8th Annual

Muscle Health Awareness Day

May 26, 2017

Program and Abstracts

health | YORK UNIVERSITÉ UNIVERSITY

David A. Hood, PhD

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

Director,
Muscle Health Research
Centre

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

Tel: (416) 736-2100 ext. 66640
Fax: 416 736-5698
Email: dhood@yorku.ca
Web: yorku.ca/dhood/

Date: May 26, 2017

To: All Participants

From: David A. Hood, MHRC Director

Welcome to the 8th Annual Muscle Health Awareness Day

The Muscle Health Research Centre at York University welcomes you to **MHAD8**, our 8th 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.

This year we welcome 8 great speakers for **MHAD8**. The focus this year is on 1) bioengineering, stem cells and gene expression, 2) muscle physiology and rehabilitation, and 3) cardiac, vascular and muscle pathophysiology, presented by a combination of both junior and senior faculty members.

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 50 poster presentations, our highest number yet!

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.

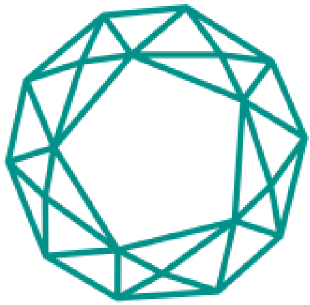
We thank all of our speakers, presenters, volunteers and sponsors for their participation, and for helping to continue to make this a successful event. Please enjoy **MHAD8**!

Sincerely,

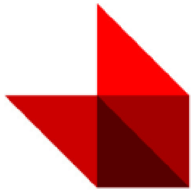
David A. Hood, PhD
Director, Muscle Health Research Centre

Conference Sponsors

Agilent Technologies



aurora SCIENTIFIC | Performance. Precision. Progress.

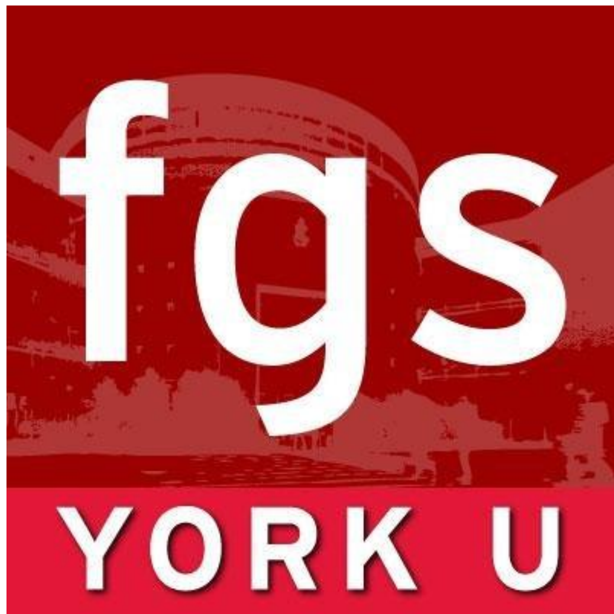


Applied Physiology, Nutrition, and Metabolism

CSEP | SCPE

THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Conference Sponsors

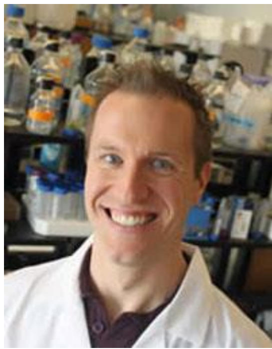


YORK U UNIVERSITÉ UNIVERSITY School of Kinesiology and Health Science

ThermoFisher SCIENTIFIC

8th Annual Muscle Health Awareness Day Speaker Profiles

	Dr. Kim Connelly, Keenan Research Centre for Biomedical Science, St. Michael's Hospital Dr. Connelly is a Scientist at the Keenan Research Centre for Biomedical Science, and is the Director of the Krembil Stem Cell Facility. His research examines the impact of stem cell based therapies in the treatment of cardiac and renal dysfunction which can arise as a result of diabetes.
	Dr. Michael De Lisio, University of Ottawa Dr. De Lisio is an Assistant Professor in the Faculty of Health at the University of Ottawa, and has established the Exercise and Stem Cell Physiology Lab (ESC Lab) there. His research is focused on defining the external cues (such as exercise) that regulate the stem cell microenvironment, and how changes to this niche affect stem cell function.
	Dr. Jefferson Frisbee, University of Western Ontario Dr. Frisbee is a Professor and Department Chair in the Department of Biomedical Physics at the Schulich School of Medicine and Dentistry at the University of Western Ontario. The aims of his research are to better understand the regulation of tissue blood flow, and the impact of cardiovascular disease on microvascular function and microvascular growth/regression.
	Dr. Vladimir Ljubicic, McMaster University Dr. Ljubicic is an Assistant Professor in the Department of Kinesiology at McMaster University, and is a Canada Research Chair (Tier II) in Neuromuscular Plasticity in Health and Disease. His research focuses on the study of proteins which play a role in remodeling the neuromuscular system and determining whether or not these proteins may be good therapeutic targets for neuromuscular disorders.

	Dr. Kei Masani, University of Toronto / UHN Dr. Masani is an Assistant Professor at the Institute of Biomaterials and Biomedical Engineering at the University of Toronto, and a Scientist at the Toronto Rehabilitation Institute in the UHN. He is interested in the study of human movement, with a focus on neuro-mechanical interactions and sensory-motor integration.
	Dr. Chetan Phadke, West Park Healthcare Centre / University of Toronto Dr. Phadke is an Assistant Professor in the Department of Physical Theraphy at the University of Toronto and a Research Scientist at the West Park Healthcare Centre. His current work focuses on spasticity research, a debilitating condition affecting people with neurological conditions, and understanding the factors that affect spasticity, how spasticity impacts function and balance and treatments to improve spasticity.
	Dr. Milica Radisic, University of Toronto / TGRI Dr. Radisic is a Professor and Canada Research Chair (Tier 2) in Functional Cardiovascular Tissue Engineering in the Department of Chemical Engineering and Applied Chemistry at the University of Toronto, as well as a Research Scientist at the Toronto General Research Institute. Her current research involves the use of stem cells and other biomaterials to engineer replacement cardiac tissue, which may then be implanted in humans
	**Dr. Jonathan Schertzer**, McMaster University Dr. Schertzer is an Assistant Professor in the Department of Biochemistry and Biomedical Sciences at McMaster University. His research interests lie in the study of the inflammatory basis of metabolic disease, and understanding how various factors propagate inflammation and alter metabolism. Further, his research also extends to the role of inflammation in myopathies.

← TO JANE ST

STEELES AVE WEST

TO KEELE ST →

← Driving from Keele Street, going West on Steeles Ave W

Driving from Highway 400, going East on Steeles Ave W to Murray Ross Pkwy

66 – Founders Road East Parking Lot
($1.75/ half hour, $10/max)

79 – Thomson Road Parking Lot
($1.75/ half hour, $15/max)

90 – Life Science Building South Lobby and Room 103
(registration / posters / talks)

24 – Shopsy's Sports Grill
(optional post-conference venue)
CASH BAR

80 – Arboretum Parking Garage
($2.50/half hour, $20/max)

York University Commons
(All TTC and GO Bus Stops)

GREEN LINES indicate quickest routes to parking lots/garages

84 - Parking Garage
($2.50/half hour, $20/max)
CREDIT CARD ACCEPTED

LEGEND
VISITOR PARKING
RESERVED PARKING
PARKING GARAGE
BLUE LIGHT EMERGENCY PHONE
PARKING INTERCOM
SECURITY
PICK-UP/DROP-OFF AREA
TTC STOPS
GLENDON-KEELE SHUTTLE & GO TRAIN SHUTTLE STOPS
VILLAGE SHUTTLE PICK-UP
ZUM BRAMPTON TRANSIT
TTC WHEEL-TRANS STOPS/ YRT MOBILITY PLUS
GO TRANSIT STOPS (EAST, WEST, NORTH)
VIVA TRANSIT STOPS
YRT STOPS
TRANSIT EXCLUSIVE ROADWAY
INFORMATION
PEDESTRIAN WALKWAYS
CONSTRUCTION ZONE
ACCESS CLOSED

YORK UNIVERSITY KEELE CAMPUS
4700 KEELE ST TORONTO ON M3J 1P3

8th Annual Muscle Health Awareness Day Program
Friday May 26, 2017
Life Science Building South Lobby and Room 103, York University

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

Session 1: Bioengineering, gene expression and stem cells (9:00-10:35)
Session Chair: Dr. Anthony Scime, *York University*

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

9:05-9:35 – Dr. Milica Radisic, *University of Toronto / TGRI*
Bioengineered heart tissue for drug discovery and therapy

9:35-10:05 – Dr. Vladimir Ljubicic, *McMaster University*
Characterizing protein arginine methyltransferase expression and function in skeletal muscle

10:05-10:35 – Dr. Michael De Lisio, *University of Ottawa*
Exercise-induced alterations in cellular systems: From bone marrow to skeletal muscle

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

Session 2: Muscle physiology and rehabilitation (11:30-12:30)
Session Chair: Dr. William Gage, *York University*

11:30-12:00 – Dr. Chetan Phadke, *West Park Healthcare Centre / University of Toronto*
The relationship between muscle spasticity and balance in persons post-stroke

12:00-12:30 –Dr. Kei Masani, *University of Toronto / UHN*
Novel method to reduce muscle fatigue using functional electrical stimulation

12:30 – 1:15 Catered Lunch (Life Science Building South Lobby);
1:15-2:00 Poster Presentations

Session 3: Cardiac, vascular and muscle pathophysiology (2:00-4:00)
Session Chair: Dr. Robert Tsushima, *York University*

2:00-2:30 – Dr. Kim Connelly, *Keenan Research Centre for Biomedical Science, St. Michael's Hospital*
Cardiac fibroblast-myocyte-matrix interactions: role of alpha 11 integrin signaling in mediating crosstalk

2:30-3:00 – Dr. Jefferson Frisbee, *University of Western Ontario*
Insights into microvascular dysfunction with metabolic syndrome: The importance of multi-scale validity

3:00-3:30 – Dr. Jonathan Schertzer, *McMaster University*
An inflammasome contributes to statin myopathy and sarcopenia

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

Poster Presentation Abstract List

Poster number	First Author (Surname)	Abstract Title	University Affiliation
1	**Afshar**	Development of a 96-well plate platform for human skeletal muscle tissue drug screening	*University of Toronto*
2	**Afshar Bakshooli**	Exercise induced autocrine myokines activate PI(3)K/AKT/P70S6K and PI(3)K/AKT/GSK3 signalling pathways in human skeletal muscle tissues	*University of Toronto*
3	**Anderson**	Grape pomace and resveratrol do not restore glucose tolerance in the ovariectomized rat	*University of Guelph*
4	**Baranowski**	Acute exercise rescues cortex BDNF signaling in high fat fed male mice	*Brock University*
5	**Beatty**	Branched-chain alpha ketoacid dehydrogenase regulation during muscle cell differentiation	*York University*
6	**Bellissimo**	Does sarcolipin ablation alter deflazacort treatment effects in *mdx* mice?	*University of Waterloo*
7	**Beyfuss**	Is p53 required for mitochondrial biogenesis with exercise?	*York University*
8	**Bhattacharya**	Decreased transcriptional corepressor p107 is associated with exercise-induced mitochondrial biogenesis in human skeletal muscle	*York University*
9	**Biafore**	The effects of a specific exercise program on shoulder function on breast cancer survivors, 6-9 months post-surgery	*York University*
10	**Bonello**	HSP 25 and 72 content in rat skeletal muscle following three types of contractions	*University of Toronto*
11	**Bott**	Proposal: Development of a mouse model for studying sex-specific effects of chronic low-grade inflammation on bone structure and bone mineral density	*Brock University*
12	**Bugyei-Twum**	Sirtuin 1 activation attenuates cardiac fibrosis by modifying Smad2/3 transactivation	*St. Michael's Hospital*
13	**Bush**	AICAR attenuates olanzapine-induced hyperglycaemia and insulin resistance in C57BL6 mice	*University of Guelph*
14	**Coleman**	The role of TDAG51 in skeletal muscle growth and regeneration	*McMaster University*
15	**Despond**	Development of a fluorescence-based assay to detect regulation changes in thin filaments containing HCM-linked actin variants	*University of Guelph*
16	**Dial**	The role of AMPK in the regulation and localization of the dystrophin-associated protein complex	*McMaster University*
17	**Dunford**	The effects of voluntary exercise and prazosin on capillary rarefaction and metabolism in streptozotocin-induced diabetic male rats	*York University*

18	**Gingrich**	The role of Xin in skeletal muscle mitochondrial function	*McMaster University*
19	**Hazlett**	Cardiorespiratory responses to CO_2 in the supine and upright postures in women throughout the menstrual cycle and men	*York University*
20	**Hirsh**	Characterizing the impact of in vivo lengthening contractions on mdx mice	*University of Toronto*
21	**Hughes**	Mitochondrial-targeted peptide SBT-20 improves mitochondrial bioenergetics in Duchenne muscular dystrophy in a mitochondrial creatine kinase dependent manner	*York University*
22	**Hunter**	Intra-mitochondrial location of the skeletal muscle perilipin 3 and 5 proteins at rest and following electrically stimulated contraction.	*Brock University*
23	**Karunendiran**	Investigation of Drosophila musculature using SHG microscopy	*University of Toronto*
24	**Kim**	Autophagy vs. mitochondrial adaptations in skeletal muscle during endurance training	*York University*
25	**Knuth**	Prior exercise training alters the response to cold stress	*University of Guelph*
26	**Lima-Rosa**	The glutathione recycling system is a regulator of contraction-induced GLUT4 translocation to the plasma membrane in C2C12 myotubes	*Sick Kids*
27	**Mann**	Potential effect of inflammation on ketoisocaproic acid induced insulin resistance	*York University*
28	**Manta**	Skeletal muscle adaptations to chronic exercise in a pre-clinical model of myotonic dystrophy type 1	*McMaster University*
29	**Miotto**	Palmitoyl-CoA mediated inhibition of mitochondrial ADP sensitivity plays a key role in promoting high-fat diet induced insulin resistance	*University of Guelph*
30	**Monaco**	Mitochondrial dysfunction in the skeletal muscle of young adults with Type 1 Diabetes: The root cause of diabetic myopathy?	*McMaster University*
31	**Nairn**	Surface EMG normalization techniques of the upper-thoracic erector spinae	*York University*
32	**Ng**	The exercise-induced activation of AMPK and p38 MAPK in a pre-clinical model of spinal muscular atrophy	*McMaster University*
33	**Ojehomon**	Developing zebrafish as an in vivo model of cardiomyopathy	*University of Guelph*
34	**Oliveira**	Perturbing mitochondrial protein import in vivo results in activation of the UPRmt	*York University*
35	**Peppler**	Subcutaneous inguinal white adipose tissue is responsive to exercise, but dispensable for the metabolic health benefit	*University of Guelph*
36	**Ramos**	Microtubule-targeting chemotherapy causes mitochondrial dysfunction in heart and skeletal muscle	*York University*

37	**Roubos**	Role of amino acid transporters during in vitro myogenesis	*University of Ottawa*
38	**Sandhu**	Phenotypic classification of cardiac actin knockouts in zebrafish using the CRISPR-Cas9 system	*University of Guelph*
39	**Sefton**	The effect of hypoxia on human 3D skeletal muscle tissues in vitro	*University of Toronto*
40	**Shaikh**	Regulatory changes in H88Y and F90Δ α-cardiac actin variants implicated in early-onset hypertrophic cardiomyopathy	*University of Guelph*
41	**Shen**	Characterizing the expression, localization and function of protein arginine methyltransferase in skeletal muscle cells	*McMaster University*
42	**Sidhu**	Can a short F-actin complex interact with important binding proteins?	*University of Guelph*
43	**Stouth**	Cellular localization and function of protein arginine methyltransferases during atrophy	*McMaster University*
44	**Tamura**	A single bout of heat stress increases Nrf2 and its target genes in mouse skeletal muscle	*York University*
45	**Townsend**	Rapid high-fat diet-induced glucose intolerance is not associated with hepatic Interleukin-6 resistance	*University of Guelph*
46	**Triolo**	Skeletal muscle disuse is associated with reductions in mitochondrial content and elevations in mitophagy in the rat hindlimb	*York University*
47	**Turnbull**	Cancer-specific cell death in response to palmitoylcarnitine is associated with increased mitochondrial hydrogen peroxide	*York University*
48	**vanLieshout**	Skeletal muscle expression and localization of protein arginine methyltransferases in response to exercise	*McMaster University*
49	**Wilkinson**	The effect of cardiolipin fatty acyl side chain composition on cytochrome c peroxidase activity	*Brock University*
50	**Xu**	Development of a novel tool to study bi-directional niche interactions in skeletal muscle	*University of Toronto*

MHAD8 Complete Abstract List

Development of a 96-well plate platform for human skeletal muscle tissue drug screening

Mohammad Afshar, Haben Abraha, Sadegh Davoudi, Mohsen Afshar, Penney M. Gilbert
Institute for Biomaterial and Biomedical Engineering, University of Toronto

Background: Three-dimensional (3D) models of human skeletal muscle (hSKM) tissue have been engineered successfully and shown to be responsive to pharmacological stimulation, but scalable processes to produce these tissues for drug screening are needed. Initial efforts to model hSKM were limited to two-dimensional (2D) muscle cell cultures. However, 2D cultures do not lend themselves to measurement of contraction force, and so are generally restricted to providing indirect measures of muscle strength. Recently, scientists have been able to engineer 3D hSKM tissues which contract upon electrical stimulation. Significantly, the effects of drugs on 3D hSKM contraction reflected their effects in vivo. One of the central benefits of these in vitro hSKM models is their utility in phenotypic drug screens. Candidate treatments for myopathic diseases (e.g. spinal muscle atrophy) can be assessed for positive muscle strength effects, while other drugs can be tested for off-target adverse effects on the skeletal muscle. However, current hSKM engineering methods are limited in scale – only two hSKM tissues can be made per mold, and measurement of contraction force is laborious. For an hSKM drug screening platform to be truly viable, it must be able to produce hSKMs in bulk and allow simple quantification of hSKM strength. **Hypothesis:** We expect that developing a custom 96-well plate capable of 3D hSKM bulk production, and simple strength quantification, will result in a platform suitable for drug screening applications. We specifically hypothesize that as seen in vivo, anabolic androgenic steroid (AAS) treatment and glucocorticoid treatment will cause hSKM strength to increase and decrease respectively. We further hypothesize that treatment with cerivastatin will cause tissue weakening, consistent with its reported myolytic effects in vivo. **Results:** Here we report the development of a human skeletal muscle microtissue (hMMT) platform, capable of producing generating hSKM tissues in bulk. The platform consists of a custom polydimethylsiloxane (PDMS) 96-well plate that holds engineered tissues through two micropost anchor points. We show that hMMTs contract in response to acetylcholine stimulation, and that this contraction induces a measurable deflection of the micropost tissue anchors. We have characterized the relationship between micropost deflection and contractile strength, and have automated post-deflection measurement using a custom Matlab program. Through confocal image analysis, we have demonstrated a time-dependent increase in muscle fiber size over 14 days of tissue culture. Finally, western blotting results suggest that increased length of tissue culture is correlated with increased levels of the maturation marker myosin heavy chain.

Exercise induced autocrine myokines activate PI(3)K/AKT/P70S6K and PI(3)K/AKT/GSK3 signalling pathways in human skeletal muscle tissues

Mohsen Afshar Bakooshli, Mohammad E. Afshar Bakooshli, Haben Abraha, Sadegh Davoudi, Penney M. Gilbert
Institute for Biomaterials and Biomedical Engineering, University of Toronto

Regular exercise has beneficial effects on physical well-being as well as to muscle health and is suggested as a non-pharmacological strategy to prevent muscle atrophy. Indeed, exercise induces a variety of changes in muscle properties such as muscle plasticity, metabolic regulation, contractile ability, fiber size, intracellular signaling, mitochondrial function, and transcriptional control. Moreover, symptoms of many muscle conditions, like Duchenne Muscular Dystrophy (DMD), do not manifest in the absence of muscle activity, and the therapeutic effects of many drugs often have synergistic effects when combined with exercise. However, the mechanisms that mediate the therapeutic effects of exercise remain obscure. In vitro models of human organs have improved our understanding of mechanisms that lead to disease as well as responses to clinical drugs. However, the majority of skeletal muscle culture models are two-dimensional platforms that are incapable of mimicking exercise and the few reports of three-dimensional (3D) models of skeletal muscle have not studied the effect of exercise in their systems. Here, we report the first 3D human skeletal muscle microtissue (hMMT)

system in a high throughput 96-well plate platform capable of mimicking muscle exercise in response to light stimuli. We show that 5 days of light induced exercise leads to higher expression of myosin heavy chain and myofiber hypertrophy compared to sedentary/control hMMTs that lead to higher force generation by exercised tissues. Our pathway analysis indicated the activation of the PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3B pathways as early as 3 hours post exercise leading to protein translation. In addition, our media supernatant studies indicated the modulation of the hMMT secretome post exercise. Moreover we show that the media from exercised hMMTs is sufficient to activate the PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3B pathways in sedentary tissues confirming the autocrine role of exercise induced myokines from myofibers. Finally we confirmed the role of IL-4 as an exercised mimetic myokine that is capable of activating protein translation pathways in sedentary hMMTs.

Grape pomace and resveratrol do not restore glucose tolerance in the ovariectomized rat
Eóin Anderson and David J. Dyck
Department of Human Health and Nutrional Sciences, University of Guelph, Guelph, ON

Objective: The loss of estrogen in females is associated with a worsening of insulin sensitivity. Natural compounds such as resveratrol (RESV) have potential insulin sensitizing effects. Grape pomace (GP) is a byproduct of winemaking, and contains RESV and other antioxidants. To our knowledge, the efficacy of RESV and GP in restoring glucose tolerance in ovariectomized (OVX) rats has not been explored. **Methods:** Phyto-estrogen free diet was administered ad libitum to sham control (SHAM) and OVX female Sprague-Dawley rats until the onset of glucose intolerance in OVX rats (12 wks). Glucose tolerance was assessed with an intraperitoneal glucose tolerance test (IPGTT). Following the induction of glucose intolerance, OVX animals were divided into treatment and control groups (n=10 each). The treatment groups received either i) a daily physiological dose of estrogen orally via a Nutella cream (5µL sesame oil/1g Nutella/kg body mass), ii) a daily dose of RESV mixed into the Nutella cream, or iii) GP supplemented at 1.5% of their diet. SHAM, OVX CON, and OVX GP each received a daily dose of untreated Nutella. Treatment continued for 6 wks followed by IPGTTs as well as an intraperitoneal insulin tolerance test. 2-3 d after the TTs, terminal surgeries were performed during which red (RG) and white gastrocnemius (WG) muscles and visceral adipose tissues were harvested before and after insulin injection. Liver was sampled post insulin injection. Tissues were analyzed for insulin signaling (total Akt, phospho-AktSer473). Visceral adipose was preserved and stained to determine adipocyte size. **Results:** OVX animals were significantly heavier than control animals at the onset of glucose intolerance and this did not change throughout the treatment period. None of the treatments restored normal glucose tolerance during the treatment. Insulin tolerance was not worsened in OVX rats, and was unaffected with treatment. No difference in liver pAkt content was found between SHAM and OVX animals. A significant increase was detected in pAkt in insulin simulated RG, WG, and visceral adipose compared to basal; however there was no effect of ovariectomy, and the interaction was not significant. There was no change in tAkt content. **Conclusion:** RESV and GP were not effective in restoring normal glucose tolerance in OVX rats.

Acute exercise rescues cortex BDNF signaling in high fat fed male mice
Bradley Baranowski[1], Willem T. Peppler[2], and Rebecca E.K. MacPherson[1]
[1]Department of Health Sciences, Brock University; [2]Human Health and Nutritional Sciences, University of Guelph

High fat diet induced obesity and insulin resistance have been directly implicated in the neuropathology of Alzheimer's disease. It is thought that high fat diets induce a reduction in neuronal plasticity through a reduction in the neurotrophin, brain-derived neurotrophic factor (BDNF). Previous work has demonstrated that acute exercise can reverse high fat diet induced alterations in cortices from obese mice, however the underlying mechanisms remain unknown. The purpose of this study was to determine the effects of a single bout of exercise on BDNF content and signaling in the prefrontal cortex from obese insulin resistant mice. Male C57BL/6 mice were fed a low (LFD, 10% kcals from lard) or a high fat diet (HFD, 60% kcals from lard) for 7 weeks. The HFD increased body mass and glucose intolerance ($p<0.05$). HFD mice underwent an acute bout of exercise (treadmill running: 15m/min, 5% incline, 120min) followed by a recovery period of 2 hours, after

which point the prefrontal cortex was collected. Prefrontal cortex from HFD mice demonstrated lower BDNF protein content and phosphorylation of the BDNF receptor (TrkB) and downstream effector cAMP response element-binding protein (CREB), as well as PGC-1alpha and ER-alpha protein content ($p<0.05$). Two hours following the acute exercise bout there was no change in BDNF protein content or mRNA expression. Further, there was no effect of exercise on the gene expression of PGC-1alpha or ER-alpha. However, TrkB and CREB phosphorylation, as well as PGC-1alpha and ER-alpha protein content were recovered ($p<0.05$). Our findings demonstrate for the first time that an acute bout of exercise can increase BDNF signaling in the prefrontal cortex of obese male mice.

Branched-chain alpha ketoacid dehydrogenase regulation during muscle cell differentiation

Brendan Beatty, Zameer Dhanani, Olasunkanmi A.J. Adegoke
Muscle Health Research Centre, York University

Skeletal muscles are critical to locomotion and whole-body substrate metabolism; their mass and function affect quality of life. Suboptimal muscle mass and function underlie or worsen chronic catabolic conditions like uncontrolled diabetes and several cancers. They are also predictive of treatment outcomes and survival. As a result, studies into mechanisms of muscle preservation and regeneration hold potential to improve patient outcomes. Muscle mass is a function of muscle cell number and protein balance. While muscle protein balance can be regulated by nutrition, especially by the branched-chain amino acids (BCAA: leucine, isoleucine and valine), the effect of nutrition on muscle cell formation and regeneration has received little attention. In addition, recent metabolomics studies have implicated metabolites of BCAAs in both the activation of anabolic signaling and in prognosis of chronic disease, but little is known about the effects of these metabolites and the pathways that generate them on muscle cell formation. The first irreversible and rate limiting reaction involved in BCAA catabolism is regulated by an enzyme complex, branched-chain alpha keto-acid dehydrogenase (BCKD). Working with rodent muscle cells, we showed that the abundance of BCKDE1-alpha subunit expression was upregulated during cell differentiation (up to 5X, $P<0.05$) without a corresponding change in its mRNA level. Myoblasts depleted of BCKDE1-alpha had impaired myotube formation and marked reduction in the expression of myofibrillar proteins. BCKD's activity is antagonistically regulated by a phosphatase (positively), and a kinase (negatively). BCKD kinase mRNA and protein expression did not change during differentiation, although BCKD phosphatase (PP2Cm) had a tendency to increase. Interestingly, depletion of BCKD kinase in myoblasts enhanced myotube formation, based on incipient myofibrillar protein accretion up to two days earlier than the controls; suggesting that increasing BCKD activity may positively regulate muscle differentiation. Collectively, these data highlight the significance of BCAA catabolism during cell differentiation and suggest that interventions that target BCKD abundance/activity hold promise for muscle regeneration.

Does sarcolipin ablation alter deflazacort treatment effects in *mdx* mice?

Catherine Bellissimo[1], Gabrielle Lugod[1], Eric Bombardier[1], Val A. Fajardo[2], A. Russell Tupling[1]
[1]Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada, [2]Department of Health Sciences, Brock University, St. Catharines, ON, Canada

Introduction: Duchenne Muscular Dystrophy (DMD) and the murine model *mdx* are degenerative diseases that are characterized by the absence of dystrophin causing membrane instability, calcium influx, progressive limb and respiratory muscle wasting, and death. Glucocorticoid steroids improve muscular strength and endurance, prolong ambulation and serve as the primary therapy for DMD. A purported effect of glucocorticoids is the stimulation of calcineurin, a calcium-dependent phosphatase that mediates adaptive skeletal muscle remodeling and increases utrophin, the functional homologue of dystrophin. Sarcolipin, a regulator of the sarco(endo) plasmic reticulum calcium ATPase (SERCA) has also been shown to increase in *mdx* mice and after glucocoirtcoids administration. Work from our lab has demonstrated that SLN is important in calcineurin stimulation and thus SLN may be involved in the positive effects of glucocorticoids in *mdx* mice. **Methods:** Four week old *mdx* and *mdx/Sln*$^{-/-}$ pups were injected with either the glucocorticoid deflazacort (DFZ) or a vehicle (VEH) alone for 7 days. Diaphragm (DIA) muscles were collected from all groups for biochemical and

histological analyses. **Results:** Preliminary data shows that DIA from *mdx* pups treated with deflazacort (*mdx* DFZ) have a significant improvement in centralized nuclei compared to *mdx* VEH and *mdx/Sln*$^{-/-}$ DFZ groups. There is a significant treatment effect of deflazacort in collagen infiltration in both *mdx* and *mdx/Sln*$^{-/-}$ groups compared to vehicle controls. Although there is no significant change in fibre type distribution or myofibre cross sectional area, there are significant improvements in fibre size variability. Type I, IIA and IIB fibres in *mdx* DFZ are more homogenous in fibre size than *mdx/Sln*$^{-/-}$ DFZ treated mice. **Conclusions:** Deflazacort treatment improves a number of classic histological markers of *mdx* mice. Centralized nuclei, a marker of regenerating fibres, is improved in *mdx* DFZ treated mice more so than *mdx/Sln*$^{-/-}$ DFZ mice. Further, deflazacort treatment improves collagen infiltration in both *mdx* and *mdx/Sln*$^{-/-}$ mice. Variability of fibre size, a hallmark of muscular dystrophy phenotype, is also improved with DFZ treatment more so in *mdx* mice than in *mdx/Sln*$^{-/-}$. At this point, it appears that SLN may be playing a role in the positive treatment effects of deflazacort treatments in *mdx* mice.

Is p53 required for mitochondrial biogenesis with exercise?

Kaitlyn Beyfuss, Avigail Erlich, David A. Hood
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Canada, M3J 1P3

The tumour suppressor protein p53, well-known for its ability to mediate oxidative stress, plays an essential role in maintaining cellular homeostasis. This is accomplished through enhanced antioxidant enzyme transcription, autophagy induction, and cellular senescence with low to moderate levels of stress, and through increased apoptosis with greater levels of stress. With certain stressors such as exercise, phosphorylation of p53 at specific residues allows for enhanced mitochondrial localization whereby it functions to maintain mitochondrial DNA integrity, thus leading to enhanced oxidative metabolism. Understanding the effects of chronic exercise on p53-regulated signaling is essential for a full comprehension on how exercise affects muscle health. To study this further, two mouse models, C57BL/6J whole body (WB) p53 wild-type (WT) and knockout (KO) mice, as well as MCK-driven p53 muscle-specific (MS) WT and KO mice were compared to assess the role of p53 in mediating muscle phenotype, mitochondrial function and endurance performance. All mice underwent a 6-week progressive treadmill training protocol with pre- and post- endurance stress tests. Phenotypic analyses determined a 25% increase in body mass in the MS WT mice relative to their WB counterparts, due to increased gastrocnemius/quadriceps mass (~55%) and epididymal fat mass (~20%). Furthermore, in the WB mice there was a trend for increased body mass in the KO mice relative to the WT mice, although both groups did display reductions in body mass with training. Tissue specific analysis was performed confirming that the increased body mass of the WB KO mice is owing to increased gastrocnemius (48%) and quadriceps (60%) mass. Though no significant effect of training was observed on muscle mass, there was a 46% reduction in the epididymal fat mass in the MS KO mice. The physiological adaptations to training were further observed indicating increased exercise capacity (60 and 75%) in the WB WT and KO mice respectively compared to their sedentary counterparts, with less of an adaptation observed in the WB KO mice (55%). The reason for this adaptation may be a result of increased mitochondrial biogenesis markers (PGC-1α, Tfam, and COV IV) and mitochondrial content measured in the trained WB WT, MS WT, and MS KO mice by 39, 25, and 29% respectively compared to sedentary counterparts. Interestingly, the trained WB KO mice had reduced mitochondrial content by 23% relative to the trained WB WT mice. Overall, the WB mice had a 30-46% greater mitochondrial content compared to the MS mice. Mitochondrial function was assessed yielding no significant differences, though trends in the trained MS mice were observed for increased respiration and reduced reactive oxygen species production in SS (state 3 and 4) and IMF (state 3) subfractions. p53-regulated signaling pathways were further examined with training and indicated a reduction in apoptosis (reduced Bax and increased Bcl-2) with no determined effect on autophagic signaling. Based on these preliminary results, p53 is not necessarily required for physiological adaptations and the signaling activation of mitochondrial biogenesis observed with training. Although both models are useful for comparison, the greater mitochondrial content and dissimilar respiration and ROS patterns observed in the whole body mice in response to training, suggests that the presence or absence of p53 systemically has a different impact on muscle plasticity.

Decreased transcriptional corepressor p107 is associated with exercise-induced mitochondrial biogenesis in human skeletal muscle

Debasmita Bhattacharya[1], Mia Ydfors[2], Meghan C. Hughes[1], Jessica Norrbom[2], Christopher G. R. Perry[1] and Anthony Scimè[1]
[1]Molecular, Cellular and Integrative Physiology and Muscle Health Research Centre, School of Kinesiology and Health Science, Faculty of Health, York University, Toronto, Canada [2]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Increased mitochondrial content and oxidative capacity are the hallmarks of exercise-induced skeletal muscle remodelling. For exercise-induced mitochondrial biogenesis, there is considerable evidence supporting the involvement of promoter activation by transcriptional coactivators. However, our knowledge regarding the role of transcriptional corepressors is lacking. Recently, the Rb family of transcriptional corepressor proteins, Rb and p107, have been shown to be involved in regulating the metabolic character of muscle and adipose tissue. Thus, we assessed their association during endurance exercise-induced mitochondrial adaptation in human skeletal muscle. We showed that p107, but not Rb, protein levels significantly decrease by 9 sessions of high-intensity interval training over a 3-week period. Further, we found that the reduction of p107 protein levels is associated with exercise-induced improved mitochondrial oxidative phosphorylation. Indeed, p107 had significant reciprocal correlations with the protein contents of each of the five mitochondrial electron transport chain complexes. These findings suggest that the transcriptional de-repression through p107 attenuation may be a novel mechanism apart from the classical model of transcriptional co-activation by which exercise stimulates mitochondrial biogenesis. It also hints that p107 might be more important than Rb in human skeletal muscle adaptation during exercise. Therefore, unravelling the role of transcriptional de-repression could be an important mechanism in understanding how exercise protects the body from metabolic diseases.

The effects of a specific exercise program on shoulder function on breast cancer survivors, 6-9 months post-surgery

Claire Biafore, Loriann Hynes, Angelo Belcastro, Michael Boni, Jamie Escallon
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, ON, Canada

Background: The most common complication following mastectomy surgery is a lack of shoulder function of the affected side, which has been linked to a decrease in quality of life. Presently, few reports are available that examine the benefits of a limited active, rehabilitative shoulder program to assist this population during their recovery period (6-9 months). **Purpose:** To evaluate the effect of implementing a specific shoulder exercise program (9 weeks) on quality of life, posture, lymphedema and range of motion for breast cancer survivors between 6-9 months post-surgery. **Study Design and Methods:** This study has been approved by York University's Human Participants Review Sub-Committee and Mount Sinai's Toronto Academic Health Sciences Network. The randomized, single blind study will consist of two groups: a) a control group (Con) (n=43) receiving one supervised exercise session at the start of the program; and b) and intervention group (Int) (n=43) receiving three supervised and guided exercise sessions throughout the nine-week program. The exercise program will target muscle flexibility, strength, endurance and include the following exercises: pectoralis stretch, lateral raises with internal rotation, bent over row and push-ups. The exercise program for the (Int) will be progressive (an increase of 5 repetitions per week) for each strengthening exercise and participant. All participants will be assessed at baseline, four weeks post-baseline and eight weeks post-baseline. Outcome measures will include: Quality of Life questionnaires (EORTC QLQ-C30, QLQ-BR23 and DASH), clinical postural evaluation, measurement of lymphedema (by a cloth measuring tape) and range of motion using a standard manual goniometer. The shoulder function for (Con) and (Int) groups will be compared at each time point by analysis of variance (2x3 between subjects and within subjects (repeated measures ANOVA) at an alpha level of 0.05. **Results (Anticipated):** Upon the completion of the study, we would expect to see an improvement in the quality of life of the participants as shoulder range of motion improves. Additionally, we expect to find an overall improvement of their postural assessment as well as a reduction in lymphedema and pain.

HSP 25 and 72 content in rat skeletal muscle following three types of contractions

John-Peter Bonello and Marius Locke
Faculty of Kinesiology and Physical Education, University of Toronto, ON Canada

Skeletal muscle responds to excessive exercise by the elevation of the cytoprotective, molecular chaperone proteins known as heat shock proteins (HSP). Although exercise is known to increase skeletal muscle HSP content, the exact aspect(s) of exercise responsible for elevating muscle HSP content remains unclear. To determine the role of different muscle contraction types, we examined HSP content in rat tibialis anterior (TA) muscle after being electrically stimulated to contract isometrically (IC) or while being actively shortened (SC) or lengthened (LC). Muscles from all groups were subjected to a total of 15 repetitions (3 sets of 5), while two additional lengthening groups underwent 5 and 10 repetitions. Contractile measures (maximal tetanic tension and peak torque) were recorded prior to, during and after stimulation. TA muscles were removed 24 hours post-stimulation and subsequently assessed for HSP content by Western Blotting. TAs subjected to SC or IC showed no significant decreases in maximal tetanic tension between pre- and post-stimulation. However, post-tetanic tension decreased by 8% ($p < 0.05$), 18% ($p < 0.05$) and 24% ($p < 0.001$) after 5, 10 or 15 LCs, respectively. Similarly, HSP 72 content showed no increase following SCs or ICs but was increased in all lengthening conditions, such that muscle HSP 72 content following 5, 10 or 15 LCs was significantly increased by 5 ($p < 0.05$), 8 ($p < 0.05$) and 18 fold ($p < 0.01$), respectively. HSP 25 content was unchanged between groups. These data suggest LCs may contribute towards the observed increased muscle HSP 72 content following exercise. In addition, as few as 5 maximal LCs may be capable of increasing muscle HSP 72 content.

Proposal: Development of a mouse model for studying sex-specific effects of chronic low-grade inflammation on bone structure and bone mineral density

Kirsten N. Bott, Sandra J. Peters, Wendy E. Ward
Department of Kinesiology and Centre for Bone and Muscle Health, Brock University, St. Catharines, ON, Canada

Chronic low-grade inflammation is associated with a wide variety of conditions and can affect the physiological function of many tissues, one of which is bone. The process of bone remodelling is mediated by osteoblasts and osteoclasts, responsible for bone formation and resorption, respectively. Circulating lipopolysaccharide (LPS) is known to stimulate low-grade inflammation eliciting an immune response and can directly modulate bone cell metabolism. Previous studies have used slow release LPS pellets in varying doses and found an upregulation of circulating proinflammatory markers (IL-1β, IL-6, and TNF-α) and compromised bone structure. However, this was either in female C57BL/6J mice or male Sprague Dawley rats and bone structure was not measured longitudinally in the same animal. The ability to track in vivo measurements of bone structure, volumetric bone mineral density (vBMD), and body composition longitudinally, in the same animal, reduces variation and allows a more precise measure of any changes in the lifespan being studied. The purpose of this study is to determine the dose-dependent and sex-specific response of trabecular bone structure and vBMD to chronic low-grade inflammation using slow-release LPS pellets. This project will be used to establish a model for chronic low-grade inflammation that compromises bone structure and vBMD. 8-week old male and female CD1 mice will receive a subcutaneous implant of placebo or LPS in the dorsal region of the neck at 8 weeks of age. This will provide a consistent, slow-release of LPS for 12 weeks (Innovative Research of America, Sarosota, FL). Mice will be randomized to one of four LPS dosages selected based on previous research: placebo (0 μg/day), low (0.133 μg/day), mid (1.33 μg/day), or high (13.3 μg/day). Proximal tibia, tibia midpoint, and lumbar vertebral body bone structure, vBMD, and body composition will be measured longitudinally at 8, 12, and 16 weeks of age using microcomputed tomography. It is hypothesized that the mid LPS treatment (1.33 μg/day) will compromise trabecular bone structure and lower vBMD in both the male and female CD1 mice with no further exacerbation with the high LPS dose (13.3 μg/day). This study will help to establish a model for low-grade inflammation, and allow researchers to investigate potential intervention strategies, such as exercise, in future studies.

Sirtuin 1 activation attenuates cardiac fibrosis by modifying Smad2/3 transactivation

Antoinette Bugyei-Twum, Christopher Ford, Robert Civitarese, Jessica Seegobin, Suzanne L. Advani, Jean Francois Desjardins, Golam Kabir, Yanling Zhang, Melissa Mitchell, Jennifer Switzer, Kerri Thai, Vanessa Shen, Armin Abadeh, Krishna K. Singh, Filio Billi, Andrew A. Advani, Richard E. Gilbert, Kim A. Connelly
Keenan Research Centre for Biomedical Science, St. Michael's Hospital; Institute of Medical Science, University of Toronto

Background: Transforming growth factor β1 (TGF-β1) is a prosclerotic cytokine involved in cardiac remodeling leading to heart failure (HF). Acetylation/de-acetylation of specific lysine residues in Smad2/3 has been shown to regulate TGF-β signaling by altering its transcriptional activity. Recently, the lysine de-acetylase sirtuin 1 (SIRT1) has been shown to have a cardioprotective effect; however SIRT1 expression and activity are paradoxically reduced in HF. **Methods and Results:** To test whether the pharmacological activation of SIRT1, using the compound SRT1720, would induce cardioprotection in a transverse aortic constriction (TAC) model, 8-weeks old male C57BL/6 mice were randomized to undergo sham surgery or transverse aortic constriction (TAC) to induce pressure overload. Post-surgery, animals were further randomized to receive SRT1720 or vehicle treatment. Echocardiography, pressure-volume loops, and histological analysis revealed an improvement in cardiac function and a reduction in deleterious LV remodeling, hypertrophy and fibrosis in TAC-operated animals treated with SRT1720. Genetic ablation and cell culture studies using a Smad-binding response element revealed SIRT1 to be a specific target of SRT1720, and identified Smad2/3 as a SIRT1 specific substrate. **Conclusion:** Overall, our data demonstrates that Smad2/3 is a specific SIRT1 target and suggests that pharmacological activation of SIRT1 may be a novel therapeutic strategy to prevent/reverse HF via modifying Smad activity.

AICAR attenuates olanzapine-induced hyperglycaemia and insulin resistance in C57BL6 mice

Natasha D. Bush, Laura N. Castellani, David C. Wright
Human Health & Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada

Olanzapine (OLZ), an antipsychotic drug used in the treatment of schizophrenia, induces undesirable side effects including hyperglycaemia, glucose intolerance, dyslipidaemia, weight gain and insulin resistance, often resulting in the development of Type 2 Diabetes (T2D). The acute effects of OLZ on blood glucose are likely caused by a reduction in insulin secretion, the development of insulin resistance in muscle and other tissues, and by increases in liver glucose production. 5'AMP-activated protein kinase (AMPK) is a cellular energy sensor activated during exercise that has been shown to increase insulin sensitivity and increase insulin-independent glucose uptake in muscle. Though having some off-target effects, 5-aminoimidazole-4-carboxamide riboside (AICAR) is a pharmacological agent that can activate AMPK in vivo. The purpose of this investigation was to determine if co-treatment with AICAR could prevent acute OLZ-induced increases in blood glucose in C57BL6 mice. AMPK and ACC phosphorylation was increased in liver, triceps muscle and epididymal adipose tissue 30 minutes following an injection of (AICAR) (250 mg/kg, IP injection) compared to vehicle-treated mice. OLZ (5 mg/kg) caused rapid (15 minutes) and sustained increases in blood glucose that was prevented with AICAR co-treatment. Treatment with OLZ attenuated the ability of insulin (0.75 U/kg, IP) to reduce blood glucose levels and this was partially prevented by AICAR. OLZ led to an exaggerated increase in blood glucose during a pyruvate tolerance test (2 g/kg, IP) and this was mitigated by AICAR. The results of the current study provide evidence that AICAR prevents OLZ-induced hyperglycemia by preventing the development of peripheral insulin resistance and by blunting increases in liver glucose output.

The role of TDAG51 in skeletal muscle growth and regeneration

Samantha K. Coleman[1], Andrew W. Cao[1], Irena A. Rebalka[1], Gabriel Gyulay[2], Richard C. Austin[2], Thomas J. Hawke[1]

[1]Pathology and Molecular Mechanisms, McMaster University, Hamilton ON, Canada [2]Department of Medicine, McMaster University, Hamilton, ON, Canada

TDAG51 (also known as pleckstrin homology like domain family A member 1– Phlda1) is a protein originally determined to be a key factor involved in T-cell receptor regulation. TDAG51 has since been found to be highly expressed in the liver, lungs and thymus. A 2007 study by Warren et al. which performed gene expression analysis in mouse models following skeletal muscle injury demonstrated an increased expression of Phlda1. With this exception, TDAG51 has not commonly been noted to be expressed in skeletal muscle, with most studies measuring protein levels under non-injured conditions. Interestingly, a 2004 paper by Toyoshima et al. found Phlda1 expression to be upregulated in response to exogenous IGF-1 administration in fibroblasts made to overexpress the IGF-1 receptor. Phlda1 was thus elucidated to be a downstream mediator of the effects of IGF-1. The exact role of Phlda1 in the IGF-1 pathway has never been further investigated, nor has this effect been observed in skeletal muscle cells or tissue. We hypothesize that TDAG51 plays an important role in muscle regeneration as a downstream player in the IGF-1 pathway. TDAG51 -/- mice generously provided by Dr. Rick Austin's lab at St. Joseph's hospital were harvested at 16 weeks of age. While these animals appeared to be phenotypically normal including normal muscle morphology, they did experience severe functional impairments. Following this, 16 week old mice WT mice were injured with cardiotoxin and harvested at 0, 6, 12, 24 and 48hrs post injury. qPCR and WB revealed an increased gene expression at 6 hours following injury and increased protein expression 12 hours following injury. This indicates that TDAG51 plays a significant role in the early stages of muscle regeneration. Finally, C2C12 cells were differentiated and treated with 10ng/µL recombinant IGF-1 protein. 2 hours following treatment the animals expression of Phlda1 was greatly increased compared to PBS treated cells.TDAG51 has been demonstrated as a novel and important component of the early regenerative process. Work is on-going to determine the localization of TDAG51 within the tissue. We hypothesize that TDAG51 is expressed in the satellite cells of the muscle, which would respond immediately to begin the regenerative process.

Development of a fluorescence-based assay to detect regulation changes in thin filaments containing HCM-linked actin variants

Evan Despond and John Dawson

Department of Molecular and Cellular Biology, Centre for Cardiovascular Investigations, University of Guelph, Guelph, ON

Hypertrophic cardiomyopathy (HCM) is a commonly inherited heart disease characterized by a thickening of the left ventricular wall and a decrease in blood volume pumped per heartbeat. Many genes and their encoded proteins have been implicated in this disease, including human cardiac actin (ACTC). One of the proposed mechanisms of disease progression is disrupted protein-protein interactions at the level of the regulated thin filament, composed of actin thin filaments, myosin thick filaments, and the regulatory proteins tropomyosin (Tm) and the troponin complex (Tn). In order to investigate whether HCM-linked ACTC variants disrupt thin filament regulation, an assay was developed with a fluorescent probe on Tm. This assay is intended to detect changes in the movement of Tm along the actin thin filament with different ACTC variants. Through titration of a myosin subfragment known as heavy meromyosin (HMM), binding curves can be produced to generate equilibrium binding constants. This information will be used to assess whether the presence of amino acid substitutions in ACTC disrupt thin filament regulation, which may ultimately lead to the development of HCM.

The role of AMPK in the regulation and localization of the dystrophin-associated protein complex

Athan G. Dial[1], Paul Rooprai[2], James S. Lally[3], Adam L. Bujak[3], Gregory R. Steinberg[3], Vladimir Ljubicic[1]
[1]Department of Kinesiology, [2]Department of Health Sciences, [3]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

The dystrophin-associated protein complex (DAPC) is composed of proteins that are highly expressed along the sarcolemma. The DAPC provides a mechanical link between the intracellular cytoskeleton and extracellular matrix. Additionally, increasing evidence shows the importance of the DAPC as a mechanosensor and signal transducer across the sarcolemma. The signalling molecule AMP-activated protein kinase (AMPK) is a powerful regulator of phenotypic plasticity. Chronic pharmacological AMPK stimulation induces the expression of DAPC components, whereas reduced AMPK activity results in DAPC dysfunction. However, a more comprehensive understanding of the influence of AMPK on the DAPC is lacking. Therefore, the purpose of this study was to investigate the role of AMPK in the expression of the DAPC in skeletal muscle. Extensor digitorum longus (EDL) and soleus (SOL) muscles representing fast glycolytic and slow oxidative tissues, respectively, were obtained from wild-type (WT) mice, as well as from mice deficient in both isoforms of the AMPK-β subunit in skeletal muscle (AMPK-MKO). Immunofluorescence imaging was performed to measure the expression and localization of DAPC components dystrophin, β-dystroglycan (β-DG), γ-sarcoglycan, (γ-SG), neuronal nitric oxide synthase (nNOS), and laminin. β-DG content was found to be ~2.2-fold higher ($p < 0.05$) in SOL compared to EDL muscles from WT animals. However, this fiber type difference was not seen in the muscles from AMPK-MKO mice. The expression of nNOS was significantly higher (+86%) in the SOL relative to the EDL muscles in AMPK-MKO animals. This fiber type specificity was not observed in WT mice. The levels of dystrophin, γ-SG, and laminin were similar between muscle types in both WT and AMPK-MKO mice, and all DAPC components were similar between genotypes. We next investigated potential regulators of DAPC transcription, including myogenin and myoD. Western blot analyses revealed that the expression of both myogenin and myoD were downregulated by 40-60% ($p < 0.05$) in EDL and SOL muscles from AMPK-MKO versus WT mice, which suggests that alternative factors may be contributing to the similar level of DAPC expression between genotypes. Indeed, computational analysis of the 3' untranslated regions of DAPC components revealed multiple regulatory elements that have been shown to affect mRNA stability in skeletal muscle, such as binding sites for RNA-binding proteins AUF1, HuR, and KSRP, as well as for microRNAs. Our results suggest that the absence of AMPK in skeletal muscle initiates a compensatory cascade of events at multiple levels of gene expression that coordinate to maintain basal levels of DAPC expression. Thus, although chronic AMPK activation induces DAPC content in skeletal muscle, it appears that AMPK is not essential for the maintenance of DAPC expression.

The effects of voluntary exercise and prazosin on capillary rarefaction and metabolism in streptozotocin-induced diabetic male rats

Emily C. Dunford, Erwan Leclair, Julian Aiken, Erin R. Mandel, Tara L. Haas, Olivier Birot, and Michael C. Riddell
School of Kinesiology and Health Science, Faculty of Health, Muscle Health Research Center and Physical Activity and Chronic Disease Unit, York University, 4700 Keele St., Toronto, ON, Canada, M3J 1P3

Type 1 diabetes mellitus (T1D) causes significant impairments within the skeletal muscle microvasculature. Both regular exercise and prazosin, an α1-adrenergic antagonist, have independently been shown to improve skeletal muscle capillarization and metabolism in healthy rats, through distinct angiogenic mechanisms. The aim of this study was to evaluate the independent and synergistic effects of voluntary exercise and prazosin treatment on capillary-to-fiber ratio (C:F) in streptozotocin (STZ)-induced diabetic rats. STZ (65mg/kg) was administered to male Sprague-Dawley rats (n=36) to induce diabetes, with healthy, non-diabetic, sedentary rats (n=10) used as controls. The STZ-treated rats were then divided into sedentary (SED) or exercising (EX, 24h access to running wheels) groups and then further subdivided into prazosin (Praz, 5 mg/kg) or water (H_2O) treatment groups, for a total of 5 treatment groups: non-diabetic- SED-H_2O (Control), STZ-SED-H_2O, STZ-EX-H_2O, STZ-SED-Praz, and STZ-EX-Praz. After 3 weeks, untreated T1D significantly reduced the C:F within both the tibialis anterior (TA) and soleus muscles in the STZ-SED-H_2O animals when compared to all other

groups ($P<0.05$). Both voluntary exercise and prazosin treatment, in STZ-treated rats, independently resulted in a normalization of C:F within the TA (1.86±0.12 & 2.04±0.03 vs 1.71±0.09, $P<0.05$) and the soleus (2.36±0.07 & 2.68±0.14 vs 2.13±0.12, $P<0.05$) muscles, when compared to STZ-SED-H_2O animals. The combined STZ-EX-Praz group resulted in the highest C:F within the TA (2.26±0.07, $P<0.05$). Voluntary exercise volume was correlated with fed blood glucose levels (r^2=0.7015, $P<0.01$), and when combined with prazosin, caused further enhanced glycemic control over the 3 week treatment period ($P<0.01$). In addition, exercise and prazosin reduced circulating nonesterified free fatty acids more than either stimulus alone ($P<0.05$). These results suggest that the distinct stimulation of angiogenesis, which occurs with both regular exercise and prazosin treatment, causes a cooperative improvement in the microvascular complications associated with T1D.

The role of Xin in skeletal muscle mitochondrial function

Molly Gingrich[1], Dhuha Al-Sajee[1], Meghan C. Hughes[2], Sofhia V. Ramos[2], Christopher G.R. Perry[2], Mark Tarnopolsky[3], Thomas J. Hawke[1]
[1]Department of Pathology & Molecular Medicine, McMaster University; [2]School of Kinesiology & Health Sciences, York University; [3]Department of Pediatrics, McMaster University

Introduction: Xin is a striated muscle protein upregulated in response to skeletal muscle damage. Xin deficiency leads to mild myopathic phenotype, impaired muscle regeneration and increased muscle fatigability. While research suggests an important role for Xin in skeletal muscle repair, Xin's function in undamaged skeletal muscle has yet to be determined. Analysis of Xin deficient skeletal muscle mitochondria has revealed both functional and structural impairments. Therefore, the objective of this ongoing study is to determine the role of Xin in skeletal muscle mitochondria. **Methods**: Research is using wild-type and Xin knockout (Xin-/-) mice with and without high-fat diet (HFD) to determine the physiological effects of Xin deficiency in the presence of a metabolic stressor. Mitochondrial respiration assays, electron microscopy, immunoprecipitation of mitochondrial cell fractions and immunofluorescent analysis are being undertaken to identify functional deficits caused by the absence of Xin as well as uncover novel binding partners for Xin. **Results**: Preliminary data suggests that Xin -/- mice display streaming of intermyofibrillar mitochondria and site-specific deficits in mitochondrial respiration, as well as a significant impairment in mitochondrial calcium retention. The introduction of a high fat diet is being used to create an additional metabolic stress, requiring further mitochondrial compensation. Recently, we have shown that Xin -/- mice experience increased fasted blood glucose compared to wild type mice. **Conclusions**: Completion of this research will help to elucidate Xin's role in resting skeletal muscle and aid in determining if Xin deficiency may be a primary contributor to the up to 20% of myopathies with currently unknown etiology.

Cardiorespiratory responses to CO_2 in the supine and upright postures in women throughout the menstrual cycle and men

Christopher Hazlett and Heather Edgell
School of Kinesiology and Health Science, York University, Toronto, Ontario

Objectives: Women experience orthostatic hypotension more often than men and experience faintness in the early follicular (EF; day 2-5) phase of the menstrual cycle more than in the mid-luteal (ML; day 18-24) phase. Interestingly, women experience lower sympathetic output during upright posture in EF compared to ML (Fu et al., 2009). The purpose of this research is to look at central chemoreflex (CCR) activation with hypercapnia while supine or upright to determine if CCR sensitivity is augmented during upright posture in EF contributing to lower sympathetic output while upright (hypocapnia occurs with tilt). **Methods:** Eight men (Height: 174.3±2.0cm, Weight: 73.9±4.1kg, Age: 22.0±1.1years) and eight women (Height: 159.4±1.9cm, Weight: 59.7±3.8kg, Age: 22.8±1.0years) breathed 2 minutes of 5% CO2 in supine or 70o upright tilt. Women were tested during EF and ML phases. Ventilation (Ve; Pneumotachometer), pulse-wave velocity (PWV; Pulse transducer) and blood pressure (BP; Finometer) were measured. **Results:** 1) In the supine position, men had a greater increase of PWV in response to CO2 compared to both EF and ML (Men: +0.7±0.2m/s, ML: -0.002±0.1m/s; EF: -0.035±0.374m/s; p=0.008 vs ML; p=0.067 vs EF). 2) Men had an augmented BP response to CO2 in the tilted position compared to supine which was not evident in EF or ML (Men: +0.03±0.73mmHg

to 4.76±0.97mmHg; ML: 1.26±0.67mmHg to 3.22±0.41mmHg; EF: +1.84±0.96mmHg to 0.17±1.71mmHg; p =0.066 vs ML; p=0.01 vs EF). 3) Women in both phases had greater Ve responses to CO_2 in tilt compared to supine (EF: +5.9±0.9L/min to +6.7±1.2L/min; ML: +6.2±1.4L/min to +8.9±1.9L/min; p=0.034).**Conclusion:** Men have either greater sympathetic activity or greater neurovascular conductance in the supine position during hypercapnia compared to women. In the upright posture men experience an augmented cardiovascular response to CO_2 which is not seen in women whereas in the upright posture women experience an augmented ventilatory response to CO_2.

Characterizing the impact of in vivo lengthening contractions on mdx mice

Danielle Hirsh, Marius Locke
Faculty of Kinesiology and Physical Education, University of Toronto

Introduction: Duchenne muscular dystrophy (DMD) is a severe, muscle wasting disease caused by mutations in the gene encoding the protein dystrophin. The absence of dystrophin in skeletal muscle causes progressive muscle wasting, the premature loss of ambulation and ultimately early mortality. Despite advances in genetic and pharmacological techniques and therapies, debate continues over the relative contributions of impaired cellular signalling and mechanical stability to the dystrophic pathology. The susceptibility of dystrophic animal models to lengthening contractions has been well established, but a definitive explanation remains elusive. This study employed the mdx mouse model of DMD to better characterize the pathological response of dystrophic muscle to lengthening contractions. Methods: The tibialis anterior muscle (TA) of male mdx and C57 mice underwent 15 electrically stimulated, lengthening contractions using a computer-controlled servomotor. After 0, 3, or 24 hours, TAs were harvested and processed for further analyses including quantification of heat shock protein (HSP) content and histological imaging. Comparisons were made between the lengthened and non-lengthened, contralateral limb of each animal, between mdx and C57 groups, and between time points. Results: Mdx mice exhibited both heavier and significantly weaker TA muscles during and after the contraction protocol ($p<0.05$), indicating deficits in both absolute and specific force. The severe impact of the protocol on dystrophic muscle was supported by haematoxylin and eosin staining of cross sections showing centralized nuclei at rest and diffuse immune infiltration, fibre rounding, and ghost fibres after the contraction protocol. Electron microscopy demonstrated typical, though minimal, z-line streaming in the lengthened C57 muscles, while the mdx muscles displayed minimal z-line streaming, but widespread myofibril disorganization and the presence of centralized nuclei. In contrast to previous lab observations in rats, neither group demonstrated a heat shock response (HSP72 or HSP25) to the exercise protocol. Conclusion: Mdx mice were weaker and more susceptible to lengthening contraction-induced damage, which did not appear to be the result of mechanical failure as evidenced by electron microscopy. This will be further explored through the examination of NF-κB and protein carbonylation in the healthy and dystrophic muscles.

Mitochondrial-targeted peptide SBT-20 improves mitochondrial bioenergetics in Duchenne muscular dystrophy in a mitochondrial creatine kinase dependent manner

Meghan C. Hughes[1,3], Sofhia V. Ramos[1,3], Nazari Polidovitch[2,3], Peter H. Backx[2,3] and Christopher G. R. Perry[1,3]
[1]School of Kinesiology and Health Science, [2]Department of Biology, [3]Muscle Health Research Center, York University, Toronto, ON, Canada

Rationale: Duchenne Muscular Dystrophy is a progressive muscle wasting disease in males resulting from mutations in the X-linked gene dystrophin. The loss of dystrophin in the dystroglycan complex causes severe muscle pathology including cardiomyopathy which can lead to early death. While the cardiac dysfunction in mouse models of this disease has been well characterized, the precise signaling events contributing to this myopathy have yet to be determined. Given that this disease is associated with markers of oxidative stress, and that other models of cardiac dysfunction are associated with impaired mitochondrial oxidative phosphorylation (respiration) and elevated oxidant emission (H_2O_2), we hypothesized that similar dysfunctions would exist in D2.B10-DMDmdx/2J mice (DMD) that could be corrected with the mitochondrial targeted peptide SBT-20. **Methods:** Left ventricular mitochondrial H_2O_2 emission (spectrofluorometry; Amplex UltraRed fluorophore)

and respiration (high-resolution respirometry; Oroboros Oxygraph) were assessed in permeabilized muscle fibre bundles (PmFB) at 4 wk of age in DMD and DBA.2J wildtype (CONT), and again at 13-14 wk in DMD following daily subcutaneous injections of SBT-20 (5mg/kg) or saline (0.9%) from 4 d for 12 weeks. H2O2 emission was measured in the presence of physiological ADP (25µM) given ADP is a critical suppressor of H2O2 emission during respiration that can be influenced by the phosphate-shuttling activity of mitochondrial creatine kinase (mtCK). The contribution of mtCK was assessed by saturating [creatine] (Cr) in vitro. **Results and Interpretation:** At 4 wk, ADP-suppression (25µM) of Complex I-supported H2O2 (5mM Pyruvate/2mM Malate) was impaired in DMD in the presence of Cr (+Cr; 36+/-4% of emission at 0µM ADP in DMD vs 12+/-1% of emission at 0µM ADP in CONT when expressed per O2 consumed; p<0.01). This was also observed in the absence of Cr (-Cr; 36+/-4 vs 17+/-2% of 0µM ADP/O2 consumed, p<0.01) suggesting H2O2 emission is elevated in DMD independent of mtCK-dependent phosphate shuttling. At this same [ADP], complex I-supported respiration was lower in DMD vs CONT in both +Cr: (30.8+/-5.0 vs 54.2+/-10.2 pmol•s-1•mg wet wt-1, p<0.05) and -Cr (35.0+/-1.2 vs 52.0+/-5.4 pmol•s-1•mg wet wt-1, p<0.01). After ~12 wk injections, SBT-20-treated DMD demonstrated greater ADP-suppression of Complex I-supported H2O2 vs CONT in +Cr (12+/-1% vs 15+/-0 % of emission at 0µM ADP/O2 consumed, p<0.01). However, there were no differences in -Cr (40+/-11 vs 42+/-13 % of 0µM ADP/O2 consumed), suggesting SBT-20 specifically improved mtCK-dependent ADP suppression of H2O2 emission. SBT-20 did not change respiration in DMD vs CONT in +Cr (111.4 +/- 12.0 vs 82.1 +/- 11.2 pmol•s-1•mg wet wt-1, p=0.10) or –Cr condition (59.6 +/- 7.4 vs 65.3 +/- 6.8 pmol•s-1•mg wet wt-1). Collectively, these findings demonstrate that SBT-20 is an effective therapeutic approach for improving the ability of mtCK to mediate ADP-suppression of H2O2 in DMD heart.

Intra-mitochondrial location of the skeletal muscle perilipin 3 and 5 proteins at rest and following electrically stimulated contraction

Hunter, M.R., Stuart, J.A., LeBlanc, P.J., Peters, S.J.
Department of Kinesiology, Centre for Bone and Muscle Health, Brock University, Ontario, Canada

The perilipin (PLIN) family of proteins associate with lipid droplets and appear to be important, regulators of fat breakdown and utilization in skeletal muscle. Previous research has demonstrated that two PLIN isoforms, PLIN3 and PLIN5, localize to mitochondria in addition to lipid droplets. The reason(s) for this are unclear, but since fatty acids released from intramuscular lipid droplets are oxidized in mitochondria, it is tempting to speculate that these PLINs could facilitate lipid droplet/mitochondrial interactions. However, this would presumably require that PLIN3/5 are in the outer mitochondrial membrane (OMM), and this has not been established. Therefore, the purpose of this study was to sub-fractionate muscle mitochondria to determine the precise intra-mitochondrial localization of PLN3/5. PLIN3/5 localization may differ between muscle rest and work; the intra-mitochondrial localization was also determined following electrically stimulated contraction-induced lipolysis. Male Long-Evans rats (5 months) were stimulated in vivo via the sciatic nerve for 30min (10ms impulses, 100Hz/3s at 10-20V; train duration 100ms) in one hindlimb, a method previously used to induce lipolysis and oxidation (Ramos et al, 2014). The contralateral hindlimb was used as a resting control. A crude mitochondrial fraction was isolated from each of the red gastrocnemius, plantaris, and soleus muscles, then further purified by centrifugation in a 60% Percoll gradient. The mitochondrial band from this step was treated with digitonin (15min; 100µg/mg mitochondria) to selectively disrupt the OMM, and centrifugation then used to generate the following two fractions: OMM/intermembrane space (IMS), and mitoplasts (inner mitochondrial membrane (IMM) and matrix (Mx)). Purity of these fractions was confirmed through western blotting markers for the OMM (TOMM22), IMS (cytochrome c), IMM (cytochrome c oxidase 4-subunit IV, COXIV) and Mx (citrate synthase). The distribution of each marker was unchanged with muscle stimulation. PLIN3/5 content was then determined in each fraction by western blotting for both rest and stimulated conditions. At rest, 70.0±0.08% of the original purified mitochondrial PLIN5 was found in the OMM/IMS fraction (34.0±0.06% was in mitoplasts, p<0.001). Interestingly though, this changed following stimulation, with only 35.0±0.1% of PLIN5 localized to OMM/IMS while a greater proportion was found in the mitoplasts (76.0±0.07%; p<0.001). PLIN3 was also predominantly in OMM/IMS at rest (92.0±0.13% vs. 18.0±0.04% in mitoplasts; p<0.001, main effect), and this was unchanged with stimulation (84.0±0.08% vs. 16.0±0.05% in the OMM/IMS vs. mitoplasts; p=0.79). This indicates that both PLINs 3 and 5 localize primarily to the OMM/IMS

fraction in resting muscle mitochondria. Electrical stimulation seemed to shift PLIN5 toward a primarily mitoplast localization. No such effect was observed with PLIN3. Taken together, these results indicate that PLIN3/5 do indeed associate with the OMM and/or IMS and that contraction affects the localization of PLIN5. It is possible that the mitochondrial location of the PLINs is related to physical interactions with the CPTI complex, which spans the OMM and IMM and facilitates importation of fatty acids for oxidation. Future work will use a co-immunoprecipitation approach to test this hypothesis.

Investigation of Drosophila musculature using SHG microscopy

Abiramy Karunendiran[1], Virginijus Barzda[2,3] and Bryan Stewart[1,4]
[1]Department of Cell and Systems Biology, [2]Department of Physics, and Institute of Optical Sciences, University of Toronto. [3]Department of Chemical and Physical Sciences, University of Toronto Mississauga, [4]Department of Biology, University of Toronto Mississauga

Nonlinear optical microscopy has been recently shown to be a superior imaging modality compared to fluorescence and electron microscopy where imaging can be done without prior staining. It provides a variety of valuable techniques that can be used to reveal structural and functional information in a biological system. Second Harmonic Generation (SHG) Microscopy particularly is a parametric process where photon absorption does not occur, preventing heating of the sample. In addition, polarization resolved imaging allows for the quantification of the microstructure of the sample. SHG is observed in non-centrosymmetric cylindrical molecules such as myosin and can be used to directly visualize muscle structure. Hence, the objective of this research is to investigate dynamic properties of sarcomere structure. Drosophila muscles were used since its structure and physiology is conserved in vertebrates. We found that the SHG response was affected by sarcomere size. Second order susceptibility values were found to change whether the muscle was in a rigor or relaxed state. Changes in the SHG response were compared in somatic and visceral muscles to observe changes in SHG response due to myofibril organization. The technique was found to observe subtle changes in the molecular arrangement of myosin in the thick filaments of sarcomere, showing it to be more sensitive than traditional fluorescence techniques.

Autophagy vs. mitochondrial adaptations in skeletal muscle during endurance training

Yuho Kim and David A. Hood
School of Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, ON, M3J 1P3 Canada

Autophagy is integral in the remodeling of skeletal muscle in response to various stimuli such as energy status, exercise, and/or other cellular stress. However, it currently remains obscure how autophagy is regulated in skeletal muscle over the course of training adaptations. In this study, we examined autophagic regulation in skeletal muscle of rats exposed to chronic contractile activity (CCA; 6 hours/day, 9V, 10 Hz continuous, 0.1 ms pulse duration) for 1, 3, and 7 days (N=8/each group). CCA resulted in muscle mitochondrial adaptation by day 7, as shown by the increase in PGC-1α, a master regulator of mitochondrial biogenesis. This was supported by significant increases in other mitochondrial markers such as COX-I and COX-IV protein levels, as well as COX activity. Notably, the ratio of LC3 II/ LC3 I, an indicator of autophagy, decreased by day 7 largely due to a significant increase in LC3 I. Furthermore, autophagic induction marker p62 appeared to be elevated on day 3 and returned to basal levels by day 7. We found that the lysosomal system is concomitantly upregulated in response to training whereby TFEB, a primary regulator of lysosomal biogenesis, increased 2-fold by day 7 along with other lysosomal markers including LAMP1, LAMP2A, and MCOLN1. Our findings suggest that, in response to chronic exercise, autophagy is upregulated prior to mitochondrial adaptations, and the lysosomal system may play an important role in mediating autophagy and mitochondrial turnover in skeletal muscle during adaptations to a higher mitochondrial content.

Prior exercise training alters the response to cold stress

Carly M. Knuth, Willem T. Peppler, Logan K. Townsend, and David C. Wright
Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada

Shivering thermogenesis is the first line of defense against cold exposure, and as skeletal muscle gradually fatigues there is an increased reliance of non-shivering thermogenesis. Brown (BAT) and beige adipose tissue, which originates within subcutaneous white adipose tissue (WAT), are the primary thermogenic tissues regulating this process, stimulated most potently by cold exposure. Exercise training has also been shown to increase the thermogenic capacity of subcutaneous WAT. Whether exercise training has an effect on the adaptations to cold stress within adipose tissue and skeletal muscle remains to be shown. To address this question, male C57BL/6 mice were subjected to either exercise training by voluntary wheel running (EX) or a sedentary (SED) intervention for 12 days. EX mice ran approximately 6km/day, which led to decreased body weight and increased glucose tolerance, while maintaining a similar pattern of food intake to SED. Mice were then further divided into groups that would be kept at room temperature (25oC) or a cold challenge of 4oC for 48-hrs, thus we examined four groups in this study; (i) SED kept at room temperature (SRT), (ii) exercise trained kept at room temperature (EXRT), (iii) SED kept at 4oC (SC), and (iv) EX kept at 4oC (EXC). Rectal temperatures (Tb) of SC and EXC were significantly lower than that of SRT and EXRT. However, EXC maintained a significantly higher Tb in comparison to SC throughout the 48-h period. The cold challenge significantly reduced body mass, this effect more pronounced in SC than EXC, accompanied by a significant reduction in inguinal WAT tissue mass in EX compared to SED. Additionally, cold and exercise had a main effect on BAT mass. The protein expression of uncoupling protein-1 significantly increased with the cold challenge in inguinal WAT, however there were no differences in BAT protein expression. Taken together our data demonstrates a unique effect of prior exercise on altering the response of adipose tissue to cold stress.

The glutathione recycling system is a regulator of contraction-induced GLUT4 translocation to the plasma membrane in C2C12 myotubes

Frederico Lima-Rosa, Philip J. Bilan, Leonardo Nogueira and Amira Klip
The Hospital for Sick Children

Exercise/skeletal muscle contraction increases the amount of glucose transporter type 4 (GLUT4) at the surface of skeletal muscle. Despite extensive studies, the exercise-derived signals that control intracellular traffic of GLUT4-containing vesicles remain undefined. Reactive oxygen species (ROS) have been shown to play a role on exercise/contraction-induced glucose uptake, although the molecular targets for ROS to promote these effects are not well defined. The glutathione recycling system (GSH/GSSG balance) is the most important element for maintaining cellular redox homoeostasis. The aim of this study was to identify the molecular signals triggered by contractions (evoked by electrical pulse stimulation - EPS) and whether a GSH/GSSG imbalance (by using 2-AAPA, a glutathione reductase inhibitor that lowers GSH) increases GLUT4 translocation to the membrane of C2C12 myotubes. Firstly, we show that both treatments increased the amount of GLUT4 at the plasma membrane. The use of 2-AAPA produced a dramatic fall in the GSH/GSSG ratio, while a smaller decrease was induced by EPS. We have begun looking at signals shared by these two conditions that might lead to GLUT4 translocation. AMPK and Akt were phosphorylated by 2-AAPA treatment and EPS. To further elucidate the contribution of the GSH/GSSG system to EPS-induced GLUT4 translocation, we increased the amount of intracellular GSH treating the cells with Glutathione-ethyl-ester (GSHee), followed by EPS. GSHee treatment reduced the EPS-stimulation of GLUT4 at the plasma membrane as well as AMPK and Akt phosphorylation. Taken together, the results suggest that ROS produced during EPS generates a GSH/GSSG imbalance which activates important signals for GLUT4 translocation to the plasma membrane.

Potential effect of inflammation on ketoisocaproic acid induced insulin resistance

Gagandeep Mann and Olasunkanmi Adegoke
Muscle Health Research Centre, School of Kinesiology and Health Sciences, York University

Branched-chain amino acids (BCAAs) have displayed metabolic benefits, and play a role in muscle protein synthesis. However, elevated levels of BCAAs and their metabolites have been linked to the pathogenesis of insulin resistance and type 2 diabetes mellitus. It has been previously demonstrated in our lab that α-ketoisocaproic acid (KIC), a metabolite of leucine, inhibits insulin-stimulated glucose uptake at 200 uM by 45%. With siRNA knockdown of the branched-chain aminotransferase 2 (BCAT2) enzyme, which catalyzes the reversible conversion of leucine to KIC, KIC's inhibitory influence on glucose transport was attenuated. This suggests that KIC is converted back to leucine in order to elicit these reductions in glucose transport, and that KIC under normal conditions is not reducing the rate of glucose transport. Thus, we aim to analyze whether or not KIC's role in insulin stimulated glucose transport in L6 myotubes will change if co-incubated with homocysteine, a proinflammatory factor because inflammation may modulate the effects of amino acids and their metabolites on insulin action. In our studies homocysteine (50 uM) suppressed insulin stimulated glucose transport by 227% (n=3, p=0.2409). As reported before, KIC suppressed insulin stimulated glucose uptake by 29% (p=0.0936). With co-incubation with homocysteine, there was a further 4.8% (p=0.7721) suppression and 50% (p=0.1124) suppression of insulin stimulated glucose transport at 50 and 500 uM of homocysteine respectively (n=4). KIC suppressed insulin stimulated Akt phosphorylation by 232% (p=0.3468), and more so when co-incubated with 500 uM homocysteine by 14.6% (n=4, p=0.2274). KIC suppressed S6K1 and S6 phosphorylation by 200-300%, this was worsened by co-incubation with homocysteine, especially at 15 uM (n=3, $p < 0.05$). Thus, the effect of KIC on insulin stimulated glucose transport and insulin signaling are worsened by the co-incubation with homocysteine. These data suggest that the effect of amino acids and their metabolites on insulin action in skeletal muscle are modulated by the inflammatory environment often seen in obesity and insulin resistant states.

Skeletal muscle adaptations to chronic exercise in a pre-clinical model of myotonic dystrophy type 1

Alexander Manta[1], Derek W Stouth[1], Irena A Rebalka[2], Jayne M Kalmar[3], Thomas J Hawke[2], Vladimir Ljubicic[1]
[1]Department of Kinesiology, McMaster University, [2]Department of Pathology and Molecular Medicine, McMaster University, [3]Department of Kinesiology & Physical Education, Wilfred Laurier University

Myotonic dystrophy type I (DM1) is the second most common muscular dystrophy (MD), and most prevalent adult form of MD. Caused by a CTG microsatellite repeat expansion in the 3' untranslated region (UTR) of the DMPK gene, muscle weakness, wasting, myotonia, and insulin resistance most prominently characterize DM1. 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, n = 10-14/group) 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). HSA-LR animals are engineered to possess a CTG expansion of ~250 repeats in the 3' UTR of the HSA gene. These mice best recapitulate the myopathy characteristic of the human disorder, and thus serve as the most heavily employed pre-clinical model of DM1. EX-DM1 animals ran 5.6 km/day, which is similar to the running volume of healthy, WT mice previously reported in the literature. Utilizing the pen test to examine muscular endurance and motor performance, the WT group exhibited a significantly higher score, as compared to SED-DM1 mice. The pen test score was normalized in the EX-DM1 group. EX-DM1 mice also demonstrated 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 chronic exercise normalized the aberrant force-frequency relationship observed between WT and SED-DM1 groups. Furthermore, EX-DM1 animals demonstrated a tendency for enhanced fatigue resistance relative to WT and SED-DM1 mice during

repetitive, electrical stimulation-evoked muscle contractile activity in situ. Finally, preliminary results suggest that chronic exercise can diminish the severity of myotonic discharges observed in the muscle of DM1 mice. 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 remodelling is warranted.

Palmitoyl-CoA mediated inhibition of mitochondrial ADP sensitivity plays a key role in promoting high-fat diet induced insulin resistance

Paula M. Miotto[1], Paul J. LeBlanc[2], and Graham P. Holloway[1]
[1]Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada. [2]Department of Health Sciences, Brock University, St. Catharines, Ontario, Canada

Excessive mitochondrial reactive oxygen species (ROS) production is a known contributor to high-fat diet induced insulin resistance in skeletal muscle. However, the exact mechanisms underpinning the rise in ROS production remain unknown. Biologically, mitochondrial ADP transport is required to support energy demands, and in turn, attenuates ROS emissions. Given that intramuscular palmitate accumulation occurs during high-fat consumption, and the lipid moiety palmitoyl-CoA (P-CoA) inhibits ADP sensitivity in vitro, we determined if a high-fat diet promotes P-CoA-mediated impairments in mitochondrial ADP sensitivity, resulting in increased ROS production and insulin resistance. We utilized wildtype (WT) and mitochondrial creatine kinase (Mi-CK) knockout (KO) mice, as we have previously established improved mitochondrial ADP sensitivity in KO mice during exercise, therefore reasoning these animals may be protected from a high-fat challenge. Mice were fed a control (10% lard) or high-fat (60% lard) diet for 2 or 4 weeks (n=8-10 per genotype/group), and glucose tolerance tests (2g / kg body weight; tail blood) verified the diet response. Mitochondrial ADP sensitivity and ROS emissions were measured in the presence of ADP and P-CoA in permeabilized muscle fibres, and muscle palmitate and insulin signaling were determined using gas chromatography and western blotting, respectively. Under control conditions, Mi-CK KO mice displayed lower muscle palmitate, greater ADP sensitivity despite the presence of P-CoA, improved glucose tolerance, and enhanced skeletal muscle insulin signaling ($P < 0.05$). The superior glucose tolerance was maintained in the KO mice after 2 weeks of a high-fat diet ($P < 0.05$), suggesting improved mitochondrial ADP sensitivity protected against high-fat diet induced insulin resistance. However, after 4 weeks of high-fat consumption, intramuscular palmitate was increased to a greater magnitude in KO mice, and as a result, KO and WT mice displayed similar impairments in P-CoA mediated inhibition of ADP sensitivity, glucose intolerance, and skeletal muscle insulin resistance ($P < 0.05$). Collectively, these data suggest that P-CoA acts as a nexus between intramuscular lipid accumulation, mitochondrial dysfunction, and insulin resistance. Specifically, these data highlight that P-CoA inhibition of ADP transport during high-fat consumption contributes to excessive ROS production and insulin resistance.

Mitochondrial dysfunction in the skeletal muscle of young adults with Type 1 Diabetes: The root cause of diabetic myopathy?

Cynthia M.F. Monaco[1], Meghan C. Hughes[2], Sofhia V. Ramos[2], Fasih Rahman[3], Christopher McGlory[4], Mark A. Tarnopolsky[5], Matthew P. Krause[3], Christopher G.R. Perry[2], Thomas J. Hawke[1]
[1]Dept of Pathology & Molecular Medicine, McMaster University, [2]School of Kinesiology & Health Sciences, York University, [3]Dept of Kinesiology, University of Windsor, [4]Dept of Kinesiology, McMaster University, [5]Dept of Pediatrics, McMaster University

Introduction/Background: Type 1 diabetes (T1D) is an autoimmune-mediated metabolic disease whereby the insulin producing beta cells in the pancreas are destroyed, resulting in insulin deficiency and hyperglycemia. While exogenous insulin therapy allows for management of the disease, it does not prevent the development of long-term diabetic complications which invariably impact the length and quality of life. Diabetic myopathy (impairments to skeletal muscle health) is an overlooked and understudied complication of T1D that is believed to expedite the progression of additional diabetic complications. This is based on the fact that skeletal muscle, by virtue of its mass, is the major site for lipid metabolism and the principle tissue in the management of blood glucose, and thus impairments to skeletal muscle health in T1D would expectedly impact disease management.

While rodent studies support physical and metabolic derangements of muscle in T1D, our understanding of the impact of T1D on skeletal muscle health in humans is next to none. Therefore, the purpose of this study was to assess the ultrastructure and metabolic function of skeletal muscle from young adults with T1D. **Methods:** Young recreationally active T1D and age-matched non-diabetic adults were recruited. Muscle biopsies were obtained by microbiopsy needle. Electron microscopy was used to assess the ultrastructure of muscle. Mitochondrial function was assessed in permeabilized muscle fiber bundles using high-resolution respirometry for mitochondrial respiration or fluorometers for reactive oxygen species (ROS) production and calcium retention capacity (CRC). **Results:** Electron microscopy revealed abnormal mitochondrial morphology and Z-line dissolution/disruption in the muscle of young T1D subjects. In line with ultrastructural alterations, the T1D subjects had lower mitochondrial respiration, elevated site-specific ROS production, and reduced CRC in comparison to the non-diabetic subjects. **Conclusions:** This data provides evidence of a previously unrecognized deficiency of mitochondrial bioenergetics in skeletal muscle of young adults with T1D. Understanding the underlying deficits of our 'largest metabolic organ' is of critical importance for identifying targeted exercise and/or pharmaceutical prescriptions that will help reduce the burden of this disease.

Surface EMG normalization techniques of the upper-thoracic erector spinae

Nairn, B.C., Simone, M., Vieira, J., & Drake, J.D.M.
York University

Introduction: Electromyography (EMG) in kinesiology involves the study of voluntary neuromuscular activation of muscles within postural tasks, functional movements, work conditions, and intervention protocols. Normalization of the EMG signal is the process by which the raw electrical activity (mV) of a task is expressed as a percentage of muscle activity obtained from a calibrated test contraction, often in the form of an isometric maximum voluntary contraction (%MVC). Normalizing EMG is necessary for comparison of EMG signals across muscles, between participants, or between days within a participant, and makes interpretation of the EMG signal biologically relevant. MVC techniques have been previously investigated in different muscles such as latissimus dorsi and lower trunk muscles; however, MVC techniques of the upper trunk, specifically at the level of the 4th thoracic vertebra (T4) remain unclear. Another concern with surface EMG is the issue of crosstalk, where signal from nearby muscles contaminates the signal of interest. Therefore, the purpose of this study was to determine which MVC technique produced the greatest level of activation from the erector spinae musculature at the T4 level (T4ES). **Methods:** A total of 30 young, healthy participants (15 M, 15 F) were recruited from the University population. Surface EMG was recorded from the dominant side of T4ES. A total of eight different manually-resisted MVC techniques were repeated twice, with the techniques being: thoracic and lumbar extension from prone, seated and standing arm raise, seated and standing row, and seated and standing lateral pulldown. Within each participant, the peak activation level from the linear envelope of the EMG signal was obtained from each trial. The peaks from the two repeats within a posture were averaged, and the maximum averaged value obtained across all eight postures was used to normalize the MVC trials. This resulted in each participant having one posture that was 100 %MVC. A 2x8 (Sex X Technique) mixed-model ANOVA was run on the dependent measure of T4ES %MVC. Alpha was set to 0.05 and posthoc analyses compared the Least Squares Means with a Tukey adjustment for multiple comparisons. **Results:** A main effect of Technique on T4ES %MVC was found ($F(7,196)=35.26$, $p<0.0001$), with the Thoracic Extension and Arm Raise (seated and standing) trials showing significantly larger values than all other techniques. No other main or interaction effects of sex or technique were found ($p>0.518$). **Discussion & Conclusions:** The results of this study found that a thoracic extension should be used for normalizing the T4ES musculature. The high activation in T4ES from the arm raise technique highlights the issue of crosstalk in surface EMG signals. This has impact for EMG analyses of multi-planar movement of the trunk and upper extremities as caution must be used when collecting and interpreting surface EMG from this level. Ongoing work is currently analyzing muscle contractions and ultrasound imaging simultaneously in order to elucidate the issue of surface EMG crosstalk this spinal level, as well as morphometry changes during muscle contractions.

The exercise-induced activation of AMPK and p38 MAPK in a pre-clinical model of spinal muscular atrophy

Sean Y. Ng, Athan G. Dial, Vladimir Ljubicic
Department of Kinesiology, McMaster University

Spinal muscular atrophy (SMA) is the leading genetic cause of infant mortality and the second most prevalent autosomal recessive disorder. SMA, which is caused by mutations in the survival motor neuron (SMN) gene, is a neuromuscular disorder (NMD) without a cure. Stimulation of key molecules that regulate skeletal muscle phenotype can be therapeutic in chronic diseases such as obesity and type 2 diabetes, as well as in some NMD contexts. Indeed, many characteristics of AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) serve to favorably maintain and remodel skeletal muscle biology. However, the expression and function of these phenotype-bending molecules have yet to be studied in a SMA. Thus, the purpose of this study was to examine these signaling pathways in the skeletal muscle of a pre-clinical model of SMA. Quadriceps (QUAD) and gastrocnemius (GAST) muscles were obtained from wild-type (WT) animals ($n = 7$), as well as from mice harboring an Smn2B/− mutation (SMA; $n = 7$) at postnatal day (p) 21. These SMA mice recapitulate the neuromuscular abnormalities characteristic of the human disorder, and display a disease onset at ~ p10 and demonstrate a mean life expectancy of ~28 days. Western blot analyses were employed to examine SMN, AMPK, p38, and PGC-1α protein levels in mixed QUAD and GAST muscle homogenates. Skeletal muscle mass was ~60–70% lower ($p < 0.05$) in SMA mice relative to their age-matched, WT littermate counterparts. SMN protein content was ~85% lower ($p < 0.05$) in the muscles from SMA mice, as compared to the WT group. Total AMPK and p38 protein content were similar between genotypes. However, phosphorylated AMPK and p38 levels were ~50% lower ($p < 0.05$) in the SMA mice. PGC-1α expression, a downstream target of AMPK and p38, was significantly reduced (−60%) in the muscle of SMA mice relative to the WT group. This decrement in PGC-1α expression was also observed using immunofluorescence techniques. Collectively, these data indicate that SMA is associated with a reduction in molecules that are important for maintaining healthy skeletal muscle, as well as for remodeling skeletal muscle phenotype. In a separate cohort of animals ($n = 1$), at p17 SMA and WT mice ran on a motorized treadmill (3 m/min) until the inability to continue exercise was determined. During the run, WT mice were exposed to incremental increases of 2 m/min every 5 minutes. SMA mice ran ~80% less ($p < 0.05$) than their WT counterparts. While exercise appeared to increase AMPK and p38 phosphorylation in both genotypes, the induction tended to be greater in the SMA animals. In summary, this study enhances our knowledge of skeletal muscle disease biology in SMA. Furthermore, preliminary data suggest that exercise may enhance signaling for SMN induction in skeletal muscle.

Developing zebrafish as an in vivo model of cardiomyopathy

Matiyo Ojehomon and John F. Dawson
Department of Molecular and Cellular Biology and the Centre for Cardiovascular Investigations, University of Guelph

Zebrafish (*Danio rerio*) has been an important animal model to the scientific community since 1960. This tropical fish has helped uncover a number of biological processes behind many human genetic diseases like cancer and muscular dystrophy. Due to its optical transparency, rapid cardiovascular development and easy gene manipulation, this organism is currently being developed to study the molecular mechanisms that lead to the development of cardiomyopathy caused by mutations in the human cardiac actin (*ACTC*) gene. With the use of Tol2 transposons and the transposase enzyme, we will be inserting human ACTC cDNA to the genome of zebrafish with the long-term goal of making zebrafish dependent on the inserted human gene by knocking down all the zebrafish cardiac actin (*zfACTC*) genes using CRISPRs. Currently, we know of two *zfACTC* genes in the zebrafish genome but using human directed sequence and functional analyses, we have uncovered a putative uncharacterized *zfACTC*. We have used whole mount in situ hybridization to support our prediction of this additional *zfactc* gene. We will also present our progress on the generation of transgenic zebrafish expressing wild type and mutant human ACTC cDNA.

Perturbing mitochondrial protein import in vivo results in activation of the UPRmt

Ashley N. Oliveira and David A. Hood
Muscle Health Research Centre, School of Kinesiology and Health Science, York University

Mitochondria rely heavily on the tight coordination of the nuclear and mitochondrial genomes. Disruption of this regulation has been shown in lower order organisms to result in a mito-nuclear imbalance in which holenzymes are unable to assume mature stoichiometry. This lack of coordination between nuclear and mitochondrially encoded subunits resulting in orphaned subunits is an activator of the mitochondrial unfolded protein response (UPRmt). The UPRmt is a highly conserved quality control mechanism that strives to achieve proteostasis by refolding or degrading misfolded proteins as well as misassembled complexes. This process is well characterized in c. elegans however, the UPRmt and its retrograde signals are poorly understood in mammals. Thus we sought to induce the UPRmt in-vivo¬ in a mammalian model to better characterize this stress response. In order to do so, C57BL/6 mice were injected once a day for 3 days with an antisense oligonucleotide (In-Vivo Morpholino) targeted to Tim23, the major channel of the inner membrane, to perturb the genomic coordination. Following Tim23 Morpholino treatment, Tim23 protein content was significantly reduced by 50%. The consequence was a 60% reduction of protein import into the mitochondrial matrix. As a result, mitochondrial chaperone cpn10 was significantly elevated by 30% and the transcription factor ATF5 also demonstrated a strong trend to increase, suggesting that the UPRmt was activated following Tim23 knockdown. Mitochondrial respiration and ROS emission was also measured to determine whether Tim23 knockdown resulted in mitochondrial dysfunction. However, no differences were found suggesting that the stress imposed is sufficient to induce the UPRmt without disrupting basic mitochondrial functions. The second aim of this study was to investigate the role of mitochondrial proteolytic byproducts in mediating retrograde signaling. Peptides released from the mitochondria following basal proteolysis were isolated and incubated with import reactions. Our results demonstrate a dose- and time-dependent inhibition of import with the addition of peptides, such that import was reduced by 48% with the addition of 6ug of peptides for 30 minutes. This suggests that mitochondrial proteolytic byproducts can exert an inhibitory effect on the protein import pathway, to inhibit excessive protein import as a negative feedback mechanism. The inhibition of import into the organelle may also serve a retrograde function, to modify nuclear gene expression and improve organelle-folding capacity, or alter the expression of protein import machinery components.

Subcutaneous inguinal white adipose tissue is responsive to exercise, but dispensable for the metabolic health benefit

Willem T. Peppler[1], Logan K. Townsend[1], Carly M. Knuth[1], Michelle T. Foster[2], David C. Wright[1]
[1]Department of Human Health and Nutritional Sciences, University of Guelph, Guelph ON N1G2W1, Canada;
[2]Department of Food Science and Human Nutrition, Colorado State
University, Fort Collins, CO 80523, USA

Exercise training has numerous beneficial effects on metabolic health, which are primarily thought to be due to adaptations within skeletal muscle. Recently, appreciation has grown for the role of adipose tissue, and evidence suggests that subcutaneous inguinal white adipose tissue (iWAT) may be critically important. After exercise, iWAT undergoes a unique phenotypic switch to expresses uncoupling protein 1 (UCP-1), which is a mitochondrial protein that uncouples respiration to dissipate excess energy through thermogenesis. Moreover, transplantation of this exercise-trained iWAT into a sedentary host has been found to activate thermogenesis and lead to improvements in whole body and skeletal muscle glucose homeostasis. Thus, we hypothesized that these contributions of iWAT may be required for the metabolic benefit of exercise. To test this, we completed SHAM or iWAT removal (lipectomy, LIPX) surgery in 8-week old male C57BL/6 mice. After approximately 3 weeks of recovery, mice remained in standard cages or were provided access to voluntary wheel running as a model of exercise training for 11 nights. SHAM and LIPX mice ran similar distances (SHAM, 4.4±0.3 km/day; LIPX, 4.8±0.5 km/day), which lead to decreases in body mass and adiposity. In both SHAM and LIPX mice voluntary wheel running led to an increased respiratory exchange ratio without changes in absolute oxygen consumption or energy expenditure. Further, voluntary wheel running improved indices of glucose homeostasis in SHAM and LIPX mice, which was characterized by lower ad libitum glucose, improved glucose tolerance,

and increased skeletal muscle protein content of hexokinase II and glucose transporter 4. Finally, the exercise induced increase in the protein content for markers of skeletal muscle mitochondrial biogenesis, PGC-1α and COXIV, was not different between SHAM and LIPX mice. Together, this data suggests that while iWAT is uniquely responsive to voluntary wheel running, it is dispensable for the metabolic health benefits.

Microtubule-targeting chemotherapy causes mitochondrial dysfunction in heart and skeletal muscle

Sofhia V. Ramos, Meghan C. Hughes and Christopher G.R. Perry

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

Rationale: A variety of chemotherapies are associated with muscle dysfunction but the mechanisms are poorly understood. Emerging evidence suggests doxorubicin (DNA targeted chemotherapy) impairs mitochondrial respiration and increases H2O2 emission. However, whether other chemotherapies create similar mitochondrial dysfunctions in muscle is unknown. An emerging model proposes that microtubules can inhibit mitochondrial ADP import/ATP export by blocking outer membrane voltage-dependent anion channels (VDAC). We hypothesized that microtubule-targeting chemotherapies (taxol, microtubule stabilizer; vinblastine, microtubule destabilizer) may alter ADP control of oxidative phosphorylation by changing VDAC-permeability to ADP. We also hypothesized that these chemotherapies would alter mitochondrial H2O2 emission considering ADP suppresses H2O2 emission from mitochondria during oxidative phosphorylation. **Methods:** Mitochondrial ADP-stimulated respiration and suppression of H2O2 emission were assessed in permeabilized muscle fibres from skeletal muscle (soleus, extensor digitorum longus; EDL) and heart left ventricle following incubation with taxol and vinblastine for 2 and 1hr respectfully, at 4◦C. **Results:** Diverse responses were observed with both microtubule stabilizing (taxol) and destabilizing (vinblastine) drugs. However, a consistent pattern emerged in heart whereby the stabilizer taxol decreased respiration (5mM ADP: control, 195.5 ± 33.6 pmol/s/mg w.w., taxol, 112.5 ± 19.6 pmols/s/mg w.w., p= 0.05) while the destabilizer vinblastine had no effect. Both stabilizer and destabilizer decreased the ability of ADP to suppress H2O2 (taxol, 15 µM (p=0.03) and 100µM (p=0.02) ADP; vinblastine, 15µM to 500µM (p=0.01) ADP). No alterations were observed in succinate-stimulated H2O2 emission protocols, demonstrating that the responses in heart were surprisingly substrate-specific. In EDL, ADP-stimulated respiration was increased at submaximal [ADP] with destabilizer (250µM ADP: control, 15.29 ± 2.10, vinblastine, 22.75 ± 2.81 pmol/min/mg d.w., p=0.05) but did not change with stabilizer. Both drugs decreased ADP-suppression of H2O2 emission (with succinate) between 50µM to 500µM ADP (p=0.01). In soleus, ADP-stimulated respiration and ADP-suppression of H2O2 did not change with either drug. **Conclusions and discussion:** Heart appears to be sensitive to microtubule-targeted chemotherapy treatments as ADP-stimulated respiration and suppression of H2O2 were impaired. Oxidative soleus may be more resistant to alteration in microtubule structure as ADP-stimulated respiration and suppression of H2O2 did not change. In contrast, glycolytic EDL responded divergently to the destabilizer whereby ADP-stimulated respiration was increased despite reduced ADP-suppression of H2O2, whereas the stabilizer had no effect on respiration but also reduced ADP-suppression of H2O2. Collectively, microtubule-stabilizing and destabilizing compounds inhibit ADP control of mitochondrial bioenergetics in heart and glycolytic skeletal muscle. Subsequent experiments will determine how these responses are related to microtubule-VDAC binding and muscle functional capacities.

Role of amino acid transporters during in vitro myogenesis

Sophia Roubos[1], Julian Nallabelli[2], Grace Niemiro[2], Russell Emmons[2], Nicole Korp[2], and Michael De Lisio[1]

[1]School of Human Kinetics, University of Ottawa, and [2]Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign

Amino acid supplementation, particularly the essential amino acid leucine, has been shown to have anabolic effects on skeletal muscle. Leucine is transported into muscle fibers by the L-type amino acid transporter 1 (LAT1) and subsequently activates mTORC1 to stimulate cell growth and proliferation pathways. Whether LAT1 is expressed on myoblasts and has direct effects on myoblasts function are still open questions. The

purpose of the present project was to examine the expression of LAT1, and other amino acid transporters, on myoblasts and to determine their role in myogenesis. Using C2C12 myoblasts, we examined the protein content and gene expression of LAT1, and other amino acid transporters, the sodium-coupled neutral amino acid transporter 2 (SNAT2), and CD98 during quiescence, proliferation, and differentiation. We also examined the effects of blocking LAT1 on myoblast proliferation and differentiation, and on mTORC1 signaling. LAT1 protein content was increased early during differentiation compared to early proliferation ($p<0.05$), while SNAT2, and CD98 protein content was not changed throughout myogenesis ($p>0.05$). Conversely, 1 day and 4 day differentiating myoblasts decreased expression of LAT1 (Day 1: 3.1 fold, Day 4: 2.8 fold; both $p<0.05$), and SNAT2 (Day
1: 1.3 fold; $p=0.051$, Day 4: 2.9 fold, $p<0.01$) when compared to proliferating myoblasts. CD98
expression was increased after 1 day in differentiation media compared to proliferating myoblasts, and myoblasts in differentiation media for 4 days (both 2 fold; both $p<0.01$). Similarly, CD98 expression increased in proliferating myoblasts compared to quiescent myoblasts (8 fold; $p<0.01$). LAT1 inhibition decreased viability of proliferating myoblasts ($p<0.05$) and impaired myoblast differentiation ($p<0.05$) compared to controls, but did not alter mTORC1 signaling. Our data demonstrate differential regulation of amino acid transporters throughout myogenesis and blocking LAT1 specifically impaired myoblast proliferation and differentiation. These data suggest that myoblasts may be directly regulated by amino acids and, as such, may also have a role in the *in vivo* regulation of skeletal muscle satellite cells.

Phenotypic classification of cardiac actin knockouts in zebrafish using the CRISPR-Cas9 system

Love P. Sandhu and John F. Dawson
Department of Molecular and Cellular Biology, Centre for Cardiovascular Investigations, University of Guelph, Guelph, ON

Cardiomyopathy is a common cause of heart failure and a growing epidemic in Canada. Two prevalent forms of cardiomyopathy are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), characterized by changes to the myocardium. The development of HCM and DCM is associated with mutations found in genes encoding muscle proteins, including cardiac actin (ACTC). 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. My overall goal is to use zebrafish to determine connections between cardiac actin mutations and cardiomyopathy development by using CRISPR-Cas9 technology to produce and characterize zebrafish cardiac actin (zfactc) knockout lines. This work will clarify which zfactc gene(s) is a cardiac isoform and will be the first characterization of the putative actc1c gene. I hypothesize that a cardiac phenotype should be observed if the knocked out zebrafish gene encodes for cardiac actin. This work will provide a zfactc knockout background for future human ACTC rescue experiments, including the characterization of cardiomyopathy-related ACTC mutants.

The effect of hypoxia on human 3D skeletal muscle tissues in vitro

Elana Sefton, Mohsen Afshar, Penney M. Gilbert
Institute of Biomaterials and Biomedical Engineering, University of Toronto

Skeletal muscle takes up the largest volume of tissue in the body representing almost 40 % of the body weight, and more than 90 % of the energy produced by muscle cells comes from the aerobic (using oxygen) pathway. Hence, an environment with low O2 availability proposes a challenge on the skeletal muscle homeostasis, and this situation will be exacerbated in cases where the demand for ATP is increased, such as during exercise. In vitro models of human organs have greatly improved our understanding of mechanisms that lead to disease as well as responses to clinical drugs. However, the majority of skeletal muscle culture models are two-dimensional platforms that are incapable of long term culture and maturation on skeletal muscle fibers in vitro. Hence three-dimensional (3D) culture model of the human skeletal muscle can better recapitulate the in vivo skeletal muscle, and can serve as a suitable platform to study the effect of hypoxia on skeletal muscle. The Gilbert Lab has established a 96-well plate micro-molded device that supports the formation and maturation of

human skeletal muscle micro tissues (hMMTs). The device is made of polydimethylsiloxane 'silicone rubber' and each well contains an oval shaped cavity in the middle and two vertical rubber posts on each side of the cavity. Human myogenic cells are mixed with extracellular matrix proteins (fibrin and matrigel) and seeded in each well where a tissue is formed within 1 week of culture time. To evaluate the impact of oxygen tension on tissue maturation, tissues are exposed to atmospheric (20% O2, 5% CO2 balanced with nitrogen) and hypoxic (3% O2, 5% CO2 balanced with nitrogen) conditions for variable days in culture. Structural and functional response of hMMTs to hypoxia are examined by: (1) Studying the muscle fiber type formation via western blotting for different myosin heavy chain subtypes (fetal, adult slow, adult fast); (2) Fiber size analysis via immunohistochemistry for sarcomeric alpha-actinin, and; (3) hMMT force generation via post deflection analysis. Finally, since hypoxia inducible factor 1 (HIF-1) has been described as the master regulator of hypoxia-mediated cellular adaptations, HIF1-alpha gene expression as well as several key proteins downstream are compared using qRT-PCR analysis. Results are compared with those from hMMTs cultured in the current standard conditions. These studies will provide valuable information as to the optimal conditions in which human skeletal muscle tissues fuse to form fibers and mature in an in vitro 3D system.

Regulatory changes in H88Y and F90Δ α-cardiac actin variants implicated in early-onset hypertrophic cardiomyopathy

Zeeshan Shaikh and John F. Dawson
Department of Molecular and Cell Biology and Centre for Cardiovascular Investigations, University of Guelph

Cardiovascular disease (CVD) impacts millions of lives worldwide with a total global healthcare cost of 17 billion dollars a year. 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 the molecular its pathogenesis, apart from its link to mutations in genes encoding sarcomere proteins, including α-cardiac actin (ACTC). My study focuses on the F90Δ and H88Y ACTC variants implicated in early-onset HCM. Previous research has shown that myosin activity is largely unchanged with these ACTC variants. I hypothesize that these ACTC variants adversely affect tropomyosin (Tm) regulation, decreasing cardiac contractility. Troponin (Tn) and Tm will be bound to ACTC variants forming regulated thin filaments (RTFs) and myosin ATPase assay and an in-vitro motility assays will generate pCa50 curves. Decreased Tm binding affinity will reduce the calcium sensitivity of F90Δ and H88Y variants. These data will contribute to a fuller understanding of the molecular pathogenesis of early-onset HCM development leading to more specific and effective treatments.

Characterizing the expression, localization and function of protein arginine methyltransferase in skeletal muscle cells

Nicole Y. Shen, Vladimir Ljubicic
McMaster University

Protein arginine methyltransferases (PRMTs) play a critical role in the development of skeletal muscle. Despite the importance of PRMTs, the expression, localization and function of these enzymes, specifically PRMT1, PRMT4 and PRMT5, during muscle development remains poorly understood. Therefore, the purpose of our study was to investigate quantitative, temporal, spatial, and functional metrics of PRMT biology in skeletal muscle cells during myogenesis. C2C12 skeletal muscle differentiation was employed as an in vitro model of skeletal muscle development. Cells were assessed during the myoblast stage, and during days 1, 3, 5, and 7 of differentiation. RT-qPCR was used to examine transcript levels, while Western blotting was used to assess protein expression, localization, and function throughout the experimental timecourse. In situ immunofluorescence was employed to investigate cell morphology and maturity. The progression of myogenesis throughout the experimental timecourse was confirmed via significant increases in embryonic myosin heavy chain and myogenin expression, two molecular markers of muscle development. PRMT1 transcript levels progressively increased throughout differentiation and were 50-120% higher ($p < 0.05$) at day 3 and 5 compared to all preceding timepoints. Similarly, PRMT1 protein expression gradually increased during

myogenesis and was 1.5-fold higher ($p < 0.05$) at day 5 of differentiation, as compared to the myoblast stage. PRMT1 protein expression followed a similar pattern of expression as myogenin, suggesting a co-regulation between these molecules during myogenic differentiation. PRMT4 transcript and protein levels were significantly lower (~20-50%) at day 5 and 7 of differentiation compared to the myoblast stage. In contrast, PRMT5 transcript and protein expression progressively increased during the timecourse and was significantly higher by day 5 of differentiation compared to the myoblast stage. Collectively, these data demonstrate PRMT-specific patterns of expression during myogenesis. Monomethylarginine content, a marker of global PRMT activity, was similar across the timecourse. This suggests that, despite enzyme-specific differentiation-induced alterations in PRMT expression, PRMTs are active methyl donors throughout myogenesis. Cell fractionation analyses revealed greater PRMT protein content within the cytosol (3-fold), as compared to the myonuclei at all timepoints of differentiation. Furthermore, PRMT1 and PRMT5 protein levels gradually increased in the cytosol during myogenesis and were significantly higher compared to the myoblast stage by day 7 of differentiation. In contrast, PRMT4 protein expression progressively increased in the myonuclei throughout the timecourse, consistent with its reported transcriptional coactivator functions. To specifically examine the role of PRMT1 during myogenic differentiation, cells were treated with TC-E 5003 (TCE), a selective inhibitor of PRMT1. In comparison to vehicle-treated cells, TCE-treated cells exhibited a deficiency in muscle differentiation, as indicated by significant reductions in myoblast fusion, and decreased myotube surface area. Altogether, our data reveal the dynamic nature of PRMTs and its importance during skeletal muscle remodelling.

Can a short F-actin complex interact with important binding proteins?
Navneet Sidhu and John F. Dawson
Molecular and Cellular Biology, University of Guelph

Actin, a cytoskeletal protein, participates in a multitude of cellular functions ranging from intracellular trafficking to muscle contraction. A rapid assembly and disassembly of actin filaments enables actin to perform complex cellular functions. This rapid turn of actin filaments is regulated by its interactions with ABPs. Despite the significance of these interactions there is no atomic resolution structure of any ABP bound to F-actin. The caveat is the tendency of actin to self-assemble under crystallisation conditions. To resolve this problem, a short, stable, non-polymerizable F-actin oligomer called ADPr-trimer was developed in the Dawson lab. My goal is to build upon the ADPr-trimer to probe interactions of F-actin with ABPs for crystal trials. Any strategy to this end involves two approaches: (1) Candidate based approach: Determining interactions of ADPr-trimer with purified ABPs (myosin subfragment1 and cofilin) and (2) Discovery based approach: Selectively retaining ABPs from cell lysates on ADPr-trimer affinity columns. Retained proteins can be identified by mass spectrometry. Identified proteins can be produced in the lab and their interactions characterised for future crystal trials. 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.

Cellular localization and function of protein arginine methyltransferases during atrophy
Derek W. Stouth, Alexander Manta, and Vladimir Ljubicic
Department of Kinesiology, McMaster University, Hamilton, ON

Protein arginine methyltransferase 1 (PRMT1), PRMT4 (also known as co-activator-associated arginine methyltransferase 1; CARM1), and PRMT5 catalyze the methylation of arginine residues on target proteins, thereby mediating intracellular processes such as signal transduction and transcriptional control. Although only a few studies have investigated PRMTs in skeletal muscle, evidence strongly suggests that these enzymes regulate skeletal muscle plasticity. However, the function of PRMTs in response to disuse-induced muscle remodelling remains unknown. Thus, our study objective was to determine whether denervation-induced muscle disuse alters the cellular localization and specific methyltransferase activities of PRMT1, PRMT4, and PRMT5 in skeletal muscle within the context of early signaling events that precede muscle atrophy. Mice were subjected to 6, 12, 24, 72, or 168 hours of unilateral hindlimb denervation (DEN). The contralateral limb served as an internal control. Western blot analyses were employed to determine nuclear and cytosolic protein expression

levels in the DEN gastrocnemius (GAST) muscle, relative to the contralateral, non-DEN, control GAST muscle across the experimental time course. Muscle mass significantly decreased by ~25% in the DEN hindlimb following 168 hours of disuse. The PRMTs exhibited remarkable enzyme-specific spatial and temporal expression in skeletal muscle in response to DEN. Nuclear PRMT1 content significantly decreased by 40% after 6 hours of DEN prior to increasing by ~2.8-fold ($p < 0.05$) after 72 and 168 hours of disuse. PRMT4 levels in the myonuclei were significantly augmented by 92% at 72 hours, before decreasing by 32% ($p < 0.05$) following 168 hours. Nuclear PRMT5 protein content increased 2.8-fold ($p < 0.05$) after 12 hours, but was significantly reduced by 43-63% following 72 and 168 hours of denervation. These unique expression profiles suggest that PRMTs have distinct functions in response to muscle disuse. Furthermore, since ~85% of PRMT protein content was found within the cytosolic compartment, it was not surprising that cytosolic PRMT levels reflected whole muscle PRMT expression. Interestingly, the levels of myonuclear peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α) protein content, a master regulator of skeletal muscle phenotype, decreased 33% ($p < 0.05$) following 6 hours of denervation, which suggests a coordinated expression of PRMT1 and PGC-1α mediated by a common upstream regulator(s). To assess PRMT methyltransferase activity, we next examined the myonuclear content of histone 4 arginine 3 (H4R3), H3R17, and H3R8, which serve as specific targets for PRMT1, PRMT4, and PRMT5 methylation, respectively. H4R3 and H3R17 marks increased 3.2- and 8.8-fold after 168 hours of DEN, whereas H3R8 methylation was elevated 1.7-fold following 12 hours of disuse. Our results suggest that alterations in PRMT1, PRMT4, and PRMT5 localization and function in response to skeletal muscle disuse are rapid and dynamic. This study provides evidence that PRMTs participate in skeletal muscle remodelling that occurs prior to, as well as during, muscle atrophy.

A single bout of heat stress increases Nrf2 and its target genes in mouse skeletal muscle

Yuki Tamura[1,2], Yu Kitaoka[3], Koichi Nakazato[2], and Hideo Hatta[3]
[1]Muscle Health Research Centre, York University, [2]Nippon Sport Science University, [3]The University of Tokyo

In recent years, we have reported that heat stress induces various skeletal muscle adaptations (e.g. mitochondria, autophagy, unfolded protein response). In this study, we explored further possibilities of muscle adaptations by heat stress treatment using transcriptome approach and subsequent bioinformatics analysis. Immediately after treatment and three hours after a single bout of heat stress treatment (exposing mouse into a hot environment chamber; 40ºC, 30 min), gastrocnemius muscles were collected and then examined changes in over 39,000 genes expression using Affimetrix microarray GeneChip. Consequently, we analyzed with bioinformatics algorithms TFactS and BioCarta to predict activated transcriptional factors and activated pathways, respectively. These computational analyses based on transcriptome data indicated that heat stress activates Nrf2 (Nfe2l2), a master transcriptional factor of antioxidant response, in skeletal muscle (TFactS: $P<0.05$; BioCarta: Fold Enrichment=7.2, $P<0.05$). We further confirmed by qPCR that heat stress increased Nrf2 (+39.4%, $P<0.05$) and its target genes such as Cat (+30.9%, $P<0.05$), Hmox1 (+182.6%, $P<0.05$), Gclc (+53.8%, $P<0.05$), Gclm (+32.1%, $P<0.05$), Gpx1 (+23.7%, $P=0.07$), Mt1a (+251.6%, $P<0.05$) and Sod1 (+27.8%, $P<0.05$) at 3h after treatment. Notably, there were non-detectable changes in oxidative stress marker (4HNE-conjugated protein) and gene expression of Nrf2-independently inducible antioxidant enzyme (Sod2) at both immediately post-treatment and 3h after treatment. Our observations suggest that the physiological significance of Nrf2 activation by heat stress may be other than defending oxidative stress.

Rapid high-fat diet-induced glucose intolerance is not associated with hepatic interleukin-6 resistance

Logan K. Townsend, Sarah K. Trottier, Willem T. Peppler, David C. Wright
Department of Human Health and Nutritional Science, University of Guelph

Background: High-fat diet (HFD)-induced hepatic insulin resistance is associated with attenuated hepatic interleukin-6 (IL-6) action. This is important as ablation of hepatic IL-6 signalling worsens systemic glucose tolerance. Hepatic IL-6 resistance may result from increased content or activity of Src homology phosphatase 2 (SHP2) in the liver, a phosphatase which dephosphorylates STAT3, a downstream IL-6 signaling intermediate. Objective: Determine if the initial development of glucose intolerance with a high fat diet is associated with the onset of liver IL-6 resistance. **Hypothesis:** HFD will rapidly induce hepatic IL-6 resistance, in association with

impairments in whole body glucose homeostasis and increased liver SHP2 content and activity. **Methods:** C57bl/6 mice (10 per group) had ad libitum access to standard chow or HFD (60% kcal from fat) for 3 d. On the 4th d, mice were fasted for 6 h before an intraperitoneal (IP) glucose tolerance test. The following day half of the mice in each group were injected IP with IL-6 (3 $\eta g \cdot kg^{-1}$ body weight) or sterile saline. Tissues were harvested 15 min post-injection. STAT3 phosphorylation and SHP2 activity were assessed via Western blotting. **Results:** Mice fed a HFD were glucose intolerant ($P<0.001$). This was not associated with attenuated hepatic STAT-3 phosphorylation following IL-6 injection. Nor was hepatic SHP2 content or activity increased in HFD mice. **Conclusions:** HFD rapidly causes systemic glucose intolerance but this is not the result of blunted hepatic IL-6 signalling.

Skeletal muscle disuse is associated with reductions in mitochondrial content and elevations in mitophagy in the rat hindlimb

Matthew Triolo, Liam D. Tryon, David A. Hood
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Canada.

During chronic muscle disuse fibre atrophy occurs concomitantly with reductions in mitochondrial content and function. The reduction in organelle content is due, in part, to depressed mitochondrial biogenesis and an elevation in its degradation processes. Although the reductions in biogenesis have been studies in depth, the processes that underlie the degradation of mitochondria have yet to be fully elucidated. The breakdown of the organelle is due to a selective form of autophagy, termed mitophagy. When a mitochondrion becomes dysfunctional, as with muscle disuse, it will dissociate from the mitochondrial network and subsequently be degraded. The goal of this work is to better understand the process of mitophagy in the context of muscle disuse. To do so, we employed a denervation protocol as a model of chronic disuse. Briefly, we unilaterally sectioned the peroneal nerve of one hindlimb, using the contralateral limb as a control in Sprague-Dawley rats. Protein measurements were made at 1, 3 and 7 days post-denervation. To study the targeting of mitochondria for degradation, a group of rats was treated with colchcine (4mg/kg/day) for 2 days prior to sacrifice in order to inhibit mitophagy, thus allowing measures of mitophagy flux to be made in subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria following 1 and 3 days of denervation. Significant 25% reductions in extensor digitorum longus (EDL) and tibialis anterior (TA) muscle mass were observed by 7 days post-denervation, along with dramatic 2-3-fold elevations in the autophagy proteins Beclin1, ATG-7 and LC3-I. However, only a 1.5-fold increase was noted for LC3-II, the lipidated precursor for autophagosome formation. This could be due increased breakdown of LC3-II, indicative of autophagy flux. Additionally, SS mitochondria had a greater elevation in mitophagy flux following 3 days, compared to 1 day, of denervation, whereas IMF mitochondria showed similar increases at 1 and 3 days post-denervation. Our data suggest that the mitochondrial decay observed during chronic muscle disuse may be due, in part, to the elevation in mitophagy flux in response to denervation, however further work is necessary to evaluate the effect of time on the breakdown of the individual mitochondrial subfractions.

Cancer-specific cell death in response to palmitoylcarnitine is associated with increased mitochondrial hydrogen peroxide

Patrick C. Turnbull, Christopher G. R. Perry
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, ON

Rationale and Hypothesis: A benchmark of cancer cells is the reliance on glycolysis characterized by decreased mitochondrial oxidative phosphorylation (eg. Warburg effect). Recent evidence suggests that fatty acids (palmitoylcarnitine; PCarn) may force a shift to mitochondrial oxidative phosphorylation in HT29 colon cancer causing cell death, yet non-cancer cells appear to be resistant. These results present the intriguing possibility that ‘lipid-therapy’ might be well tolerated by healthy cells while retaining potency in cancer. However, the mechanism of lipid-induced cancer cell death is not known. Glutathione is the main antioxidant found within the cell, we hypothesize that cell survival is determined by heterogeneity of basal intracellular glutathione. We theorize that cells with low basal glutathione will be more susceptible to elevated levels of mitochondrial H2O2 from beta-oxidation of PCarn, whereas cells with higher glutathione will be more resistant

to elevated levels of H_2O_2. **Experimental approach:** We developed a live cell kinetic assay to measure H_2O_2 emission in real time; using this assay we exposed three different cancer lines (HT29 colorectal adenocarcinoma, MCF7 breast adenocarcinoma and HepG2 hepatocellular carcinoma) and one non-cancerous cell line (CCD841 colon epithelial cells) to increasing amounts of PCarn (25µM, 50µM 100µM) for 2, 24 and 48hrs. As a measure of cell proliferation, we adapted a BCA protein assay to digest adhered cells in-well as a measure of total cell content. **Results:** After 48hr incubation of 100µM PCarn, H_2O_2 emission increased in MCF7 (20%) and HT29 (170%), which corresponded to decreased protein contents of 20% and 50% respectively, thereby demonstrating a graded relationship between degree of H_2O_2 emission and reduction in cell content. HepG2 demonstrated increased H_2O_2 emission after 48hr incubation with 25µM (150% increase) and 50µM PCarn (218% increase) with a 50% reduction in protein content in both cases. In the non-cancerous CCD841, we noticed an increase in H_2O_2 (115%) emission, despite no change in protein content. Collectively, this data suggests that increases in H_2O_2 production through PCarn oxidation is well tolerated by non-cancerous cells, however cancer cells are unable to withstand the concomitant increase in mitochondrial H_2O_2 emission albeit in response to different PCarn concentrations. Future work will determine the manner by which these cancer-specific redox-cell death relationships are regulated by glutathione buffering.

Skeletal muscle expression and localization of protein arginine methyltransferases in response to exercise

Tiffany L. vanLieshout, Derek W. Stouth, Vladimir Ljubicic
Department of Kinesiology, McMaster University, Hamilton, ON L8S 4L8, Canada

Protein arginine methyltransferase 1 (PRMT1), PRMT4, and PRMT5 catalyze the methylation of arginine residues on target proteins. In turn, these marked proteins mediate a variety of biological functions. By regulating molecules that are critical to the remodelling of skeletal muscle phenotype, PRMTs may influence skeletal muscle plasticity. Thus, our study tests the hypothesis that the intracellular signals required for muscle adaptation to exercise will be associated with the induction of PRMT expression and activity. C57BL/6 mice were assigned to one of three experimental groups: sedentary (SED), acute bout of exercise (0PE), or acute exercise followed by 180 minutes of recovery (3PE). The mice in the exercise groups performed a single bout of treadmill running at 15 m/min for 90 minutes. The extensor digitorum longus (EDL) and the soleus (SOL) muscles were utilized for RT-qPCR and Western blot assays, while the gastrocnemius (GAST) muscle was employed to isolate nuclear and cytosolic compartments for protein localization analyses. AMPK activation status was 1.7-2.2 fold higher ($p < 0.05$) immediately post-exercise (0PE) in the EDL and SOL muscles, and returned to baseline levels at 3PE. Furthermore, PGC-1α mRNA expression was elevated by 10-13-fold ($p < 0.05$) in both muscles at 0PE and 3PE, which demonstrates that the experimental design utilized in this study was effective at evoking an intracellular milieu indicative of the exercise response. The level of whole muscle PGC-1α protein content was similar between SED, 0PE, and 3PE. In muscles from the SED group, PRMTs exhibited fiber type- and enzyme-specific gene expression patterns at the mRNA and protein levels. PRMT1 and PRMT5 mRNA expression was similar between muscles, while PRMT4 transcript levels were significantly lower (-40%) in SOL compared to EDL muscles. PRMT1 and PRMT5 protein content was 90% and 140% higher in SOL relative to EDL muscles, respectively ($p < 0.05$), whereas in contrast PRMT4 displayed similar protein expression between muscle types. PRMT mRNA and protein content were similar between SED, 0PE, and 3PE. Assessment of muscle monomethylarginine (MMA) content was performed in order to examine total PRMT activity, while asymmetric dimethylarginine (ADMA) levels were assayed as a marker of PRMT1 and PRMT4 activity, and symmetric dimethylarginine (SDMA) content was analyzed to assess PRMT5 function. The presence of all three methylarginine species was significantly higher (40-100%) in the SOL muscles compared to the EDL muscles. As observed with PRMT gene expression, MMA, ADMA, and SDMA were similar between SED, 0PE, and 3PE. Analysis of GAST nuclear and cytosolic fractions demonstrated that PRMT protein expression was significantly higher in the cytosolic compartment versus the myonuclei. Cellular PRMT localization was similar between SED, 0PE, and 3PE. Collectively, this study reveals characteristics of PRMT biology that may important for the exercise-induced remodelling of skeletal muscle.

The effect of cardiolipin fatty acyl side chain composition on cytochrome c peroxidase activity

Jennifer Wilkinson, Sebastian Silvera and Paul J. LeBlanc
Brock University

Duchenne Muscular Dystrophy (DMD) is a debilitating disease resulting in rapid muscle wasting through apoptosis. Apoptosis is related to decreased efficiency of the electron transport chain of mitochondria, leading to increased reactive oxygen species (ROS). Secondly, dystrophic muscle experiences elevated intracellular calcium. Calcium dysregulation and elevated ROS result in the release of pro-apoptotic factors like cytochrome c. It is accepted that peroxidation of cardiolipin (CL) fatty acyl chains is integral in the early steps of apoptosis. This results in the transformation of cytochrome c from an oxidase to a peroxidase that exacerbates CL damage, resulting in cytochrome c release. CL in dystrophic muscle are less unsaturated compared to healthy controls. Additionally, bovine cytochrome c peroxidase activity is altered by CL fatty acyl composition. However, it is unclear the role of CL fatty acyl composition in mediating cytochrome c peroxidase activity in dystrophic muscle. Synthetic liposomes composed of phospholipids to mimic the dystrophic mitochondrial membrane environment will be created to test the effect of CL side chain composition on peroxidase activity. It is hypothesized that the level of CL saturation will alter cytochrome c peroxidase activity.

Development of a novel tool to study bi-directional niche interactions in skeletal muscle

Bella Xu[1,2], Mohsen Afshar Bakooshli[2], Alison P. McGuigan[1,2], Penney M. Gilbert[2,3,4]
[1]Department of Chemical Engineering and Applied Chemistry, University of Toronto; [2]Institute of Biomaterials and Biomedical Engineering, University of Toronto; [3]Department of Biochemistry, University of Toronto; [4]Terrence Donnelly Centre for Cellular and Biomolecular Research, Toronto

In acute skeletal muscle loss caused by surgery, accidents or recovery from medical conditions involving secondary loss of muscle mass, a complete functional recovery is unlikely to be achieved by innate repair. The interactions between muscle regeneration and inflammation have been assumed for decades, and recently the surprising level of coordination between them has been revealed. However, understanding spatial and temporal links between immune system before and after muscle injury is limited by the lack of effective models to recapitulate complicated muscle environment *in vitro,* while enabling simple stratification and acquisition of data from different cell populations. Here, we describe an engineered model Tissue Roll for Analysis of Cellular Environment and Response (TRACER) that provides a simple strategy to control culture heterogeneity, but simultaneously preserves complex cell-cell interplay that is not possible in traditional co-culture system. Importantly, spatially distinct cell populations can be easily and rapidly isolated on demand for analysis. TRACER has enabled recapitulation of many spatial aspects of tumor organization *in vivo.* Now, TRACER is validated to mimic muscle niche interactions based on known knowledge, and then explore bidirectional signaling between muscle progenitors and immune system during muscle tissue maintenance and repair. Identifying the role of inflammation in skeletal muscle tissue will provide new therapeutic targets to improve muscle growth or regeneration following injury.

Passport Program

Interact with and collect a sticker from each of the following event sponsors at their promotional table. Once you have interacted with and collected a sticker from each company (5 stickers total), bring this completed page to the registration desk to be entered into a draw for a cash prize! The draw will take place at the end of the day, following the presentation of the poster awards.

Agilent Technologies

aurora SCIENTIFIC | Performance. Precision. Progress.

ThermoFisher SCIENTIFIC

NOTES

NOTES

NOTES