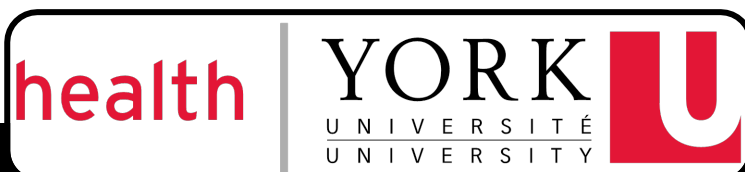


9th Annual
Muscle Health Awareness Day
May 25, 2018

Program and Abstracts



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





Date: May 25, 2018

To: All Participants

From: David A. Hood, MHRC Director

David A. Hood, PhD

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Canada Research Chair
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School of Kinesiology &
Health Science

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Welcome to the 9th Annual Muscle Health Awareness Day

The Muscle Health Research Centre at York University welcomes you to **MHAD9**, our 9th annual “*Muscle Health Awareness Day*”, designed to bring together scientists, faculty members, graduate students and post-doctoral fellows to discuss issues related to skeletal and cardiac muscle physiology, metabolism, adaptation, development and disease.

We are pleased to welcome 8 great speakers for **MHAD9**. The focus this year is on 1) muscle and exercise metabolism, 2) neuromuscular physiology and pathology, and 3) cardiac and vascular function and dysfunction.

Our goal is to give graduate students an opportunity to network and present their work in an informal, yet educational manner. This year we have more than 50 poster presentations to showcase.

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

We thank all of our speakers, presenters, volunteers and sponsors for their participation, and for helping to continue to make this a successful event. Please enjoy **MHAD9**!

Sincerely,

A handwritten signature in blue ink, appearing to read "David A. Hood".

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

9th Annual Muscle Health Awareness Day Program

Friday May 25, 2018

Life Science Building South Lobby and Room 103, York University

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

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

Session Chair: Dr. Michael Riddell

9:00-9:05 – Dr. David Hood, York University

Welcome and Introduction

9:05-9:35 – Dr. Keir Menzies, University of Ottawa

NAD⁺ metabolism and novel therapeutic strategies in muscle health and disease

9:35-10:05 – Dr. Daniel Moore, University of Toronto

Dietary protein in active populations: from minimum requirements to optimal intakes

10:05-10:35 – Dr. Charles Thornton, University of Rochester

Molecular pathology and physiology of myotonic dystrophy

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

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

Session Chair: Dr. Janessa Drake

11:30-12:00 – Dr. Tessa Gordon, University of Toronto

Peripheral nerve regeneration - a stimulating topic for muscle health

12:00-12:30 – Dr. Audrey Hicks, McMaster University

Fatigue in adults with Multiple Sclerosis: Does exercise help or hurt?

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

1:30-2:00 Poster Presentations

Session 3: Cardiac and Vascular Physiology (2:00-4:00)

Session Chair: Dr. Peter Backx

2:00-2:30 – Dr. Bobby Yanagawa, St. Michael's Hospital

Systematic review and meta-analysis to inform valvular and coronary surgery

2:30-3:00 – Dr. Chris Ellis, University of Western

Regulating the Distribution of Oxygen Supply within Skeletal Muscle

3:00-3:30 Dr. Tara Haas, York University

Rethinking capillaries - Key determinants of metabolic health?

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

9th Annual Muscle Health Awareness Day

Speaker Profiles



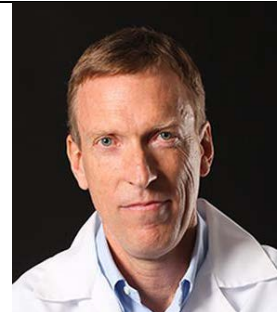
Dr. Keir Menzies, University of Ottawa

Dr. Keir Menzies is an Assistant Professor in the Interdisciplinary School of Health Sciences. Dr. Menzies' research examines new metabolic signaling pathways to help identify and develop translational treatment strategies for aging and neuromuscular diseases. He explores signaling networks that control mitochondrial function in muscle and other important organs affecting whole body metabolism.



Dr. Daniel Moore, University of Toronto

Dr. Daniel Moore is an Assistant Professor in the Faculty of Kinesiology and Physical Education at the University of Toronto. His research focuses on the interplay between exercise and nutrition across different population groups. He has previously studied the effects of different nutrients on aging muscle as well as how they help young adults' muscles recover after exercise



Dr. Charles Thornton, University of Rochester

Dr. Charles Thornton is a Professor of Neurology at the University of Rochester School of Medicine and Dentistry. His research focuses on Myotonic Dystrophy, repeat expansion diseases, and therapeutic development for neuromuscular disorders.



Dr. Tessa Gordon, The Hospital for Sick Children

Dr. Tessa Gordon is a Scientist at the Hospital for Sick Children. Her research aims to promote functional recovery after peripheral nerve injuries. She specifically examines nerve regeneration and the mechanisms that underlie poor axon regeneration, and she explores strategies to overcome the time-dependent decline in regenerative capacity of motor and sensory neurons.



Dr. Audrey Hicks, McMaster University

Dr. Audrey Hicks is a Professor in the Kinesiology Department at McMaster University, and is an Associate Chair of the undergraduate program. Her research examines the health and rehabilitative benefits of engaging in regular exercise. Furthermore, Dr. Hicks works to develop best practice physical activity guidelines for special populations.



Dr. Bobby Yanagawa, St. Michael's Hospital

Dr. Bobby Yanagawa is an Assistant Professor in the Department of Medicine at the University of Toronto and surgeon in the Division of Cardiovascular Surgery at St. Michael's Hospital. His research focuses on basic, translational, and clinical studies of ischemic and valvular heart disease, and cardiac arrhythmias.



Dr. Christopher Ellis, Western University

Dr. Christopher Ellis is a Professor and Graduate Chair in the Department of Medical Biophysics at the University of Western Ontario. Dr. Ellis does research in systems biology, bioengineering and computing in Mathematics, Natural Science, Engineering and Medicine. His research examines oxygen saturation dependent regulation of microvascular oxygen supply by erythrocytes.



Dr. Tara Haas, York University

Dr. Tara Haas is Professor in the School of Kinesiology and Health Science at York University. Her research focuses on the molecular control of blood vessel growth (angiogenesis) in skeletal muscle and adipose tissue. Furthermore, she examines these mechanisms in the context of exercise, peripheral artery disease, and obesity/diabetes.

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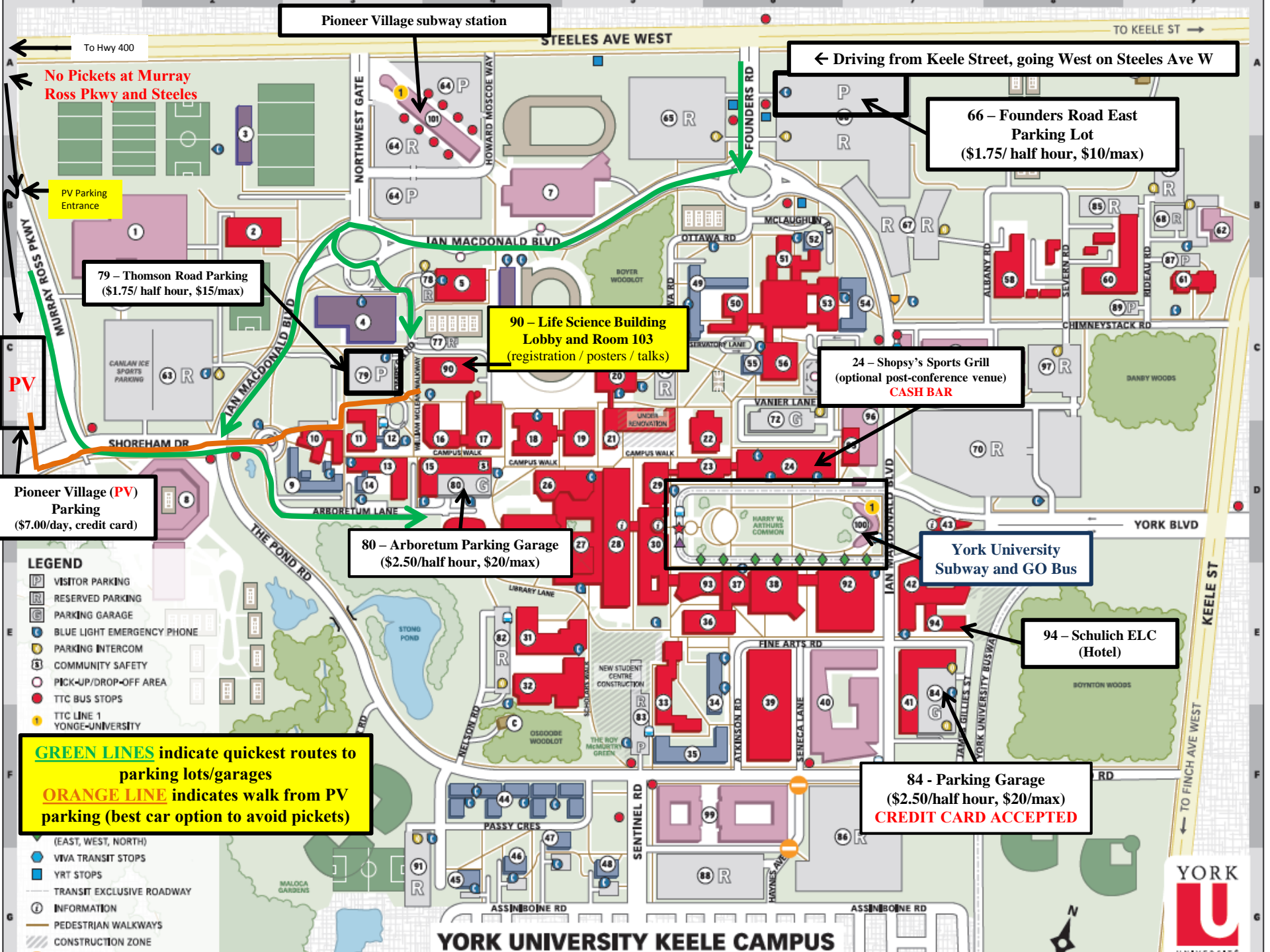


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YORK UNIVERSITY KEELE CAMPUS



Poster Presentation Abstract List

Poster Number	First Author (Surname)	Abstract Title	University Affiliation
1	Abou Sawan	Whole eggs but not egg whites ingestion induces mechanistic target of rapamycin (mTOR) co-localization with the lysosome after resistance exercise in trained young men	<i>University of Toronto</i>
2	Alshamali	Investigating Potential Mechanisms Involved in the Effects of Statins on Frailty and Musculoskeletal Dysfunction	<i>University of Guelph</i>
3	Bhattacharya	A novel energy sensing mechanism that synchronizes myogenic progenitor cell metabolism	<i>York University</i>
4	Birnbaum	Acute and Persistent Diaphragmatic Weakness after Myocardial Infarction	<i>University of Guelph</i>
5	Bott	Synergist ablation of soleus and gastrocnemius via tenotomy results in fibre type specific atrophy in mouse tibialis anterior	<i>Brock University</i>
6	Boyer	Erk1/2 signaling is essential to maintain the satellite cell pool and for muscle regeneration.	<i>Cincinnati Children's Hospital Medical Center / Howard Hughes Medical Institute</i>
7	Brennan	Elucidating Pathomechanisms and Evaluating Therapies for RYR1-related Myopathies	<i>University of Toronto</i>
8	Chandrakumar	Muscle and Bone Adiposity in Postmenopausal Women with Osteoporosis	<i>University Health Network</i>
9	D'Aquila	Enhanced muscle contractile kinetics and glucose metabolism in skeletal muscle by altering fiber type: A novel function of teneurin C-terminal associated peptide (TCAP)-1.	<i>University of Toronto</i>
10	De Ciantis	High Fat Diet Promotes Perfusion Recovery and a Pro-Regenerative Phenotype in the Ischemic Skeletal Muscle	<i>York University</i>
11	Despond	Investigation of the HCM-linked cardiac actin variant A331P	<i>University of Guelph</i>
12	Dial	Myostatin as a potential mediator of myopathy in young adults with type 1 diabetes	<i>McMaster University</i>
13	Dimonte	Motion & Muscle Activation Patterns During an Extreme Conditioning Protocol	<i>York University</i>
14	Dovijarski	The effect of Retinoic acid on Protein Import Machinery in C2C12 cells	<i>York University</i>

15	Duncan	Does exercise intensity influence protein requirements of endurance athletes?	<i>University of Toronto</i>
16	Edgett	Lack of endothelial cell-specific erythropoietin impairs fasting blood glucose	<i>University of Guelph</i>
17	Fung	Comparison Between HR-pQCT and Diagnostic Ultrasound-Acquired Achilles Tendon Cross-Sectional Area	<i>University of Toronto</i>
18	Gingrich	Xin as a novel regulator of mitochondrial morphology and bioenergetics in skeletal muscle	<i>McMaster University</i>
19	Green	A Peripherally-Restricted 5-HT _{2A} Serotonin Receptor Antagonist, Xylamidine, Enhances Brown Adipose Tissue (BAT) Function and Improves Glucose Homeostasis	<i>York University</i>
20	Hannaian	Immediate as compared to delayed protein ingestion may improve adaptations to short-term variable intensity exercise training in recreationally active males	<i>University of Toronto</i>
21	Hughes	In Duchenne muscular dystrophy, mitochondrial bioenergetic impairments are linked to dysfunctions in creatine-dependent energy exchange	<i>York University</i>
22	Jenkins	Do Brief Daily Bouts of Stair Climbing Exercise Improve Cardiorespiratory Fitness?	<i>McMaster University</i>
23	Ma	Genetic ablation of the serotonin reuptake transporter (SERT) leads to obesity but does not inhibit brown adipose tissue (BAT) function	<i>McMaster University</i>
24	Mann	The Effect of Ketoisocaproic Acid on Insulin Stimulated Glucose Transport in Skeletal Muscle Cells is Modulated by Inflammatory Factors	<i>York University</i>
25	Manta	Skeletal muscle adaptations to chronic exercise training in a murine model of myotonic dystrophy type 1	<i>McMaster University</i>
26	Marrow	Investigating the effectiveness of acute and repeated ischemic preconditioning on the enhancement of 5-km cycling performance	<i>University of Guelph</i>
27	Mazzulla	Daily protein requirements are elevated in resistance-trained men after exercise	<i>University of Toronto</i>
28	Mora	The Effect of a Chemotherapy Drug Cocktail on Myotube Morphology and Protein Metabolism	<i>York University</i>
29	Ng	Acute exercise-signaling in a type II spinal muscular atrophy mouse model	<i>McMaster University</i>
30	Ogilvie	Splenectomy-induced cardiac remodeling and diastolic dysfunction	<i>University of Guelph</i>

31	Ojehomon	Outcome of E99K ACTC Gene Expression in Zebrafish	<i>University of Guelph</i>
32	Pooni	Accuracy of wrist-worn activity monitors during different forms of physical activity	<i>York University</i>
33	Qiu	ENU Based Mutagenesis for Discovering Suppressors of Nematine Myopathy	<i>University of Toronto</i>
34	Rajna	Alpha-linolenic acid and linoleic acid differentially regulate the skeletal muscle secretome of obese Zucker rats	<i>University of Guelph</i>
35	Ramos	Microtubule destabilizing chemotherapy may prevent the induction of apoptosis in glycolytic muscles utilizing in-vitro and in-vivo methodologies	<i>York University</i>
36	Rezvan	Different Endothelial Cell Capacity to Respond to Angiogenic Stimuli in Male and Female Mice	<i>York University</i>
37	Roubos	The influence of exercise and weight loss on muscle remodeling during colon cancer induction in mice	<i>University of Ottawa</i>
38	Rudnicki	Endothelial-specific Foxo1 depletion prevents obesity-related disorders by increasing vascular metabolism and growth	<i>York University</i>
39	Sandhu	Characterization of cardiac actin gene switch in zebrafish using CRISPR-Cas9 technology	<i>University of Guelph</i>
40	Sarfaraz	Comparing the effects of lipophilic and non-lipophilic pharmaceuticals on markers of atrial fibrillation in heart failure	<i>University of Guelph</i>
41	Shaikh	M-class α -cardiac actin variants linked with early-onset hypertrophic cardiomyopathy affect actomyosin regulation	<i>University of Guelph</i>
42	Sidhu	F-actin derived ADPr-actin trimer: A platform for determining atomic structure of F-ABP complexes	<i>University of Guelph</i>
43	Soendergaard	3 weeks of oral glutathione supplementation improves skeletal muscle insulin sensitivity in humans	<i>University of Copenhagen</i>
44	Teng	A Study of the DCM-linked Cardiac Actin Variant T126I.	<i>University of Guelph</i>
45	Tinline-Goodfellow	Excess Habitual Protein Consumption May Lead to an Overestimation of Protein Requirements by Stable Isotope Methodology in Resistance Trained Athletes	<i>University of Toronto</i>
46	Triolo	The effect of age and exercise on muscle mitochondria and lysosomal biogenesis	<i>York University</i>
47	Tripathi	Role of a β -catenin-Smad7 complex at muscle creatinine kinase regulatory region.	<i>York University</i>

48	Turnbull	Cancer-Specific Cell Death in Response to Palmitoylcarnitine is Caused by Elevated H ₂ O ₂ Emission and Corresponding Glutathione Depletion	<i>York University</i>
49	Virdi	Role of Syntaxin 1A in Cardiac Excitation-Contraction Coupling	<i>York University</i>
50	Watson	Examining the effects of low-dose lithium feeding on GSK3-beta, soleus muscle mass, TNF-alpha and IL-6 protein expression following tenotomy surgery	<i>Brock University</i>
51	Williamson	Higher protein intakes enhance whole body protein metabolism and exercise performance in endurance-trained males	<i>University of Toronto</i>
52	Xiong	Is perceived fatigue related to physical performance in adults with MS?	<i>McMaster University</i>
53	Yu	All-cause mortality risk greatest in metabolic syndrome combinations with elevated blood pressure	<i>York University</i>
54	Zhang	GCN5 regulates dystrophin expression in skeletal muscle through YY1 acetylation	<i>University of Ottawa</i>

MHAD9 Complete Abstract List

Whole eggs but not egg whites ingestion induces mechanistic target of rapamycin (mTOR) co-localization with the lysosome after resistance exercise in trained young men

Sidney Abou Sawan¹, Stephan van Vliet², Daniel W.D. West¹, Evan L. Shy², Joseph W. Beals², Scott A. Paluska², Nicholas A. Burd², and Daniel R. Moore¹

¹University of Toronto, Toronto, Canada, ²University of Illinois at Urbana-Champaign, Urbana, IL, USA.

We have recently demonstrated that ingestion of whole eggs induces a greater myofibrillar protein synthesis (MPS) response when compared to isonitrogenous egg whites over 5 h of recovery from resistance exercise in young men. Given that a coordinated anabolic response is necessary for the regulation of MPS with exercise and protein ingestion, our aim was to determine whether whole eggs or egg whites ingestion influenced post-exercise mechanistic target of rapamycin complex 1 (mTORC1) protein co-localization in human skeletal muscle. In crossover trials, 10 healthy resistance-trained men (21±1 y; 88±3 kg; body fat: 16±1 %; means±SEM) completed lower body resistance exercise (4 sets of 10 repetitions at 80% of 10-RM for both leg press and leg extensions) before ingesting whole eggs (18 g protein, 17 g fat) or egg whites (18 g protein, 0 g fat) in scrambled form. Muscle biopsies were obtained before exercise and 120 and 300 min after egg ingestion to assess protein co-localization of anabolic signaling molecules by immunofluorescence. After resistance exercise, there were similar decreases in TSC2 co-localization with Rheb (P<0.01) mirrored by a reciprocal increases in mTOR co-localization with Rheb (P<0.01) at 120 and 300 min of exercise recovery in response to whole eggs and egg whites ingestion. mTOR co-localization with LAMP2 significantly increased at 120 and 300 min of post-exercise recovery after whole eggs ingestion (P<0.01). There was no change in mTOR co-localization with LAMP2 (P=0.12) in response to egg whites ingestion. These findings suggest greater mRNA translation initiation, as demonstrated by mTOR co-localization with the lysosome, after whole egg consumption during post-exercise recovery compared with egg whites. This greater anabolic potential is presumably due to the yolk matrix of the whole egg, which contains bioactive compounds (such as lipids, vitamins, minerals etc.) that may mediate this enhanced translational potential during acute recovery from resistance exercise.

Investigating Potential Mechanisms Involved in the Effects of Statins on Frailty and Musculoskeletal Dysfunction

Razan Alshamali*, Jordynn Klein*, Tiffany VanLieshout**, Vladimir Ljubicic ** and Jeremy Simpson*

Dept. of Human Health and Nutritional Sciences, University of Guelph

Statins are the most widely used drugs to lower elevated LDL levels and prevent CVD. Some clinicians worry that statins may induce premature musculoskeletal dysfunction and frailty in elderly cardiovascular patients. The main objective of this study was to investigate a relationship between statin administration and frailty. A secondary objective of this study was to determine the effects of lipophilicity on statin associated muscle symptoms (SAMS). We hypothesized that statin-treated rats would exhibit musculoskeletal dysfunction that would be positively correlated with frailty. Further, we hypothesized that rats treated with the lipophilic statin (atorvastatin) would exhibit exacerbated frailty and musculoskeletal dysfunction relative to the hydrophilic statin (rosuvastatin); perhaps elucidating a central mechanism of action due to its ability to cross the blood-brain barrier. **Methods:** 29 male Wistar rats (2.07-2.25 years old) were administered either atorvastatin, rosuvastatin, or a placebo once daily for 5 weeks. A 37-item frailty index was used to evaluate frailty, and a maximum grip strength test was conducted to evaluate musculoskeletal function. **Results:** Statin-treated rats exhibited a

reduction in cardiovascular frailty symptoms as compared to control-condition rats. However, there were no significant differences in any other parameter of frailty. There were statistically significant differences between treatment groups in grip strength, where atorvastatin treated rats showed a diminished max grip strength in comparison to rosuvastatin treated rats. No correlations were observed between frailty and maximum grip strength. **Discussion:** Statin treatment does appear to improve cardiovascular frailty (one of the indices of frailty tested) in a geriatric population. However, these results indicate that statin treatment does not appear to be associated with neither global frailty nor musculoskeletal dysfunction. Moreover, Atorvastatin treated rats showed a greater decline in max grip strength may suggest a more central mechanism of action to statins and musculoskeletal dysfunction.

A novel energy sensing mechanism that synchronizes myogenic progenitor cell metabolism

Debasmita Bhattacharya^{1,2}, Dr. Anthony Scime^{1,2}

¹*Molecular, Cellular and Integrative Physiology and Muscle Health Research Centre.* ²*Stem Cell Research Group, Faculty of Health, York University, Toronto, Canada*

The regulation of energy generation is integrated into the control mechanisms that govern stem cell fate decisions during proliferation, self-renewal and differentiation. This is highlighted by dynamic metabolic changes that regulate cell cycle maintenance and progression. However, it is uncertain for how glycolysis and Oxphos work together to regulate the yield of ATP, the bio-synthesis of macromolecules, and the production of reducing equivalents that are necessary for cell division. In this study we have uncovered a novel control mechanism for energy generation by an unknown function of the cell cycle regulator and co-transcriptional repressor p107. We found that p107 directly interacts at the D-loop promoter region of mitochondrial DNA leading to repression of mitochondrial-encoded proteins. Intriguingly, during myogenic progenitor cell (MPC) proliferation, it acts as an energy sensor of the cellular NAD⁺/NADH ratio. Indeed, it compartmentalizes to the mitochondria to repress its gene expression when the NAD⁺/NADH ratio is reduced, but manifests an opposite affect when the ratio is increased. This is further highlighted by knockout p107 that did not exhibit an effect on mitochondrial gene expression, although reflected difference in NAD⁺/NADH. Importantly, the energy sensing capacity of p107 is coupled to ATP generation by the mitochondria in MPCs. This role for p107 is related to its interaction with the NAD⁺ dependent deacetylase Sirt1, which is crucial to maintain mitochondrial quality and function. Activating or inhibiting Sirt1 activity influences p107 mitochondrial localization underscoring the significance of their relationship. Our data showcases an unforeseen role for a cell-cycle regulator in arbitrating the MPC metabolic demand through mitochondrial function. This new paradigm adds to our understanding of how the interplay between glycolysis and Oxphos control energy generation in proliferating MPCs.

Acute and Persistent Diaphragmatic Weakness after Myocardial Infarction

Shara Birnbaum, Mathew J. Platt, Andrew J. Foster, Coral L. Murrant, Jeremy A. Simpson

Department of Human Health and Nutritional Science, University of Guelph, Guelph Ontario, Canada

Introduction: Diaphragmatic weakness is a salient feature of heart failure that contributes to exercise intolerance, dyspnea and reduced quality of life. However, while several studies confirm that diaphragmatic weakness is present in end-stage heart failure, little is known about the incipient cause and pathogenesis prior to end-stage heart failure. It is theorized that diaphragmatic weakness is induced by a chronic mechanical load imposed by pulmonary edema. Yet, pulmonary edema has not been explored in the early phases of heart failure; thus, it is unknown firstly if pulmonary edema is present early in the progression of heart failure, and secondly if this edema contributes to diaphragmatic weakness during this time. As such, the diaphragm is not

therapeutically targeted during the initial stages of heart failure. **Purpose:** Therefore, the purpose of this study was to investigate the early changes to diaphragm function in a myocardial infarction (MI) model of heart failure. **Hypothesis:** We hypothesized that diaphragmatic weakness commences during the acute phase post-MI, after the presence of transient pulmonary edema. **Methods:** MI was induced in CD-1 male mice through permanent ligation of the left anterior descending coronary artery. Cardiac, pulmonary and diaphragmatic parameters were measured using a variety of techniques (e.g. invasive hemodynamics, echocardiography, audible pulmonary rales, maximal inspiratory pressures) at 1, 3, 7, 14 and 28 days following MI. **Results:** Here, we report pulmonary edema began at 3 days, with no change to diaphragmatic load or strength. Pulmonary edema peaked at 7 days post-MI, and was accompanied by a decrease in pulmonary compliance. At this time, there was an elevation in inspiratory pressure during eupneic breathing, which is strong evidence that the decrease in lung compliance induced an increase in diaphragmatic load. The increased diaphragmatic load dissipated by 14 days when pulmonary edema also subsided. Subsequently, maximal diaphragmatic strength was reduced, beginning at 7 days post-MI and remaining impaired until study endpoint at 28 days post-MI. **Conclusion:** Taken together, these data suggest that diaphragmatic weakness is not specific to end-stage heart failure, but rather is observed acutely after MI. Further, injury is likely a result of decreased pulmonary compliance and the resultant increase to diaphragmatic load. Thus, future research should identify the mechanism for early diaphragm deficiencies, as opposed to exclusively treating diaphragm weakness during end-stage heart failure. Identifying the incipient changes to the diaphragm post-MI may inform novel therapeutic avenues to impede diaphragmatic weakness before it develops.

Synergist ablation of soleus and gastrocnemius via tenotomy results in fibre type specific atrophy in mouse tibialis anterior

Kirsten N. Bott^{1,2}, Colton Watson^{2,3}, Cameron F. Leveille^{2,3}, Adam J. MacNeil^{2,3}, Sandra J. Peters^{1,2}, Paul J. LeBlanc^{2,3}, Val A. Fajardo^{2,3}

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

Examining the molecular mechanisms regulating muscle mass is critical for developing potential therapeutic strategies to prevent muscle loss. We have previously utilized a simultaneous muscle overload/unload model, whereby tenotomy of soleus and gastrocnemius tendons mechanically overloads plantaris (hypertrophy) while unloading soleus (atrophy) after two weeks. In this model, the foot of the tenotomized leg appears to be held in dorsiflexion, however the muscle mainly responsible for dorsiflexion the tibialis anterior (TA) has not yet been characterized in this model. Previous research demonstrating that dorsiflexion immobilization results in significant muscle atrophy of TA due to an increase in inflammation and polyubiquitylation. Therefore, the present study examined whether TA muscles from the tenotomized leg would also display significant muscle atrophy, and whether increased inflammation and polyubiquitylation would contribute to the muscle loss. We also examined glycogen synthase kinase 3 (GSK3) activation, given this enzyme's role in promoting polyubiquitylation and muscle atrophy. Ten male C57BL/6N mice ($28.1 \pm 0.8\text{g}$) were subjected to soleus and gastrocnemius tenotomy (TN) in one leg, while the other leg was subjected to a sham surgery to serve as an internal control (CON). Two-weeks post-surgery mice were euthanized and TA muscles were collected. The TA muscles in the TN leg were 12% smaller compared to the CON leg ($p < 0.01$), suggesting muscle atrophy. Using immunofluorescent fibre typing, it was found that this atrophy was due to a selective reduction in myofibre cross-sectional area of type IIB (CON $4091 \pm 275 \mu\text{m}^2$ vs. TN $3599 \pm 193 \mu\text{m}^2$, $p < 0.01$) and type IIX-IIB (CON $3281 \pm 243 \mu\text{m}^2$ vs. TN $2933 \pm 262 \mu\text{m}^2$, $p < 0.05$) fibres, without any significant reductions in total number of

myofibres or fibre type distribution. H&E staining showed some centralized nuclei with no signs of mononuclear cell infiltration in the TN TA. Correspondingly, no significant changes in mRNA expression of IL-6 and TNF α inflammatory cytokines were observed in TN TA. However, we did observe a 1.9-fold ($p < 0.05$) increase in polyubiquitylated proteins and higher GSK3 activation as revealed by a 30% reduction in phosphorylated GSK3 (relative to total GSK3; $p < 0.05$) in the TN TA compared to CON. In conclusion, TA muscle undergoes a selective atrophy of fast-glycolytic fibres in response to soleus and gastrocnemius TN, and this may be due to GSK3 activation and polyubiquitylation. Future studies should investigate whether GSK3 represents a viable therapeutic target in this model.

Erk1/2 signaling is essential to maintain the satellite cell pool and for muscle regeneration.

Justin G. Boyer¹, Ph.D., Xing Fu¹, Jeffery D. Molkentin^{1,2} Ph.D.

¹*Division of Molecular Cardiovascular Biology, Heart Institute, Cincinnati Children's Hospital Medical Center.* ²*Howard Hughes Medical Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, 240 Albert Sabin Way, Cincinnati, OH 45229, USA*

Mitogen-activated protein kinases, including extracellular signal-regulated kinase 1/2 (Erk1/2) are highly conserved kinases that regulate a number of cellular activities. Previous in vitro work suggested a role for Erk1/2 signaling during myogenesis, leading us to hypothesize that these kinases play a critical role in muscle regeneration. To address this hypothesis we generated satellite cell-specific Erk1/2 knockout mice (Erk1/2 scKO) by first crossing Erk1 global knockout mice (Erk1^{-/-}) to Erk2 floxed mice, which were then crossed with an inducible Pax7Cre-ERT2 mouse. Additionally, we crossed Erk1/2 scKO mice with the R26NG GFP reporter mouse so that satellite cells could be tracked in vivo. Treating Erk1/2 scKO and control mice with tamoxifen for 4 weeks resulted in the complete loss of satellite cells in adult Erk1/2 double knockout mice. We next assessed muscle regeneration in these mice by injecting cardiotoxin in the tibialis anterior (TA) muscle 3 days after the first tamoxifen injection. Muscles were harvested 4 days post-cardiotoxin injury or 7 days after beginning the tamoxifen regimen. At this time point, Erk1/2 scKO mice had the same number of satellite cells in the uninjured TA muscle as controls. Erk1/2 scKO;R26NG injured muscles were indistinguishable from controls by examining H&E stained histological sections 4 days after cardiotoxin injury. However, immunohistochemistry analysis revealed that, unlike in control animals where we observed satellite cells surrounding and fusing with necrotic fibers, no GFP positive signal was observed surrounding injured fibers in Erk1/2 scKO;R26NG animals. These data suggest that Erk1/2-deficient satellite cells die upon activation and certainly do not proliferate and expand. Indeed, in a separate experiment, we treated Erk1/2 scKO mice with tamoxifen and harvested muscles 10 days post-cardiotoxin injection. Here, injured TA muscles from Erk1/2 scKO animals were completely devoid of any newly regenerating myofibers. Together, these results show that Erk1/2 are required for proper satellite cell function. Evaluating the role of Erk1/2 in muscle regeneration and in satellite cell recruitment might hold significant implications for muscular dystrophy therapy development.

Elucidating Pathomechanisms and Evaluating Therapies for RYR1-related Myopathies

Stephanie Brennan, Sundeep Malik, Linda Groom, James J. Dowling, and Robert Dirksen

The Hospital for Sick Children, University of Toronto, University of Rochester Medical Center

Congenital myopathies are a genetically heterogeneous group of neuromuscular disorders that typically present in childhood and exhibit an estimated prevalence of 1:26,000. Mutations in the skeletal muscle ryanodine receptor 1 gene (RYR1) have been identified as the most common cause of congenital myopathies. Associated symptoms can range from severe neonatal presentations to adult onset disease and include significant lifelong

disabilities. Currently there are no treatment options available for RYR1 -related myopathies, and the lack of treatment strategies is due in part to the absence of appropriate animal models that fully recapitulate the range of clinical phenotypes observed in patients. Thus, it is of fundamental importance to develop an animal model that accurately depicts the complexity of congenital RYR1-related myopathies that will allow us to correctly identify successful treatment plans. This project aims to identify potential therapeutics using a novel Ryr1 mutant mice model of recessive RYR1 myopathies. Upon full characterization of the novel recessive mouse model and with the help of our collaborating laboratories, we will test isolated compounds that show improvement in ryr1 zebrafish models with the intention of successfully improving the disease phenotypes of these mice. We predict that this novel compound heterozygous Ryr1 mutant mouse line will provide the first model of recessive RYR1 myopathy and will enable us to identify the first translatable therapeutics for this severe neuromuscular disorder of childhood.

Muscle and Bone Adiposity in Postmenopausal Women with Osteoporosis

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Background: Muscle, bone, and fat originate from the same mesenchymal stem cell lineage. Bone and muscle have shown to interact, but little has been examined in terms of fat interactions with each of bone and muscle. It has previously been shown that commitment to adipocytes could compromise bone and muscle cellular populations. Clinical studies demonstrated the presence of fat within bone and muscle using MRI. Hypotheses: We hypothesized that increased bone marrow adiposity and increased muscle adiposity are related to one another, and that this relationship is associated with osteoporosis, a condition that is associated with abnormalities in bone metabolism. **Methods:** Postmenopausal women 60-85 years of age were recruited from physician clinics across Ontario. All participants completed a bone density scan of the hip and spine to diagnose osteoporosis. Muscle adiposity was measured with a fast spin-echo MRI at the 66% site of the lower leg. Fat segmentation was achieved using a semi-automated iterative threshold seeking algorithm that has previously shown reproducibility (precision error <5%). Peripheral quantitative computed tomography measured marrow density of the distal tibia (a surrogate of marrow fat) by separating out cortical, subcortical and trabecular bone. Basic anthropometrics were also obtained. **Statistical analyses:** Muscle adiposity from MRI was regressed on bone marrow density, adjusting for age, height, and weight. Models were further examined with an interaction with osteoporosis status. Significant interactions were probed to determine where significance lied. **Results:** In 170 women, for every 10 mg/cm³ lower marrow density (indicative of more fatty marrow), there was a correspondingly larger percentage of muscle fat at the calf: 3.63(1.10-6.06)%, which remained significant after accounting for age, height and weight. Interaction of this relationship with osteoporosis status was significant (p<0.001). Upon probing this interaction, the relationship was found only in women with osteoporosis (r=9.00(4.11,13.89)), but not in those without osteoporosis (r=0.36(-2.12,2.83)), for each 10 mg/cm³ lower marrow density. **Conclusions:** Fat from bone marrow and muscle may be related to the same phenomenon, which is likely also responsible for osteoporosis. More research should focus on abnormalities in mesenchymal cell commitment to fat to address potential clinical populations associated with abnormal bone or muscle metabolism.

Enhanced muscle contractile kinetics and glucose metabolism in skeletal muscle by altering fiber type: A novel function of teneurin C-terminal associated peptide (TCAP)-1.

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Muscle function and metabolism are intrinsically linked, as evidenced by metabolic syndromes that result in poor muscle function and degradation. Skeletal muscle is one of the most important sites of glucose metabolism, as it is responsible for 40% of glucose-associated energy requirements and 80% of tissue glucose uptake under insulin-stimulated conditions, yet despite this, our understanding of muscle energy metabolism and muscle function is not well understood. We now show that teneurin C-terminal associated peptide (TCAP)-1 is novel metabolic regulator that enhances muscle function. In rats, TCAP-1-treated muscles had enhanced baseline contractile kinetics, as established by increased peak twitch-force production and half-relaxation rate, and decreased contraction velocity. These contractile parameters were also enhanced under fatigue conditions compared to vehicle-treated muscle. Associated with these findings, was an increase in myosin heavy chain (MHC)-I expression with an associated decrease MHC-II-type fibers in skeletal muscle suggesting a transition from glycolytic to oxidative type fibers. TCAP-1 also increases glucose uptake in skeletal muscle, in vivo and in the established murine skeletal muscle cell line, C2C12. Concomitant with this, is an increase in NADH production, indicating enhanced cellular and mitochondrial metabolism. Using C2C12 cells as a model to investigate the TCAP-1-mediated cellular mechanism, TCAP-1 induced a rapid biphasic peak of cytosolic calcium that corresponds the timeline of the TCAP-1-mediated increase in inositol triphosphate (IP3) levels, but rapidly returns to baseline. This calcium profile was abolished when the 2-APB inhibitor for the sarcoplasmic reticulum (SR)-bound IP3 receptor, was applied. The increase in cellular metabolism, along with the rapid decrease observed in cytosolic calcium suggests a role with the mitochondria as they require significant calcium importation during activation. TCAP-1 induced significant mitochondrial membrane hyperpolarization that corroborated the calcium release from the SR, indicating calcium import into the mitochondria. Typically, mitochondrial activation is upregulated by calcium influx as calcium has stimulatory roles in the TCA cycle and the electron transport chain. In confirmation of this possibility, TCAP-1 treated cells induced increased expression of succinate dehydrogenase, a rate-limiting enzyme of the TCA cycle, and overall cellular ATP concentrations. Thus, this work for the first time demonstrates that TCAP-1 has a novel role in skeletal muscle function and energy metabolism, that can be used to improve muscle health and enhance muscle performance.

High Fat Diet Promotes Perfusion Recovery and a Pro-Regenerative Phenotype in the Ischemic Skeletal Muscle

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Peripheral artery disease (PAD) is characterized by an attenuated angiogenic response and unresolved inflammation that impairs muscle regeneration in the ischemic muscle. These myopathies are compounded by obesity-associated metabolic co-morbidities; however, the underlying mechanisms remain unknown. We hypothesized that a high fat diet attenuates angiogenesis (capillary growth) and promotes unresolved inflammation that diminishes the regenerative capacity of the ischemic muscle. C57BL/6 male mice were fed high fat (HF) or normal chow (NC) diets (8-9 weeks). Intravital microscopy of the EDL muscle was used to measure real-time capillary hemodynamics in 1 group of mice. Under resting conditions, the capillaries of HF mice exhibited greater red blood cell velocity and O₂ saturation compared to NC. A 2nd group underwent unilateral common femoral artery ligation (~90% blood flow reduction). Blood-flow was assessed by Laser-

Doppler imaging and muscle was collected at 4 or 14 days post-ligation surgery. Blood-flow recovery was greater in HF mice at days 7, 11 and 14 compared to NC mice. At day 4, mRNA of pan-macrophage marker (Emr1) and anti-inflammatory macrophage marker (Mrc1) were equally upregulated in the ischemic muscle of NC and HF mice. At day 14, capillary marker (Pecam1) was increased in ischemic muscle of both NC and HF mice. Unexpectedly, Emr1 levels remained elevated only in NC mice, and this corresponded with higher levels of pro-fibrotic mRNA (Col1a1) compared to HF mice. Our data suggest that adaptations to a moderate duration HF diet in young mice result in a more favorable regenerative microenvironment that potentially predicts an improved recovery of the ischemic muscle.

Investigation of the HCM-linked cardiac actin variant A331P

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Cardiovascular disease is the leading cause of death worldwide, and a great burden on the Canadian economy. Of the variety of cardiovascular diseases identified, the most commonly inherited is cardiomyopathy. One particular subset, known as hypertrophic cardiomyopathy (HCM), is characterized by an increase in left ventricular size, and a decrease in cardiac performance. The generally accepted hypothesis for the cause of HCM is an increase in calcium sensitivity that over-stimulates muscle fibers, leading to their enlargement and eventual fibrosis. Many genes have been implicated in the pathogenesis of HCM, including α -cardiac actin (ACTC). To date, 12 HCM-linked mutations have been discovered in Actc1, encoding for proteins with a variety of amino acid changes. One particular ACTC variant, known as A331P, goes against the general hypothesis of HCM progression. Unregulated filaments show little to no change in intrinsic characteristics or protein-protein interactions from control actin. With the addition of regulatory proteins, however, A331P-ACTC shows a decrease in calcium sensitivity and contractility compared to WT-ACTC, despite being identified as an HCM-linked variant. Finally, in vivo expression of A331P-ACTC in mouse hearts failed to develop an HCM phenotype, making definitive conclusions harder to draw. Preliminary results from my research agree that A331P-ACTC causes a decrease in calcium sensitivity, adding further evidence that the generally accepted HCM hypothesis may be incomplete. Additional experiments will shed more light on the impact of A331P-ACTC on regulated thin filaments, and how this amino acid change impacts the regulatory capabilities of tropomyosin and the troponin complex.

Myostatin as a potential mediator of myopathy in young adults with type 1 diabetes

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Type 1 diabetes mellitus (T1D) is characterized by lack of insulin due to the autoimmune destruction of pancreatic beta-cells. Individuals with T1D are at a high risk of comorbidities including nephropathy and cardiovascular disease. Evidence is now emerging that muscle is also adversely affected in the T1D condition, i.e., myopathy. Myostatin, a negative regulator of muscle growth, is elevated in a number of disease states associated with impaired muscle health including cancer cachexia, muscular dystrophy and type 2 diabetes. Furthermore, myostatin inhibition in rodent models of lipodystrophy and obesity resulted in measurable improvements in glucose handling and insulin sensitivity. However, the characterization of myostatin in the skeletal muscle of humans with T1D has yet to be reported in the literature. Therefore, the purpose of this

ongoing study is to examine the expression of myostatin as a potential mediator of myopathy in those with T1D. Young adults (18-28 yrs old) with/without T1D (n = 12) underwent micro-punch biopsy of the vastus lateralis muscle and venous blood sampling in order to measure skeletal muscle and serum myostatin expression, respectively. Muscle protein expression was measured via immunoblotting, while serum myostatin was measured by ELISA. Our results to date reveal an elevated myostatin in the skeletal muscle of T1D individuals (P=0.16). Additionally, elevated skeletal muscle myostatin was significantly and positively correlated with body fat % (R2 = 0.545, p < 0.05) while being negatively correlated with production of mitochondrial protein content (R2 = 0.351, p < 0.05) in all cohorts. In those with type 1 diabetes, serum myostatin was positively correlated with HbA1c levels (R2 = 0.399, p = 0.09), but was inversely correlated with myostatin in muscle (R2 = 0.652, p < 0.05) Our data is the first reported evidence of elevated skeletal muscle myostatin in individuals with T1D. Ongoing experiments are involved in unraveling the expression of downstream factors mediating myostatin action in the skeletal muscle of those with T1D.

Motion & Muscle Activation Patterns During an Extreme Conditioning Protocol

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Extreme Conditioning Protocols (ECPs) have increased in popularity in recent years. ECPs as defined by American College of Sports Medicine (ACSM), involve high repetition, vigorous intensity workouts performed in a specific order and duration, with self-selected short rest between and within sets. The high intensity and competitive atmosphere has led many to conclude that such workouts are inherently dangerous and impose an increased risk of injury with minimal evidence. The spine is the second most common site for injury during an ECP, surpassed only by the shoulder [1]. However previous studies have used self-reported questionnaires instead of quantitative kinematic and/or kinetic measures. The purpose of this study is to quantify muscle activity and kinematics of experienced participants while they complete an ECP. **METHODS** Males (n=10) and females (n=10) (29.5 ± 3.9 years) experienced in ECPs were recruited to participate in the study. Participants were screened for a history of musculoskeletal injury (none in last 2 years) and by a certified CrossFit™ coach for correct form in squat, deadlift and dumbbell push press. Muscle activations during the ECP were collected with electromyography (EMG) from eight muscles bilaterally: thoracic and lumbar erector spinae (TES, LES), rectus abdominis (RA), external oblique, internal oblique, gluteus medius, biceps femoris, and rectus femoris muscles. Maximum voluntary contractions (MVCs) were collected to provide normalizing data. Kinematics were collected using a total of 46 smart markers (3D Investigator™, Northern Digital Inc., Ontario, Canada). 16 markers were used on four rigid bodies at the spine levels C7, T6, T12 and S2, and 30 markers were used on ten rigid bodies to the track limbs and head. Maximum range of motion in spinal flexion, extension, lateral bend and axial twist was captured to provide normalizing data. The protocol consisted of 5 rounds of deadlifts, front squats and dumbbell push press completed as quickly as possible with self-prescribed rest. Perceived level of discomfort was measured using a 100mm visual analog scale (VAS) before and after each round (6 total VAS scores). Heart rate was monitored with a Polar M400 and chest strap to provide an indication of intensity.

RESULTS During the deadlift, RA mean activity increased in round 5 (p=0.01). LES and TES showed a decrease in mean activity between rounds 1 and 5 (p=0.001). Both upper and lower thoracic segments displayed an increase in flexion in the 5th round compared to the 1st (44.2% (3.7) to 51.2% (1.4) of total flexion range, p=0.009; 63.0% (6.4) to 67.2% (8.6), p=0.02). The lumbar spine did not show any significant levels of flexion. Hip and knee flexion tended to decrease between round 1 and 5, although only changes in hip flexion reached significance. Left hip flexion decreased from 112.5° (14.4) to 102.7° (14.3), p<0.001) and right hip flexion decreased from 108.8° (19.5) to 102.7° (14.3), p=0.01. During the squat, there was a general decrease in activity

of lumbar and thoracic erector spinae muscles when comparing round 1 to round 4 ($p < 0.01$). The lower thoracic and lumbar segments tended towards greater extension by the 5th round (28.5% (1.0) to 25.4% (1.2) of total flexion range, $p < 0.001$; 28.7% (6.8) to 21.5% (1.2), $p < 0.001$). Left hip flexion increased from 7.1° (9.6) to 9.5° (10.9), $p = 0.002$ and right hip flexion increased from 7.9° (9.6) to 9.7° (7.3), $p = 0.002$. Discomfort ratings increased from 0.65mm (0.5) in the 1st round to 15.6mm (12.3) in the 5th round (> 10 mm change indicates clinically relevant pain level). The largest changes in discomfort were between 3rd and 4th rounds, and the 4th and 5th rounds. Participants completed the first round with a heart rate of 76.9% (8.6) of their maximum and ended the last round at 90.0% (4.5). **DISCUSSION / CONCLUSIONS** Both squat and deadlift form changed with increasing round. During the deadlift, participants adopted a more straight legged, bent over posture. With an increase in thoracic flexion and decrease in LES and TES activity, it is likely that the posterior passive structures of the thoracic spine were under strain during later rounds [2]. During the squat, hip and knee angles increased in flexion while the lower thoracic and lumbar spine segments adopted a more upright posture. Hooper et al. found a decrease in knee angle and an increase in hip flexion during an ECP involving squats [3]. A possible explanation of the small differences in our findings may be due to the ECP-experienced participants in this study being more accustomed to performing while fatigued and possibly better able to adopt a more spine-sparing form with increasing fatigue (in the squat). However, additional research is needed to understand whether the changes in the deadlift are from the participants taking greater risks by adopting a less conservative form. Although the discomfort scores did reach clinically significant levels of back pain by round 4 continuing into round 5, the results must be interpreted with caution as participants were not asked to discern between injurious pain and exercise induced discomfort. Therefore, it appears that experienced individuals can self-select an appropriate amount of rest to maintain high exercise intensity, with only small changes in posture and muscle activation.

The effect of Retinoic acid on Protein Import Machinery in C2C12 cells

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The mitochondrial protein import machinery (PIM) plays an important role in maintaining optimal mitochondrial health. PIM components function to allow the import of nuclear-derived proteins that are essential for mitochondrial processes, such as mitochondrial transcription factor A (Tfam). Exercise is a known method for improving mitochondrial import by the induction of cytosolic molecular chaperones, the up-regulation of PIM components, and the increase in matrix chaperonins. However, whether dietary nutrients can also upregulate PIM components in mitochondria is not fully elucidated. Thus, the purpose of this project was to evaluate the effect of retinoic acid (RA) on skeletal muscle import machinery and the import of Tfam. Mouse C2C12 myoblasts were plated on 6-well plates and allowed to differentiate into myotubes. Myotubes were treated with either vehicle (DMSO), or the retinoic acid (RA) isomers 9-cis RA or All-trans RA (ATRA). Following treatment, Tfam, translocase of the outer membrane 20 and 40 (Tom20, 40), translocase of the inner membrane 23 (Tim 23), and heat shock protein chaperones 60 and 70 (Hsp-60, mtHsp70) protein levels were measured. Treatment of myotubes with either of the RA isomers resulted in significant increases in the percentage of imported Tfam (20-22%), as well as the expression of Tim23 (90-100%), Tom20 (39-53%) and Tom40 (40-45%). The levels of Hsp60 and mtHsp70 were more moderately elevated. Our data suggest that retinoic acid isomers can promote the upregulation of PIM component machinery, which could explain the increases in the percentage of Tfam imported into the mitochondria. Thus, RA isomers may be useful adjuncts to contractile activity in stimulating the import of precursor proteins into mitochondria, thereby facilitating mitochondrial biogenesis.

Does exercise intensity influence protein requirements of endurance athletes?

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Increased protein requirements in endurance athletes are due in part to the need to replenish exercise-induced amino acid oxidative losses. Amino acid oxidation is increased during periods of low glycogen availability, which could suggest that higher intensity exercise (e.g., >90% HRmax), which is primarily carbohydrate-dependent, may increase post-exercise protein requirements. Utilizing the minimally invasive indicator amino acid oxidation technique, we hypothesized that the oxidation of our indicator amino acid would be greater following high as compared to low-intensity exercise, reflecting a heightened amino acid requirement. Following 2 days of controlled diet (1.4g protein/kg/d) and training (15km total), seven endurance-trained males (31±3y, 78±6kg, 63±9ml/kg/min) completed a 10-km treadmill run at a low (70±3% HRmax, 50±7% VO₂peak) or high (91±1% HRmax, 74±8% VO₂peak) intensity. Following exercise, participants consumed eight hourly meals containing sufficient energy and 0.93g protein/kg/d provided as a crystalline amino acid mixture modelled on the composition of egg protein. Phenylalanine and tyrosine were consumed in excess to ensure the indicator amino acid ([1-¹³C]phenylalanine, consumed over the final 4h) was directed towards excretion after maximization of whole body protein synthesis. Breath ¹³CO₂ enrichment (IRMS) and CO₂ production (indirect calorimetry) were measured at metabolic and isotopic plateau for determination of ¹³CO₂ excretion (F¹³CO₂). Urinary phenylalanine enrichment (LCMS) was measured for determination of phenylalanine flux and oxidation (PheOx). The rate of carbohydrate oxidation measured by indirect calorimetry was greater during high compared to low-intensity exercise (3.3±0.4g/min vs. 1.3±0.2g/min, respectively; p<0.05). PheOx was similar after high- and low-intensity exercise (7.4 ± 12.6 vs. 7.7 ± 1.4 umol/kg/h, respectively; p>0.05). Phenylalanine flux was greater following high- compared to low-intensity exercise (61.2 ± 7.1 vs. 68.4 ± 13.5 umol/kg/h, respectively; p=0.06), indicative of higher rates of whole-body protein synthesis (p<0.05) and breakdown (p=0.06). In summary, our findings suggest that protein requirements of endurance-trained athletes are similar following a 10-km run performed at a low or high intensity. However, increasing exercise intensity enhances protein turnover in recovery from endurance exercise.

Lack of endothelial cell-specific erythropoietin impairs fasting blood glucose

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Classically, erythropoietin (EPO) is known as the master regulator of erythropoiesis. However, recent evidence supports nonhematopoietic roles for EPO including cytoprotection, cell proliferation and regulation of energy homeostasis. Disruption of EPO signaling in nonerythroid tissues via knockout of the EPO receptor promotes obesity; however, the mechanism(s) by which EPO regulates energy homeostasis remains unknown. We have identified an endothelial cell-specific EPO knockout (EPOEndo-KO) mouse model that has elevated 12-hour fasting blood glucose (wild-type = 5.5±1.0 mmol/L, EPOEndo-KO = 6.6±1.1 mmol/L). In addition, EPOEndo-KO mice have less resting glycogen in glycolytic muscles and more in the liver compared to wild-type mice. Thus, our objective was to understand EPO signaling in regulating whole-body glucose homeostasis. We hypothesized that loss of EPO would alter enzymes involved in glucose mobilization and storage in the liver and glycolytic muscle. To do this we fasted wild-type and EPOEndo-KO mice for 12 hours (20:00h-8:00h). Liver and limb muscles were harvested and immediately frozen in liquid nitrogen prior to immunoblot analysis.

Endothelial cell-specific deletion of EPO was created by crossing LoxP-EPO with Tie2-Cre mice. EPO^{Endo} KO mice followed Mendelian genetics and had normal hematocrit and body weight. In fasted EDL, EPO^{Endo} KO mice had more phosphorylated Akt(Ser473), glycogen synthase (Ser641) and AMPK(Thr172), but less phosphorylated GSK-3 β (Ser9) compared to wild-type mice, suggesting more inhibition of glycogen synthesis. In fasted liver, there was more phosphorylation of glycogen synthase (Ser641), suggesting less glycogen synthesis, potentially contributing to higher fasting blood glucose levels. Here we are the first to demonstrate that loss of EPO plays a role in regulating blood glucose.

Comparison Between HR-pQCT and Diagnostic Ultrasound-Acquired Achilles Tendon Cross-Sectional Area

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Background: High-resolution peripheral quantitative computed tomography (HR-pQCT) scans are used to assess bone architecture at appendicular sites. In distal tibia scans, the Achilles tendon (AT) can be identified by its high linear attenuation compared to surrounding fat. Recently, soft tissue analysis algorithms for HR-pQCT have enabled the segmentation of muscle and tendon from distal tibia scans. **Objectives:** To 1) assess the precision of HR-pQCT-derived AT cross-sectional area (HR AT-CSA) 2) validate HR AT-CSA measures against ultrasound-derived AT-CSA (US AT-CSA). **Methods:** Women and men ≥ 50 years of age with no history of ankle fractures or tendinopathy participated in this observational study. Both HR-pQCT (0.082 mm isotropic voxel size) and US scans (B-mode) were performed following standardized protocols on the non-dominant leg. HR AT-CSA was computed by applying the soft-tissue algorithm to semi-automatically drawn contours of the leg. US AT-CSA was obtained 22.5 mm proximal from the mid-aspect of the medial malleolus, a site that approximates the scan site of HR-pQCT. Analysis-reanalysis of 30 HR-pQCT and US images were performed to measure precision, quantified by: root mean square coefficients of variation (RMSCV), least significant change (LSC), and agreement via type 2,1 intraclass correlation for single observers (ICC2,1). Agreement between modalities for AT-CSA was determined using Pearson correlation and Bland-Altman analysis. Paired-sample t-test assessed the hypothesis that no significant difference exists between HR- and US-derived AT-CSA. **Results:** Among 78 women and 20 men with HR-pQCT scans (age: 63 \pm 10 years, HR AT-CSA: 0.54 \pm 0.11 cm²), only 45 received US scans (US AT-CSA: 0.55 \pm 0.13 cm²). US showed a lower RMSCV (1%) compared to HR-pQCT (3%). LSC for HR-pQCT and US were 0.04 cm² and 0.01 cm² respectively. ICC2,1 for US (0.998) was higher than HR-pQCT (0.959). Pearson correlation between HR and US AT-CSA was 0.916 (p<0.01). On average, there were no statistically significant differences between the imaging modalities on quantifying AT-CSA, t(43) = -0.91, (p = 0.37). **Conclusion:** HR-pQCT distal tibia scans can be used to derive precise and accurate measures of AT-CSA. Our results may warrant the future use of HR-pQCT for quantifying AT-CSA, but HR-pQCT should be further validated against magnetic resonance imaging, prior to doing so.

Xin as a novel regulator of mitochondrial morphology and bioenergetics in skeletal muscle

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Introduction: Xin is a striated muscle specific, cytoskeletal adaptor protein important for muscle regeneration. However, Xin's function within non-injured skeletal muscle remains largely unknown. We recently localized Xin to the mitochondria and peri-mitochondrial regions of skeletal muscle. Therefore, the objectives of this ongoing study are to investigate the effect of Xin deficiency on skeletal muscle mitochondrial structure and function, and to identify novel Xin binding partners within mitochondria. **Methods:** Wild-type (WT) and Xin knockout (Xin^{-/-}) mice were fed a standard or high-fat diet (HFD-60%kcal fat) for 8 weeks to determine the physiological effects of Xin deficiency in the presence of a metabolic stress. Glucose tolerance testing, histological analysis and electron microscopy were undertaken to identify deficits caused by the absence of Xin. **Results:** HFD-fed Xin^{-/-} mice displayed a ~1.5-fold (46.4%+ 7.5; p<0.05) increase in intermyofibrillar mitochondrial content and a ~2.8-fold (186.3%+ 9.6; p<0.05) increase in size, associated with mitochondrial swelling, streaming and loss of cristae. Xin^{-/-} mice had increased fasted blood glucose levels (Xin^{-/-}: 15.8+1.042, WT: 10.7+0.652; p<0.05) and reduced glucose tolerance (AUC Xin^{-/-}: 51.4+1.7, WT: 31.1+3.1; p<0.05). Despite significant deficiencies in mitochondrial structure and glucose handling, HFD-fed Xin^{-/-} mice did not gain greater body or fat mass relative to WT mice. **Conclusions:** Xin^{-/-} mice exhibited abnormal muscle mitochondrial morphology and dysregulated glucose handling, independent of changes in body weight. Current experiments involve protein-protein interaction assays to determine novel mitochondrial binding partners to improve our understanding of the role(s) of Xin in skeletal muscle mitochondria. Our studies unraveling the importance of Xin may uncover it as an unidentified source for the 20% of human myopathies with unknown etiology

A Peripherally-Restricted 5-HT_{2A} Serotonin Receptor Antagonist, Xylamidine, Enhances Brown Adipose Tissue (BAT) Function and Improves Glucose Homeostasis

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Recently, inhibiting peripheral serotonin (5-HT) synthesis was found to increase brown adipose tissue (BAT) thermogenesis and to prevent the development of diet-induced obesity, glucose intolerance, insulin resistance and hepatic lipid deposition (doi:10.1038/ncomms7794 and 10.1038/nm.3766). The current study investigated if 1) 5-HT directly inhibits BAT via a receptor-mediated mechanism, 2) which 5-HT receptor is present in BAT and 3) if 5-HT receptor antagonism improves metabolism. In murine brown adipocytes (BAs), 10 nM 5-HT reduced Ucp1 promoter activity and UCP1 protein levels via an extracellular receptor-mediated mechanism. RNA-Seq analysis determined 5-HT_{2A} as the highest expressed 5-HT receptor in human BAs and in silico analyses predicted that pharmacological inhibition of 5-HT_{2A} would induce a thermogenic program. In vitro, receptor antagonists and genetic ablation eliminated all 5-HT_{2A} activity. In vivo, a single injection of a peripherally-restricted 5-HT_{2A} antagonist (Xylamidine) prevented 5-HT-induced impairments in BAT-mediated energy expenditure and improved glucose tolerance. Chronic administration of Xylamidine to chow-

fed mice for 5-weeks improved BAT function, while treating high fat diet (HFD)-fed mice for 10 weeks with Xylamidine attenuated body mass gains, improved glucose tolerance and lowered liver triglyceride levels. Therefore, 5-HT_{2A} antagonism improves BAT function and represents a novel therapeutic strategy for treating obesity and metabolic syndrome.

Immediate as compared to delayed protein ingestion may improve adaptations to short-term variable intensity exercise training in recreationally active males

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High intensity cycle training can increase muscle power and aerobic capacity in as little as a week. Dietary protein ingestion enhances muscle protein synthesis during the immediate and overnight post-exercise periods to support muscle remodeling and recovery. The aim of this study was to determine if immediate (IMM) as compared to delayed (DEL) protein ingestion supports a greater acute recovery of exercise performance during successive days of variable intensity training (VIT) and/or supports greater chronic training adaptations. Recreationally active participants (IMM= 20±2yr, 74±6kg, 51.9±3.4ml·kg⁻¹·min⁻¹ VO₂peak; DEL= 21±3yr, 72±6kg, 52.3±3.7ml·kg⁻¹·min⁻¹ VO₂peak; means±SD) performed five consecutive days of a practical VIT (i.e. Loughborough Intermittent Shuttle Test) the evening prior to consuming a beverage providing carbohydrate and whey protein (IMM; 0.7g and 0.3g/kg, respectively) or carbohydrate alone (DEL; 1g/kg). After the ~10-h overnight recovery period, participants consumed the reciprocal beverage. Exercise performance was assessed before each VIT (i.e. exercise recovery) and 2 days after the final VIT (i.e. training adaptation) by vertical jump height (VJ), knee extensor isometric maximal voluntary contraction (MVC), anaerobic peak (PP) and mean power (MP) by Wingate, and aerobic capacity (VO₂peak) by multi-stage beep test. Null-hypothesis testing yielded no differences in performance recovery on sequential days of training. After one week of VIT, there were increases (main effects for time, P<0.05) for MVC (IMM= ~10%; DEL= ~9%) and distance traveled in the beep test (IMM= ~14%; DEL= ~10%) and a decrease in PP (IMM= ~8%; DEL= ~7%). Magnitude-based inferential statistics revealed possible small-to-moderate benefits for IMM compared to DEL for VJ (ES=0.31) and MP (ES=0.5) and likely large effects for VO₂peak (ES=0.82) and MVC (ES=1.16) after training. A practical VIT simulating team sport exercise increases exercise capacity in recreationally active males in 5 days. Consuming protein immediately after evening VIT may support greater short-term training adaptations than delayed ingestion.

In Duchenne muscular dystrophy, mitochondrial bioenergetic impairments are linked to dysfunctions in creatine-dependent energy exchange

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Introduction: Mitochondrial creatine kinase (mtCK) is crucial for shuttling high energy phosphate to the cytoplasm in the form of phosphocreatine, and for attenuating reactive oxygen species (ROS) production. Diseases characterized by altered mitochondrial bioenergetics are increasingly associated with mtCK defects. Our recent findings demonstrate that Duchenne muscular dystrophy (DMD) exhibits impaired mitochondrial energy production and elevated H₂O₂ (mH₂O₂) emission. The present investigation sought to determine if these deficits are associated with impaired mtCK. **Methods:** mH₂O₂ emission and respiration were assessed in permeabilized muscle fibre bundles from heart and diaphragm muscle of D2.mdx mice (52 weeks). Experiments

were performed in the presence of 20mM Cr (+Cr) to maximally activate mtCK and the more efficient PCr/Cr energy shuttle, and in the absence of creatine (-Cr), to force the mitochondria to rely solely on the less efficient ATP/ADP diffusion. **Results:**The capacity for mH₂O₂ emission in the absence of ADP (State II) was unchanged in DMD heart and lower in DMD diaphragm in both +Cr (96.0 pmol/sec/mg dry wt in DMD vs 498.0 pmol/sec/mg dry wt in WT, p<0.05) and -Cr (50.2 DMD vs 312.9 WT, p<0.05). 25 μ M ADP was added to evaluate the ability of ADP to lower mH₂O₂ emission (state III). ADP attenuation of mH₂O₂ was impaired in +Cr (HEART: 69% of state II mH₂O₂ in DMD vs 61% in WT, p=0.06; DIAPHRAGM: 75% vs 54%, p<0.05) but improved in -Cr in the heart (69% DMD vs 98% WT, p<0.05), and unchanged in diaphragm. State III respiration was also evaluated using 25 μ M ADP. In +Cr, respiration was impaired in the heart (48.0 pmol/sec/mg wet wt in DMD vs 75.6 pmol/sec/mg wet wt in WT, p<0.05) and diaphragm (43.4 vs 47.8, p<0.05). -Cr, respiration was improved in DMD heart (60.0 vs 29.8 pmol/sec/mg wet wt, p<0.05) and unchanged in diaphragm. **Conclusions:**These findings demonstrate an impairment in mtCK in DMD and that a partial compensatory mechanism exists to improve the alternative mtCK-independent energy transfer system. Future research will determine if mtCK impairments are mediated by ROS-inhibition.

Do Brief Daily Bouts of Stair Climbing Exercise Improve Cardiorespiratory Fitness?

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Introduction: Few Canadians meet the current recommendation of 150 minutes per week of moderate- to vigorous-intensity physical activity. There is renewed interest in the potential for practical, time efficient strategies to improve cardiorespiratory fitness (CRF). Low-volume sprint interval training (SIT) has been shown to elicit similar improvements in CRF compared to longer duration moderate-intensity exercise, despite a much smaller time commitment. There is also increasing recognition that breaking up periods of sedentary time throughout the day is associated with improvements in health. The concept of “exercise snacking” refers to separating a single session of exercise into multiple shorter sessions of exercise spread throughout the day (Francois et al. *Diabetologia*. 57:1437-1445, 2014). **Purpose:** We examined whether a training program involving brief bouts of stair climbing spread throughout the day would improve CRF as measured by peak oxygen uptake (VO₂peak). We hypothesized that 6 wk of training, 3 d/wk, would improve CRF compared to pre-training and a non-training control group. **Methods:** Healthy young sedentary adults were randomly assigned to either a training or control group (n=12 each). The intervention involved three sessions of ascending a three-flight stairwell (60 steps), with each session separated by 1-4 h. The intervention was modeled after a previous 6-wk training study in our lab that showed a ~1-MET improvement in CRF when three bouts of stairclimbing were performed once daily over a ~5-min period with 2-min of recovery, in addition to a brief warm-up and cool-down (Allison et al. *Med Sci Sports Exerc*. 49:298-307, 2017). **Results:** Reproducibility of CRF was assessed in 12 participants who performed two baseline VO₂peak tests separated by \geq 96 h. The intraclass correlation was 0.97 and the coefficient of variation was 2.0%. There was a significant time by group interaction such that VO₂peak was higher in the intervention group compared to control following the training period (P<0.05). However, the absolute increase in VO₂peak in the exercise group was modest (1834 \pm 328 to 1929 \pm 322 ml/min) and the ~5% change was not significantly different compared to pre-training. Peak power output increased after training in the intervention group by 8% (160 \pm 32 to 173 \pm 22), and was higher than the control group post-training (P<0.05). **Conclusion:** The stair climbing exercise snacking protocol was feasible and well-tolerated by previously sedentary individuals, but insufficient to increase CRF in the training group.

We speculate that the longer recovery time between bouts reduced the overall metabolic stress as compared to traditional SIT protocols. Future research should investigate different exercise snacks in terms of duration, frequency and timing and examine the effect on CRF and other health-related indices.

Genetic ablation of the serotonin reuptake transporter (SERT) leads to obesity but does not inhibit brown adipose tissue (BAT) function

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BACKGROUND: Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants that inhibit the serotonin reuptake transporter (SERT) and consequently increase serotonin receptor signalling. Furthermore, some SSRIs are associated with an increased incidence of obesity and metabolic disease. Recently, our research team found that Tph1 inhibition, the rate-limiting enzyme of peripheral serotonin (5-HT) synthesis, led to improved metabolism via increased energy expenditure from brown adipose tissue (BAT), thus implicating peripheral serotonin (5-HT) as a negative regulator of BAT and metabolic health. Previous studies have demonstrated that genetic elimination of SERT (SERT KO) in mice leads to late-onset obesity, insulin resistance, and hepatic lipid accumulation. Others have observed reduced thermogenic gene expression in BAT but did not examine the energy expenditure of these mice. Thus, we hypothesized that SERT KO mice are prone to obesity because of elevated extracellular 5-HT, reduced thermogenic gene expression and decreased BAT energy expenditure. **METHODS:** Using RNA-Seq and qPCR, SERT expression was measured in human and murine brown adipocytes. WT and SERT KO littermates were housed in either room temperature (RT; 22°C) or thermoneutrality (TN; 30°C). Body composition was monitored weekly starting at 6 weeks of age. To assess BAT function, mice received an acute injection of a β -adrenergic receptor agonist (CL-316,243). Subsequently, oxygen consumption and was measured. Furthermore, mice were placed in metabolic cages to assess feeding behaviour, activity levels, and energy expenditure. **RESULTS:** We confirmed SERT expression in both white and brown adipocytes and that, in murine brown adipocytes, SERT is functionally active and sensitive to SSRIs. Furthermore, SERT KO mice exhibited higher adiposity compared to wildtype (WT), even from a young age (6 weeks). Surprisingly, we found SERT KO mice had greater BAT activity and energy expenditure. However, SERT KO also had greater caloric intake and a net positive caloric balance. At TN, when BAT activity is minimal, SERT KO mice had even greater weight gain and adiposity. **CONCLUSION:** SERT KO mice had increased weight gain and adiposity despite higher BAT activity and energy expenditure. Furthermore, this increased weight gain and adiposity is seen at a young age and even in conditions where BAT is inactive, which may be explained by the increased caloric intake. Therefore, obesity in SERT KO mice appears to be independent of direct inhibitory effects of 5-HT on BAT but is likely due to increased appetite.

The Effect of Ketoisocaproic Acid on Insulin Stimulated Glucose Transport in Skeletal Muscle Cells is Modulated by Inflammatory Factors

Gagandeep Mann & Dr. Olasunkanmi Adegoke

Recent studies have linked elevated levels of branched-chain amino acids and their metabolites to the pathogenesis of insulin resistance and type 2 diabetes. We previously showed that α -ketoisocaproic acid (KIC), a metabolite of leucine, inhibits insulin-stimulated glucose uptake but only if branched-chain aminotransferase 2 (BCAT2), the enzyme that catalyzes the reversible conversion of leucine to KIC, is present. Inflammation is a

major component of insulin resistance, so the effect of KIC on insulin resistance may be modified by the inflammatory environment. **Objective:** Here, we examined whether or not KIC's role in insulin stimulated glucose transport in L6 myotubes would be modified by co-incubation with homocysteine, a pro-inflammatory factor. **Results:** KIC (200 μ M) suppressed insulin stimulated glucose uptake by 25%. There was a further 7% and 34% suppression of insulin stimulated glucose transport by co-incubation with [50] and [500] μ M of homocysteine, respectively. Interestingly, co-incubation of muscle cells with KIC and homocysteine increased AKTSer473 phosphorylation. KIC suppressed S6K1 and S6 phosphorylation by 43% and 64% respectively, this was worsened by co-incubation with homocysteine, especially at 15 μ M. To produce a more robust inflammatory response we incubated the myotubes with KIC in the presence of additional pro-inflammatory factors. KIC with 10ng/ml of tumor necrosis factor- α , 50 μ M of homocysteine, and 10ng/ml of interleukin-6, resulted in a further decrease of 40% in insulin-stimulated glucose uptake compared to incubation with KIC alone. To explore the mechanism of this effect we examined the phosphorylation status of factors implicated in insulin resistance. IKIC alone resulted in a 38% decrease in insulin-stimulated S6K1Thr389 phosphorylation. There was also an 80% decrease in insulin-stimulated IRS-1Ser612 phosphorylation, and a further 16% suppression of IRS-1Ser612 with co-incubation of KIC with pro-inflammatory factors. Strikingly, the effect of KIC on insulin-stimulated glucose uptake in the presence of inflammation was attenuated in the absence of BCAT2 (33% with BCAT2 present vs 19% in the absence of BCAT2), suggesting that in the presence of inflammation the effect of KIC is mediated by its conversion back to leucine. **Conclusions:** These results suggest that the effect of amino acids and their metabolites, especially KIC on insulin action in skeletal muscle are modulated by the inflammatory environment seen in obesity and insulin resistant states. However, the effect of KIC appears to be from its conversion to leucine, as opposed to KIC exerting the effect itself. Ultimately, interventions that modulate BCAA metabolism, especially within the context of inflammation, can have implications on the management of insulin resistance and its comorbidities.

Skeletal muscle adaptations to chronic exercise training in a murine model of myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant trinucleotide repeat disease with multisystem involvement, which is most prominently characterized by skeletal muscle weakness, wasting, myotonia, and insulin resistance. Recent studies investigating the therapeutic efficacy of exercise training in DM1 patients demonstrate that chronic physical activity can elicit some functional benefits. Enhancing our understanding of exercise-induced alterations in DM1 biology may assist in the discovery of effective lifestyle and/or pharmacological interventions to mitigate DM1. Thus, the purpose of this investigation was to examine exercise-induced skeletal muscle plasticity in a pre-clinical model of DM1. Three groups of mice (3-6 months old) were utilized: i) sedentary human skeletal actin-long repeat (HSA-LR) mice (SED-DM1), ii) HSA-LR mice with volitional access to a home cage running wheel for 6-8 weeks (EX-DM1), and iii) sedentary wild-type mice (WT). EX-DM1 animals ran 5.6 km/day, which is less than the running volume of healthy, WT mice previously reported in the literature. Utilizing the pen test latency to failure to examine muscular endurance and motor performance, the WT group exhibited a significantly higher score, as compared to SED-DM1 mice. Pen test performance was normalized in the EX-DM1 group. Chronic exercise rescued forelimb grip strength, which was 24% lower ($p < 0.05$) in the SED-DM1 animals versus the WT group. Direct, in situ stimulation of the plantar flexor muscle group revealed that daily, volitional physical activity normalized the aberrant force-

frequency relationship observed between WT and SED-DM1 groups. Furthermore, muscles from EX-DM1 animals demonstrated a tendency for enhanced fatigue resistance relative to WT and SED-DM1 mice. Chronic exercise also ameliorated several signature metrics indicative of DM1, including myotonia and aberrant chloride channel levels. Electromyography tracings revealed an exercise-induced decrease by 70-85 % ($p < 0.05$) in the duration of myotonia in the medial gastrocnemius and triceps muscles respectively. These data were complemented by the normalization of muscle chloride channel content and its restoration at the sarcolemma. Additionally, this coincided with the reduction in the prevalence of CUG repeat foci-positive myonuclei in EDL by 27% and 45% decrease in sequestered muscleblind-like 1 in the EX-DM1 animals, as compared to their SED-DM1 littermates. Collectively, our data suggest that chronic physical activity elicits numerous favorable adaptations in DM1 biology. Examination of the underlying molecular mechanisms driving this beneficial muscle remodeling is warranted

Investigating the effectiveness of acute and repeated ischemic preconditioning on the enhancement of 5-km cycling performance

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Introduction: Ischemic preconditioning (IPC) is a phenomenon where brief, non-lethal, ischemia protects organs from subsequent stress (e.g. ischemic-reperfusion injury) or toxins (e.g. doxorubicin). IPC elicits a biphasic pattern of protection: an early phase lasting 1-2 h and a second phase appearing 12-24 h later and lasting 48-72 h. IPC is a highly conserved physiological process which occurs in all tissue types and across species, with most research focusing on severe stresses. Emerging data suggests IPC can be used to improve athletic performance; however, the efficacy remains controversial. **Objective:** To investigate whether IPC enhances cyclist performance. **Hypothesis:** The acute phase of IPC will increase cyclist speed and average power output during a 5-km time trial which will be further enhanced when combined with the prolonged phase. **Methods:** 6 trained cyclists were exposed to a randomized, crossover study consisting of: 1) control (no IPC), 2) one IPC application (at 15 min), 3) two IPC applications (at 24 h and 15 min) and 4) three IPC applications (at 48 h, 24 h and 15 min) prior to a 5-km cycling time trial. IPC application consisted of 3 bouts of 5-min cycles of bilateral circulatory occlusion and reperfusion to the upper thighs. Using a two-tailed one-way repeated measures ANOVA, we assessed total time to completion and average power output between control and three intervention groups. **Results:** No difference in 5-km completion time was observed between control and intervention groups ($P = 0.30$). Further, we found no significant difference in average power output following acute and repeated IPC application ($P = 0.28$). **Conclusions:** Surprisingly, contrary to our hypothesis, IPC did not enhance 5-km time trial cycling performance in either phase. As such, future research should focus on characterizing the dose-response and timing of IPC through both phases to investigate the optimal efficacy for cycling performance.

Daily protein requirements are elevated in resistance-trained men after exercise

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Introduction: Dietary protein ingestion increases whole-body net balance (NB; algebraic difference between protein synthesis [PS] and breakdown [PB]) to support resistance exercise-induced anabolism. Maximizing NB while minimizing oxidation (OX) during post-exercise recovery would be the most efficient means to consume

dietary protein. The indicator amino acid oxidation (IAAO) technique provides a non-invasive estimate of the protein intake that maximizes PS and NB. Therefore, the purpose of this study was to utilize IAAO to determine protein requirements in trained men after exercise and to determine a protein intake that maximizes whole-body NB and minimizes OX. Methods: After 2-d dietary (1.2 g protein·kg⁻¹·d⁻¹) and exercise control, 7 resistance-trained men (24±3 y; 80±7 kg; 11±5% body fat; bench press=117±17 kg; leg press=338±50 kg; habitual protein intake=2.3±0.5 g·kg⁻¹·d⁻¹; mean±SD) performed a bout of whole-body resistance exercise. Following exercise, participants consumed 8 hourly isocaloric meals providing a variable amount of protein (0.2-3.0 g·kg⁻¹·d⁻¹) and sufficient energy and carbohydrate. Protein was provided as crystalline amino acids modeled after egg protein with the stable isotope L-[1-¹³C]phenylalanine as the indicator amino acid. Breath and urine samples were taken at isotopic steady-state to determine phenylalanine OX and flux (Q; estimate of PB), respectively. Breath ¹³CO₂ excretion (F¹³CO₂; reciprocal of whole-body PS) and NB were analyzed using mixed model biphasic linear regression with the breakpoint defined as the estimated average requirement (EAR) for dietary protein. NB was determined by the difference between PS (Q-OX) and PB. Total amino acid oxidation was estimated from the ratio of urinary urea to creatinine (U/Cr). Results: F¹³CO₂ demonstrated a robust biphasic response (R²=0.75; P<0.01) with an EAR of 2.0±0.38 g protein·kg⁻¹·d⁻¹ (mean±95%CI). Whole-body NB was maximized (R²=0.53; P<0.01) at a protein intake corresponding to 2.0±0.6 g·kg⁻¹·d⁻¹, with a linear increase (R²=0.70; P<0.01) in U/Cr across the range of protein intakes. Conclusions: The EAR of ~2.0 g·kg⁻¹·d⁻¹, which maximizes whole-body NB and minimizes total amino acid oxidation, for resistance-trained men after exercise is greater than the IAAO-derived estimate in non-exercising men (~0.93 g·kg⁻¹·d⁻¹) and is at the upper range of current general protein recommendations for athletes (1.2-2.0 g·kg⁻¹·d⁻¹). Based on previous research, the capacity to enhance whole-body NB appears to be greater than that required to maximize muscle PS in resistance-trained athletes accustomed to a high habitual protein intake.

The Effect of a Chemotherapy Drug Cocktail on Myotube Morphology and Protein Metabolism

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Affecting nearly all cancer patients today, cachexia is a condition characterized by weight loss and fatigue resulting from decreases in both muscle mass and function. Previous reports have suggested a causative link between chemotherapy treatment and cachexia. With the mechanisms related to this association unclear, this study investigates the effects of a common chemotherapy drug cocktail on myotube morphology and protein metabolism. Combining multiple chemotherapy drugs has been reported previously to bring about combined effects that result in lessening of side effects and increased effectiveness. We first examined the mechanisms of a cisplatin, 5-fluorouracil and leucovorin drug cocktail on differentiation of myoblasts into myotubes. Myoblasts exposed to the drug cocktail on day 0 of differentiation resulted in greater than 70% cell death by day 2. In an attempt to rescue this deficit, interventions to increase both cell number and cell density were employed, but the resulting cell death was unchanged. Following these findings, we turned to explore the effects of the chemotherapy drug cocktail on myotubes differentiated for 4 days. Compared to myotubes treated with vehicle, those treated with the drug cocktail showed dysmorphic shape and shrinkage followed by a 75% decrease in MHC (n=2, p=0.0051), 80% decrease in troponin (n=2, p<.001) and ~50% decrease in tropomyosin (n=2, p=0.1031) by day 6 of differentiation. To explore the reasons for the low abundance of myofibrillar proteins, we examined levels of p-AKT, p-S6 and p-S6K1 in myotubes treated with the drug cocktail. Compared to myotubes treated with vehicle, myotubes treated with the drug cocktail showed ~65% decrease in p-AKT (n=2, p=0.0029), ~80% decrease in p-S6 (n=2, p=0.0251) and ~70% decrease in p-S6K1 (n=1). Taken together, this data shows that a chemotherapy drug cocktail prevents myotube formation and decreases the abundance of myofibrillar proteins through pathways of reduced protein synthesis and

degradation. These findings suggest that the reported efficacy of a chemotherapy drug cocktail comes at a cost to the health of skeletal muscle.

Acute exercise-signaling in a type II spinal muscular atrophy mouse model

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Spinal muscular atrophy (SMA) predominantly affects motoneurons and muscle, resulting in skeletal muscle dysfunction and wasting. SMA is caused by mutations in the survival motor neuron 1 (SMN1) gene resulting in the deficiency of the crucial survival motor neuron protein (SMN). Prescribed exercise is an emerging therapy for SMA, however its molecular mechanisms are largely undefined. Stimulating molecules that regulate muscle phenotype is beneficial for neuromuscular diseases, such as Duchenne muscular dystrophy and amyotrophic lateral sclerosis. AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinase (p38), and peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) are examples of such proteins that are responsive to exercise, which serve to favorably maintain and remodel skeletal muscle biology. However, the expression and function of these phenotype-altering molecules in SMA are unclear. Thus, the purpose of this study was to determine the effect of a single bout of exercise on the activation of AMPK, p38, and PGC-1 α in the skeletal muscle of SMA mice. P17 SMA mice ran on a motorized treadmill (3 m/min) until the inability to continue exercise was empirically determined. Sedentary age-matched wild-type (WT) and SMA mice were also used as controls. Exercise elicited a ~2-fold increase ($p < 0.05$) in QUAD muscle AMPK and p38 activation status relative to sedentary SMA mice. PGC-1 α protein levels were unaffected by a single bout of physical activity in SMA mice. Interestingly, PGC-1 α nuclear translocation was evident 3 hours post exercise in the slower and more oxidative soleus muscle from SMA mice ($P < 0.05$). Collectively, these data indicate that acute exercise can induce AMPK and p38 signaling, as well as downstream targets such as PGC-1 α , in skeletal muscle in SMA mice.

Splenectomy-induced cardiac remodeling and diastolic dysfunction

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BACKGROUND: The spleen, either due to trauma, severe infection, or various causes of splenomegaly, may be surgically removed. In the US alone, ~22,000 splenectomies are performed each year. From a surgical point of view, splenectomy is associated with excellent outcomes in the near-term, however, epidemiological studies express that splenectomy increases the risk for the development of cardiovascular disease and associated comorbidities. This suggests that the spleen plays a contributing role in maintaining cardiovascular homeostasis. Recent investigations demonstrate the function of the spleen in the modulation of inflammation in cardiac disease states. **METHODS:** In splenectomized and sham male Wistar rats, cardiac function was assessed at 5 and 9 weeks following surgery using echocardiography and invasive hemodynamics. Furthermore, histology was used to assess left ventricular hypertrophy and fibrosis, and alternatively activated (M2-like) macrophage subsets in the heart using Picro-sirius Red and CD206 staining, respectively. **RESULTS:** By 5 weeks post-splenectomy, there was no evidence of any changes in cardiac function or remodeling. In contrast, by 9 weeks post-surgery diastolic dysfunction (elevated LVEDP, dP/dt min, and Tauw) was evident without systolic

decompensation (conserved EF, dP/dt max). This was associated with a significant increase in interstitial fibrosis and cardiomyocyte hypertrophy at 9 weeks post-surgery. Additionally, there was a decline in CD206 positive macrophage populations in the heart, which was concurrent with pathological remodeling. **CONCLUSIONS:** Splenectomy in healthy rodents caused a decline in resident cardiac CD206 positive macrophages, with development of diastolic dysfunction, interstitial fibrosis, and cardiomyocyte hypertrophy without evidence of systolic decompensation. CD206 positive macrophages are important in myocardial remodeling and the resolution of inflammation under cardiovascular stress, however, our current understanding of the physiological purpose of these resident macrophage populations at steady state is limited. The spleen appears to be required for maintaining CD206 positive macrophage populations in the heart, and the loss of this population is associated with fibrotic changes in the heart and the development of diastolic dysfunction, demonstrating an underappreciated role of the spleen in cardiac homeostasis.

Outcome of E99K ACTC Gene Expression in Zebrafish

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Cardiomyopathy is a disease of the heart muscle, of which hypertrophic (HCM) and dilated cardiomyopathy (DCM) are the two most common types. HCM is estimated to affect 1 in 200 individuals, making it the most commonly inherited heart disease. Mutations in sarcomere proteins has been accepted as the main cause of cardiomyopathy, and cardiac actin (ACTC), needed for contraction of the heart, is one of them. To date, there are 16 known mutations found in ACTC that have been linked to cardiomyopathy. Some of these mutations have been characterized using an in vitro system, but only a few have been studied in vivo. Expressing these variants in a suitable in vivo system will help compliment the results that have been obtained from in vitro experiments. One ACTC variant in particular, E99K, has been studied in both systems, and is known for its rare apical hypertrophy, and thickening of the left ventricle and interventricular septum. Due to their optical transparency and rapid cardiovascular development, my lab works with zebrafish as an in vivo model system for cardiomyopathy. With the use of the Tol2 transposon system, I have generated a transgenic line expressing the E99K ACTC variant in the heart with the help of a cardiac myosin light chain promoter. eGFP was also expressed under the control of the B actin promoter, which acts as a control to indicate fish positive for the E99K ACTC variant. Intercrossing E99K fish resulted in embryos having a reduced heart rate and about 50% mortality during a seven-day period. The embryos also show the consequences of cardiac defect like developmental delay, edema, and bent or shortened tails. Additionally, the hearts of adult E99K transgenic zebrafish were shown to be bigger in size when compared to wildtype zebrafish. The E99K line will serve as an example of how to characterize the other ACTC variant that I will be expressing in the zebrafish genome and in the future, could be used in therapeutic development to treat cardiomyopathy. This will also provide more understanding into how ACTC mutations cause cardiomyopathy and further increase the knowledge in the cardiovascular field.

Accuracy of wrist-worn activity monitors during different forms of physical activity

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Physical activity monitors have become increasingly popular in recent years and can be effective tools for increasing physical activity levels. The accuracy of these devices at measuring heart rate (HR) and energy expenditure (EE) remains an active area of investigation. However, most research groups simply examine accuracy during steady-state aerobic exercise. The objective of this study was to assess the accuracy of 2 consumer-based, wrist-worn activity monitors during 4 different forms of physical activity. The Fitbit Charge 2 and Garmin vivosmart HR+ were assessed for accuracy in measuring HR, EE (kcal), METs, and VO₂ in 20 healthy adults (Age: 27.5 ± 6.0 yr; BMI: 22.5 ± 2.3 kg/m²; 11 females) during a VO₂ max test, resistance exercise, activities of daily living, and intermittent high-intensity exercise. Both activity monitors were compared to research-grade devices (Cosmed K4b2/K5, Polar H7 and A300) to determine percent relative error (%RE). Fitbit and Garmin demonstrated reasonable accuracy at measuring HR, but overall displayed a negative bias. Fitbit estimated HR with a %RE of -4.7% ± 19.6, while Garmin estimated HR with a %RE of -3.3% ± 16.7 across all activities. EE estimates from Fitbit and Garmin differed significantly with Fitbit displaying a greater negative bias (Fitbit: -19.3% ± 28.9; Garmin: -1.6% ± 30.6, P<0.001) across all activities. Fitbit displayed significantly higher error when activities were performed on a cycle ergometer compared to Garmin (Fitbit: -40.5% ± 30.2; Garmin: -7.9% ± 27.6, P<0.001). METs and VO₂ estimates from Fitbit also displayed a negative bias (METs: -14.6% ± 30.7; VO₂: -14.5% ± 30.0), similar to EE estimates. Although the Fitbit Charge 2 and Garmin vivosmart HR+ demonstrated acceptable HR accuracy with a small negative bias, Fitbit significantly underestimated EE during all activities, especially during activities performed on the cycle ergometer.

ENU Based Mutagenesis for Discovering Suppressors of Nemaline Myopathy

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Nemaline myopathy (NM) is a childhood onset, genetically heterogeneous muscle disease that is one of the most common non-dystrophic myopathies. The disease presents with muscle weakness, impaired gross motor development, respiratory failure and the presence on muscle biopsy of abnormal protein accumulations within the muscle fibers known as nemaline bodies. Recessive mutations in NEBULIN are the most common cause of NM, accounting for >50% of all NM cases. Currently, there are no treatments or therapies available for the treatment of NM patients. Nebulin is one of the largest genes in the human genome, encoding for a 600-900 kDa protein. It has been cited to have multiple functions such as affecting sarcomeric structure and contractility, stabilizing and specifying thin filament length, and modulating both the calcium sensitivity of force response and actomyosin cross-bridging kinetics. The most common NM mutation is deletion of NEB exon 55 (NEBex55). There is variability in clinical presentations and severity of NEBex55 patients, ranging from typical to severe. The presence of such heterogeneity in a disease population sharing the same NEB genotype suggests the presence of genetic modifiers. Using a previously developed NEBex55 mouse model, which recapitulates

clinical features of nemaline myopathy, we have conducted a forward genetic screen through ENU mediated mutagenesis to identify potential suppressors of the nemaline myopathy phenotype seen in the mouse model. So far in our ENU mutagenesis screen, we have not been able to identify a mutation which can suppress the nemaline myopathy phenotype in mice, however we have been able to identify potential novel mutations which are able to induce a nemaline myopathy like phenotype in heterozygous mice. We are currently investigating these novel mutations to gain further insight into the molecular mechanisms of nebulin, which are still unclear. Through learning more about nebulin and its role(s), we aim to identify a potential pathway that can be a target for therapy.

Alpha-linolenic acid and linoleic acid differentially regulate the skeletal muscle secretome of obese Zucker rats

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Evidence shows that proteins secreted from skeletal muscle influence a broad range of metabolic signaling pathways. We previously reported that essential polyunsaturated fatty acids (PUFA) improved whole-body glucose homeostasis in obese Zucker rats; however, the mechanisms underlying these benefits remain enigmatic. While PUFA and obesity influence skeletal muscle function, their effects on the secretome are unknown. The aim of this work was to determine if improvements in whole-body glucose homeostasis in obese Zucker rats fed diets supplemented with either linoleic acid (LA) or alpha-linolenic acid (ALA) for 12-wks are related to changes in the skeletal muscle secretome. Secreted proteins were identified with a predictive bioinformatic analysis of microarray gene expression from red tibialis anterior (TA) skeletal muscle. Approximately 130 genes were differentially expressed (false discovery rate = 0.05) in obese rats compared to lean controls. The expression of 15 genes encoding secreted proteins was differentially regulated in obese controls, obese LA-supplemented and obese ALA-supplemented rats compared to lean controls. Five secreted proteins (Col3a1, Col15a1, Pdgfd, Lyz2, and Angptl4) were differentially regulated by LA and ALA. Most notably, ALA supplementation reduced Angptl4 gene expression compared to obese control and obese-LA supplemented rats, and reduced circulating ANGPTL4 serum concentrations. ALA also influenced Angptl4 gene expression and ANGPTL4 secretion from differentiated rat L6 myotubes. Altogether, the present data indicates that obesity has a greater global impact on skeletal muscle gene expression than either essential PUFA; however, LA and ALA may exert their metabolic benefits in part by regulating the skeletal muscle secretome.

Microtubule destabilizing chemotherapy may prevent the induction of apoptosis in glycolytic muscles utilizing in-vitro and in-vivo methodologies

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The voltage-dependent anion channel (VDAC) is believed to be a critical regulator of mitochondrial respiration and H₂O₂ emission by modulating ADP permeability into the mitochondria. Furthermore, under metabolically stressful conditions, VDAC participates in the formation of the mitochondrial permeability transition pore (mtPTP) which is responsible for the release of apoptotic factors to activate caspases. An emerging model proposes that microtubules can regulate mitochondrial function by altering tubulin-VDAC interaction, where tubulin physically blocks the VDAC channel limiting ADP diffusion. However, the degree to which tubulin

regulates VDAC-dependent mitochondrial bioenergetics in muscle is unknown. Utilizing *in-vitro* (n=8) and *in-vivo* (n=10) approaches in glycolytic extensor digitorum longus (EDL) and white gastrocnemius (WG) muscles respectively, we hypothesized that a microtubule destabilizer (vinblastine), previously shown to manipulate microtubule architecture, will reduce the tubulin-VDAC interaction and increase the ability of ADP to stimulate mitochondrial respiration and attenuate H₂O₂ emission. In addition, we aimed to determine how tubulin-VDAC interactions relate to mtPTP formation. Using histochemistry and proximity ligation assays following *in-vitro* EDL single fiber destabilization, microtubule organization appeared less dense and tubulin-VDAC interaction demonstrated no change (α -tubulin; control = 984 ± 54.52 , vinblastine; 942.28 ± 348.57 number of spots; β -II-tubulin; control = 469 ± 161.10 , vinblastine = 472 ± 127.31 number of spots). After *in-vitro* incubations and *in-vivo* injections with vinblastine, ADP-stimulated respiration, and attenuation of H₂O₂ emission was unaltered. This data shows that vinblastine did not alter tubulin-VDAC interaction and disrupt ADP diffusion through VDAC. Under a calcium challenge, the WG muscle retained more calcium before the mtPTP opened. This may suggest that vinblastine had off-target effects on mtPTP, preventing the initiation of apoptosis. Reductions in mitochondrial activated caspase 3 (saline= 3.04 ± 0.30 , vinblastine = 2.23 ± 0.22 , RFU/min/ μ g protein p=0.09), caspase 9 (saline = 2.51 ± 0.27 , vinblastine = 1.74 ± 0.22 RFU/min/ μ g protein, p=0.03) and cytosolic activated caspase 8 (saline = 14.89 ± 1.39 , vinblastine = 9.89 ± 1.17 RFU/min/ μ g protein, p=0.04) support our interpretations of this data with lower activity following *in-vivo* destabilizing injections.

Altering microtubule organization was associated with an increased calcium retention capacity and delay in mPTP opening despite no change in ADP's control of mitochondrial bioenergetics. However, these events were not dependent on tubulin-VDAC interactions suggesting vinblastine chemotherapy acts through an alternative mechanism to inhibit the induction of apoptosis. Such inhibition might be detrimental to the cell given apoptosis is a necessary program required for cellular regeneration following stress.

Different Endothelial Cell Capacity to Respond to Angiogenic Stimuli in Male and Female Mice

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Background: Capillary growth through the process of angiogenesis is essential to maintain a healthy status of adipose tissue during its expansion. Previous data from our lab indicate that female mice showed higher adipose angiogenesis than males in response to a high-fat diet. This leads us to hypothesize that endothelial cells from females have a higher angiogenic capacity compared to male cells. Therefore, we aimed to examine sex-related differences in the response of endothelial cells to an angiogenic stimulus using vascular endothelial growth factor A (VEGF), the main pro-angiogenic factor. **Methods:** Visceral adipose tissue was harvested from 10 weeks-old male and female C57BL/6J mice and was used for RNA analysis, endothelial sprouting assays and for endothelial cell isolation. **Results:** Gene expression analysis showed similar mRNA levels of the endothelial cell markers, *Pecam1* and *Vwf* and the angiogenic factor, *Vegfa* in whole adipose tissue from male and female mice. Although significantly higher expression of estrogen receptor 1 (*Esr1*) was detected in female endothelial cells, no difference was observed in mRNA levels of angiogenic-related genes, *Vegfa* and *Vegfr2* compared to male cells. Consistent with higher angiogenesis observed in female adipose tissue, VEGF-stimulated endothelial cell outgrowth from 3D collagen adipose explant cultures indicated that 85% of female explants developed new capillary sprouts whereas new sprouts were observed only in 50% of male explants. However, the sprouting area and cell density was greater in male compared to female explants, which suggested a higher number of proliferating endothelial cells. In a serum-stimulated cell proliferation assay, male endothelial cells growth was 1.5 fold greater than that of females cells. **Conclusion:** These data unexpectedly demonstrate that male endothelial cells have higher capacity to proliferate in response to VEGFA compared to

female cells. Thus, these findings indicate that the higher vascularization of adipose tissue previously observed in high-fat-fed female mice may not result from sex-differences in the proliferative capacity of endothelial cells. Further experiments will examine contributions of different signaling pathways such as estrogen receptor 1 and investigate potential sexual dimorphism in the angiogenic environment of male and female adipose tissues

The influence of exercise and weight loss on muscle remodeling during colon cancer induction in mice

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Diet and exercise have been recommended to reduce the risk of colorectal cancer (CRC) in obese individuals. However, studies have not been conducted to assess the influence of these weight loss interventions on muscle remodeling during CRC progression. Our aim was to investigate the effects of weight loss with or without exercise on markers of muscle remodeling in a mouse model of CRC. We hypothesized that exercise plus weight loss would increase muscle mass, muscle stem/progenitor cell content, and reduce markers of inflammation and apoptosis compared to weight loss alone. Mice consumed a high-fat diet (HFD) to induce obesity or a control (CON) diet. Subsequently, mice received injections of azoxymethane to induce CRC. Then, weight loss was induced in HFD mice by placing them on the CON diet and those mice either remained sedentary (HFD-SED) or began treadmill exercise (HFD-EX). After 40 weeks, mice were sacrificed and analyzed for markers of muscle remodeling. HFD-SED and HFD-EX had increased lean mass ($p < 0.05$ vs. CON), and HFD-EX had increased tibialis anterior (TA) weight ($p < 0.05$ vs. CON). The proportion of medium-sized fibers increased ($p < 0.05$) in HFD-EX, but there were no differences in overall cross-sectional area, myonuclei per fiber, or myonuclear domain. HFD-SED had increased fibrosis ($p < 0.05$ vs. HFD-EX) and adiposity ($p < 0.05$ vs. CON). The number of proliferating satellite cells (SCs) and fibroadipogenic progenitors (FAPs) was greater in HFD-EX ($p < 0.05$ vs. CON). There were no differences in quiescent or differentiating SCs. Additionally, p-NF κ B was reduced following exercise ($p < 0.05$). Findings suggest that a HFD, followed by a weight loss intervention with exercise reduces fibro/fatty muscle degeneration, and increases stem/progenitor cell content. These findings provide the rationale for exercise interventions for maintaining muscle quality during weight loss in CRC.

Endothelial-specific Foxo1 depletion prevents obesity-related disorders by increasing vascular metabolism and growth

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Impaired capillary growth contributes to the pathogenesis of diet-induced adipose tissue dysfunction. However, the molecular mechanisms orchestrating this process remain unknown. The transcription factor Forkhead Box O1 (FoxO1) is a major regulator of the angiogenic capacity of endothelial cells and compelling evidence indicate that endothelial FoxO1 dysregulation in adipose tissue coincides with obesity-related disturbances. This led us to hypothesize that FoxO1 signaling is implicated in the response of endothelial cells to high-fat diet and impairment of adipose angiogenesis associated with obesity impacting whole-body homeostasis. Methods: To generate endothelial cell-specific Foxo1 deficient mice (Foxo1^{IEC-D} mice), we crossbred Foxo1 floxed

(Foxo1f/f) and Pdgfb-creERT2 mice that express tamoxifen-activated Cre recombinase in endothelial cells. Cre-mediated recombination of Foxo1 was induced in adult mice with 5 consecutive intraperitoneal (i.p.) injections of 200 μ L of tamoxifen (15 mg/mL). Cre-;Foxo1f/f littermates were used as control mice in this study. Foxo1 mRNA levels were analyzed in endothelial cells isolated from white adipose tissue by qPCR analysis. To verify the effects of endothelial-Foxo1 on diet-induced obesity, male Foxo1iEC-D and Foxo1f/f mice (6-8 weeks-old, n=7/group) were fed a high-fat (HF) diet (58% fat) for 16 weeks. Results: Foxo1 mRNA was ~60% lower in microvascular endothelial cells from Foxo1iEC-D mice. When fed a high-fat diet, Foxo1iEC-D mice showed increased capillary expansion in multiple organs, but particularly in adipose depots. These mice also displayed a lean appearance, lower glycemia and increased glucose tolerance. Consistent with a healthier adipose tissue expansion, visceral adipose tissue from Foxo1iEC-D mice exhibited smaller adipocytes with improved insulin signaling and adipokine production compared to littermate controls but no change in the expression of browning markers Ucp1 and Prdm16. Mechanistically, Foxo1 depletion accelerated metabolic activity of microvascular endothelial cells by increasing expression of glycolytic markers and glycolysis, which may account for the alteration in whole-body glucose homeostasis. Conclusions: These findings delineate the pivotal role of FoxO1 in controlling endothelial metabolic and angiogenic adaptations in response to high-fat diet and further reveal a contribution of the endothelium to whole-body metabolism.

Characterization of cardiac actin gene switch in zebrafish using CRISPR-Cas9 technology

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Cardiomyopathy is a common cause of heart failure, a growing epidemic in Canada. Two prevalent forms of cardiomyopathy are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), which are characterized by changes to the heart muscle (myocardium). The development of HCM and DCM has been associated with mutations found in genes encoding muscle proteins, including cardiac actin (ACTC1). Anatomical and histological descriptions of cardiomyopathy are largely determined, but our understanding of the molecular basis of cardiomyopathy development remains incomplete. To address this gap in understanding, an in vivo model is required to study the underlying molecular mechanisms leading to the development of cardiomyopathy. Generating a model for cardiovascular disease will allow scientists to target the associated dysfunctions with precision therapies leading to improved treatments and reducing the global burden of heart failure. I will take the first step toward our overall goal of using zebrafish to determine connections between cardiac actin mutations and cardiomyopathy development by using CRISPR-Cas9 system to produce and characterize cardiac-specific actin genes in zebrafish (zfactc genes) knockout lines. Three zfactc genes have been identified through literature and phylogenetic analysis - zfactc1a, cardiofunk (zfacta1b), and zfactc1c. Research about the role of cardiac actin in cardiomyopathy development using zebrafish is translatable to humans because zebrafish and human ACTC proteins share an incredible 99% sequence identity. To validate zfactc genes as cardiac-specific, I have used CRISPR-Cas9 to produce and characterize zebrafish zfactc knockout lines. Phenotype characterization of individual CRISPR-injected embryos show four trajectories: (1) no clear dysfunction, (2) gradual decline towards death, (3) interval cardiac phenotypes, and (4) sudden death following a cardiac phenotype. Examples of cardiac phenotypes include blood accumulation, pericardial edema, and heart rate differences. Preliminary results suggest that there is a genetic switch in development of zebrafish between zfacta1b and zfactc1a at approximately 3 dpf. zfacta1b is believed to be more highly expressed post-fertilization and the pre-dominant isoform in the heart during the first 3 dpf and then after 3 dpf, zfactc1a becomes more highly expressed becoming the predominant isoform in the heart. This work will provide a zfactc

knockout lines to determine the roles of zfactc genes in cardiac development and for future human ACTC rescue experiments

Comparing the effects of lipophilic and non-lipophilic pharmaceuticals on markers of atrial fibrillation in heart failure

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Introduction: Heart failure (HF) is the inability of the heart to pump the amount of blood needed to meet the body's oxygen demands. Compared to healthy individuals, HF patients have a greater risk of developing an arrhythmia called atrial fibrillation (AF). However, the exact mechanism(s) by which HF causes AF are unknown. The purpose of this study was to gain insight into central mechanisms, which involves changes in the central nervous system that may cause the development of AF in HF. We designed an experiment to treat a model of HF (the spontaneously hypertensive rat; SHR) using drugs with varying lipophilicities, as lipophilic drugs can cross the blood-brain barrier and exert central effects. We hypothesized that a lipophilic drug will reduce AF duration and atrial fibrosis more than a non-lipophilic drug. This finding would suggest the involvement of a central component in the development of AF in HF. **Methods:** Aged SHRs were divided into two treatment groups. Rats were dosed daily with 15mg/kg of either candesartan (lipophilic; n=8) or entresto (non-lipophilic; n=8) for four months. Surface electrocardiography (ECG) data was assessed for the presence of AF, which was defined as a noisy ECG baseline lasting longer than five seconds. Subsequently, the atria were removed, fixed in formalin and stained with picosirius red to quantify fibrosis. **Results:** ECG analysis did not reveal any significant differences in the duration of AF episodes ($705.5 \pm 222.2s$ entresto; $728.1 \pm 207.4s$ candesartan) or the number of AF episodes/SHR ($4 \text{ episodes} \pm 1.2/\text{SHR}$ entresto; 3 ± 1.7 episodes/SHR candesartan) between the two treatment groups. The percentage of left atrial fibrosis also did not differ between the groups ($13.24 \pm 7.03\%$ entresto, $17.14 \pm 5.12\%$ candesartan). **Discussion:** In this study so far, we report no histological or ECG differences between the candesartan or entresto treated SHRs, suggesting there may not be a difference in their ability to reduce AF markers. However, histological analysis of the right atria remains to be done, and may clarify any distinctions between the two treatments. The right atrium contains the sinoatrial node, which directs electrical impulses in the atria. Fibrosis promotes AF by interfering with the conduction of these electrical impulses. Therefore, reductions in right atrial fibrosis may be a better indicator of decreased susceptibility to AF. Overall, the current data does not suggest the involvement of a central component in regulating AF in HF, as the lipophilic drug was not better at reducing AF parameters than the non-lipophilic

M-class α -cardiac actin variants linked with early-onset hypertrophic cardiomyopathy affect actomyosin regulation

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Cardiovascular disease (CVD) impacts millions of lives, accounting for 23.5% of deaths worldwide. A commonly inherited CVD is a disease of the heart muscle called cardiomyopathy. Hypertrophic cardiomyopathy (HCM) is defined by an increase in ventricular wall thickness resulting in the abnormal relaxation of the heart, impeding systole. HCM expression is variable and little is known about its molecular pathogenesis, apart from its link to mutations in genes encoding sarcomere proteins, including α -cardiac actin (ACTC). My study focuses on the F90 Δ and H88Y ACTC variants implicated in early-onset HCM. These are found within the myosin binding site on actin, and are classified as M-class variants. Research has shown that

direct actin and myosin (actomyosin) interactions remain largely unchanged with these ACTC variants. The prevailing hypothesis in the field, is the HCM is caused by an increase in cardiac contractility partly due to an increase in calcium (Ca²⁺) sensitivity. I hypothesize that F90Δ and H88Y ACTC variants interfere with tropomyosin (Tm) regulation, leading to improper inhibition of myosin binding resulting in increased Ca²⁺ sensitivity. Troponin (Tn) and Tm will be bound to ACTC variants forming regulated thin filaments (RTFs) and changes in regulation should affect their Ca²⁺ sensitivity in the presence of myosin. Myosin ATPase and in vitro motility assays will be performed to generate pCa curves, comparing variant RTF's Ca²⁺ sensitivity with wild type (WT) RTFs. Decreased Tm binding affinity should increase the Ca²⁺ sensitivity of F90Δ and H88Y RTFs resulting in a shift of their pCa curves relative to WT RTFs. These data will contribute to a better understanding of the molecular pathogenesis of HCM, bridging the gap between our physiological understanding of this disease and the molecular changes which occur due to it.

F-actin derived ADPr-actin trimer: A platform for determining atomic structure of F-ABP complexes

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The multifunctional actin protein forms highly dynamic or stable filaments that allow it to perform biological functions ranging from intracellular trafficking to muscle contraction. These actin dynamics are critical for actin's function and are regulated by interactions with several actin binding proteins (ABPs). Despite the significance of these interaction, the field lacks atomistic details of interaction of F-actin with ABPs. Therefore, a non-polymerisable F-actin-derived structure called ADPr-trimer was developed to act as a scaffold to study atomic interactions of F-actin with ABPs. The aim of this project is to generate heterocomplexes of ADPr-trimer with F-ABPs for structural work. A candidate-based approach was employed to determine interactions of ADPr-trimer with purified ABPs (myosin subfragment1, cofilin and gelsolin). Gelsolin was shown to interact with ADPr-trimer and the gelsolin-ADPr-trimer complex (GS:3mr) was purified for crystal trials; however, the complex failed to yield crystals. With advances in EM imaging, we are considering Single particle cryo-EM as a viable alternative for resolving structure of GS:3mr. In addition, a discovery-based approach involving pull-downs of ABPs from cell lysates using ADPr-trimer affinity columns coupled with mass spectrometry is being employed to identify ADPr-trimer binding proteins. Identified proteins can be produced in the lab and their interactions characterised for future structural work. This research will provide a foundation to further explore the field of F-actinomics and will impact our understanding of regulation of actin dynamics in cells – a process critical to several life processes

3 weeks of oral glutathione supplementation improves skeletal muscle insulin sensitivity in humans

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Background: Oxidative stress can lead to skeletal muscle insulin resistance and is therefore suggested to be involved in the pathogenesis of type 2 diabetes (T2D). The body's defense system against oxidative stress relies on antioxidants, of which glutathione (GSH) is a critical regulator of the redox environment of the cell. Patients with T2D and obese insulin resistant subjects have a decreased ratio of reduced GSH to oxidized GSH (GSSG) in plasma and skeletal muscle. Intravenous GSH infusion has been found to improve skeletal muscle glucose uptake by ~25% and the redox environment in patients with T2D, but the effect of oral GSH supplementation on skeletal muscle insulin sensitivity is not known. Therefore, we aimed to investigate if 3 weeks of oral GSH

supplementation would improve insulin sensitivity and if so, could this potential effect be linked to a more beneficial redox environment in the skeletal muscle cell. **Methods:** 10 patients with T2D and 10 healthy matched control subjects participated in the study (age: 60 ± 1 years, BMI: 32 ± 2 , VO_2 peak 28 ± 1 ml \cdot min $^{-1}\cdot$ kg $^{-1}$). In a double-blinded randomized manner, 11 subjects (6 T2D and 5 controls) received 1000mg GSH/day and 9 subjects (4 T2D and 5 controls) received 1000mg placebo/day for 3 weeks. Insulin sensitivity was measured with the hyperinsulinemic euglycemic clamp technique and muscle biopsies were obtained for determination of mitochondrial hydrogen peroxide (H₂O₂) emission by high-resolution fluorometry (Oxygraph-2k; Oroboros, Innsbruck, Austria). **Results:** Insulin sensitivity increased in the GSH supplementation group (6.0 ± 1.8 vs. 6.7 ± 1.9 mg \cdot min $^{-1}\cdot$ kg body weight $^{-1}$, $P<0.05$), but tended to decrease in the placebo group (5.7 ± 2 vs. 5.1 ± 1.2 mg \cdot min $^{-1}\cdot$ kg body weight $^{-1}$, $P<0.1$). Basal H₂O₂ emission and BMI did not change, but VO_2 peak decreased significantly as a main effect of time (GSH: 28 ± 2 vs. 27 ± 2 , placebo: 28 ± 1 vs. 26 ± 2 ml \cdot min $^{-1}\cdot$ kg $^{-1}$). **Discussion:** Our results suggest that only 3 weeks oral GSH supplementation can improve insulin sensitivity. Surprisingly, we observed a decrease in VO_2 peak as a main effect of time. The reason for this is unknown, but could indicate that GSH may have the potential to counteract a decrease in insulin sensitivity induced by a decrease in VO_2 peak. If so, the observed decrease in VO_2 peak may actually mask the true effect of GSH on insulin sensitivity. The mechanism behind the improved insulin sensitivity does not appear to be due to a lower basal H₂O₂ emission capacity, but may be due to an increased ratio of reduced GSH to GSSG.

A Study of the DCM-linked Cardiac Actin Variant T126I.

Zi Teng and John F. Dawson

Cardiomyopathy refers to diseases resulted from the malfunctioning of the heart muscles, with dilated cardiomyopathy (DCM) being the most prevalent subset. DCM is a disease of the sarcomere that results in weakened left ventricle muscle; this decreases the efficiency of the heart to pump blood. T126I is a α -cardiac actin (ACTC) protein variant found exclusively in a patient with DCM. The aim of this study is to determine how a change in residue 126 of ACTC from threonine to isoleucine can impact the rate of actin protein polymerization and muscle contraction at the level of regulated thin filaments (RTFs). I hypothesized that the T126I-ACTC variant will have an increased critical concentration and rate of polymerization as seen with other ACTC variants located on the same helix of the actin protein. Additionally, I hypothesized that T126I RTFs will exhibit a decrease in calcium sensitivity as observed in RTFs containing DCM-linked protein variants. Four assays were performed and the results opposed my original hypotheses. It was shown that T126I-ACTC has a decreased critical concentration and an increase in calcium sensitivity. This indicates that this variant is more stable in the F-actin form and requires less calcium to elicit the same results compared to bovine ACTC.

Excess Habitual Protein Consumption May Lead to an Overestimation of Protein Requirements by Stable Isotope Methodology in Resistance Trained Athletes

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Resistance-trained individuals generally consume protein above their daily requirements, despite these requirements being elevated compared to sedentary individuals. High habitual dietary protein consumption may upregulate enzymatic activity in metabolic pathways involved in utilization of amino acids for energy, increasing both amino acid oxidation and urinary nitrogen excretion. We aimed to determine the impact of a high habitual protein diet on estimates of protein requirements by the stable-isotope indicator amino acid oxidation (IAAO) technique. Healthy resistance-trained participants [$n=5$ males (25y, 73.0kg, 9.9% body fat,

2.69g protein/kg/d) n=3 females (23y, 62.7kg, 21.4% body fat, 2.03g protein/kg/d)] were randomized to consume a high (H) and low (L) protein diet while performing whole body resistance exercise every other day. During H, participants consumed 2.2g protein/kg/d for 2 days prior to determining whole body phenylalanine metabolism after exercise on day 3 (H1). During L, participants consumed 2.2g protein/kg/d for 2 days prior to consuming 1.2g protein/kg/d for 5 days. Phenylalanine metabolism was measured on days 3, 5 and 7 (L1, L2, and L3, respectively). Protein was provided in the form of crystalline amino acids containing the stable isotope L-[1-13C] phenylalanine to determine phenylalanine metabolism. Steady state $^{13}\text{CO}_2$ excretion ($F^{13}\text{CO}_2$), the reciprocal of whole body protein synthesis, was determined from breath $^{13}\text{CO}_2$ enrichment and CO_2 production and phenylalanine flux (Q) was determined from urinary [1-13C] phenylalanine enrichment. There was an overall significant difference for mean $F^{13}\text{CO}_2$ values over the four metabolic trials ($p=0.024$). Preliminary results from a subset of N=3 males revealed that Q was greater on L1 vs. L2 and L3 ($p<0.05$). Preliminary findings from this study suggest high habitual dietary protein intake may increase amino acid oxidation and attenuate whole body protein synthesis when decreasing to a moderate protein intake. Adaptation to a lower protein intake may require up to 5 days and could influence previous estimations of protein requirements by IAAO in athletes who habitually consume excess dietary amino acids.

The effect of age and exercise on muscle mitochondria and lysosomal biogenesis

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Mitochondrial content in skeletal muscle is determined by a balance between organelle synthesis (i.e biogenesis) and breakdown (i.e mitophagy). Mitophagy involves the breakdown of dysfunctional mitochondria within lysosomes, and it is known that endurance exercise increases signaling towards both biogenesis and mitophagy. However, the degree of signaling is reduced in aged muscle. While mitochondrial biogenesis in response to exercise has been investigated extensively in aging models, mitophagy and the role of the lysosomes in mediating this process has yet to be studied in terms of aging and exercise. It is also known that aged muscle exhibits lysosomal dysregulation, as evident by the buildup of non-degraded material within lysosomes, termed lipofuscin. Thus, we evaluated the lysosomal biogenesis in response to exercise in the gastrocnemius muscle of young (4-6 months) and aged (22-24 months) mice, utilizing a TFEB-promoter luciferase reporter transcription assay, and subsequently subjected the animals to rest or to incremental acute exercise to exhaustion. The tibialis anterior muscles were used for 1) measurement of mitochondrial respiration and ROS production and 2) nuclear and cytosolic fractionation to evaluate protein localization in response to exercise. Aged mice displayed reduced muscle mass and ran 40% less than their young counterparts in response to exercise, but displayed similar post-exercise lactate levels. Permeabilized muscle fibres of the aged mice showed 20-to-30% reductions in Complex I- and Complex II-stimulated basal and active respiration, as well as elevations in basal and active H_2O_2 emission rates supported by these complexes. Basally, aged muscle had lower mitochondrial protein (COX-I and COX-IV), TFEB-promoter activity and TFEB protein, but elevated nuclear TFEB. Aged skeletal muscle also displayed elevations in the lysosomal proteins LAMP1, LAMP2, and HSC-70. This lysosomal accumulation observed in aged muscle may be explained by either a build-up of non-degraded lysosomes, or the transcriptional activity resulting from elevated nuclear-TFEB protein. In response to exercise, the muscle from young and aged animals displayed significant 1.6-fold and 2.4-fold increases in TFEB-promoter activity, along with by 2.5-fold and 1.5-fold elevations in nuclear TFEB protein, respectively. Our data suggest that exercise is capable of increasing the signaling towards lysosomal biogenesis in both young and aged skeletal muscle, and this could help alleviate lysosomal dysfunction in aged muscle.

Role of a β -catenin-Smad7 complex at muscle creatinine kinase regulatory region.

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Recent reports indicate that Smad7 promotes skeletal muscle differentiation (myogenesis). We previously documented a non-canonical role of Smad7 during myogenesis as a nuclear coactivator, that is independent of its role in TGF- β signaling. To further characterize the myogenic function of Smad7, we carried out interactome analysis in myogenic cells. Smad7 affinity purification coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis identified β -catenin as a component of the Smad7 interactome. Biochemical analysis demonstrated that aa575-683 of β -catenin was sufficient for the Smad7 interaction. Using reporter gene analysis and chromatin immunoprecipitation, we observed that Smad7 and β -catenin are cooperatively recruited to the Ckm promoter to facilitate its transcriptional activation in myogenic cells. Depletion of endogenous Smad7 and β -catenin using siRNA technology reduced Ckm promoter activity indicating their mutual role in its activation during myogenesis. Deletion of the Smad7 interaction domain of β -catenin substantially reduced the effect of Smad7 on the Ckm promoter indicating that Smad7 recruitment requires β -catenin. Exogenous expression of β -catenin aa575-683 abolished the function of β -catenin on the Ckm promoter. Further interactome analysis revealed a novel interaction of a Mediator subunit (MED 13) with Smad7 and perturbation of β -catenin expression abrogated that interaction. Collectively, these studies highlight a novel role of a Smad7- β -catenin interaction at the Ckm gene suggesting a key role in the program of myogenic gene expression underlying skeletal muscle development and regeneration

Cancer-Specific Cell Death in Response to Palmitoylcarnitine is Caused by Elevated H₂O₂ Emission and Corresponding Glutathione Depletion

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Introduction: Many cancers rely on glycolysis rather than mitochondrial oxidative phosphorylation (Warburg effect). Recent evidence suggests that palmitoylcarnitine (PCarn) incubations increase mitochondrial oxidative phosphorylation in colorectal adenocarcinoma and causes apoptosis, yet non-cancer cells appear resistant. The mechanism of PCarn-induced cancer cell death is unclear but may be related to the cell's ability to buffer elevated H₂O₂ during oxidative phosphorylation. We hypothesize that cancers with low glutathione, the main intracellular antioxidant, will be susceptible to PCarn-induced mitochondrial H₂O₂, whereas cancers with higher glutathione will be resistant. **Methods:** We exposed HT29 colon adenocarcinoma, MCF7 breast adenocarcinoma and non-cancerous CCD841 epithelial cells to PCarn (50 μ M, 100 μ M) for 24hrs and 48hrs. We measured the effect of PCarn on clonogenic survival, caspase-3 activity, mitochondrial H₂O₂ emission and reduced (GSH) and oxidized (GSSG) glutathione. **Results:** At 24hrs, HT29 cells (glutathione: 41 μ mol/g protein) had a decrease in clonogenic survival in response to PCarn which corresponded with increased H₂O₂ emission as well as decreased GSH at 100 μ M PCarn ($p < 0.05$). This relationship was not observed in MCF7 cells (glutathione: 104 μ mol/g protein) as at 24hrs PCarn did not affect clonogenic survival, caspase activity, glutathione or H₂O₂ emission. Non-cancerous CCD841 cells (glutathione: 35 μ mol/g protein) increased H₂O₂ emission following 24hrs of PCarn incubation ($p < 0.05$) but did not demonstrate changes in clonogenic survival, caspase activity or glutathione. Co-incubation of HT29 cells with PCarn and the antioxidant N-acetylcysteine was able to partially rescue the clonogenic survival of HT29 cells by 48hrs, while co-incubation with the glutathione depleting agent L-buthionine-sulfoximine with PCarn was able to sensitize the previously resistant CCD841 cells to PCarn and further sensitize HT29 cells at both 24hrs and 48hrs ($p < 0.05$). **Discussion and**

Conclusion: This suggests that PCarn decreases clonogenic survival in low-glutathione HT29 colon adenocarcinoma but not in high-glutathione MCF7 breast adenocarcinoma or non-cancerous CCD841 colon epithelial cells. While both CCD841 and HT29 had elevated H₂O₂ following PCarn incubation, HT29 had the highest baseline H₂O₂ emission rate suggesting that any increase in H₂O₂ emission was able to cause irreparable damage to the cells through oxidizing glutathione and leading to apoptosis, while depleting glutathione was able to sensitize the previously resistant CCD841 cells and further sensitive HT29. This highlights the importance of glutathione in protecting cells from mitochondrial derived H₂O₂ following PCarn incubation.

Role of Syntaxin 1A in Cardiac Excitation-Contraction Coupling

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SNARE proteins and their role in exocytosis have been thoroughly studied since their discovery in the 1990s. Specifically, the SNARE protein, syntaxin1A (STX1A), is expressed in various tissues and cells, including neurons, pancreatic endocrine cells, and cardiac myocytes. However, the role of STX1A in the heart remains unclear. Our studies explore the potential role of STX1A in cardiac excitation-contraction coupling. Tamoxifen-inducible C57BL/6 mice expressing the α -myosin heavy chain promoter (α MHC) driving the mutated estrogen (Mer) construct fused with Cre recombinase gene (MerCreMer) were crossed with STX1A flox/flox mice. To generate cardiac-specific STX1A knockout mice (STX1A KO), α MHC-MerCreMer/STX1A flox/flox mice were injected with tamoxifen (40 mg/kg) over 4 days and were monitored for 6 weeks. Noninvasive echocardiography showed STX1A KO mice underwent transient systolic dysfunction which persisted for 3 weeks. Ejection fraction and fractional shortening decreased in STX1A KO mice immediately after the 4th tamoxifen injection (0 week post-injection) which returned to control levels by the 3rd week. Systolic left ventricular (LV) volume and diameter were higher at 0 week. No changes in these parameters were observed in wild-type mice injected with tamoxifen or Cre expressing mice. There were no significant alterations in heart rate, stroke volume, or LV diastolic volume or diameter. LV posterior wall thickness measurements and whole heart morphometry showed no signs of hypertrophy in STX1A KO hearts. Invasive hemodynamics data revealed no change in LV pressure during systole or diastole over this time frame. Rate of pressure generation (dP/dt systole) was also found to remain unchanged, whereas the rate of relaxation (dP/dt diastole) was reduced in STX1A KO hearts which persisted over the 3 week period. Echocardiography measurements also showed significant delay between the R-wave of the ECG and onset of contraction suggesting excitation-contraction uncoupling in STX1A KO mice. The observations of this study are indicative of STX1A's role in the maintenance of normal excitability and contraction of cardiac myocytes.

Examining the effects of low-dose lithium feeding on GSK3-beta, soleus muscle mass, TNF-alpha and IL-6 protein expression following tenotomy surgery

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Skeletal muscle allows for movements and posture and accounts for 40-50% of body mass. It is also a highly metabolic tissue and therefore reductions in skeletal muscle mass can impose significant burdens on quality of life and overall health. Glycogen synthase kinase 3-beta (GSK3-beta) is a negative regulator of skeletal muscle mass with its atrophic effects mediated through enhanced polyubiquitination and inhibition of protein synthesis, myoblast fusion, and muscle hypertrophy. Furthermore, GSK3-beta enhances muscle inflammation further

exacerbating these effects; and we have recently shown that pro-inflammatory cytokines TNF-alpha and IL-6, are significantly elevated in the tenotomized soleus (a model of unloaded muscle atrophy). Here, we fed mice a low dose of lithium, a natural inhibitor of GSK3-beta to determine whether GSK3-beta inhibition could attenuate the muscle atrophy and muscle inflammation observed in the tenotomized soleus. Specifically, 9 male C57BL/6J mice (3-5 months) were randomly assigned to control (n=4) or treatment (n=5) groups, with the treatment group receiving LiCl supplemented in their drinking water (10 mg/kg/day) for a 6-week period. At 4 weeks into the feeding schedule, all mice were subjected to the tenotomy surgical procedure, which mechanically unloads the soleus muscle. Following surgery, mice were maintained on lithium supplementation for 2-weeks to allow for adaptation to the tenotomy before being euthanized for tissue collection and analysis. As expected, we saw significant inhibition of GSK3-beta with the LiCl treatment resulting in a reduction in phosphorylation status ($p = 0.01$). Moreover, soleus muscle atrophy was observed in both the control (-39%) and lithium supplemented (-48%) mice following surgery; however, while a main effect of surgery ($p < 0.0001$) was detected, there was no significant interaction. Nevertheless, a main effect of diet was observed, which suggested larger overall muscle mass in the lithium supplemented mice ($p = 0.01$). With respect to inflammation, there was increase of ~20% in IL-6 and TNF-alpha expression ($p = 0.004$, each) in the soleus following tenotomy, however contrary to our initial hypothesis, low dose lithium had no effect on these levels compared to control. In conclusion, low dose lithium feeding inhibits GSK3-beta and increases overall soleus muscle mass, but does not attenuate the reduction in soleus muscle mass and increase in IL-6 and TNF-alpha in response to tenotomy surgery. Future studies will increase sample size and utilize histological and immunofluorescent fibre type staining to more precisely determine changes in muscle size and fibre type distribution.

Higher protein intakes enhance whole body protein metabolism and exercise performance in endurance-trained males

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Dietary amino acids are important for both the repair and rebuilding of body proteins and the replenishment of exercise-induced oxidative losses. Current athlete recommendations are based primarily on the protein intake required to maintain nitrogen (i.e. protein) balance rather than one that optimizes whole body protein metabolism and maintains exercise performance. **PURPOSE:** To determine how a range of protein intakes, including a new tracer-derived safe intake, altered protein metabolism and exercise performance during a period of controlled training. **METHODS:** Using a double blind randomized crossover design, 10 male endurance-trained runners (~32y; ~65 ml O₂/kg/min; ~62 km/wk) completed 3 trials, each consisting of 4 days of controlled training (20, 5, 10, 20 km days 1-4, respectively). Controlled diets provided 6-9 g/kg/d of carbohydrate and 0.80 g protein/kg/d from whole foods that was supplemented with 0.12 (LOW), 0.40 (MOD), and 1.03 (HIGH) g of crystalline amino acids/kg/d modelled after egg protein. Oral [15N] glycine was ingested on the 1st and 4th day to determine whole body protein synthesis (S), breakdown (B), and net balance (NB). Maximum voluntary isometric contraction (MVC), 5-km Time Trial (5kmTT) and peak force (Jump) were tested 2 days before and immediately after the controlled diet and training. **RESULTS:** S and B were not altered by training or protein intake. NB was negative in LOW and positive in HIGH with a dose-response between conditions (HIGH > MOD > LOW, $p < 0.05$). Inferential statistics revealed that for MVC, HIGH likely (probability 87%) had a moderate benefit over LOW (ES=0.57) and likely (probability 77%) a small benefit over MOD (ES=0.42). For the 5kmTT, HIGH likely (probability 79%) had a moderate benefit over LOW (ES=0.57) and a possible (probability 69%) small benefit over MOD (ES=0.26). No differences were found for

Jump performance. **CONCLUSION:** Endurance trained males consuming adequate carbohydrate maintained exercise performance and enhanced whole body protein metabolism when consuming >1.2g/kg/d of dietary protein. Our data suggest that training quality and post-exercise recovery would be optimized in endurance-trained runners who consume dietary protein towards at the higher end of current ACSM recommendations (i.e. 1.2-2g/kg).

Is perceived fatigue related to physical performance in adults with MS?

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Fatigue is one of the most debilitating symptoms of multiple sclerosis (MS), negatively affecting both activities of daily living and quality of life. The purpose of this study was to examine the relationship between the level of perceived fatigue and physical performance, and to determine the effect of a mental challenge on performance outcomes. Eighteen individuals were recruited (10 diagnosed with MS, and 8 as age-, and sex- matched controls). The MS group was categorized into either “high fatigue” or “low fatigue”, based on scores on a MS fatigue scale (MFIS). Maximal voluntary contraction (MVC) was measured in the handgrip muscles of the dominant arm. Participants performed a submaximal fatigue task which required to hold 50% of their MVC for as long as possible before and after completing a mentally challenging (Stroop) or unchallenging task. Visual analogy scales (VAS) were used to monitor perceived fatigue throughout the testing session. No significant difference was found in either the strength or endurance measures between any of the groups. Compared with the MS group, physical VAS scores were lower in controls at baseline, but higher after the submaximal fatigue tasks. In conclusion, adults with MS have similar handgrip strength and time to task failure on a submaximal task as controls. Despite approximately half of the participants with MS being categorized as “high fatigue”, the subjective sensation of fatigue did not impact their strength or endurance compared to those categorized as “low fatigue” or the controls. Further, the perception of physical fatigue in people with MS does not appear to be as sensitive to physically demanding tasks as in controls.

All-cause mortality risk greatest in metabolic syndrome combinations with elevated blood pressure

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Objective: 1) To identify the most prevalent metabolic syndrome (MetS) combinations and 2) To evaluate the associations between different MetS combinations with all-cause mortality. **Methods:** A merged sample of 81,665 adults from 7 U.S. population cohorts was used. MetS was defined by the revised National Cholesterol Education Program criteria. **Results:** Clustering of all five risk factors was the most prevalent MetS combination present in 13.1% of men and 16.8% of women with MetS. As expected, having more MetS factors was generally more strongly associated with all-cause mortality (4 or 5 MetS factors, HR range = 1.63 to 3.38, $p = 0.06$ to 0.0001) than those with 3 or less MetS factors (HR = 0.93 to 2.76, $p = 0.75$ to 0.0001). Further, all MetS combinations with elevated blood pressure as a component was significantly associated with mortality. In fact, elevated blood pressure alone, without any other risk factors was significantly associated with mortality in men (HR, 95% CI = 1.66, 1.48-1.86, $p < 0.0001$) and women (HR = 1.67, 1.42-1.97, $p < 0.0001$). In contrast, waist circumference, glucose or triglycerides alone was not associated with increased mortality risk, while HDL was only significant in men (HR = 1.39, 1.18-1.63, $p < 0.0001$). **Conclusion:** In a large-scale U.S. population, different combinations of MetS components vary substantially in their associations with all-cause mortality.

MetS combinations with elevated blood pressure are more strongly associated with greater mortality risk and identify the importance of blood pressure as a key component of MetS.

GCN5 regulates dystrophin expression in skeletal muscle through YY1 acetylation

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Protein lysine acetylation is a reversible post-translational modification (PTM) regulating protein structure and function. As a protein acetyltransferase, GCN5 plays a critical role in gene expression via genome wide modification of histones and DNA binding proteins, affecting chromatin accessibility. Here, we demonstrate that the muscle-specific knockout of Gcn5 (Gcn5skm^{-/-}) leads to reduced expression of the key cellular structural protein dystrophin resulting in a myopathic phenotype. Gcn5skm^{-/-} mice exhibit features similar to those seen in mice lacking dystrophin expression, aka mdx mice, a mouse model of muscular dystrophy. YY1 (Yin-Yang 1) plays a role in expression of long mRNA transcripts. Analysis of the dystrophin promoter led us to examine the role of GCN5 in the YY1-dependent regulation of dystrophin transcription. GCN5 was found to mediate the acetylation of two lysine residues in YY1, as identified with high-resolution tandem mass spectrometry. Finally, gain- and loss-of-function mutations in YY1 confirmed that Gcn5-dependent acetylation of YY1 is a necessary critical switch to positively regulate the expression of dystrophin in skeletal muscle. In summary, GCN5 positively regulates dystrophin expression via the acetylation of YY1 which may lead to new pharmaceutical developments with applications in certain forms of muscular dystrophy.

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