure is a global pandemic, directly affecting the lives of over 600,000 Canadians. Approximately half of all heart failure patients have preserved ejection fraction (EF >50%). Yet, all therapeutic options for heart failure patients target heart failure with reduced ejection fraction (HFrEF; EF<50%).

Hemin, a substrate and inducer of heme-oxygenase, is explored as a novel therapeutic treatment due to its cytoprotective properties, which may prevent the progression of heart failure with preserved ejection fraction (HFpEF). We hypothesize that hemin therapy will prevent the development of diastolic dysfunction, concentric hypertrophy, and excessive cardiac fibrosis.

Methods: 8-week-old male Wistar rats were subjected to splenectomy surgery to induce a HFpEF-like syndrome, or sham surgery. Rats were randomized to hemin- and vehicle-treated groups at 5-weeks post-splenectomy or sham surgery and received treatment for every 3 days for 4 weeks. Hemin was prepared and administered at a dose of 50 μmol/mL/kg body weight. At 9-weeks post-surgery, left ventricle structure, function, and fibrosis were evaluated by B-mode and M-mode echocardiography, invasive hemodynamics, and histology using Picrosirius red, respectively.

Results: At 9 weeks post-surgery, echocardiography indicated no change in end diastolic dimensions, end systolic dimensions, or posterior wall thickness. In contrast to our hypothesis, concentric hypertrophy of the left ventricle was not observed in either hemin- or vehicle-treated splenectomised rats. At the same time point, invasive hemodynamics revealed that hemin treatment prevented the development of diastolic dysfunction, as shown by left ventricle end diastolic pressure (LVEDP) and maximal rate of relaxation (dP/dt), which were not elevated in this hemin-treated HFpEF-like model compared to vehicle-treated splenectomy rats. Additionally, histological analysis of the left ventricle showed that hemin therapy prevented excessive cardiac fibrosis in the splenectomy hemin-treated in comparison to the splenectomy vehicle-treated group.

Conclusion: Splenectomy in otherwise healthy male rats, induces a HFpEF-like phenotype characterized by diastolic dysfunction, preserved systolic function, and fibrosis. For the first time, we show that hemin

therapy prevents diastolic dysfunction and cardiac fibrosis in our model of HFpEF. These findings are the important first steps for the use of hemin as a potential therapeutic for the treatment of diastolic dysfunction. Future investigations encourage exploring the efficacy of hemin in large animal models of HFpEF and human clinical trials with HFpEF patients.

NEXT GENERATION AMPK ACTIVATION ELICITS ADAPTIVE GENE EXPRESSION IN THE SKELETAL MUSCLE OF DYSTROPHIC ANIMALS

Sean Y Ng, Ian Diffey, Hannah Liang, Vladimir Ljubicic

Department of Kinesiology, McMaster University, Hamilton, ON

Duchenne muscular dystrophy (DMD) is a life-limiting neuromuscular disorder characterized by muscle weakness and wasting. Early proof-of-concept studies have demonstrated that the dystrophic phenotype can be mitigated with the pharmacological stimulation of AMP-activated protein kinase (AMPK) via utrophin- and autophagy-dependent mechanisms. However, first-generation AMPK-inducers such as AICAR have failed to translate to clinical populations. Thus, the identification of novel, safe, and efficacious molecules that stimulate AMPK is of particular importance. The purpose of the present study was to evaluate the cellular and molecular responses to a new generation AMPK activator in dystrophic muscle. Wild-type (WT) and dystrophic (mdx) animals were treated with a single dose (5 mpk) of the orally bioactive AMPK activator, MK-8722, via gavage and were euthanized 3-, 6-, or 12-hours post-administration. WT and mdx animals treated with a vehicle (Veh) solution served as control groups. The tibialis anterior muscles were harvested and processed for immunoblotting and qPCR analyses. MK-8722 administration significantly elevated phosphorylated AMPK and ACC protein levels in the skeletal muscle of WT and mdx animals. AMPK-induction resulted in a ~4-fold increase ($P < 0.05$) in utrophin expression 12-hours post-gavage in dystrophic muscle. Concomitantly, PGC-1α mRNA was also significantly increased. Autophagy markers, p62 and the LC3II:LC3I ratio, were lower ($P < 0.05$) in mdx animals compared with WT mice and were unchanged with MK-8722 treatment. AMPK-specific ULK1 phosphorylation was ~4-fold greater ($P < 0.05$) in mdx animals given MK-8722 compared with Veh. MK-8722 did not affect the mTORC1/ULK1 signaling axis, which suggests that the compound stimulates autophagy via AMPK but independently of the mTORC1 network. This is the first study to demonstrate cellular and molecular responses to a new-generation AMPK activator in dystrophic skeletal muscle. Our results confirm that acute pharmacological targeting of AMPK augments disease-mitigating mechanisms and prefaces further investigation of the chronic effects of novel AMPK activators in DMD.

INTRON RETENTION: WHAT IS IT AND HOW DO WE EVALUATE IT?

Northrup V[1], Perez LJ[2], Edgett B[1,3], Simpson J[3], Brunt K[1]

[1]Department of Pharmacology, Dalhousie University, [2]Department of Veterinary Clinical Medicine, University of Illinois, [3]Department of Human Health and Nutrition, University of Guelph

Intron retention (IR) has been identified as a form of alternative splicing. IR has functions in tissue specific protein diversity, splicing regulation and control of gene expression. The ability to easily detect IR could add to our ability to understand genetic regulation of our genomes. We used erythropoietin (EPO) to evaluate

IR and gain a better understanding of its regulation in response to hypoxia and ischemia. HTB16 (Glioblastoma), CHP212 (Neuroblastoma), hCMEC/D3 (Blood brain barrier) and HEK293 (kidney) cell lines were placed in hypoxic (1% oxygen), ischemic (1% oxygen, no serum or glucose) or normoxic conditions (18% oxygen) for 24 hours and RNA extracted. Quantitative polymerase chain reaction (qPCR) was performed with two sets or primers for EPO (intron (within exons) and non-intron (across splice sites)) and housekeeping genes for normalization. Overall gene expression and ratio between intron and non-intron were determined using analysis of variance (ANOVA). EPO expression was increased in HTB16, CHP212 and HEK293, but not hCMEC/D3 cell lines in response to ischemia but not hypoxia. When comparing intron vs. non-intron primers there was a shift towards more intron retaining transcripts, indicating an induction of the gene, in HTB16, CHP212 and hCMEC/D3 but not in HEK293. IR of the EPO gene is occurring in brain cell lines, but not in kidney cell lines. This could indicate a tissue specificity of IR that adds complexity to EPO expression. IR should be considered when examining the expression of a gene.

MITOCHONDRIAL MAINTENANCE IN AGED HUMAN RIGHT ATRIAL TISSUE FOLLOWING ISCHEMIA-REPERFUSION INJURY

Ashley N. Oliveira[1], Shamini Murugavel[2], Bobby Yanagawa[2] and David A. Hood[1]

[1]Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, ON, Canada, M3J 1P3.
[2]Division of Cardiac Surgery, St. Michael's Hospital, Toronto, ON, Canada, M5B 1W8.

Aging is associated with a number of structural, metabolic and biochemical changes, many of which affect the heart. As a highly oxidative tissue, mitochondria are crucial to the viability of the myocardium. Thus, the maintenance of these organelles is vital to supporting healthy myocardial aging, however little is known about mitochondrial turnover in the aging human heart. Autophagy is the process through which damaged cellular components are degraded by the lysosome, and is associated with pro-cell survival outcomes following stress such as ischemia-reperfusion (IR). However, autophagy is downregulated with age in several tissues, so it remains unclear how the intersection of age can influence the autophagy response to insults such as IR. To evaluate mitochondrial turnover and autophagy, samples from right atria were collected from young (≤50 years) and aged (≥70 years) patients undergoing coronary artery bypass surgery (CABG). Tissue was collected prior to and following cardioplegic arrest during surgery, providing a clinical model of ischemia-reperfusion injury. Patients were matched for hypertension status, dyslipidemia, as well as prescribed medications. Mitochondrial content was not significantly different between the young and aged patients, however a 32% reduction in UQCRC2, a subunit of complex III was seen in the aged. Interestingly, in response to IR increases in VDAC and COX-I protein content were observed in the young, but not the aged patients, indicating a reduced responsiveness to IR with age. Furthermore, age-induced reductions in Parkin and NIX protein suggest an impairment in mitochondrial recycling, or mitophagy, which may be leading to an accumulation of dysfunctional mitochondria in the aged patients. Following IR, our data suggest that, in the young cohort autophagy is reduced as Beclin-1 decreased by 63% and no changes were observed in either p62 or LC3-II:LC3-I ratio. However, this response was blunted in the aged cohort, further supporting an inability to respond to IR injury with age. These findings suggest that modest mitochondrial remodeling occurs with age in the heart, accompanied by an age-associated reduction in the ability of the cardiac cell to mount an appropriate response to IR stress.

IMPAIRED SKELETAL MUSCLE REGENERATION IN TYPE 1 DIABETES MELLITUS IS CHARACTERIZED BY A DYSFUNCTIONAL SPHINGOLIPID PATHWAY RESPONSE

Jacob M. Ouellette, Michael D. Mallender, Dylan J. Hian-Cheong, Stephen J. Trumble, Thomas J. Hawke, and Matthew P. Krause

Department of Kinesiology, Faculty of Human Kinetics, University of Windsor, Windsor, Canada, N9B 3P4. Department of Biology, Baylor University, Waco, United States, 76798. Department of Health Sciences, McMaster University, Hamilton, Canada, L8S 4L8.

Type 1 diabetes mellitus (T1DM) impairs regenerative capacity of skeletal muscle, one of several aspects of diabetic myopathy. It was hypothesized that accumulation of lipid species within the muscle (lipotoxicity) could cause this impairment. Diabetes causes increased lipid deposition in skeletal muscle, yet the lipid response following muscle tissue damage has not been investigated. To assess the effect of T1DM on lipid deposition in regenerating skeletal muscle, wild-type (WT) and T1DM (Akita) mice (n=5) received cardiotoxin (CTX) injections into the left tibialis anterior (TA). TAs were collected 5 days post-CTX and subsequently cryosectioned and stained with BODIPY 493/503 to visualize lipids. Microscope images were analyzed for proportion of area positive for lipid via thresholding. Injured Akita muscle was found to have more total lipid as compared to uninjured (control) Akita, and both injured and control WT muscle ($p<0.05$). Next, individual lipid species were assessed via liquid chromatography-mass spectrometry across a time-course of muscle regeneration. CTX-injured quadriceps of 22 WT and 21 Akita mice were collected at 1, 3, 5, and 7 days post-CTX (n=4-6) and analyzed for 45 different lipid species including sphingosine-1-phosphate (S1P), a sphingolipid critical in stimulating satellite cell activity. Injured WT quadriceps were found to have significantly elevated concentrations of S1P as compared to control particularly at 5 days post-CTX ($p<0.05$), however this response was blunted in the Akita quadriceps. No differences in concentrations of the precursors of S1P, sphingomyelin, dihydroceramide, ceramide, and sphingosine, were found. Next, S1P-regulating enzymes, sphingosine kinase (SphK1) and S1P lyase (SPL) were assessed via SDS-PAGE and western blotting. Elevated SPL expression was found in Akita injured muscle ($p<0.05$) while no changes were found in SphK1 expression or associated ERK1/2 signaling, suggesting that S1P breakdown is increased in T1DM. These data indicate that at early time points, impaired muscle regeneration in T1DM is due to SPL-catalyzed S1P breakdown.

USING EXTRACELLULAR VESICLES TO PREDICT INDIVIDUAL RESPONSE TO EXERCISE IN OBESE YOUTH

Taiana M. Pierdoná*§, Alexandria Martin*, Patience Obi*§, Samira Seif*§, Benjamin Bydak*§, Ashley Eadie#, Keith Brunt#, Jonathan M. McGavock¶*, Martin Sénéchal†, Ayesha Saleem*§

Faculty of Medicine, Department of Pharmacology, Dalhousie University, Saint John, New Brunswick
¶ Department of Pediatrics and Child Health, Max Rady College of Medicine, University of Manitoba
† Cardiometabolic Exercise and Lifestyle Lab, Faculty of Kinesiology, University of New Brunswick
* Diabetes Research Envisioned & Accomplished in Manitoba (DREAM), Children's Hospital Research Institute of Manitoba (CHRIM)
§ Faculty of Kinesiology and Recreation Management, University of Manitoba, Manitoba, Canada

Exercise is associated with various health benefits, including the prevention and management of obesity and cardiometabolic risk factors. However, a strong heterogeneity in the adaptive response to exercise training exists. Differential response to exercise training might be mediated by myokines (proteins, nucleic acids,

metabolites) that can be released directly into the systemic circulation or packaged within extracellular vesicles (EVs). The objective of this study was to evaluate if changes in EVs after acute aerobic exercise (AE) were associated with the responders phenotype following 6-week resistance exercise training. This is a secondary analysis of plasma samples from the EXIT trial (clinical trial #02204670). Eleven sedentary obese youth (15.7±0.5 years, BMI ≥ 95th percentile) underwent an acute bout of AE (60% heart rate reserve, 45 min). Blood was collected before exercise [time (AT) 0 min], during [AT15, 30, 45 min], and 75 min after exercise [AT120]. Afterward, youth participated in 6-week resistance training program, and were categorized into responders (RE) or non-responders (NRE) based on changes in insulin sensitivity (above or below 50 percentile). Primary outcome: EVs were isolated using size exclusion chromatography (Izon®). EV protein concentration, size, zeta potential and protein markers associated with exosomes were analyzed in a single-blind fashion. Overall, there was a general increase in EV production with AE in both groups. Average EV size was significantly larger in RE (~147 nm) as opposed to NRE (~127 nm) at baseline (AT0) and throughout the AE bout. EV size distribution analysis revealed the highest yield of EVs (200 - 250 nm in size) in the RE group, whereas NRE group expressed more EVs between 50 - 100 nm in size ($p<0.05$). The preferential expression of smaller EVs (usually associated with exosomes) in the NRE occurred in tandem with ~36% higher protein levels of exosome markers, TSG101 and CD63 ($p<0.05$). While no difference in average zeta potential (stability) was observed between groups, both groups had the most stable EV populations at AT30. Lastly, RE had significantly higher concentrations of total EV protein than the NRE at AT15 ($p <0.05$). Our data suggest that obese children that respond to exercise produce larger EVs with higher protein content at rest and during an acute bout of exercise prior to training. The relationship between the larger EV cargo components and ability to respond to exercise has yet to be fully elucidated. Funded by DREAM Catalyst and Research Manitoba Grants.

ACCURACY OF ENERGY EXPENDITURE MEASUREMENTS BY ACTIVITY MONITORS IN TYPE 1 DIABETES AND CONTROL SUBJECTS

Rubin Pooni, MSc[1], Sarah M. McGaugh, MSc[1], Riddhi Patel, MFSc[1], Michael C. Riddell, PhD[1]

[1]School of Kinesiology and Health Science, York University, Toronto, ON

Physical activity monitors have become increasingly popular in recent years and researchers have started integrating these devices into artificial pancreas (AP) systems to improve the management of type 1 diabetes (T1D). The accuracy of these devices at measuring energy expenditure (EE) may be important for the function of AP systems. Most research to date has determined the accuracy of activity monitors during steady state aerobic exercise in healthy individuals. In this study, the accuracy of the Fitbit Ionic and Garmin vívosmart 3 at measuring EE was assessed against indirect calorimetry (Cosmed K5) during 5 forms of non-steady state activities in individuals with and without T1D. Fourteen adults (Age: 25.8 ± 8.1 year; BMI: 24.1 ± 3.4 kg/m2; 8 T1D) performed a VO2 peak test, resistance exercise, activities of daily living, and high-intensity interval training on treadmill and cycle ergometer, while wearing a Fitbit and Garmin watch. A significant difference in accuracy was displayed by Garmin between individuals with T1D and healthy controls (T1D: 22.6 ± 35.4%; Control: -15.6 ± 24.0%, $P<0.0001$), but no such difference was exhibited by Fitbit (T1D: 13.5 ± 35.8%; Control: 19.5 ± 37.0%). In summary, the Garmin vívosmart 3 overestimates EE during physical activity in individuals with T1D as compared to healthy controls. This difference may need to be addressed before integrating these devices into AP systems.

PERSISTENT NECROSIS AND IMPAIRED REGENERATION RELATED TO ELEVATED PAI-1 IN AGED SKELETAL MUSCLE

Fasih A. Rahman, Sarah A. Angus, Kyle Stokes, Phillip Karpowicz & Matthew P. Krause

Department of Kinesiology & Department of Biomedical Sciences, University of Windsor, Windsor, Canada, N9B 3P4

Skeletal muscle is highly adaptive and has the ability to regenerate following damage. The regenerative capacity of skeletal muscle declines with age, while observations have confirmed increased accumulation of extracellular matrix (ECM) proteins, termed fibrosis. Many of the mechanisms relating fibrosis to impaired skeletal muscle regeneration remain unexplored, particularly in aged muscle. This study examined the temporal expression and localization of important markers of ECM remodeling protein, plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinase-9 (MMP-9), and ECM markers in relation to macrophage infiltration during early regeneration timepoints. The regeneration process was studied in young (3mo) and aged (18mo) C57BL/6J mice at 3, 5, and 7 days following cardiotoxin-induced damage to the tibialis anterior muscle. Immunohistochemical analyses were performed to determine regenerative capacity, ECM remodeling, and macrophage infiltration in response to PAI-1, MMP-9 and ECM proteins. The regenerative capacity of aged muscle was limited as indicated by reduced muscle mass, cross-sectional myofiber area, and embryonic myosin heavy chain (eMHC) expression. Greater intracellular PAI-1 expression was found in aged regenerating myofibres 5 and 7 days following damage. Aged muscle also showed significantly greater extramyocellular PAI-1 and accumulation of collagen Type-I isoform in necrotic regions 3 and 5 days following damage. This was concomitant with delayed macrophage (F4/80+ cells) infiltration in regenerating regions, and reduced colocalization with MMP-9. These results demonstrate that ECM remodeling is impaired during the early stages following muscle damage, a result of elevated expression of major inhibitor of ECM breakdown, PAI-1. Furthermore, the suppression of macrophage infiltration and MMP-9-macrophage colocalization indicates an inability to breakdown the ECM, and thus, initiate the regeneration process in aged muscle. This study provides a foundation for the study of the mechanism underlying the impairment of aged muscle regeneration.

THE ROLE OF PGC-1α IN THE EXPRESSION OF CARDIOLIPIN SYNTHESIS ENZYMES IN SKELETAL MUSCLE

Brandon J. Richards and David A. Hood

School of Kinesiology and Health Science, and the Muscle Health Research Centre, York University, Toronto, Ontario, Canada

INTRODUCTION: Cardiolipin (CL), a phospholipid found almost exclusively within the inner mitochondrial membrane, plays a vital role in electron transport chain (ETC) function. Recent research has suggested that peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis, may be important for the synthesis of CL. Previous work in our lab has demonstrated that the mRNA levels of CTP:PA-cytidylyltransferase-1 (CDS-1) and acyl-CoA:lysocardiolipin acyltransferase-1 (ALCAT1) decrease and increase, respectively, in PGC-1α knockout (KO) mice, while other enzymes that have a fundamental role in the synthesis of CL remain unaffected. However, previous research has also established that the mRNA level encoding an enzyme does not always correspond to the protein level that is observed. Therefore, the purpose of this study was to measure the protein content of enzymes that play a critical role in the synthesis of CL in PGC-1α KO and WT mice. METHODS: Western blots were performed to determine the content of cardiolipin synthase (CLS),

ALCAT1, and tafazzin (Taz). To do this, the tibialis anterior muscle was extracted from PGC-1α KO and wildtype (WT) mice, homogenized, and then Western blot procedures were performed as previously described. RESULTS: Taz protein content was significantly reduced ($p \leq 0.05$) in KO, relative to WT mice. Taz is important for converting immature CL to its mature form, which then can be embedded into the mitochondrial membranes. Previous research has shown that the ratio of immature CL to mature CL in healthy individuals is below 0.18, whereas in patients with Barth Syndrome (Taz deficiency), this ratio is around 0.96. A ratio above 0.18, as seen in Barth Syndrome patients, may lead to abnormal mitochondria, reduced ETC function, irregular supercomplex formation, and altered apoptotic activity. In contrast, ALCAT1 and CLS protein content were not significantly affected by the lack of PGC-1α. CONCLUSION: PGC-1α KO mice have significantly less Taz protein, which is suggestive of the synthesis of less mature CL. Together, these findings suggest that, while PGC-1α may not influence the total CL level, the proportion of immature-to-mature CL may depend on PGC-1α, and this could be a contributor to the decline in mitochondrial function observed in the absence of this important mitochondrial transcriptional regulator.

CREATINE SUPPLEMENTATION EXHIBITS SEX DIFFERENCES IN WHITE ADIPOSE TISSUE AND INCREASES MITOCHONDRIAL MARKERS IN FEMALE RATS

Chantal R. Ryan, Tyler Dunham, Jensen Murphy, Brian D. Roy, Rebecca E.K. MacPherson

Department of Health Sciences, Brock University, St. Catharines, ON, L2S 3A1, Canada

Activation of white adipose tissue (WAT) thermogenesis, known as WAT browning, has emerged as an attractive approach to treat obesity and obesity-related diseases. Traditionally BAT or browning WAT has been defined by the expression of uncoupling protein 1 (UCP1), a mitochondrial protein that uncouples respiration from ATP production. However, recent work has highlighted creatine metabolism or futile cycling as a potential thermogenic pathway. Creatine-driven futile cycling is a catabolic process which results in ATP oxidation by mitochondrial creatine kinase, resulting in the phosphorylation of creatine and increases in ADP concentrations that drive thermogenic respiration. The purpose of the present study was two-fold: 1) to determine the effects of creatine supplementation on markers of WAT browning, and 2) to determine if creatine supplementation exhibits sex differences. Thirty-two Sprague-Dawley rats (16 male, 16 female) were randomly assigned to one of four experimental groups: control, 2.5g/L, 5g/L and 10g/L of creatine monohydrate (CM) and 1% sucrose via drinking water. Rats had ad libitum access to their drinking water and a standard chow diet throughout the study. Following the 8-week intervention, brown interscapular adipose (BAT) and white subcutaneous inguinal (iWAT) fat depots were collected. Western blotting analysis demonstrated no significant changes in BAT samples for both males and females. Males had lower cytochrome C than females with 10g/L of creatine monohydrate ($p<0.05$). In iWAT, mitochondrial markers (COXIV, PDH, citrate synthase, and cytochrome C) and GAMT exhibit no changes in males, however, a decrease in UCP-1 at 5g/L compared to control was observed ($p<0.05$). Females exhibit a lower control PDH content than males ($p<0.01$) in iWAT, accompanied by an increase in PDH levels at all doses of CM compared to control ($p<0.05$). Furthermore, in females COXIV and cytochrome C presented significant increases with 5g/L and 10g/L, respectively ($p<0.05$). Both males and females did not present with body weight differences despite dose differences. This study presents novel work demonstrating that female WAT exhibits less mitochondrial markers than males under control conditions, however it demonstrates the potential for CM supplementation to increase female adipose thermogenicity to a similar level observed in males.

INFLAMMATION-MEDIATED ACTIVATION OF INOS TRIGGERS ENERGY CRISIS IN SKELETAL MUSCLE TO PROMOTE ATROPHY

Derek T. Hall#, Jason Sadek#, Bianca Colalillo, Sergio Di Marco, and Imed-Eddine Gallouzi.

#Authors contributed equally to this work

Dept. of Biochemistry, McGill University

Cachexia syndrome develops in patients with chronic inflammatory diseases such as cancer and sepsis and is characterized by progressive muscle wasting. Inducible nitric oxide synthase (iNOS) has been suggested to be an effector of cachectic muscle wasting. However, the mechanism by which iNOS promotes atrophy and whether it represents a viable target for therapy remains unexplored. In this study, we demonstrate that under cachectic conditions iNOS triggers mitochondrial dysfunction in muscle fibers. Also, we identify iNOS-mediated inhibition of oxidative phosphorylation (OXPHOS) as the leading cause of reduced mitochondrial respiration. Under these conditions muscle fibers reduce their bioenergetic capacity, leading to the onset of energetic stress, activation of AMPK, suppression of mTOR, and, ultimately, atrophy. All these effects were reversed by the clinically developed iNOS inhibitor GW274150 (GW). Importantly, iNOS impairment prevents loss of strength, activation of AMPK, and atrophy in both the C26 model of cancer cachexia and an LPS-induced model of septic cachexia. Therefore, our data demonstrate a critical role for iNOS activity in the onset of cachectic muscle wasting and provide a proof-of-principle that iNOS inhibitors, such as GW274150, could represent a novel therapeutic for the treatment of cachexia.

ASSOCIATING MUSCLE FUNCTION WITH PHASES OF REACTIVE STEPPING PHASES YOUNG, HEALTHY ADULTS

Tyler M. Saumur[a-c] , Jacqueline Nestico[a,b], George Mochizuki[d], Stephen D. Perry[a,b,e,f] , Avril Mansfield[a,c,e], Sunita Mathur[a,b,e]

[a]Toronto Rehabilitation Institute – University Health Network; [b]Rehabilitation Sciences Institute, University of Toronto; [c]Evaluative Clinical Sciences, Hurvitz Brain Sciences Program, Sunnybrook Research Institute; [d]School of Kinesiology and Health Science, York University; [e] Department of Physical Therapy, University of Toronto; f - Department of Kinesiology and Physical Education, Wilfrid Laurier University

Improving muscle strength is often a goal of exercise training to decrease falls risk. If we can increase function of specific muscles associated with reactive stepping – one of the only strategies that can lead to successful stabilization following a large challenge to balance – we may be able to further decrease falls risk. Accordingly, this study aimed to determine the relationship between lower limb muscle strength and explosive force with force plate-derived timing measures of reactive stepping during small and large balance perturbations. Nineteen young, healthy adults (27.6 ± 3.0 years of age; 10 F: 9 M) responded to 6 small (~8-10% of body weight) and 6 large perturbations (~13-15% of body weight) using an anterior lean-and-release system (causing a forward fall), where they were instructed to recover balance in as few steps as possible. Foot-off, swing, and restabilization times were extracted from the force plates using centre of pressure measures. In addition, peak isokinetic torque, isometric torque, and explosive force of the knee extensors/flexors and plantar/dorsiflexors were measured using isokinetic dynamometry. Correlations were run based on a priori hypotheses and corrected for the number of comparisons (Bonferroni) for each variable.

We found that plantar flexor explosive force had a significant negative correlation with foot-off time ($r = -0.515$, $p = 0.024$) during small perturbations. In addition, knee extensor explosive force was negatively correlated with swing time for both small ($r = -0.571$, $p = 0.011$) and large perturbations ($r = -0.582$, $p = 0.009$). Lastly, knee flexor peak isometric torque was associated with restabilization time following large perturbations ($r = -0.459$, $p = 0.048$), however this was not statistically significant after correcting for multiple comparisons. Overall, these findings suggest that there are specific aspects of muscle function that are related to the different phases of reactive stepping in young adults. Exercise training aimed at decreasing falls risk should consider targeting strength and explosive force in these muscle groups based on the phase of reactive stepping during which these deficits are present. Future work should aim to confirm these findings in clinical populations where falls are prevalent.

MAKING THE MOST OF THE COVID CRISIS – MINING ONLINE CARDIAC DATABASES

Iryna Savinova[1], Ashley L. Eadie[2,3], Nadya Romanova[1], Keith R. Brunt[2,3], and Jeremy A. Simpson[1,2]

[1]Department of Human Health and Nutritional Sciences, University of Guelph, ON, Canada; [2]IMPART Team Canada Investigator Network; [3]Department of Pharmacology, Dalhousie Medicine New Brunswick, NB, Canada

Introduction: Heart failure is a major cause of morbidity and mortality worldwide. Despite therapeutic advances in the past few decades, 5-year survival rates are still ~50%, suggesting new treatment strategies are required to further improve survival. While heart failure is generally regarded as impairment in left ventricle (LV) function, emerging data shows right ventricle (RV) function is equally, if not more, impaired despite the incipient stress being applied to the LV. To address this concern from a molecular perspective and continue research during the COVID crisis, we shifted our study to mining online public microarray databases. Our objective was to investigate changes in cardiokine gene expression in animal models and patients with heart failure, using a bi-ventricular approach.

Methods: Microarray gene expression data was obtained from the Gene Expression Omnibus (GEO) databases. The following search terms were explored in obtaining relevant microarray datasets: heart, ventricle, heart failure, humans, and mice. Final studies were selected based on their inclusion of microarray data for myostatin, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP).

Results: We screened over 100 studies, of which, 67 met the inclusion criteria of investigating the ventricles separately. Of these, 54 examined the LV, 10 examined the RV and only 3 compared both ventricles. A final total of 11 studies remained when further selecting for only mouse and human data. In healthy mice prior to stress, myostatin expression is significantly greater in the RV than in the LV. Upon the application of a cardiac stress (e.g., myocardial infarction), myostatin increases in the RV while the LV remains a non-responder. ANP and BNP expression increases in both ventricles. While human RV data is limited in sample number (n=1), human LV data is consistent with that of the rodents in which ANP and BNP expression increases, while myostatin expression does not change.

Conclusion: This data reveals that the LV and RV differ at a molecular level, with the RV being the predominant cardiokine-secreting region, both in health and in response to disease. Thus, LV-centric research is overlooking key changes that occur in the RV that affect overall cardiac function. To ensure these changes are accounted for in developing novel treatments for heart failure, it is critical for future studies to adopt a multi-chamber approach to evaluating cardiac physiology.

THE ROLE OF ATF5 IN SKELETAL MUSCLE UPRMT REGULATION FOLLOWING ENDURANCE EXERCISE

Mikhaela Slavin and David A. Hood

Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Canada, M3J 1P3

During a bout of exercise, contractile activity produces stress signals within skeletal muscle cells that promote the accumulation of misfolded proteins within the mitochondrion. The subsequent proteotoxicity activates a cytoprotective program called the Mitochondrial Unfolded Protein Response (UPRmt). The UPRmt attenuates global transcription in general, but increases that of specific chaperones and proteases to improve mitochondrial folding capacity and preserve its function. The activating transcription factor 5 (ATF5) has been found to be an important inducer of this stress pathway in mammalian cells and is responsible for the transcription of key UPRmt proteins, such as mitochondrial heat shock protein 70 (mtHSP70), heat shock protein 60 (HSP60) and Lon protease (LonP). The function and regulation of the UPRmt and whether ATF5 could have a role in the maintenance of mitochondrial function and turnover during exercise-induced stress is not known. Thus, we aim to analyze the role of the transcription factor ATF5 in the regulation of the mitochondrial and UPRmt responses following acute exercise, and in response to exercise training adaptations. The objectives of this project are to: 1) investigate the role of ATF5 in basal mitochondrial maintenance, 2) study the impact of ATF5 on the UPRmt gene expression response to acute exercise, and 3) evaluate the function of ATF5 in mediating mitochondrial adaptations to long-term endurance training. We hypothesize that ATF5 is necessary for basal mitochondrial maintenance, the induction of vital UPRmt proteins, and efficient mitochondrial function and turnover following exercise. Furthermore, we hypothesize that trained muscle will experience attenuated activation of the UPRmt, as an adaptation to regular exercise. The significance of this work is to elucidate the transcriptional control that ATF5 exerts in maintaining muscle mitochondrial content, in UPRmt activation, and mitochondrial biogenesis, to help muscle adapt to exercise stress.

UNDERSTANDING THE ROLE OF ACTIN'S C-TERMINUS IN ACTOMYOSIN FORCE GENERATION

Karl Steffensen and Dr. John Dawson

University of Guelph

Movement is essential for life. While an individual might move to find food or breathe, movement at the cellular level facilitates this larger scale motility. Dynamic protein-protein interactions between actin and myosin generate the forces needed for these cellular and larger scale movements. Despite the abundance and prevalence of actin, the details of its highly flexible C-terminus remain a mystery due to a lack of structural information, but there are hints of a dynamic role within actomyosin force generation. Proteolytic cleavage of actin's two C-terminal residues reduces myosin's force generating ATPase activity, while fluorescence studies indicate conformational changes at the C-terminus upon myosin binding. Furthermore, constraining actin's C-terminus with chemical crosslinking restricts myosin force generation proportional to the number of crosslinks formed. To determine the role of actin's C-terminus in actomyosin force generation, I am developing an optimized purification of crosslinked actin oligomers to produce actin filaments composed exclusively of crosslinked dimers (F-dimer) or trimers (F-trimer). The biochemical properties of these filaments are being studied through myosin ATPase and in vitro motility (IVM) assays. In addition, cryo-

EM with direct electron detectors will be used to develop high resolution structures of crosslinked actomyosin, revealing the conformation of and interactions with actin's C-terminus. Characterizing the biochemical function and dynamic structure of actin's C-terminus will provide a deeper understanding of how actin interacts with myosin to generate forces for movement in essential life processes.

MOLECULAR TRANSDUCERS OF HUMAN SKELETAL MUSCLE REMODELING UNDER DIFFERENT LOADING STATES

Tanner Stokes[1], James A. Timmons[2], Hannah Crossland[3], Thomas R. Tripp[4], Kevin Murphy[1], Chris McGlory[5], Cameron J. Mitchell[6], Sara Y. Oikawa [1], Robert W. Morton [1], Bethan Phillips[3], Steve Baker [7], Phillip J. Atherton [3], Claes Wahlestedt[2] and Stuart M. Phillips[1]

[1]Department of Kinesiology, McMaster University, Hamilton, Ontario, CANADA
[2]University of Miami Miller School of Medicine, Miami, FL, USA
[3]School of Medicine, Royal Derby Hospital, University of Nottingham, Derby, UK
[4]Faculty of Kinesiology, University of Calgary, Calgary, Alberta, CANADA
[5]School of Kinesiology and Health Studies, Queens University, Kingston, Ontario, CANADA
[6]School of Kinesiology, University of British Columbia, British Columbia, CANADA
[7]Physical Medicine and Rehabilitation, Department of Medicine, McMaster University, Hamilton, Ontario, CANADA

Skeletal muscle adaptation to increased and decreased loading is highly heterogeneous between individuals and the underlying molecular regulators that underpin this heterogeneity are unclear. Efforts to define a core molecular signature related to hypertrophy have been thwarted by studies that average molecular responses across a relatively small number of individuals. We hypothesized that a within-subjects' approach that addresses physiological heterogeneity would more reliably identify transcripts that are important for muscle growth. Twelve young men undertook 10 weeks of unilateral lower-limb resistance exercise training (RT) and 2 weeks of contralateral immobilization (IMB) so that a more consistent differential physiological response to alterations in muscle loading would be observed. Gene expression was quantified using Affymetrix HTA 2.0 microarrays using RNA extracted from muscle samples obtained following RT and IMB. We employed a novel analytical strategy that enabled analysis of the untranslated regions (UTR) of a gene in addition to the full-length transcript. We then established the relationship between differentially regulated genes and changes in leg lean mass using three of our previous independent exercise studies (total n=100). Of the 11,628 genes that passed quality control, 18% had at least 1 full-length transcript regulated (FDR<5%, FC>1.2; 1435 up-regulated and 649 downregulated) and 141 genes correlated with lean muscle mass gains in a consistent manner across all independent datasets (either at the full-length transcript, 5'UTR or 3'UTR). Many of the genes in this growth signature have established roles in muscle plasticity, including the apelin receptor and FOXO3; however, our analysis also revealed many novel regulatory events. For instance, the 3'UTR signals of BCAT2 and FKBP1A – but not the full-length transcripts – were strongly correlated with an increase in muscle size. Our data are the first to demonstrate that UTR regulatory events covary with lean mass transition in humans and underscore the importance of probing regulation at specific gene regions rather than the full-length transcript.

ENABLING SKELETAL MUSCLE REPAIR AND FUNCTIONAL RECOVERY FOLLOWING DENERVATION-INDUCED INJURY USING ULTRASOUND MEDIATED GENE DELIVERY (UMGD)

Jaryd Te[1], Michael Kuliszewski[1], Judy Correa[1], Howard Leong-Poi[1,2], Jane Batt[1,3]

[1]Keenan Research Center for Biomedical Science, St Michaels Hospital, ,[2]Division of Cardiology, St Michaels Hospital, [3]Interdepartmental Division of Critical Care Medicine, University of Toronto.

Background/Rationale/Significance: Skeletal muscle is essential for mobility and its health relies upon innervation. Peripheral nerve injury due to accidental limb trauma can denervate target skeletal muscle, causing immediate loss of muscle function, atrophy, and development of fibrosis. Delayed re-innervation can result in permanent functional loss, leading to a myriad of physical, financial, social, and psychological burdens on affected individuals. To date, there are no practical and/or preventative therapies to sustain the regenerative capacity of long-term denervated muscle; however, novel techniques such as Ultrasound-mediated Gene Delivery (UMGD) are theorized to be a localized, targeted, and effective treatment strategy.

Experimental Design: We denervate the gastrocnemius and soleus (calf) muscles in the rat by performing unilateral transection of the tibial nerve; the innervated contralateral limb serves as an internal control. We then conduct UMGD on the denervated hindlimb. Briefly, minicircle DNA plasmids coupled to carrier microbubbles are administered intravenously 24 hours following nerve transection. The ultrasound transducer is then swept over the denervated muscles, cavitating microbubbles and allowing for minicircle uptake and expression in target cells. We harvest muscle two weeks following UMGD and assess atrophy (gross weight, myofibre type-specific cross sectional area), vascularity, fibrosis, as well as satellite cell and Fibro-adipogenic progenitor (FAP) content.

Results: We have first characterized a long-term model of denervation in the rat, including developing a method for identifying Fibro-adipogenic Progenitors (FAPs), a resident interstitial cell population that are key effectors of muscle regeneration, repair, and pathogenesis. These preliminary experiments revealed significant atrophy at two weeks post-denervation, thus informing the optimal timepoint to assess UMGD efficacy. After two weeks, we report successful delivery of three myogenic genes (IGF-1, VEGF, and Ang-1) using UMGD. Furthermore, delivered animals exhibited mild increases in gross weight, Type II fibre CSA, and overall vascularization compared to empty minicircle-delivered controls.

Conclusions: We have shown that UMGD is a viable model for targeted gene delivery to skeletal muscle through partial prevention of the atrophy and vascular loss induced by denervation injury. In the clinic, such preventative effects may be sufficient to sustain denervated muscle's receptivity to reinnervation, subsequently allowing afflicted individuals to fully regain functional capacity and resume a healthy and productive life.

INSULIN-STIMULATED GLUT4 TRANSLOCATION IS REDUCED BY PALMITATE AND LINKED TO DEFECTIVE RAC1-INDUCED ACTIN REMODELLING

Victoria Tokarz, Humayon Akhuanzada and Amira Klip

Cell Biology Program, The Hospital for Sick Children, Toronto

Introduction: Glucose enters muscle cells through GLUT4 glucose transporter, which redistributes to the cell surface in response to insulin (GLUT4 translocation). GLUT4 delivery to the plasma membrane is

coordinated by two parallel signalling pathways downstream of PI3-kinase: activation of Akt and insulin-responsive Rabs, and Rac1-dependent actin remodelling. Reduced GLUT4 translocation is a common feature in insulin-resistant animals and humans, but the responsible molecular mechanisms are surprisingly unknown. Importantly, whereas impaired Akt phosphorylation is not uniformly associated with muscle insulin resistance in vivo, selective loss of skeletal muscle Rac1 impairs insulin-stimulated glucose uptake and Rac1-dependent signalling is impaired during obesity and T2D.

Rationale & Hypothesis: Rac1 has emerged as a key regulator of muscle GLUT4 translocation that is susceptible to dysfunction during insulin resistance. However, the mechanisms underlying impaired Rac1-dependent signalling are unknown. Muscle cell cultures are useful to investigate the subcellular events underlying Rac1 dysregulation. We hypothesize that defective Rac1-dependent actin remodelling may work alone or in concert with defective Akt to reduce GLUT4 translocation in response to palmitate (a saturated fatty acid that causes muscle insulin resistance).

Methods: L6 myoblasts overexpressing myc-tagged GLUT4 and the human insulin receptor were treated with PA for 18h. Cultures were serum starved for 3h, stimulated with insulin (0.1 nM, 15min) and processed for the quantitative detection of surface GLUT4, analysis of actin dynamics, and G-LISA assay of Rac1 activity.

Results: A PA concentration curve identified that insulin resistance of GLUT4 translocation precedes reductions in insulin-stimulated Akt phosphorylation. Under these conditions, the Rac1 response to insulin failed (despite elevated basal Rac1 activity compared to control) and a concomitant loss of insulin-stimulated actin remodelling was observed. In PA-treated myoblasts, overexpression of constitutively active Rac1 evoked only few and aberrant dorsal ruffles and failed to restore GLUT4 translocation, suggesting dysregulation of downstream actin remodelling proteins. The cooperative activities of cofilin and Arp2/3 are required for actin remodelling, and PA-treated myoblasts: i) failed to activate (de-phosphorylate) cofilin in response to insulin and ii) show mis-localization of the Arp2/3 subunit ARPC2 to the cytoplasm (while controls showed membrane localization). Concomitant with these defects in the cytoskeleton and related proteins, we observed dispersion of intracellular GLUT4 beyond its usual tight perinuclear localization, which may exacerbate the impairment in GLUT4. translocation.

Conclusions: PA-induced insulin resistance of GLUT4 translocation in myoblasts is possibly a consequence of defective actin remodelling. The defect persists in the presence of active Rac1, implicating downstream actin remodelling proteins like cofilin and Arp2/3 as putative elements whose dysfunction contributes to impaired GLUT4 translocation during insulin resistance.

Significance: Currently, no diabetic therapy targets skeletal muscle (the main consumer of dietary glucose), nor GLUT4 translocation, an established defect occurring early and probably defining muscle insulin resistance. Targeting Akt is not a viable or logical approach. Instead, identifying more precise, downstream targets for chemical manipulation in the GLUT4 translocation pathway may offer unique opportunities for therapy.

REGULATION OF THE AUTOPHAGY-LYSOSOME SYSTEM IN RESPONSE TO HINDLIMB DENERVATION

Triolo, M., Slavin, M., Moradi, N., & Hood, D.A.

Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, Canada.

During chronic muscle disuse, fibre atrophy occurs due to decreases in protein synthesis and elevations in the degradation of proteins and intracellular organelles. One system that regulates degradation is the autophagy-lysosome pathway, which targets damaged proteins and organelles by autophagosomes, followed by delivery of these to the lysosomes for degradation. The regulation of these processes is poorly characterized during muscle disuse, and very little is understood about the regulation of the lysosomes as an end stage for degradation. Thus, the objective of this work is to better understand the autophagy-lysosome system during muscle disuse. Accordingly, we employed a hindlimb denervation protocol in which we unilaterally sectioned the peroneal nerve of one hindlimb, using the contralateral limb as a control in Sprague-Dawley rats for 1,3 or 7 days. We observed significant 10% and 30% reductions in tibialis anterior (TA) mass by 3- and 7-days post-denervation, respectively. Significant elevations in the autophagy proteins (Beclin1, ATG7, p62, LC3-II) were measured at 3 days post-denervation, and further elevated at 7-days post-denervation. To investigate the changes in autophagy flux, we treated a subset of animals with colchicine (4mg/kg/day) for 2 days. Autophagy flux was enhanced 1.3-fold at 1-day post-denervation, further elevated 1.9-fold following 3-days of denervation and subsequently reduced by 30% following 7 days in comparison to time-matched controls. We also observed significant elevations in the lysosomal proteins LAMP1, LAMP2, and V-ATPase. To uncover why, we measured TFEB protein, a transcription factor that regulates the expression of genes responsible for the lysosomal biogenesis. The nuclear localization of TFEB was enhanced by 70% after 1 day, while TFEB protein was elevated by 50% at 7 days, serving to promote the expression of lysosome-associated genes. Cumulatively, these data suggest that the intrinsic activity of the autophagosomal breakdown pathway is sufficient in the early time course. However, the upregulation in the autophagy-lysosome system, concomitant with reductions in flux indicate an inability to efficiently remove autophagy-bound substrates with prolonged denervation.

MULTI-EXON SKIPPING AS A POTENTIAL THERAPY FOR NEMALINE MYOPATHY

Senthuri Vithy, Lacromioara Fabian

University of Toronto

Nemaline Myopathy (NM) is a non-dystrophic congenital myopathy which currently has no therapy. The gene Nebulin (Neb) accounts for 50% of all cases of NM, with most patients being compound heterozygous for two different mutations in Neb. Patients with NM have significantly reduced levels of nebulin. Nebulin is a giant protein that is mainly expressed in the skeletal muscle, specifically in the sarcomere. Most of the protein consists of repetitive repeats that help nebulin bind to the thin filament. A potential therapy is to delete repeats that harbor the pathogenic mutations to express a truncated but functional nebulin. This is based on the idea that mutations in NEB lead to no protein due to the repeats in the proteins being out of frame. I am using CRISPR-cas9 as a tool to delete a whole repeat in the genome of a zebrafish model of NEB related NM. The zebrafish model, due to a splice donor site mutation in exon 46 in NEB, has significantly reduced expression of NEB, motor deficiency and short survival. My project is to test the concept of exon skipping as a therapeutic strategy to produce a truncated but functional nebulin in zebrafish.

CLAMPING SKELETAL MUSCLE PO2 ELIMINATES HYPERINSULINEMIC MICROVASCULAR BLOOD FLOW RESPONSE

Brenda N Wells, Meghan E Kiley, Gaylene M Russell McEvoy, Hamza Shogan, Graham M Fraser

Cardiovascular Sciences, Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland

Hypothesis: Increased blood flow in response to insulin is partially mediated by local metabolic demand coupled with tissue oxygen concentration.

Methods: Two protocols were used to address the hypothesis using 9 anesthetized Sprague-Dawley (171-204g) rats with arterial and venous catheters inserted to maintain the cardiovascular state. The extensor digitorum longus (EDL) muscle was isolated and reflected onto the stage of an inverted microscope. Intravital video microscopy sequences were recorded during baseline and during hyperinsulinemic euglycemia in both protocols. Euglycemia was achieved through simultaneous intravenous infusion of insulin (2U/kg/hr) and variable rates of 50% glucose until normoglycemic levels were reached. In protocol 1, the EDL was reflected over a glass stage insert and isolated from room air, whereas in protocol 2, the muscle was reflected over a semi-permeable membrane interfacing the EDL with a gas exchange chamber. Using this chamber, oxygen (O2) levels were oscillated at one minute intervals from 7%-12%-2%-7%, with stable 5% carbon dioxide concentrations. Analysis of microvascular capillary flow was conducted using custom MATLAB software yielding measurements of red blood cell supply rate (SR) and oxygen saturation (SO2). Animal protocols were approved by Memorial University's Institutional Animal Care Committee.

Results: No significant differences in glucose infusion rates were required to achieve euglycemia between the two protocols; similarly no differences in blood glucose at baseline or euglycemia between protocols were observed. In protocol 1, SR increased to 11.2 cells/s during euglycemia, compared to 8.0 cells/s at baseline ($p = 0.0255$), as expected. Imposed O2 challenges in protocol 2 caused significant changes in SO2 at 12% and 2% concentrations ($p < 0.037$); SO2 at each oxygen level was the same at baseline and in euglycemia. In protocol 2, SR decreased at 12% O2 and increased at 2% O2 during the oscillation, compared to 7% O2, under both baseline and euglycemic conditions. SR responses to oxygen oscillations during euglycemia mirrored those at baseline at each concentration ($p > 0.996$).

Conclusions: The gas exchange chamber was effective at manipulating tissue oxygen levels, observed through profound SO2 changes during chamber oscillations at both baseline and euglycemic conditions. Our results suggest the increase in muscle blood flow observed in response to insulin, as seen in protocol 1, is eliminated if tissue oxygen environment is fixed at given oxygen concentrations as demonstrated in protocol 2. Project funded through a CIHR project grant awarded to GM Fraser.

GSK3 INHIBITION WITH LOW DOSE LIHTIUM SUPPLEMENTATION AUGMENTS FATIGUE RESISTANCE IN SOLEUS MUSCLES

K.C. Whitley[1,2], S.I. Hamstra[1,2], R.W. Baranowski[1,2], C.J.F. Watson[3], R.E.K. MacPherson[3], A.J. MacNeil[3], B.D. Roy[1,2], R. Vandenboom[1,2], V.A. Fajardo[1,2] *

[1]Department of Kinesiology, Brock University, St. Catharines, ON [2]Centre for Bone and Muscle Health, Brock University, St. Catharines, ON [3]Department of Health Sciences, Brock University, St. Catharines, ON

Calcineurin is a Ca2+ dependent serine/threonine phosphatase that dephosphorylates nuclear factor of activated T-cells (NFAT), allowing for NFAT entry into the nucleus. In skeletal muscle, calcineurin activation promotes the slow oxidative phenotype leading to fatigue resistance and mitochondrial biogenesis with an increase in proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) expression. Glycogen synthase kinase 3 beta (GSK3β) is a serine/threonine kinase, and previous in vitro and ex vivo studies have shown that GSK3β inhibits calcineurin signalling by re-phosphorylating NFAT preventing its entry into the nucleus. However, whether GSK3β regulates calcineurin signalling in vivo, particularly in skeletal muscle, remains unknown. Here, we tested whether GSK3β inhibition with low dose lithium chloride (LiCl) supplementation (10 mg/kg/day for 6 weeks) in adult male C57BL/6J mice would enhance calcineurin signalling and augment muscle fatigue resistance in soleus and extensor digitorum longus (EDL) muscles. LiCl treatment inhibited GSK3β with a significant elevation in serine9 phosphorylation in both soleus (+1.8-fold, $p = 0.007$) and EDL (+1.3-fold $p = 0.04$) muscles. However, an apparent activation of calcineurin signalling was only found in the soleus with a trending decrease in NFAT dephosphorylation (-60%, $p = 0.08$) and a significant increase in PGC-1α (+1.5-fold, $p = 0.05$). Similarly, the soleus muscle ($p = 0.04$) but not the EDL ($p = 0.26$) displayed a significant enhancement in fatigue resistance. In conclusion, our findings suggest that GSK3β inhibition with low dose LiCl supplementation activates calcineurin and promotes muscle fatigue resistance in the soleus but not EDL.

INTERRUPTION OF SEDENTARY TIME WITH INTERMITTENT WALKING OR BODY WEIGHT SQUATS IMPROVES SKELETAL MUSCLE DIETARY AMINO ACID UTILIZATION WITH MINIMAL IMPACT ON ANABOLIC-RELATED INTRAMUSCULAR SIGNALING

Eric Williamson[1], Nathan Hodson[1], Jenna B. Gillen1, Stephanie Estafanos[1], Julia M. Malowany[1], Dinesh Kumbhare[2], Daniel R. Moore[1]

[1]University of Toronto, Toronto, ON [2]Toronto Rehabilitation Institute, Toronto, ON

Low physical activity (e.g. reduced daily steps) reduces the ability of dietary amino acids (AA) to support skeletal muscle remodeling through regulation of intramuscular anabolic signaling. Interrupting prolonged sitting with short bouts of intermittent exercise enhances postprandial glycemic control but has unknown effects on sensitizing skeletal muscle to AA and muscle anabolic signaling. PURPOSE: To determine the ability of interrupting prolonged sitting with short exercise bouts to enhance incorporation of dietary AA into myofibrillar protein and activate anabolic intramuscular signaling pathways. METHODS: Twelve participants (7 males and 5 females; ~23y; ~40.0mlO_2/kg/min; ~25.1kg/m2; ~4676 steps/d) completed three 7.5 hr trials in a randomized order consisting of prolonged sitting (SIT), sitting with intermittent walking (WLK; 2 min at 3.1mph every 30 min) or sitting with intermittent squatting (SQT; 15 'chair stands with calf raise' every 30 min). Two mixed-macronutrient meals (~55:30:15% carbohydrate:fat:protein), enriched to 15% with ring-[2H5]phenylalanine or ring-[13C6]phenylalanine, were provided to mimic breakfast and lunch. Skeletal muscle biopsies were obtained ~30 min following the last bout of exercise (4.5h after 'lunch') in each trial and at the start of one trial (baseline). Changes in myofibrillar AA enrichment (LC/MS/MS, ΔMyo) and phosphorylation of proteins (immunoblotting) involved in protein synthesis (mTORC1 & ERK1/2 pathways) were determined. RESULTS: According to a priori comparisons (paired one-tailed T-test), ΔMyo tended to be greater with SQT (0.038±0.003MPE; P=0.10) and WLK (0.047±0.006MPE; P=0.06) compared to SIT (0.032±0.004MPE). Fold change in RPS6Ser240/244 phosphorylation was greater in SQT compared to SIT (7.6±2.7 vs. 1.6±0.45 fold, p=0.026). All other targets measured (4EBP1Thr37/46,

eEF2Thr56, mTORSer2448, ERK1/2Thr202/Tyr204, p38MAPKThr180/Tyr182) were unaffected (P>0.05). CONCLUSION: Interrupting prolonged sitting with short bouts of exercise appears to improve the utilization of dietary AA for de novo myofibrillar protein synthesis. This effect may be mediated in part by enhanced mTORC1-related signaling with resistive-type squats but not with brief walking.

THE EFFECTS OF EXERCISE TRAINING ON RESTING METABOLIC RATE IN YOUTH WITH OVERWEIGHT OR OBESITY

Winnie W. Yu[1], SoJung Lee[2], Hala Tamim[1], Jennifer L. Kuk[1]

[1]School of Kinesiology and Health Sciences, York University, Toronto, ON, Canada
[2]Division of Sports Medicine, Graduate School of Physical Education, Kyung Hee University, Yongin, Republic of Korea

Objectives: The present study will examine 1) the effects of aerobic and/or resistance exercise training without dietary restriction on resting metabolic rate (RMR) and 2) whether changes in body composition are associated with changes in RMR in youth with overweight or obesity. Methods: 140 sedentary boys and girls (≤18 years, BMI percentile > 85%) were randomly assigned to either a control group (n=18) or one of 3 exercise modalities: aerobic (n=51), resistance (n=50) or a combination of aerobic and resistance (n=21). RMR was measured by indirect calorimetry with a ventilated hood and body composition was measured by DXA and MRI. Results: Changes in absolute RMR did not differ between different exercise modalities versus control (p>0.05). Significant decreases in fat mass (FM) were observed in the aerobic (-1.9 ± 0.4 kg) and resistance groups (-1.0 ± 0.4 kg) whereas all groups decreased in visceral fat (-0.2 ± 0.02 kg) compared to control. Increase in fat free mas (FFM) was only seen in the combined group (2.3 ± 0.4 kg) whereas increases in skeletal muscle were observed in both resistance (1.2 ± 0.2 kg) and combined (1.5 ± 0.3 kg) groups versus control. Changes in FFM, but not FM, visceral fat or skeletal muscle was a significant determinant of RMR change independent of exercise modality (p=0.04). Conclusion: Although the type of exercise performed was not associated with different changes in RMR, findings from the study support that changes in FFM is a modest predictor of RMR change in youth with overweight or obesity.