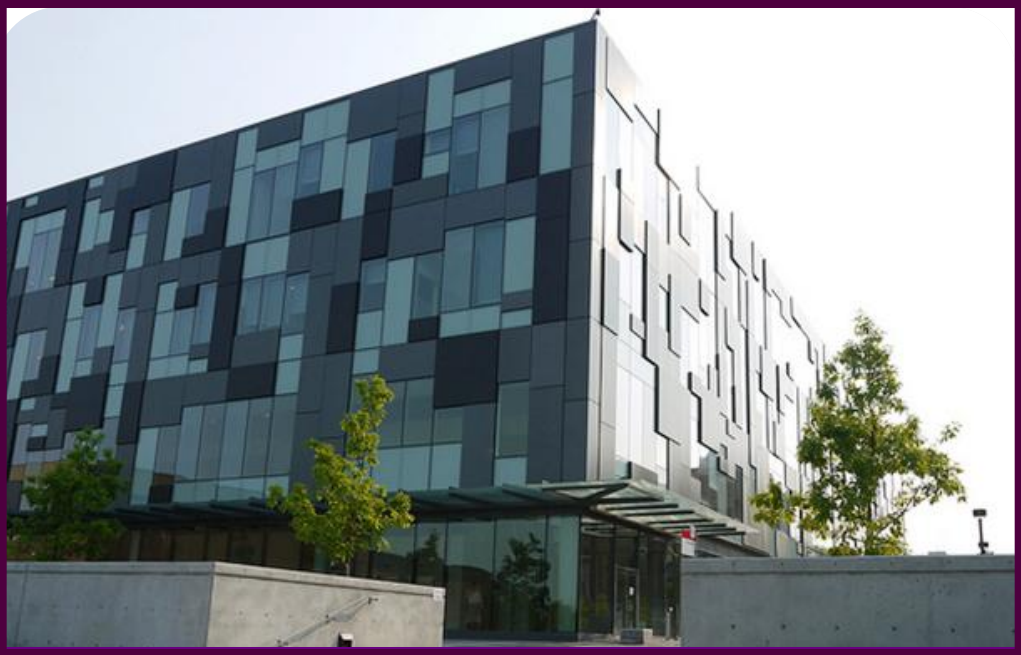
4th Annual
Muscle Health Awareness Day
May 17, 2013



York University

Program and Abstracts

Muscle Health Research Centre (MHRC)

Leading research on muscle adaptations.

YORK UNIVERSITÉ UNIVERSITY

Faculty of Health

Date: May 17, 2013

To: All Participants

From: David A. Hood, MHRC Director

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/

Welcome to the 4th Annual

Muscle Health Awareness Day

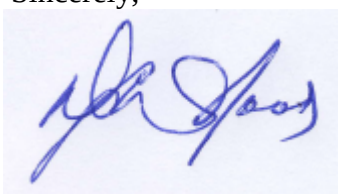
The Muscle Health Research Centre at York University welcomes you to **MHAD4**, our fourth annual "Muscle Health Awareness Day", designed to bring together scientists, faculty members, graduate students and post-doctoral fellows to discuss issues related to muscle physiology, metabolism, adaptation, development and disease.

We have 10 great speakers for **MHAD4**. This year, we have reached further afield. Our participants included faculty members not only from Southern Ontario, but also from upstate New York and Michigan. The focus this year is on 1) muscle lipid metabolism, 2) muscle physiology and pathology, 3) motoneuron-muscle interactions and 4) muscle blood flow.

Our goal is to highlight the work of both junior and senior faculty members, and to give graduate students an opportunity to network and present their work in an informal, yet educational manner. As usual, any feedback for improvement is welcome as we continue to develop and refine the MHAD as a yearly event.

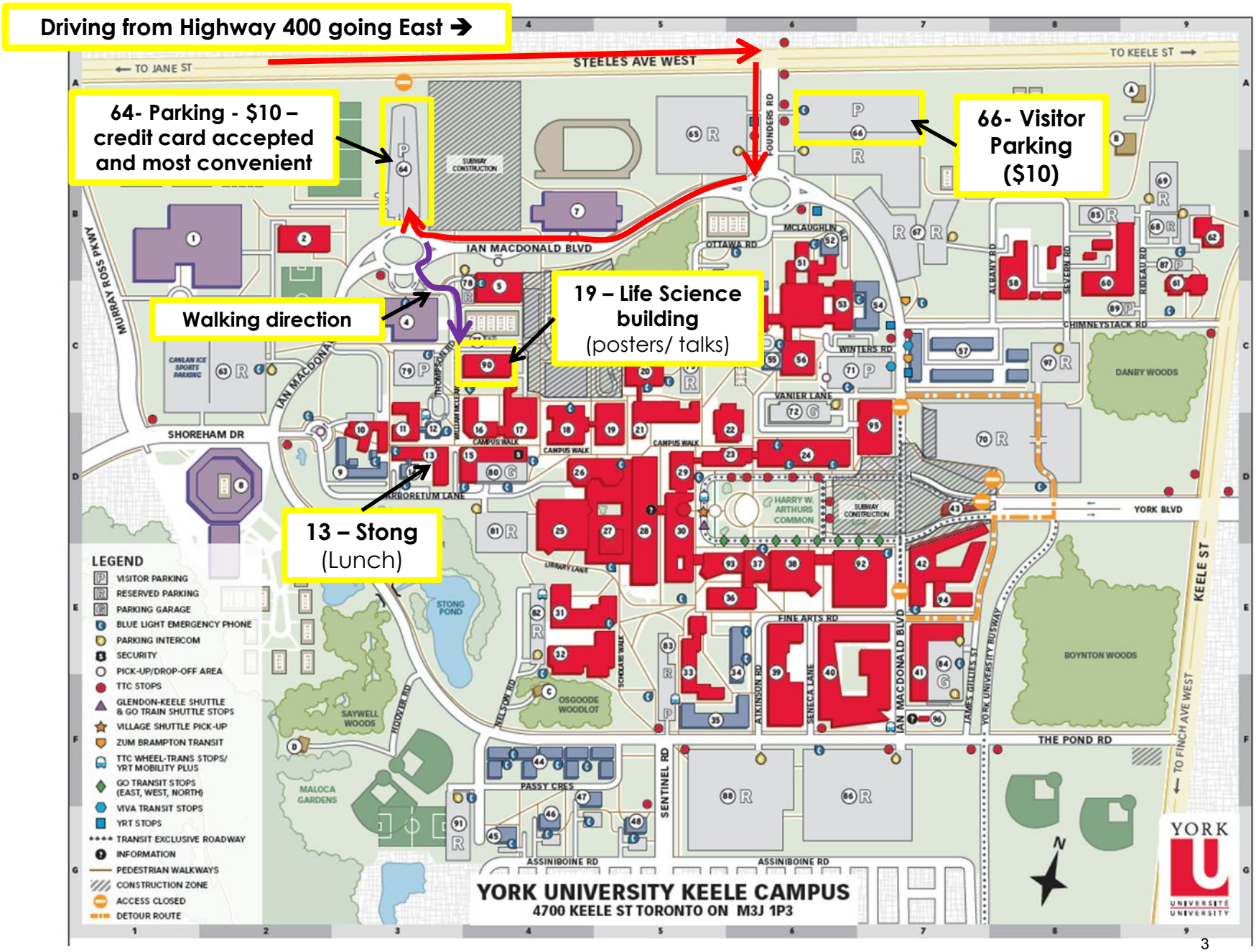
We thank all of our speakers, presenters and volunteers for their participation and for helping to make this a successful event. We hope that you enjoy **MHAD4**!

Sincerely,



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

Sponsorship for the 4th Annual MHAD has been generously provided by:


Driving from Highway 400 going East ➔
64- Parking - $10 – credit card accepted and most convenient
66- Visitor Parking ($10)
Walking direction
19 – Life Science building (posters/ talks)
13 – Stong (Lunch)
TO JANE ST
STEELES AVE WEST
TO KEELE ST
IAN MACDONALD BLVD
MURRAY ROSS PKWY
SHOREHAM DR
ARBORETUM LANE
CAMPUS WALK
VANIER LANE
WINTERS RD
CHIMNEYSTACK RD
DANBY WOODS
YORK BLVD
KEELE ST
FINE ARTS RD
THE POND RD
PASSY CRES
ASSINIBOINE RD
SENTINEL RD
BOYNTON WOODS
SAYWELL WOODS
MALOCA GARDENS
STONG POND
OSGOODE WOODLOT
HARRY W. ARTHURS COMMON
SUBWAY CONSTRUCTION
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
DETOUR ROUTE
YORK UNIVERSITY KEELE CAMPUS
4700 KEELE ST TORONTO ON M3J 1P3
YORK UNIVERSITÉ UNIVERSITY

4th Annual Muscle Health Awareness Day Program
May 17, 2013 – Life Sciences Building Rm.103, York University

8:15 – 9:00 Registration, Poster mounting, and light Breakfast

Session 1: Lipids in Muscle (9:00-10:20)

Chair: Dr. Michael Connor, York University

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

9:05-9:30 – Dr. Jeff Horowitz, University of Michigan
Effects of exercise and diet on muscle lipid metabolism and insulin resistance

9:30-9:55 – Dr. Lawrence Spriet, University of Guelph
Sarcolemmal and mitochondrial membrane fat transport proteins in human skeletal muscle.

9:55-10:20 – Dr. David Williamson, University of Buffalo
Lipid regulation of muscle cell cycle

10:20 – 11:15 Break (POSTER Presenting and Viewing)

Session 2: Muscle physiology and pathology (11:15-12:30)

Chair : Dr. Christopher Perry, York University

11:15-11:40 – Dr. Bernard Jasmin, University of Ottawa
Rationally designing novel therapeutics for Duchenne muscular dystrophy: From basic science discoveries to pre-clinical and clinical studies

11:40-12:05 - Dr. Rene Vandenboom, Brock University
Force Potentiation in Skeletal Muscle: From Mechanism to Function

12:05-12:30 - Dr. Robert Dirksen, University of Rochester
Store-operated Ca2+ Entry and Muscle Fatigue: Mice Run Better with SOCCs

12:30 – 2:30 Lunch (Orange Snail, Stong College); 2:00-2:30 POSTERS

Session 3: Neuroscience and Muscle Blood Flow (2:30-4:15)

Chair: Dr. Robert Tsushima, York University

2:30-2:55 – Dr. Jayne Kalmar, Wilfrid Laurier University
PICs, Pitfalls, and Potentials: Studying Motor Neuron Properties in Humans

2:55-3:20 – Dr. John Grande, Hospital for Sick Children
Calcium-dependent morphological heterogeneity at the neuromuscular junction

3:20-3:45 – Dr. Geoff Pickering, Western University
Stabilizing angiogenesis in ischemic muscle

3:45-4:10 – Dr. Michael Tschakovsky, Queen's University
Individual Differences in Cardiovascular Support of Exercising Muscle: Group Means Are Not Enough!

4:10- Dr. David Hood
Poster Awards, Concluding Remarks

4th Muscle Health Awareness Day - Poster Presentations			
Poster #	**First Author (last name)**	**Title**	**University affiliation**
1	Aiken	**MHAD POSTER AWARD FINALIST - MSc** Exercise training stimulus triggers the expression of the oncoprotein Human Double Minute-2 in human skeletal muscle	York University
2	Abbasi	**MHAD POSTER AWARD FINALIST - MSc** ERp44 Expression During Zebrafish and Mouse Embryogenesis and Heart Development	University of Toronto
3	Anillo	Effects of cardiomyopathy mutations in subdomain 1 of α-cardiac actin (ACTC) on actomyosin contraction	University of Guelph
4	Arpino	**MHAD POSTER AWARD FINALIST - PhD** The Robust Angiogenic Response to Hindlimb Ischemia in Mice Yields a Microvasculature that is Structurally and Functionally Abnormal	Western University
5	Beaudry	Voluntary wheel running alleviates glucose intolerance and insulin insensitivity induced by high corticosterone and high-fat feeding in rats	York University
6	Bentley	Individual vasodilatory response heterogeneity during progressive forearm exercise: evidence for vasodilator phenotypes	Queen's University
7	Castellani	Interleukin-6 and adipose tissue insulin resistance during the recovery from exercise	University of Guelph
8	Chen	Effect of Endurance Exercise on Mitochondrial Function in WT and Sirtuin-1 KO mice	York University
9	Crilly	Circadian regulation of mitochondrial content in skeletal muscle	York University
10	Dunford	**MHAD POSTER AWARD FINALIST - PhD** Regular Exercise Improves Insulin Sensitivity and Glucose Tolerance in Rats Fed a High-Fat Diet Combined with Elevated Glucocorticoids.	York University
11	Foley	**MHAD POSTER AWARD FINALIST - MSc** Training induced adaptation of interhemispheric inhibition	Wilfrid Laurier University
12	Green	Effects of AMPK Activation on C2C12 Cells Depleted of mtDNA	York University

13	Herbst	Human Omega-3 Supplementation Alters Mitochondrial Membrane Composition and Respiration Kinetics Independent of OXPHOS protein content	University of Guelph
14	Izaddoustdar	Increased atrial arrhythmia susceptibility induced by intense chronic exercise requires TNFα	University of Toronto
15	Jain	High-fat diet-induced mitochondrial biogenesis is regulated by mitochondrial derived reactive oxygen species activation of CaMKII	University of Guelph
16	Kitaoka	Compensatory Energy Transfer Pathways in Glycogen storage disease type V (McArdle disease)	McMaster University
17	Kuntz	Systemic Inflammation and Resistance Training in Young and Older Men and Women	McMaster University
18	Liu	Regulation of exercise induced endothelial sprout formation by FoxO1/3/4	York University
19	Ljubicic	Chronic metformin treatment induces beneficial adaptations in dystrophic skeletal muscle	University of Ottawa
20	Ludzki	Mitochondrial transcription factor A is redistributed to the mitochondria in ZDF rats	University of Guelph
21	MacPherson	**MHAD POSTER AWARD FINALIST - PhD** Unlike adipose tissue, skeletal muscle PLIN phosphorylation is not necessary to initiate lipolysis	Brock University
22	Maeda	Programmed Cell Death 4 (PDCD4) is a critical regulator of muscle cell differentiation	York University
23	Mandel	The role of TIMP-1 on vascular remodeling in response to hind-limb ischemia	York University
24	Matravadia	**MHAD POSTER AWARD FINALIST - PhD** Impact of LA and ALA supplementation on insulin sensitivity and mitochondrial function in obese Zucker rats	University of Guelph
25	Memme	Denervation-induced adaptations in autophagy and mitochondrial morphology proteins.	York University
26	Miadovnik	Effects of Sport-Specific, Intermittent High-Intensity Exercise on Post-Exercise Heart Rate Variability and Glycemia in Young Athletes with Type 1 Diabetes.	York University
27	Natividad	Expression and characterization of SNARE proteins in cardiac myocytes and fibroblasts: role in constitutive ANP and BNP secretion	York University

28	Paglialunga	Divergent tissue specific mitochondrial respiration and reactive oxygen species (ROS) emissions rates with high-fat feeding in skeletal muscle and white adipose tissue	University of Guelph
29	Powers	Cortical mechanisms of fatigue and stability following a concussion	Wilfrid Laurier University
30	Ravel-Chapuis	The RNA-binding protein Staufen1 is increased in DM1 skeletal muscle and promotes alternative pre-mRNA splicing.	University of Ottawa
31	Root-McCaig	**MHAD POSTER AWARD FINALIST - MSc** The Regulation of the Hepatokine Follistatin in Mammalian Liver in Response to an Acute Injection of Epinephrine	University of Guelph
32	Shahsavar	Vitamin D3 deficiency increases cellular stress and death in ALS	York University
33	Simnett	The Effect of TBC1D1 Ablation on Metabolism in Rats	University of Guelph
34	Singh	The role of ß-catenin on apopoptotic susceptibility and matrix turnover in the heart after myocardial infarction.	University of Toronto
35	Smith	Force potentiation in the absence of myosin regulatory light chain phosphorylation is concurrent with elevations in resting cytosolic Ca2+.	University of Waterloo
36	Taheri-Shalmani	Is vitamin D3 at 50x the adequate intake toxic in ALS?"	York University
37	Tryon	Effect of denervation on the expression of Tfam in rat skeletal muscle	York University
38	Uchida	Myocyte-derived VEGF regulates vascular adaptations to increased blood flow in skeletal muscle	York University
39	Vandenberk	Contaminating effects of other motor neuron properties on estimates of persistent inward currents in humans	Wilfrid Laurier University
40	Wales	ChIP-exo analysis reveals novel and conserved MEF2 target genes in C2C12 and cardiomyocytes	York University
41	Walsh	Neurotrophic Growth Factor Response to Lower Body Resistance Training in Older Adults	Queen's University
42	Wang	Myocyte-derived VEGF regulates adaptations to increased, but not decreased blood flow in the skeletal muscle vasculature	York University

43	Wang	ERp44 deficiency in mice, zebrafish and mouse embryonic stem cell derived cardiomyocytes display aberrant Ca2+ homeostasis, ER stress-induced apoptosis, and cardiomyopathy	University of Toronto
44	White	A comparison of inflammatory and functional effects of different cold-water immersion protocols for recovery from high-intensity sprint exercise	University of Toronto
45	Zaharieva	Exploring the process of psychosocial development and glycemic control in youth with type 1 diabetes mellitus (T1DM) attending a unique diabetes sports camp.	York University

ERp44 Expression During Zebrafish and Mouse Embryogenesis and Heart Development

Cynthia Abbasi1*, Dingyan Wang1*, Suzan El-Rass2, Judy Quiang2, Peter Backx1, Brian Cox1, Xiao-Yan Wen2 and Anthony O. Gramolini1
1Department of Physiology & 2Department of Medicine, University of Toronto, Toronto, Ontario, Canada

Endoplasmic reticulum resident protein 44 (ERp44), also known as thioredoxin domain-containing protein 4 (TXNDC4) is a novel unfolded protein response (UPR)-induced endoplasmic reticulum (ER) protein that is a member of the Protein Disulphide Isomerase (PDI) family. Zebrafish ERp44 protein has 78% homology and mouse ERp44 protein has 93% homology to human ERp44 protein and are abundantly expressed during embryonic development as demonstrated by RNA in situ hybridization. Previous proteomic results have shown an eight-fold increase of ERp44 in various models of heart disease. Thus, we were interested in examining the role and expression of ERp44 in the heart and embryonic development and postnatal life. To investigate the role of ERp44 in cardiac development in vivo, we have knocked down the zebrafish ERp44 gene in developing zebrafish embryos by gene-specific morpholinos (MOs) and generated an ERp44 knockout mice by gene trapping. RT-PCR and immunoblotting demonstrated that zebrafish ERp44 is wildly expressed during embryonic development and adult tissues. The ERp44 knockout mouse was developed using a LacZ reporter gene inserted into the first intron of the ERp44 locus by gene trapping. LacZ staining, immunofluorescence, and western blot were the three main techniques used to investigate the ERp44 expression during embryogenesis and heart development in zebrafish and the mice. Microinjections of both gene-specific MOs resulted in retarded embryonic development, pericardial edema and slowed blood circulation. Blood retention was prevalently observed in MO-injected embryos. Heart chambers were enlarged and cardiac looping was abnormal. The survival rate of the ERp44 adult knockout (KO) mice is significantly low (<0.05%) and a high embryonic lethality is observed with majority of dead KOs being observed at E12.5. LacZ expression was observed during embryogenesis (E7.5 – E14.5). At embryonic stage E7.5-E11.5, LacZ expression was found in the whole body embryo and a higher expression was observed in the heart. LacZ expression in embryos declined from E13 to E19.5. However, strong expression was still observed in both the ventricle and atria of an E12, postnatal day1, and day7 heart. Interestingly, ERp44 expression was decline in the myocytes of the adult heart and localized to the epicardium myocardium region. Cardiomyocytes and fibroblasts were isolated from neonatal and adult mice and double stained with lacZ ß-galactosidase MarkerGene TM fluorogenic substrate FDG, cardiac cell marker anti-TroponinT and fibroblast marker anti-Vimentin. We found a high LacZ expression in the neonatal cardiomyocytes and fibroblasts but declining expression in the adult cardiomyocytes and fibroblasts. The above results have also been confirmed with western blot. ERp44 is expressed in various muscle cell types during embryonic development and in postnatal life. These results suggest that ERp44 plays a significant role in early embryonic stages and may be critical for heart development and functioning in early embryonic development.

Exercise training stimulus triggers the expression of the oncoprotein Human Double Minute-2 in human skeletal muscle

Julian Aiken1, Dara Slopack1, Fares Gouzi2, Jacques Mercier2, Tara L. Haas1,
Maurice Hayot2, Thomas Gustafsson3, Olivier Birot1 and Emilie Roudier1
1Angiogenesis Research Group, York University, Toronto, ON, Canada
2Clinical Physiology, University of Montpellier, Montpellier, France
3Clinical Physiology, Karolinska Institute, Stockholm, Sweden

High expression levels of Human Double Minute-2 (Hdm2) are often associated with an increased risk of cancer. Hdm2 is well established as an oncoprotein exerting various tumorigenic effects. Conversely, the physiological functions of Hdm2 in non-tumour cells and healthy tissues remain largely unknown. We have previously demonstrated that exercise training stimulates expression of Mdm2, the murine analogue

of Hdm2, in rodent skeletal muscle and that Mdm2 was required for exercise-induced muscle angiogenesis (Roudier et al., FASEB J 2012). Here we show that exercise training stimulated the expression of Hdm2 protein in human skeletal muscle from +38 to +113%. This robust physiological response was observed in 60-70% of the subjects tested, including men and women, as well as young and senior subjects. In young male subjects, post-training changes in Hdm2 were correlated positively with changes in Platelet Endothelial Cell Adhesion Molecule-1, an indicator of the level of muscle capillarisation. Interestingly, a concomitant decrease in the tumour suppressor FoxO1 levels did not occur with training although Mdm2/Hdm2 is known to inhibit FoxO1 expression in diseased skeletal muscle. This could suggest that Hdm2 has different targets when stimulated in a physiological context and that exercise training could be considered therapeutically in the context of cancer in combination with anti-Hdm2 drug therapies in order to preserve Hdm2 physiological functions in healthy tissues.

Effects of cardiomyopathy mutations in subdomain 1 of α-cardiac actin (ACTC) on actomyosin contraction

Maria Anillo and John F. Dawson
University of Guelph

Cardiovascular disease is a global health problem. To prevent failure, the heart compensates by undergoing morphological and functional changes to the left ventricular myocardium, manifesting as either hypertrophic (HCM) or dilated (DCM) cardiomyopathies. Mutations in genes encoding sarcomeric proteins are linked to the development of primary cardiomyopathies. Thus far, the α-cardiac actin (ACTC) gene has 12 known mutations leading to HCM and 2 contributing to DCM. Of particular interest are four ACTC missense mutations (E99K, R95C, H88Y, F90del) located in subdomain 1 of actin protein subunits at the proposed actomyosin interface. These mutations may form weaker actomyosin interactions that lead to decreased force generation and transmission, paving the way to cardiomyocyte remodeling. To determine the effects of ACTC protein variants on actomyosin interaction, ACTC protein variants are expressed with baculovirus/Sf21 cells and purified by affinity chromatography. The intrinsic properties of the ACTC variants are then determined, including protein stability and F-actin polymerization measurements using circular dichroism and pyrene fluorescence. Actomyosin interactions are tested using in-vitro motility (IVM) assays and NADH-coupled ATPase assays. The results of this will help elucidate the molecular interactions contributing to HCM development and better management of cardiomyopathy disease states.

The Robust Angiogenic Response to Hindlimb Ischemia in Mice Yields a Microvasculature that is Structurally and Functionally Abnormal

John-Michael Arpino, Zengxuan Nong, Hao Yin, Fuyan Li, Stephanie Milkovich, Christopher G. Ellis, J. Geoffrey Pickering.
Department of Medical Biophysics, Schulich School of Medicine and Dentistry, Robarts Research Institute, University of Western Ontario, London, ON, Canada, N6A 5K9

Background: The development of new blood vessels in ischemic tissues is critical to tissue regeneration and occurs vigorously in the mouse following hindlimb ischemia. However, the extent to which a capillary network is functionally restored is unknown. This is partly because strategies for evaluating the microvascular flow in regenerating tissue are limited. Methods/Results: We have developed a novel system for evaluating neo-microvascular structure, remodeling, and function, following extensor digitorum longus (EDL) muscle obliteration and regeneration induced by infarction. We subjected male C57BL/6 mice to proximal femoral artery excision and undertook intravital video microscopy of the surface microvasculature of the extensor digitorum longus (EDL) muscle. Microvascular flow was delineated over 28 days using UV-fluorescence (330-385 nm) imaging and following intravenous injection of FITC-Dextran (460-490 nm). Microvascular flow was captured live at a video frame rate of

21 frames/second. This revealed a complete absence of microvascular flow along the entire surface of the EDL muscle for 4 days following femoral artery excision. On day 5, perfusion in the distal muscle started, but in dilated channels that were up to 6-fold wider than normal capillaries with very slow flow rates, ranging from 0-47% of red blood cell flow in baseline control capillaries (136±133 μm/sec). By day 7, flowing microvessels existed throughout the EDL surface and the markedly dilated vessels were no longer evident. By day 14, microvascular length density returned to baseline (0.0135±0.0003 μm microvssel/μm2), however branch prevalence was elevated (3.2±06 vs 2.1±0.4 branch points/mm of microvessel, $P<0.05$). As well, large-caliber arteriolar vessels were found running parallel to the EDL surface, uncharacteristic of control networks, and some of which were seen to flow directly into venous structures, bypassing a capillary network. Finally, mean microvascular flow increased to only 77.7% of baseline capillary flow (458±228 um/sec) and displayed less regional variability than in non-ischemic muscle. Conclusion: Following mouse femoral artery excision, a flowing microvascular network forms after an extended period of no-flow, but with vessels that initially bear no resemblance to capillaries. These channels rapidly remodel into a highly branched microvascular network with aberrant flow patterns. Thus, despite the return of flow to ischemic muscle, the microvasculature is abnormal. This vital limitation to post-natal angiogenesis needs to be considered when designing strategies for tissue regeneration.

Voluntary wheel running alleviates glucose intolerance and insulin insensitivity induced by high corticosterone and high-fat feeding in rats

Beaudry JL, Dunford, E, Zaharieva, D and Riddell MC.
School of Kinesiology and Health Science, York University, Toronto, ON.

Corticosterone (CORT) and a high-fat diet (HFD) both induce severe insulin resistance however, rarely in concert have they been investigated. Regular exercise alleviates insulin resistance and preserve β cell mass. Here we examined the short-term effect of these stressors and voluntary exercise on β cell dynamics in male Sprague-Dawley rats (~6 weeks) given CORT pellets (400 mg/rat) or wax pellets (control) and placed on a HFD or standard diet (SD). After one week of treatment, fasted blood glucose levels in CORT-HFD rats were significantly elevated (>11 mM) and animals were more insulin resistant than controls indicated by HOMA-IR levels (15.1±1.64 vs 1.0±0.12, $p<0.05$), whereas HOMA-β indicated lower β cell function compared to CORT-SD animals (3.72±0.64 vs 1.64±0.22, $p<0.05$). β and α cell mass were 1.5- and 0.6-fold higher, respectively, in CORT-HFD treated animals compared to controls (both $p<0.05$). Voluntary wheel running showed that CORT-HFD animals had significantly less visceral adiposity (19.9±1.46 vs 29.43±2.25g/kg, $p<0.05$) than controls. Compared to sedentary, exercising animals also had improved fasting glycemia (13.64±2.097mM vs 20.36±0.73mM, $p<0.05$) and improved glucose tolerance (600.1±121.5 vs 988.4±137.9 AUC following oral glucose load, $p<0.05$). Insulin sensitivity was significantly improved in running CORT-HFD animals compared to sedentary CORT-HFD animals (53.1±12.0 vs 92.3±26.4mmol/L). We conclude that CORT and HFD induce severe insulin resistance that overwhelms β cell function. However, exercise intervention in this model greatly improves glucose and insulin tolerance resulting in better glucose control and body fat composition.

Individual vasodilatory response heterogeneity during progressive forearm exercise: evidence for vasodilator phenotypes

Robert F. Bentley, J. Mikhail Kellawan, Jackie S. Moynes, Veronica Poitras, Jeremy J. Walsh, Michael E. Tschakovsky. Queen's University, Kingston, Ontario, K7L 3N6

PURPOSE: To determine whether vasodilator response phenotypes are present during a perfusion pressure induced perturbation to exercising muscle oxygen delivery (O2D). METHODS: 10 healthy male subjects (19.5±0.4 yrs) completed two trials of progressive handgrip exercise to exhaustion (2.5kg increments every 3.5 mins) in each forearm above and below heart level (forearm arterial perfusion

pressure (FAPP) Δ 29.5±0.97mmHg). Forearm blood flow ((FBF (ml/min); brachial artery Doppler and echo ultrasound), mean arterial blood pressure (MAP (mmHg); finger photoplethysmography) and O2D (ml/O2/min; venous effluents) were measured at the end of each work rate (WR). RESULTS: Group level, Δ FBF was compromised beyond the 5kg WR in above vs. below. There was no vasodilatory (P=0.21) or exercise pressor (P=0.63) response, and submax O2D, submax and peak VO2 and peak WR were compromised by reduced FAPP (all P<0.05). In contrast, individual responses revealed compensatory vasodilators (n=6) and those who did not (n=4). Vasodilators blunted the FAPP-evoked reduction in submax O2D and VO2 compared to non-vasodilators (P<0.05), and experienced less of a compromise to peak WR (P<0.05). CONCLUSIONS: In the current model, vasodilatory response phenotypes exist, which determine hypoperfusion susceptibility and the degree to which aerobic metabolism and exercise performance are compromised. NSERC.

Interleukin-6 and adipose tissue insulin resistance during the recovery from exercise

Laura Castellani1, Christopher G.R. Perry2, Jared Root-McCaig1, and David C. Wright1
1 Department of Human Health and Nutritional Sciences, University of Guelph, Guelph Ontario, Canada
2 School of Kinesiology and Health Science, York University, York Ontario Canada

Interleukin-6 (IL-6) expression is increased in adipose tissue after exercise, however, the functional significance of this increase is unknown. The current study aimed to define the relationship between increases in IL-6 signalling and adipose tissue metabolism following a single bout of exercise. Male C57BL/6J mice ran for 2-hours on a motorized treadmill (15 metres/minute, 5% incline). Immediately following exercise IL-6 mRNA expression was elevated in epididymal white adipose tissue (eWAT). This was accompanied by a subsequent increase in IL-6 protein content and SOCS-3 mRNA 4 hours after exercise. At this time point circulating IL-6 levels were not elevated. To ascertain a functional association between elevated IL-6 signalling and adipose tissue metabolism we assessed in vivo insulin signalling. Insulin-stimulated Protein Kinase B (PKB) phosphorylation was blunted in eWAT from mice that had run 4 hours previously compared to sedentary controls and this was associated with an attenuated reduction in plasma glycerol and fatty acid levels following insulin injection. Insulin-stimulated PKB phosphorylation was intact in triceps muscle and liver. Our results demonstrate an association between adipose tissue IL-6 signalling and the development of adipose tissue insulin resistance during the recovery from exercise. This may be advantageous in the provision of fatty acids to liver and skeletal to be used as a fuel source, while sparing glucose for glycogen synthesis.

Effect of Endurance Exercise on Mitochondrial Function in WT and Sirtuin-1 KO mice

Chris C.W. Chen, Heather N. Carter and David A. Hood
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Canada, M3J 1P3

The purpose of this study was to examine whether the expression of SirT1 regulates mitochondrial protein import in muscle and whether changes in protein import are related to mitochondrial content and function. We hypothesized that exercise (an increase in energetic demand) protects muscle function, in part by stimulating SirT1 activity, thereby improving mitochondrial protein import. Protein import was measured using WT (n=4) and SirT1 KO mice (n=5) that underwent a voluntary wheel running protocol for 10 weeks. We compared the amount of precursor protein import in intermyofibrillar (IMF) mitochondria isolated from the hindlimb muscles of control and trained mice. Training led to augmentations in protein import after 10 weeks of training in both WT and Sirtuin-1 KO mice, respectively. This was accompanied by an increase in mitochondrial yield and whole muscle cytochrome c oxidase activity. Furthermore, training led to improvements in State 3 (active) respiration and reductions in reactive oxygen species (ROS) production. Our findings suggest that endurance exercise can rescue mitochondrial protein import and content when Sirtuin-1 is deficient. Future research will examine exercise-related changes in mitochondrial protein import machinery components of WT and Sirtuin-1 KO mice.

Circadian regulation of mitochondrial content in skeletal muscle

Matthew J. Crilly, Jonathan M. Memme, Stephen Pastore and Dr. David A. Hood
Muscle Health Research Centre, York University

Circadian rhythms are known to govern a wide variety of biological processes, however, their influence in regulating mitochondrial content remains unclear. The present study sought to investigate the regulation that circadian rhythms may exert on mitochondria within skeletal muscle under basal conditions. This was accomplished by removing the soleus muscle at four distinct time points throughout the day (12am, 6am, 12pm, 6pm) and determining the concentration of proteins crucial for organelle biogenesis and mitophagy in conjunction with measuring COX enzyme activity. Within muscle, mitochondria exhibit circadian variation which is characterized by a 19% (6pm vs. 12am) increase in COX enzyme activity. Furthermore, there appears to be a reciprocal relation between mitochondrial concentration and nuclear PGC1α levels. Although circadian rhythms demonstrate a modest influence on biogenesis markers, there is a more pronounced effect on mitophagy-related proteins. Circadian induction of mitophagy was observed to reach a peak during the afternoon, characterized by marked 2-3 fold increases in LC3II protein during the inactive period. Our data suggest that circadian rhythms regulate mitochondrial content within skeletal muscle through variations in both organelle biogenesis as well as mitophagy.

Regular Exercise Improves Insulin Sensitivity and Glucose Tolerance in Rats Fed a High-Fat Diet Combined with Elevated Glucocorticoids.

Emily C Dunford*, Jacqueline L Beaudry, Ashley Peckett, Dessi P Zaharieva, Michael C Riddell
School of Kinesiology and Health Science, York University, Toronto, ON.

Type 2 diabetes mellitus (T2DM) is associated with elevated free fatty acids (FFAs) and glucocortocoids (GCs), which are naturally occurring stress hormones. Independently, chronically elevated FFAs and GCs are known to reduce skeletal muscle insulin signaling and interfere with glucose uptake, ultimately contributing to the development of T2DM. We have established a new rodent model of T2DM that combines exogenous GC with a high fat diet (HFD) that rapidly induces severe insulin resistance and diabetes with two weeks of treatment. It is known that volitional exercise improves insulin sensitivity and peripheral glucose uptake in T2DM, but the effectiveness of exercise in this new model of GC-induced diabetes is unknown. We hypothesize that exercise will facilitate improved insulin signaling and glucose tolerance in this model. Sprague-Dawley rats were administered exogenous corticosterone pellets and a HFD (60%), and separated into two groups, sedentary and exercise, with the exercise group given voluntary exercise wheels. Elevated GC and HFD animals have decreased type IIb muscle fibre mass and elevated intramuscular triglycerides. At the molecular level, total IRS-1 protein content was greatly decreased (92%; $p<0.01$) with a trend towards reduced phosphorylation of Akt S473 in the gastrocnemius muscle which could account for the severe insulin resistance. When voluntary wheel running was administered, the glucose intolerance and insulin resistance was ameliorated. We conclude from these findings that the improved insulin sensitivity could have been mediated through the combined increases in muscular mass of the epitrochlearis (2-fold; $p<0.0018$), tibialis anterior (15%; $p<0.002$) and plantaris (15%; $p<0.06$) muscles.

Training induced adaptation of interhemispheric inhibition

R. C. A. Foley and J. M. Kalmar
Wilfrid Laurier University

Mirror activation is the unintended contraction of contralateral homologous muscles of the opposite hand during a voluntary unimanual contraction. Changes in interhemispheric inhibition (IHI) play a role in the acquisition of unimanual and bimanual tasks. PURPOSE: The purpose of this study was to determine whether alterations in IHI would contribute to reductions in mirror activation during initial learning and

subsequent training of a novel unimanual task. METHODS: The protocol consisted of alternating right and left hand ramps to 50% maximal index finger abduction force. A task was devised that made force production by the left first dorsal interosseous muscle (FDI) more difficult if there was an unintended contraction of the right FDI. This was achieved by subtracting right force from the left force output. Participants were randomly assigned to a control (CON, n=9) or a training group (TRAIN, n=9). We hypothesized that IHI would increase and mirror activation would decrease after three days of training. Paired-pulse transcranial magnetic stimulation (10ms and 40ms interpulse intervals) was used to quantify IHI of the left motor cortex by the right. IHI was assessed 500ms prior to 5% of maximal contractions of the left FDI. RESULTS: The TRAIN group, who were told that right force was subtracted from left force, had higher mirror activation than CON at the onset of the first day of training. Lower mirror activation was not correlated with higher IHI at this time point. Mirror activation was lower initially in the CON group but was not significantly reduced on the first day. After the 3 training days, the TRAIN group had a further reduction in mirror activation where the CON group had no further reduction ($p=0.07$). IHI increased following 3 additional days of training and was significantly correlated with a decrease in mirror activation ($r=.811$, $p=.008$). CONCLUSION: When participants were aware of the strategy required to maximize force, mirror activation was immediately reduced during this novel unimanual task, due to increased IHI.

Effects of AMPK Activation on C2C12 Cells Depleted of mtDNA

Alex E. Green, David A. Hood
Muscle Health Research Centre, School of Kinesiology and Health Science, York University

Mitochondria are the primary site of cellular energy production. The structure and function of mitochondria depend on the coordinated expression of both the nuclear genome and the maternally-inherited mitochondrial genome. The mitochondrial genome is contained in multiple copies of 16.1 kb mitochondrial DNA (mtDNA). Each molecule contains the required information to encode 13 essential electron transport chain (ETC) proteins. When mtDNA is depleted, this results in mitochondrial depletion syndrome (MDS). This is characterized by a decrease in ETC activity and a disruption of cellular energy homeostasis. It particularly affects cell types that depend on oxidative phosphorylation for energy production, such as skeletal muscle, heart and brain. 5' AMP-activated protein kinase (AMPK) is an energy sensing kinase that can initiate the formation of new mitochondria, as well as the degradation of old or dysfunctional organelles. We hypothesized that treating cells harbouring low numbers of mtDNA with an AMPK activator (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AICAR) would ameliorate the mitochondrial dysfunction and improve mtDNA copy number. Thus, we developed murine myoblasts (C2C12 cells) depleted up to 87% of control levels of mtDNA by long-term treatment with ethidium bromide. We characterized the depletion of these cells and selected clones containing 34% (Dep-34) and 13% (Dep-13) of control mtDNA levels. We treated these cells for 24 hours with 1mM AICAR to activate AMPK. AICAR decreased cell proliferation by 49%, 58%, and 56% in control, Dep-34, and Dep-13 cells, respectively. AICAR increased mitochondrial mass and membrane potential in control cells, had no effect in Dep-34 cells, and decreased both in Dep-13 cells. Furthermore, reactive oxygen species, as measured by DCF fluorescence, decreased in control cells, did not change in Dep-34 cells, and increased in Dep-13 cells following AICAR treatment. Thus, the activation of AMPK improved mitochondrial mass and function in the presence of normal, healthy levels of mtDNA, but had deleterious effects on organelle function and content under conditions in which mtDNA was severely depleted. The downstream mechanisms responsible for these effects within the biogenesis or mitophagy pathways remain to be determined.

Human Omega-3 Supplementation Alters Mitochondrial Membrane Composition and Respiration Kinetics Independent of OXPHOS protein content

Eric A.F. Herbst, Christopher Gerling, Jamie Whitfield, Kazutaka Mukai, Adrian Chabowski, Lawrence L. Spriet, and Graham P. Holloway
University of Guelph

Supplementation of Omega-3 PUFAs has been hypothesized to change membrane composition and therefore have a potential effect on mitochondrial respiratory function and reactive oxygen species (ROS) production. To test this, human subjects (n=18) were placed on fish oil supplements for 12 wks with a total intake of 2g EPA and 1g DHA per day. Skeletal muscle biopsies were taken prior to (Pre) and following (Post) supplementation and used to examine changes in mitochondrial membrane phospholipid composition, mitochondrial pyruvate and ADP kinetics, mitochondrial ROS emission, OXPHOS protein content, and lipid peroxidation. Omega-3 incorporation into mitochondrial membranes increased ~300%, particularly in phosphatidylcholine and phosphoethanolamine phospholipid fractions, without altering total mitochondrial phospholipid content. In permeabilized muscle fibres, pyruvate supported respiratory kinetics were unaltered by Omega-3 supplementation, and total cellular expression of OXPHOS proteins were not altered, suggesting an absence of mitochondrial biogenesis or changes in electron transport chain function. In contrast, ADP titrated in the presence of pyruvate demonstrated increased sensitivity to ADP (decreased Km) independent of the presence of creatine, suggesting changes at the level of adenine nucleotide transporter (ANT) or changes in ATP synthase function/sensitivity. However, creatine also lead to enhanced submaximal and maximal respiration (Vmax) as well, suggesting enhanced activity of the creatine kinase shuttle. In addition, Omega-3 supplementation increased the maximal propensity for in vitro mitochondrial ROS emission ~30% without changing lipid peroxidation in vivo, as determined by 4HNE content. These data suggest that Omega-3 supplementation effects mitochondrial membrane structure and improves maximal ADP kinetics in human skeletal muscle, while future work will determine whether changes in ADP sensitivity are a result of changes in the ANT protein or post-translational modification of components of the electron transport chain.

Increased atrial arrhythmia susceptibility induced by intense chronic exercise requires TNFα

Roozbeh Aschar-Sobbi*1,2, Farzad Izaddoustdar*1,2, Adam S. Korogyi1,2, Gerrie P. Farman3, David Dorian1, Jeremy A. Simpson4, Jari M. Tuomi5,#, Brian Cox1,6, Paul Dorian7,8,9 and Peter H. Backx1,2.
1Department of Physiology, University of Toronto, Ontario, Canada
2University Health Network, University of Toronto, Ontario, Canada
3Department of Physiology and Biophysics, Boston University, Boston, Massachusetts
4Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada
5Department of Physiology and Pharmacology, Western University, London, Ontario, Canada
6Program in Developmental and Stem Cell Biology, Hospital for Sick Children Research Institute, Toronto, Ontario, Canada
7Division of Cardiology, University of Toronto, Toronto, Ontario, Canada
8Department of Medicine, University of Toronto, Ontario, Canada
9St. Michaelâ€™s Hospital, Toronto, Ontario, Canada
#Present Address: Northern Ontario Medical School, Sudbury, Ontario, Canada
*These authors contributed equally

Atrial fibrillation (AF) is the most common arrhythmia and results in reduced quality of life, elevated risk of stroke and increased mortality. Recent evidence has linked long term intense endurance exercise to a 4–5 fold increase in AF incidence, despite improving ventricular function and decreasing mortality rates. The underlying mechanism for this link is unknown. We found that chronic intense swimming or running increased AF vulnerability in mice without promoting ventricular arrhythmias, in association with

lowered heart rates and improved ventricular function. Six weeks of exercise also caused macrophage infiltration, fibrosis, and slowed conduction in atria, but not in ventricles. Since acute bouts of exercise induced marked elevations in both atrial pressure and serum levels of the mechanosensitive inflammatory cytokine, tumor necrosis factor-α (TNFα) we explored the possible involvement of TNFα in the exercise-induced atrial remodeling. TNFα inhibition (with Etanercept) or TNFα gene ablation completely prevented atrial remodeling and AF inducibility, whereas heart rate reductions and ventricular remodeling were not affected. These results establish that exercise induces atrial remodeling and increases AF vulnerability in a TNFα-dependent manner.

High-fat diet-induced mitochondrial biogenesis is regulated by mitochondrial derived reactive oxygen species activation of CaMKII

S. S. Jain1, S. Paglialunga1, A. Ludzki1, E. A. Herbst1, J. S. Lally1, C. Vigna2, A. R Tupling2, P. Schrauwen3, J. Hoeks3, A. Bonen1 and G. P. Holloway1
1Department of Human Health & Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada
2Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada
3Department of Human Biology, Maastricht University, Maastricht, The Netherlands

Calcium/calmodulin dependent protein kinase (CaMK) activation induces mitochondrial biogenesis in response to increasing cytosolic calcium concentrations. Calcium leak from the ryanodine receptor is also regulated by reactive oxygen species (ROS), which are increased with high-fat feeding. Therefore, we examined whether ROS-induced CaMKII-mediated signaling induced skeletal muscle mitochondrial biogenesis in selected models of lipid oversupply. In obese Zucker rats and in high fat-fed rodents, in which muscle mitochondrial content was upregulated, CaMKII phosphorylation was increased independent of changes in calcium uptake, as sarco(endo)plasmic (SR) reticulum Ca2+-ATPase (SERCA) protein expression or activity were not altered, implicating altered SR calcium leak in the activation of CaMKII. In support of this, we find that high-fat feeding increased mitochondrial ROS emission and hydrogen peroxide induced SR calcium leak from the ryanodine receptor and activation of CaMKII. Moreover, administration of a mitochondrial-specific antioxidant (SkQ) prevented high-fat diet-induced phosphorylation of CaMKII as well as the induction of mitochondrial biogenesis. Altogether these data suggest that increased mitochondrial ROS emission is required for the induction of SR-calcium leak, activation of CaMKII, and the induction of mitochondrial biogenesis in response to excess lipid availability.

Compensatory Energy Transfer Pathways in Glycogen storage disease type V (McArdle disease)

Yu Kitaoka1, Daniel I Ogborn1, Nicholas J Mocellin1, Uwe Schlattner2, Mark A Tarnopolsky1.
1McMaster University, Hamilton, ON, Canada, 2University Joseph Fourier, Grenoble, France

McArdle disease (MD) is well known as a metabolic myopathy due to myophosphorylase deficiency. We examined monocarboxylate transporters (MCT) and creatine kinase (CK) protein content in skeletal muscle from 12 patients with MD and 12 age-matched controls, to evaluate potential cellular adaptations to compensate for the loss of glycogenolysis. MD muscle had higher protein content of MCT1 and mitochondrial CK (mt-CK), with no changes in the activities of citrate synthase (CS) and cytochrome c oxidase (COX) and voltage-dependent anion channel (VDAC) protein, as compared with controls. These results suggest that lactate uptake via MCT1 could be important to increase the pyruvate availability, and that mt-CK could be important to maintain phosphocreatine concentration and thus local ATP availability for MD patients, under conditions of reduced energy flux due to inborn errors of metabolism. Collectively, the above data suggest that the proteins related to extra-muscular fuel uptake and intra-muscular energy transduction are up-regulated without change in mitochondrial mass in MD muscle.

Additionally, our findings support the effectiveness of carbohydrate for MD patients, and also highlight a potential role for creatine supplementation in the treatment of this population.

Systemic Inflammation and Resistance Training in Young and Older Men and Women

Alexander Kuntz1, Cameron Mitchell1, Megan Tanzos1, Devonna Truong1, Melissa Merrow1, Sara Oikawa1, Mark Tarnopolsky2, Stuart Phillips1
Department of Kinesiology1, Medicine2, McMaster University, Hamilton, Ontario

Background: Systemic inflammation is associated with an increased risk of numerous chronic diseases. Endurance exercise has been demonstrated to ameliorate systemic inflammation; however, the link with resistance exercise is not well understood and inflammation may affect the hypertrophic response to resistance exercise.
Objective: To investigate the relationship between systemic inflammation and resistance training in young and older, men and women.
Methods: 68 untrained subjects consisting of 16 young males (YM 22.4±2.1yr, BF%=17±7), 19 young females (YF 23.9±3.4yr, BF%=33 ±9), 17 older males (OM 74.4±5.3yr, BF%=28±6) and 16 older females (OF68.0±3.0yr, BF%=41±7) performed resistance exercise thrice weekly for a total of twelve weeks. Resting venous blood draws were taken before and after the twelve weeks of training in addition to five post exercise blood draws occurring at 0, 15, 30 and 60 minutes after a resistance exercise bout. Inflammation was evaluated by measuring the concentrations of interleukin-6 (IL-6) and tumor necrosis factor–alpha (TNF-α). Body composition was assessed using dual energy x-ray absorptiometry (DEXA).
Results: There was an effect of age with greater resting, baseline IL-6 and TNF-α concentrations in the older subjects, relative to young ($p<0.001$) and a sex effect demonstrated by the greater resting, baseline TNF-α concentrations compared to the males ($p=0.011$). Resistance training did not change the resting inflammatory biomarker concentrations. There was a negative correlation between resting, baseline concentrations of IL-6 ($r=-0.343$, $p=0.021$) and TNF-α ($r=-0.360$, $p=0.016$) with gains in fat-free mass.
Conclusions: 12 weeks of resistance exercise (3x/week) is not effective at ameliorating systemic inflammation. Levels of systemic inflammatory markers may influence the response to resistance exercise; this thesis requires further investigation.

Regulation of exercise induced endothelial sprout formation by FoxO1/3/4

Sammy T.K. Liu, Dara Slopack, Emilie Roudier, and Tara L. Haas
School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada

Forkhead Box “O” (FoxO) transcription factors have been shown to negatively regulated the process of capillary growth, but their influence on endothelial sprout initiation is not known. Mice with endothelial cell (EC) directed deletion of FoxO1/3/4 (FoxOΔ) have accelerated angiogenesis in response to repeated bouts of endurance exercise. We hypothesize this occurs due to FoxO regulation of genes such as Delta-like1 (Dll1) and Tyrosine-protein kinase receptor (Tek), which are involved in sprout formation and capillary maturation. EC with a deletion of FoxO1/3/4 showed decreases in Dll1 and Tek mRNA by 0.35 and 0.50 fold, respectively. Dll1 expression was significantly down-regulated in mouse gastrocnemius muscle after 7 and 14 days of treadmill running, coinciding with the period of FoxO down-regulation and capillary sprouting. In contrast, Tek expression was bi-phasic, increasing two-fold in animals trained for 1 and 14 days compared to sedentary counterparts. In FoxOΔ mice, basal Dll1 mRNA was reduced compared to wildtype, and there was no further decrease with training. The up-regulation of Tek seen after 14 days of exercise was abolished in FoxOΔ animals. Our results indicate that Dll1 and Tek are novel FoxO targets, and their regulation with repeated exercise bouts may assist in the process of sprout formation and subsequent capillary maturation.

Chronic metformin treatment induces beneficial adaptations in dystrophic skeletal muscle

V. Ljubicic, J-F. Couvrette, J-M. Renaud, L.A. Megeney and B.J. Jasmin
Department of Cellular and Molecular Medicine and Centre for Neuromuscular Disease, Faculty of Medicine, University of Ottawa, and the Ottawa Hospital Research Institute, Ottawa, ON, Canada

DMD is the most severe inherited neuromuscular disorder for which there is no effective treatment. Our pre-clinical studies in mdx mice demonstrated that pharmacological activation of PPARβ/δ or AMPK elicits a fiber type shift towards the slower, more oxidative (SO) phenotype together with utrophin A upregulation thereby attenuating the dystrophic pathology (HMG, 18:2640, 2009; HMG, 20:3478, 2011; AJP Cell 302:C110, 2012). The challenge now is to identify agonists of these pathways that have more immediate clinical relevance. The purpose of this study was to investigate whether AMPK stimulation via metformin (MET), a front-line drug in the treatment of type 2 diabetes, induces beneficial muscle remodeling. In C2C12 cells, MET augmented expression of PGC-1α, a master regulator of the SO myogenic program, as well as utrophin A levels. MET treatment of mdx mice for 6 weeks resulted in an increase in PGC-1α in fast skeletal muscle with a concomitant reduction of the transcriptional corepressor RIP140. Expression of utrophin A was also augmented after MET administration in vivo. The data clearly suggest that MET-induced reciprocal changes in PGC-1α and RIP140 expression are linked to elevations in utrophin A content. As these adaptations are expected to be highly beneficial to dystrophic fibers, we propose that MET represents a novel and promising therapeutic avenue for DMD.
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Mitochondrial transcription factor A is redistributed to the mitochondria in ZDF rats

Alison C. Ludzki, Brennan K. Smith, Jamie S.V. Lally, Brendon J. Gurd, Arend Bonen & Graham P. Holloway, Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario

INTRODUCTION: Mitochondrial transcription factor A (Tfam) is a nuclear-derived protein with a central role in maintaining mitochondrial DNA transcription, replication, and stability. It was recently identified that the subcellular location of Tfam is dynamically regulated during exercise, while similar control of Tfam remains to be determined in a model of insulin resistance. Therefore, we measured the subcellular distribution of Tfam in the insulin resistant ZDF rat at 6 weeks of age. RESULTS: Blood glucose (+2 fold) and insulin (+5 fold) concentrations were increased ($P<0.05$) in the ZDF rat, confirming the presence of insulin resistance. Total cellular protein of Tfam was reduced (-21%) in the ZDF rat. In contrast, Tfam protein was increased in both subsarcolemmal (SS; +26%) and intermyofibrillar (IMF; +47%) mitochondria. Despite this increase, there were no changes in total cellular markers of mitochondrial content (citrate synthase activity, COXIV protein and mitochondrial DNA abundance), or in the accumulation of OXPHOS proteins within isolated SS and IMF mitochondria, suggesting Tfam accumulation within mitochondria did not induce biogenesis. This was further supported by an absence of observed biogenesis in transmission electron microscopy images in either SS or IMF regions. Given the apparent absence of mtDNA replication or transcription we aimed to determine if Tfam translocated to mitochondria in response to oxidative stress as a potential mechanism to stabilize mtDNA. To elucidate this potential relationship, isolated soleus muscles from Sprague-Dawley rats were transiently (20 min) incubated with exogenous H_2O_2 or saline (control). Following H_2O_2 exposure, Tfam protein accumulated within both SS (+41%) and IMF (+49%) mitochondria, indicating ROS is a potential regulator of the dynamic localization of mitochondrial Tfam measured with insulin resistance. CONCLUSIONS: Mitochondrial Tfam redistributes to the mitochondria in insulin resistant ZDF rats. ROS may act as a signal for this redistribution. Therefore, increased mitochondrial Tfam is likely a compensatory adaptation to maintain mitochondrial stability in situations of cellular redox stress.

Unlike adipose tissue, skeletal muscle PLIN phosphorylation is not necessary to initiate lipolysis

REK MacPherson, R Vandenboom, BD Roy, and SJ Peters
Centre for Bone and Muscle Health, Department of Kinesiology, Brock University, St. Catharines, ON, L2S 3A1

Skeletal muscle lipid droplet coat proteins (perilipins or PLINs) are considered to be key regulators of lipolysis, however the mechanisms of this regulation in skeletal muscle remain unknown. In adipose tissue, the access of lipases to the lipid droplet depends on PLIN1 phosphorylation, however PLIN1 is not expressed in skeletal muscle and phosphorylation of the remaining PLINs has yet to be investigated. Therefore, we investigated the isolated and combined effects of epinephrine and contraction on PLIN and ATGL interactions as well as phosphorylation. Briefly, isolated rat solei were assigned to one of four 30min experimental conditions: 1) rest; 2) intermittent tetanic stimulation (150ms volleys at 60Hz with a train rate of 20 tetani per min (25oC)); 3) 5 nM epinephrine; 4) intermittent tetanic stimulation and 5nM epinephrine. Immunoprecipitation of serine phosphorylated proteins followed by western blotting for PLIN2, PLIN3, PLIN5, or ATGL revealed that only PLIN2 is not phosphorylated under any of the experimental conditions. This is the first study to show that in whole skeletal muscle both PLIN3 and PLIN5 are serine phosphorylated, but this is unchanged following either adrenergic and/or contractile stimulation. Further, we found that ATGL phosphorylation did not change from rest with any of the conditions. Oil red O staining of muscle sections for lipid content shows a 46% decrease with contraction, a36% decrease with epinephrine, and a 62% decrease with both (n=4). Co-immunoprecipitation revealed that the ATGL to CGI-58 interaction increased by 43% with contraction, 13% with epinephrine, and 52% with both, however this did not reach statistical significance. PLIN2, PLIN3, and PLIN5 all interact with ATGL, this interaction is unchanged following the experimental conditions. Our results show that in skeletal muscle PLIN2 is not phosphorylated with lipolytic stimulation and that PLIN3, PLIN5, and ATGL phosphorylation is not increased with either epinephrine stimulation or contraction. This indicates that, unlike in adipose tissue, PLIN phosphorylation does not seem to be necessary for skeletal muscle lipolysis.

Programmed Cell Death 4 (PDCD4) is a critical regulator of muscle cell differentiation

Abdikarim Abdullahi, Amir Gilad, Zameer Dhanani, Naomi Maeda, and Olasunkanmi A.J. Adegoke
School of Kinesiology and Health Science, York University, Toronto, ON Canada

The mammalian target of rapamycin complex 1/S6 ribosomal protein kinase 1 (mTORC1/S6K1) pathway is involved in regulating mRNA translation and skeletal muscle mass. It does this in part by inhibiting the tumor suppressor protein, programmed cell death 4 (PDCD4). In C2C12 and L6 muscle cells, we showed that PDCD4 abundance was high on day 1 and then decreased as myoblasts differentiated into myotubes ($p<0.05$). siRNA-mediated knockdown of S6K1 reversed the decrease in PDCD4 abundance and significantly decreased myosin heavy chain 1 (MHC 1) protein abundance, suggesting that PDCD4 regulation was vital for differentiation. Indeed, cells depleted of PDCD4 had reduced MHC abundance, showed delayed myoblast fusion and abnormal myotube formation. On days 3 and 4 of differentiation, myotubes depleted of PDCD4 showed 40-60% reductions in myotube protein synthesis. This study unravels a link between PDCD4 and muscle cell differentiation, and suggests that this mTORC1/S6K1 substrate may be of therapeutic significance for muscle recovery following injury or atrophy. Funded by NSERC.

The role of TIMP-1 on vascular remodeling in response to hind-limb ischemia

E.R. Mandel and T.L Haas
School of Kinesiology and Health Science, York University, Toronto, ON Canada

Vascular remodeling, defined as lasting structural changes to the vessel wall, occurs in response to hemodynamic stimuli. Sprouting angiogenesis and arteriogenesis are two forms of vascular remodeling that allow for the restoration of blood flow to an ischemic area. Both forms of vascular remodeling require the coordinated action of matrix metalloproteinases (MMPs) and their endogenous inhibitors tissue inhibitor of metalloproteinases (TIMPs). We hypothesize that a loss of TIMP-1 will shift the TIMP-MMP balance in favor of MMPs, thereby increasing vascular remodeling in response to hind-limb ischemia. TIMP-1 knock out mice (KO) and wild-type (WT) c57BL/6 mice will undergo femoral artery ligation or sham surgery and will recover for 4, 7, 14 or 21 days. Over the course of recovery, hind-limb blood flow measurements will be made. At the end point, collateral diameters and blood flow will be measured, after which hind-limb muscles will be isolated for further analysis. Preliminary work from WT mice exposed to hind-limb ischemia revealed a small but significant increase in TIMP-1 mRNA and a large increase in MMP-2 mRNA ($p<0.05$ n=4-6), 4 days following ligation. In TIMP-1 KO mice, there is a significant increase in soleus and extensor digitorum longus (EDL) muscle permeability 4 days following femoral artery ligation. This increase correlates with a trend towards an increase in MMP activity of KO but not WT mice. Blood flow recovery post hind-limb ischemia remains significantly lower than baseline after 21 days of recovery in the TIMP-1 KO mice ($p<0.01$, n=6-21), while blood flow is recovered to pre-ligation levels by day 7 in WT mice. A large portion of the recovery likely is due to alterations in the femoral artery collateral arteries. Blood flow through the collaterals is noticeably higher in TIMP-1 KO mice after 14 and 21 days of recovery, compared to sham operated animals. Furthermore, there appears to be an increase in collateral arterial diameter in the ligation compared to control leg after 21 days of recovery. These data indicated that, contrary to our hypothesis, recovery of hind-limb blood flow due to angiogenesis and arteriogenesis appears hindered in TIMP-1 KO compared to WT mice.

Impact of LA and ALA supplementation on insulin sensitivity and mitochondrial function in obese Zucker rats

Sarthak Matravadia, Eric A. Herbst, Swati S. Jain, David M. Mutch and Graham P. Holloway
University of Guelph

INTRODUCTION: Omega-6 (n6) polyunsaturated fatty acids (PUFAs) are thought to be pro-inflammatory and negative in the context of insulin sensitivity, while in contrast omega-3 (n3) fish oil improves insulin sensitivity. However in both cases the mechanisms responsible for these alterations remain to be determined, but may stem from alterations in mitochondrial bioenergetics and oxidative stress. Therefore, we aimed to investigate the role of essential dietary n3 and n6 PUFAs on skeletal muscle insulin sensitivity and mitochondrial function. METHODS: Five week-old lean and obese Zucker rats were fed either control, n3 [α-linolenic acid (ALA)] or n6 [linoleic acid (LA)] enriched diets for ten weeks. Whole-body insulin sensitivity and skeletal muscle insulin signaling was measured. Subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondrial subpopulations were isolated for functional analyses, as well as for the analysis of electron transport chain (ETC) proteins and markers of oxidative stress. RESULTS: Both n3 and n6 diets improved whole-body and skeletal muscle insulin sensitivity in obese rats. Compensatory increases in mitochondrial respiration and OXPHOS protein content were apparent in SS mitochondria from both untreated and ALA supplemented obese animals, while SS mitochondria from LA supplemented animals were not different. In contrast, no changes were observed in IMF mitochondria in any experimental condition. Regardless of these differences in mitochondrial bioenergetics, both ALA and LA normalized 4-hydroxynonenal content, a marker of lipid peroxidation. CONCLUSION: Both LA and ALA improved insulin resistance and oxidative stress in the obese state, but they appear to work independent of positive changes within mitochondrial bioenergetics.

Effects of Sport-Specific, Intermittent High-Intensity Exercise on Post-Exercise Heart Rate Variability and Glycemia in Young Athletes with Type 1 Diabetes.

Lisa Miadovnik*, Michael Riddell, Robert Gumieniak, Chip Rowan, Dessi Zaharieva, Veronica Jamnik
School of Kinesiology & Health Science, York University, Toronto, Ontario

Type 1 diabetes (T1D) is associated with hypoglycemia and premature autonomic disturbance (using heart rate variability [HRV]) – both which have been implicated in sudden death. Chronic exercise improves HRV in individuals with T1D, however the acute effects of a single bout of exercise on subsequent nocturnal HRV remain unknown. We recruited hockey players with and without T1D in order to examine how a single bout of intermittent high-intensity exercise (IHE) impacts nocturnal HRV. HRV was analyzed from 12am–6am following 3 events; a hockey game, an IHE bout on a cycle ergometer, and a non-exercise day. Continuous glucose monitors recorded blood glucose (BG). Lab exercise led to a mean BG decrease of 2.3 ± 3 mmol/L, while the change during a hockey game was insignificant (mean Δ: -0.2 ± 4.2 mmol/L). There was 1 incidence of nocturnal hypoglycemia which occurred after a hockey game (lasting 225 min). No significant differences in HRV were noted between nights, with a trend toward improved HRV after the non-exercise day in all participants. More favourable HRV profiles (higher SDNN, RMSSD, and HF) were observed in T1D participants across all nights ($p > 0.05$). SDNN, used as an estimate of overall HRV, was 50% greater for those with T1D ($p<0.05$), but only after the non-exercise day. Given that a decrease in SDNN has been associated with sudden cardiac death, this increase in SDNN may have a protective benefit in individuals with T1D.

Denervation-induced adaptations in autophagy and mitochondrial morphology proteins.

Jonathan M. Memme, Matthew J. Crilly, Liam D. Tryon, Sobia Iqbal and David A. Hood.
Muscle Health Research Centre, School of Kinesiology and Health Sciences, York University

Chronic muscle disuse induced by denervation results in a reduction of muscle mass as well as mitochondrial content and function. Expression of factors contributing to this physiological response, namely autophagy proteins, has not been well documented in response to denervation during the time frame preceding 7 days. Therefore, we set out to investigate the acute effects of chronic muscle disuse by utilizing a unilateral peroneal nerve axotomy, thus removing the neural stimulus to the tibialis anterior (TA) muscle, in male Sprague Dawley rats. Following a denervation period of either 8 hours, 16 hours, 1 day, 3 days, 5 days or 7 days, TA muscles from both the denervated and contralateral sham-operated leg were excised and used to measure changes in proteins involved in mitochondrial fission and fusion, autophagy, and mitochondrial biogenesis. Chronic disuse resulted in 14% and 39% reductions in muscle mass following 3 and 7 days respectively, with similar decreases in mitochondrial content of 25% and 40%. Autophagy proteins LC3-II and p62 increased slightly by 11-32%, whereas Atg7 and Beclin-1 increased more substantially by 1.5- and 4-fold respectively. As well, the ratio of fission proteins (Fis1 and Drp1) relative to fusion proteins (Mfn2 and Opa1) in isolated mitochondria exhibited an increase by 7 days. Tfam protein expression was measured as a marker of mitochondrial biogenesis, however no significant change occurred following 7 days of denervation. Overall, chronic muscle disuse resulted in an increase in the expression of factors involved in the fragmentation of the mitochondrial networks, along with an up-regulation of the expression of autophagy proteins, thereby contributing to a decrease in mitochondrial content in order to remove damaged or worn-out organelles.

Expression and characterization of SNARE proteins in cardiac myocytes and fibroblasts: role in constitutive ANP and BNP secretion

Nikki Natividad and Robert Tsushima
Department of Biology, York University, Toronto ON M3J 1P3

The hormones atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are stored and secreted in cardiomyocytes within the atria and ventricle of the endocrine heart in Golgi-derived secretory granules within the cell. To date, there is still little information reported regarding the mechanism of their release. Recent studies have identified certain proteins involved in intracellular membrane fusion events (SNARE proteins) in the trafficking of these granules. While SNARE proteins have been identified to control regulated release of ANP, it has yet to be determined which SNARE proteins play a role in the constitutive pathway. In this study, we characterized the age-dependent expression profiles of three SNARE proteins (syntaxin 5A, syntaxin 18, and SNAP29) in rat atrial and ventricular tissue and cells implicated to play a role in constitutive exocytosis. We found that syntaxin 5A protein levels increased with age, while syntaxin 18 and SNAP29 levels declined. We also examined the effect of cardiac hypertrophy on neonatal ventricular myocytes using fetal bovine serum (FBS) or phenylephrine, and transforming growth factor-β to induce the transformation of fibroblasts to myofibroblasts. The effects of hypertrophy on SNARE protein expression in ventricular cells were similar to those seen as age increased, parallel to an increase in ANP and BNP expression. To examine the differences between constitutive and regulated secretion, media and whole cell ANP and BNP secretion levels were measured in untreated ventricular myocytes and myocytes treated with endothelin, respectively. This is the first study examining the SNARE proteins involved in the constitutive secretion of ANP and BNP. Our findings suggest that the SNARE proteins syntaxin 5A, syntaxin 18 and SNAP29 may be involved in constitutive exocytosis of these hormones.

Divergent tissue specific mitochondrial respiration and reactive oxygen species (ROS) emissions rates with high-fat feeding in skeletal muscle and white adipose tissue

Sabina Paglialunga 1, Alison Ludzki 1, and Graham P Holloway 1
1 Department of Human Health and Nutritional Sciences, University of Guelph

Background: Consuming a high-fat (HF) diet increases the risk of developing type II diabetes mellitus, obesity as well as a host of other chronic diseases. In skeletal muscle, insulin resistance, mitochondrial oxidative stress and ROS emissions are exacerbated under HF conditions. Meanwhile, white adipose tissue (WAT) under goes a gross expansion upon HF feeding altering WAT function. The effect of HF feeding and adipocyte dysfunction on mitochondrial bioenegetics and ROS emission in WAT has not been thoroughly investigated. Therefore, the aim of the current study is to determine mitochondrial respiration and ROS emissions rates in WAT of mice under control and HF-fed conditions. Study design: Male C57Bl6 mice (n=4-5, 15 weeks of age) were placed on a standard chow (10% kcal fat) or a HF diet (60% kcal fat) for 3 or 8 weeks. Subcutaneous (SAT) and visceral (VAT) adipose tissue depots as well as red gastrocnemius skeletal muscle fibres were used for analysis. Mitochondrial respiration was measured by high-resolution respirometry. WAT mitochondrial ROS emissions were determined by a fluoremetric assay supported by complex I (pyruvate+malate) and glycerol-3-phosphate (G3P) dehydrogenase substrates. Plasma insulin and lipid analysis remain to be determined. Results: HF feeding significantly increased weight gain compared to chow mice. As anticipated, skeletal muscle mitochondrial respiration was upregulated upon HF feeding. Similarly, VAT mitochondrial respiration was increased with the HF diet at both 3 and 8 weeks, however this was not true for SAT. In contrast, both pyruvate+malate- and G3P- supported ROS emissions were significantly lower in SAT after 8 weeks of chow feeding compared to 3 weeks of chow feeding. Moreover, 3 weeks of HF feeding also reduced SAT pyruvate+malate supported ROS emissions (-37%, $p<0.05$ vs chow). In addition, when normalized to a mitochondrial content marker, VAT G3P-supported ROS emissions were reduced as well upon HF feeding at both 3

weeks (-35%, $p<0.05$) and 8 weeks (-26%, $p=0.06$). Conclusions: The present study highlights the importance of age and diet on adipose tissue metabolism. Depot specific changes were observed with respect to mitochondrial function suggesting site-specific adipose tissue remolding or mitochondrial biogenesis. Surprisingly, HF feeding tended to lower mitochondrial WAT ROS emissions, suggesting a metabolic adaptation to counter the HF insult in adipose tissue. On-going studies will further elucidate this mechanism.

Effects of Mitophagy Inhibition on Mitochondria in Muscle Cells

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

Energy production in mitochondria is attained by a sequence of redox reactions within the electron transport chain (ETC), a process termed oxidative phosphorylation. Intracellular reactive oxygen species (ROS) are a by-product of this process, and are produced in excess when the organelle becomes compromised. Elevated ROS can lead to further organelle damage, leading to mitochondrial dysfunction and loss of membrane potential ($\Delta\Psi m$). Preservation of $\Delta\Psi m$ is critical to the utility of this organelle since it provides the proton-motive force for ATP production. Mitophagy, an essential mitochondrial quality control mechanism, allows for the clearance of dysfunctional organelles. The population of mitochondria is a balance between synthesis (biogenesis) and mitophagy. Endurance exercise has been consistently shown to induce mitochondrial biogenesis. This effect is reproduced in cellular models using chronic contractile activity (CCA). The up-regulation of key mitochondrial markers such as cytochrome c oxidase (COX) are a result of CCA, and indicative of biogenesis. In contrast, the mechanisms of mitophagy in mammalian skeletal muscle have not been well described and little is known about the interaction between mitochondrial biogenesis and mitophagy. The purposes of this study are to understand the role of mitophagy in regulating mitochondrial content and quality, and how this mechanism is influenced by CCA. A chemical inhibitor of lysosomal function, Bafilomycin A1 (BafA), works to avert the binding of the autophagosome to the lysosome in the terminal stages of mitophagy, and is used to assess autophagic flux. We employed a cell culture model using murine C2C12 myotubes exposed to a regimen of CCA (3 hrs x 4 days) in the presence of BafA or vehicle. We then examined various assessments of mitochondrial content and quality. CCA increased COX subunit IV (COXIV) and COX activity by 2-3 fold, indicating organelle biogenesis. A key autophagic marker LC3 presents in two distinct migrating forms, the cytosolic LC3I and its lipidated, phagosome-associated form LC3II. Since conversion of LC3I to LC3II is associated with an increase in the phagosome machinery, it is a useful index of autophagy. BafA (3nM) treatment inhibited autophagic flux as determined by an 8-fold increase in LC3II levels. The inhibitor had no effect on COX activity, but it resulted in reduced state III and state IV respiration. Fluorescent live cell imaging of mitochondria from BafA-treated myotubes revealed a greater density of fragmented organelles. Hence, inhibition of autophagic flux to the lysosome resulted in an accumulation of dysfunctional organelles. CCA also increased LC3II levels, but decreased PINK1, a mitochondrial kinase that allows for the targeting of mitochondria for degradation. Thus, future work is required to continue to unveil the role of chronic exercise in regulating the pathway of mitochondrial degradation in muscle cells.

Cortical mechanisms of fatigue and stability following a concussion

K. Powers, MSc(c), M. Cinelli, PhD & J. Kalmar, PhD
Wilfrid Laurier University, Department of Kinesiology and Physical Education

Altered cortical excitability may impair voluntary muscle activation, increase sense of force and challenge the balance control system. Balance is impaired for 3-5 days post-concussion while cortical hypoexcitability may persist for months. PURPOSE: To determine the relationship between persistent cortical changes and initial balance deficits after concussion. METHODS: Nine concussed (CONC)

varsity football players (20.0 ± 1.1 yrs) were matched with nine non-concussed control (CONT) teammates (20.3 ± 1.4 yrs). Static stability was measured 3-5 days following a concussion using a force plate (45s trials at 50Hz) to assess changes in centre of pressure (COP) displacement and velocity with eyes open (EO) and eyes closed (EC). Transcranial magnetic stimulation was used to assess cortical excitability ~10 days post-concussion. Maximal voluntary activation was calculated using cortical twitch interpolation and sense of force was determined using constant force sensation contractions. CONC and CONT were tested at the same time points. RESULTS: During EO, anterior-posterior RMS of COP velocity (A-P vCOP) was larger ($p=0.029$) for CONC (0.015 ± 0.004 m/s) compared to CONT (0.010 ± 0.003 m/s), implying that CONC have impaired balance control. Collectively, all participants' A-P vCOP were negatively correlated ($r=-0.54$, $p=0.032$) with voluntary activation, suggesting that a decreased ability to activate muscle is associated with a reduced ability to control A-P vCOP during standing. This difference between the groups' A-P vCOP was not observed in the medial-lateral (M-L) vCOP. With EC, A-P vCOP was greater ($p=0.001$) for the CONC (0.026 ± 0.008 m/s) than CONT (0.014 ± 0.008 m/s) and A-P vCOP was negatively correlated ($r=-0.61$, $p=0.012$) with the first rate constant of the double-exponential equation fit to the force-sensation contraction. This relationship suggests that sense of force deficits may contribute to impaired A-P balance. This is likely due to the up-regulation of other sensory information in the absence of vision; however, this strategy is not as effective post-concussion. CONCLUSION: Changes in voluntary activation and force sensation during the recovery phase following a concussion may contribute to impaired A-P balance control during the initial phase.

The RNA-binding protein Staufen1 is increased in DM1 skeletal muscle and promotes alternative pre-mRNA splicing.

Ravel-Chapuis A, Bélanger G, Yadava RS, Mahadevan MS, DesGroseillers L, Côté J, Jasmin BJ.
Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, K1H 8M5, Canada.

In myotonic dystrophy type 1 (DM1), dystrophia myotonica protein kinase (DMPK) mRNAs with expanded CUG repeats (CUGexp) aggregate in the nucleus and become toxic to cells by sequestering and/or misregulating RNA-binding proteins, resulting in aberrant alternative splicing. In this study, we find that the RNA-binding protein Staufen1 is markedly and specifically increased in skeletal muscle from DM1 mouse models and patients. We show that Staufen1 interacts with mutant CUGexp mRNAs and promotes their nuclear export and translation. This effect is critically dependent on the third double-stranded RNA-binding domain of Staufen1 and shuttling of Staufen1 into the nucleus via its nuclear localization signal. Moreover, we uncover a new role of Staufen1 in splicing regulation. Overexpression of Staufen1 rescues alternative splicing of two key pre-mRNAs known to be aberrantly spliced in DM1, suggesting its increased expression represents an adaptive response to the pathology. Altogether, our results unravel a novel function for Staufen1 in splicing regulation and indicate that it may positively modulate the complex DM1 phenotype, thereby revealing its potential as a therapeutic target.

The Regulation of the Hepatokine Follistatin in Mammalian Liver in Response to an Acute Injection of Epinephrine

Jared Root-McCaig, Eric Herbst, Laura Castellani, Zhaongxiao Wan, David C. Wright
University of Guelph

Follistatin is an endogenous inhibitor of myostatin. Recent work has suggested that follistatin expression is increased in the liver following exercise. However, little information is known regarding the specific mechanisms that may mediate this effect. The purpose of this project was to examine the relationship between exercise, epinephrine and CREB in the regulation of liver follistatin mRNA. An acute bout of exercise induced an ~70% increase in follistatin mRNA in mouse liver immediately following 2 hours of treadmill running (15 m/min, 5% incline). The increase in follistatin returned to sedentary levels 2 hours

following exercise. The increase in follistatin was mirrored by increases in the phosphorylation of AMPK and CREB. Similarly, 2 hours following a bolus injection of epinephrine (2 mg/kg bw) follistatin expression was robustly increased ~ 6 fold and this was preceded by increases in the phosphorylation of CREB and AMPK. Our data suggests that exercise-induced increases in epinephrine and subsequent activation of AMPK and CREB may mediated exercise induced increases in liver follistatin expression.

Vitamin D3 deficiency increases cellular stress and death in ALS

Shayan Shahsavar, Siavash Taheri-Shalmani, Jesse Adam Solomon, Alexandro Gianforcaro, and Mazen Jamal Hamadeh
School of Kinesiology and Health Science, York University, Toronto, ON.

Background: We previously demonstrated that dietary vitamin D3 (D3) restriction hastened the decline in functional capacity following disease onset in the G93A mouse model of amyotrophic lateral sclerosis (ALS). ALS is a progressive neuromuscular disease characterized by the degeneration and death of motor neurons.
Objective: In this pilot study, we analyzed the quadriceps of G93A mice following dietary D3 restriction at 2.5% the adequate intake for vitamin D receptor and markers of intracellular calcium trafficking (parvalbumin, calretinin, calbindin d28k), endoplasmic reticulum (ER) stress (SERCA2, CHOP) and apoptosis (caspase 12 cleaved/pro ratio – casp12, bax/bcl2 ratio). Methods: Beginning at age 25 d, 42 G93A mice were provided food ad libitum with either adequate (AI; 1 IU D3/g feed; 12 M, 11 F) or deficient (DEF; 0.025 IU D3/g feed; 10 M, 9 F) D3. At age 113 d, the quadriceps were analyzed for protein content. Differences were considered significant at $P \leq 0.10$. Results: In females, DEF had 20% lower calretinin ($P = 0.079$), 23% lower calbindin d28k ($P = 0.062$), 98% higher SERCA2 (0.072), 56% higher casp12 ($P = 0.082$), and 111% higher bax/bcl2 ratio ($P = 0.029$) vs. AI. DEF males had 67% higher CHOP ($P = 0.036$) vs. AI. Conclusion: Dietary D3 at 2.5% the AI reduces calcium buffering capacity and increases prolonged ER stress-induced apoptosis in female, but not in male, G93A mice. We hypothesize a reciprocal interaction between ER stress and calcium buffering. (Supported by NSERC and Faculty of Health, York University)

The Effect of TBC1D1 Ablation on Metabolism in Rats

Genevieve M. Simnett, Swati S. Jain, David C. Wright, Arend Bonen, Graham P. Holloway
Department of Human Health & Nutritional Sciences, University of Guelph, Guelph, Canada

Introduction: Tre-2/USP6, BUB2, cdc16 domain family 1 (TBC1D1), a Rab-GTPase-Activating protein, has recently emerged as a potential mediator of glucose metabolism in skeletal muscle. Stimulated by both insulin and contraction, TBC1D1 has been proposed to regulate GLUT4 translocation to the plasma membrane allowing subsequent glucose uptake. This study seeks to investigate the role of TBC1D1 in whole-body metabolism. Methods: Respiratory Exchange Ratios (RER) of TBC1D1 knockout (KO) and wildtype (WT) Sprague Dawley rats (n=4) were measured by indirect calorimetry during the light and dark cycles. Intraperitoneal insulin tolerance tests (IPITT) and glucose tolerance tests (IPGTT) were performed on KO and WT rats (n=9) following a 4 hour fasting period. Serum insulin concentrations were measured before and 10 minutes following an IP glucose injection (n=6). Results: TBC1D1 KO rats demonstrated higher ($p<0.05$) blood glucose levels under basal conditions and impaired glucose tolerance (higher peak blood glucose and area under the curve) compared to WT rats. In contrast to impaired glucose tolerance, whole body insulin tolerance was improved in KO rats. TBC1D1 ablation resulted in reduced insulin levels under basal conditions and following glucose administration, accounting for the divergent glucose and insulin tolerance. In addition, RER was significantly reduced in KO rats in both the light and dark cycles, which coincided with an increase in fat oxidation (KJ/min) and a reduction in carbohydrate oxidation. Conclusion: Ablation of TBC1D1 in rats causes impaired glucose tolerance and enhanced insulin sensitivity, suggesting that TBC1D1 KO rats have an impaired pancreatic capacity to secrete insulin. In addition, TBC1D1 appears to play a role in the regulation of whole-body metabolism as

its ablation causes a shift towards increased lipid utilization. Additional investigations of the detailed mechanisms of these processes are warranted to further elucidate TBC1D1 function and regulation.

The role of β-catenin on apopoptotic susceptibility and matrix turnover in the heart after myocardial infarction.

Kaustabh Singh1,5, Keith R. Brunt3, Jun Wu5, Kota Hatta2,5, Anton Mihic2,5, Shu-Hong Li5, Lu Sun5, Saeid Amini-Nik4 Benjamin A. Alman4, Richard D. Weisel5, Ren-Ke Li1,2,5.
1Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto ON.
2Institute of Medical Sciences, University of Toronto, Toronto ON.
3Department of Pharmacology, Dalhousie Medicine, Saint John, NB. 4Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto ON.
5Division of Cardiovascular Surgery, Division of Experimental Therapeutics, Toronto General Research Institute, University Health Network.

Background: Following a heart attack (or myocardial infarction), a large population of cardiomyocytes undergo cell death. This loss of beating cardiomyocytes is followed by detrimental remodelling in the injured tissue which can lead to heart failure. β-catenin is a member of the cadherin family of proteins, and is found at cell-cell junctions or within the intracellular compartment. Membrane-bound β-catenin transduces mechano-transduction signals, while cytosolic β-catenin translocates to the nucleus and activates TCF driven genes in response to Wnts. In wound healing, β-catenin has been known to increase cell survival and promote desirable tissue repair. However, its role in the heart has not been well characterized. Methods and Results: To test whether β-catenin levels are affected during MI, WT mice were subjected to coronary ligation and the protein expression of β-catenin was measured over time. β-catenin was found to be elevated at 3 and 7 days following injury. Analyzing the tissue samples at their cytosolic and nuclear levels revealed that β-catenin levels were higher in the nucleus when compared to sham tissue. This suggests an increase in transcriptional co-activation of Wnt-related canonical signaling following MI during normal conditions. The specific role of β-catenin in cardiomyocytes was investigated by employing transgenic mice harboring a floxed mutation for β-catenin. To specifically target this gene in myocytes, we crossed these mice with transgenic mice that have a Muscle Creatine Kinase-Cre (MCK-Cre) driven promoter. The resulting mice have reduced expression of β-catenin in cardiomyocytes and skeletal muscle. These mice were found to have significantly lower cardiac function, as measured by echocardiography following myocardial infarction. This change in heart function was corraborated with trichrome histlogical analysis on the injured sections, in which scar area and thinning were significanly greater. This suggested that β-catenin is important for mantaining both cardiac performance and tissue remodelling following myocardial infarction. Future work will focus on characterizing the underlying mechanisms regulating these changes, which will be geared towards improving cardiac outcome following heart attacks. Conclusions: β-catenin protein expression and its abundance in the nucleus was elevated following myocardial infarction in the heart. Mice with reduced expression of β-catenin in cardiomyocytes were correlated with poor cardiac remodelling. Up regulating β-catenin nuclear activity and signaling in response to MI can help develop new therapeutic targets for preventing congestive heart failure.

Force potentiation in the absence of myosin regulatory light chain phosphorylation is concurrent with elevations in resting cytosolic Ca2+.

Ian C. Smith1, Rene Vandenboom2, A. Russell Tupling1.
1 Department of Kinesiology, University of Waterloo
2 Department of Kinesiology, Brock University

INTRODUCTION: Brief activity of fast twitch skeletal muscle induces elevations in submaximal force during subsequent contractions. This phenomenon, known as potentiation, is commonly attributed to

phosphorylation of the myosin regulatory light chain (RLC). However, mounting evidence suggests that there is a secondary potentiation-causing mechanism, complementary to RLC phosphorlyation, which may be Ca2+-related. The purpose of this study was to examine if brief muscle activation can alter the cytosolic Ca2+ concentration such that it could contribute to potentiation. METHODS: Mouse lumbrical muscles, which do not exhibit RLC phosphorylation, were loaded with Ca2+-sensitive fluorescent indicators, fura-2, indo-1 or furaptra, to detect changes in resting cytosolic Ca2+ and the amplitude and time course of Ca2+ transients during the development and dissipation of potentiation at 37ºC. RESULTS: Despite significant increases ($P<0.05$) in twitch force, and faster ($P<0.05$) rates of force production and relaxation in the potentiated state, there were no detectable differences in the amplitude or time course of the calcium transients. Conversely, resting cytosolic Ca2+ increased ($P<0.05$) during potentiation and was positively and temporally correlated with the development and dissipation of the activity-induced twitch enhancements (all $R2>0.90$ except force during potentiation development ($R2 =0.66$)). CONCLUSIONS: The temporal overlap between elevations in resting cytosolic Ca2+ and enhancements in twitch performance suggests that changes in the cytosolic Ca2+ concentration that are subthreshold for force development can affect the characteristics of a subsequent contraction. This provides a potential means by which resting Ca2+ can enhance twitch contractility in the absence of RLC phosphorylation, though the precise mechanism remains equivocal.

Is vitamin D3 at 50x the adequate intake toxic in ALS?"

Siavash Taheri-Shalmani, Shayan Shahsavar, Alexandro Gianforcaro, Jesse Adam Solomon, and Mazen Jamal Hamadeh
School of Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, ON

Background: We previously showed that dietary vitamin D3 (D3) at 50x the adequate intake (AI) increased functional capacity in the transgenic G93A mouse model of amyotrophic lateral sclerosis (ALS), with females exhibiting signs of D3 toxicity. ALS is a progressive neuromuscular disease resulting in the death of upper and lower motor neurons. Objective: In this pilot study, we analyzed the quadriceps of G93A mice following dietary D3 supplementation at 50x the AI for vitamin D receptor and markers of intracellular calcium trafficking (parvalbumin, calretinin, calbindin d28k), endoplasmic reticulum (ER) stress (SERCA2, CHOP) and apoptosis (caspase 12 cleaved/pro ratio, bax/bcl2 ratio). Methods: Beginning at age 25 d, 41 G93A mice were provided food ad libitum with either adequate (AI; 1 IU D3/g feed; 12 M, 11 F) or high (HiD; 50 IU D3/g feed; 10 M, 8 F) D3. At age 113 d, the quadriceps were analyzed for protein content. Differences were considered significant at $P \leq 0.10$. Results: In females, HiD had 20% lower calbindin d28k ($P = 0.066$), 122% higher SERCA2 ($P = 0.011$), 35% lower CHOP ($P = 0.068$), and 228% higher bax/bcl2 ratio ($P = 0.023$) vs. AI. HiD males showed no significant differences vs. AI. Conclusion: Dietary D3 at 50x the AI decreases ER stress in the quadriceps of female G93A mice, but increases apoptosis, which could be due to D3 toxicity. We hypothesize a vitamin D-mediated dynamic crosstalk between ER stress and calcium trafficking. (Supported by NSERC and Faculty of Health, York University)

Effect of denervation on the expression of Tfam in rat skeletal muscle

Liam D. Tryon, Steven F. Pastore and David A. Hood
School of Kinesiology and Health Science, York University

Chronic muscle disuse, such as that produced by long-term denervation, results in a loss of muscle mass in addition to reductions in mitochondrial content and function. However, acute alterations in the expression of factors regulating mitochondrial content, namely mitochondrial transcription factor A (Tfam), have yet to be elucidated following denervation. Thus, to investigate changes in Tfam expression, we employed the in vivo transfection of a Tfam promoter-luciferase reporter construct into rat tibialis anterior muscle denervated for 0, 8, 16, 24, 72 or 120 hours. Tfam transcription was reduced by 40-70%

between 8 and 72 hours of denervation, and returned to control levels by 120 hours. This was not reflected by a reduction in Tfam mRNA content within 24 hours of denervation. These changes in Tfam transcription and mRNA preceded alterations in mitochondrial content and muscle mass, first noted by 72 and 120 hours, respectively. Whole muscle Tfam protein content was unaltered during this acute denervation, however mRNA levels of a mitochondrial DNA-encoded transcript (an indirect marker of Tfam activity) were reduced by 30-40% of control levels at 72 and 120 hours of denervation. Our data suggest that denervation results in early reductions in gene expression regulating mitochondrial content, which are associated with decrements in muscle mass during disuse conditions. Further investigation of the upstream signals governing Tfam expression and function in denervated skeletal muscle is warranted.

Myocyte-derived VEGF regulates vascular adaptations to increased blood flow in skeletal muscle

C. Uchida1, E. Nwadozi1, A. Hasanee1, E. Gee1, S. Olenich2, I.M. Olfert2, T.L. Haas1. 1School of Kinesiology and Health Science, York University, Toronto, ON, Canada. 2Division of Exercise Physiology, University of West Virginia, Morgantown, WV, USA

Vascular endothelial growth factor (VEGF) is a key regulator of vascular remodeling, and can be produced by both mesenchymal and endothelial cells. Skeletal muscle angiogenesis can be initiated by either metabolic or hemodynamic stimuli. Skeletal myocyte VEGF is required for exercise-induced angiogenesis. We hypothesized that muscle-derived VEGF is not required for vascular adaptations to increased blood flow. Myocyte-specific VEGF deleted (mVEGF-/-) mice or wildtype littermates were treated with prazosin for 14 days to induce a sustained increase in blood flow. The baseline capillary to fiber ratio, vascular area and number of small smooth muscle actin positive vessels were reduced in the EDL muscles of mVEGF-/- vs. WT littermates ($p<0.01$, n=3-7 per group). Prazosin treatment resulted in an increase in vascular area in the EDL of WT but not mVEGF-/- mice, suggesting that angiogenesis was inhibited by the muscle VEGF deletion ($p<0.05$, n=3 per group). Preliminary evidence also indicates that arteriolar remodeling may not occur in the mVEGF-/- mice treated with prazosin, as the number of large smooth muscle actin positive vessels tended to increase in the WT but not mVEGF-/- mice (n=3 per group). Our results show that lack of myocyte-derived VEGF impairs development of an appropriate microvascular network and prevents vascular adaptations to increased blood flow within skeletal muscle. Funded by NSERC and the Heart and Stroke Foundation of Canada.

Contaminating effects of other motor neuron properties on estimates of persistent inward currents in humans

Vandenberk, M., & Kalmar, J.
Wilfrid Laurier University

Persistent inward currents (PIC) play an important role in setting the input-output gain of motor neurons. In humans, these currents are estimated by calculating the difference between synaptic input at motor unit recruitment and derecruitment (ΔF) derived from paired motor unit recordings. The purpose of this study was to assess the contribution of other intrinsic properties (spike threshold accommodation and spike frequency adaptation) to ΔF estimates of PIC in human motor units by using ramps with varying rates of rise and duration. The relationship between reciprocal inhibition (RI) and PIC was also used to estimate the contribution of PIC relative to other motor neuron properties that result in nonlinear motor unit firing behaviour. It was hypothesized that slower rates of ramp rise and longer ramp durations would inflate ΔF estimates of PIC, and RI & PIC values would only be correlated during the ramp with the fastest rate of rise and shortest duration when spike threshold accommodation and spike frequency adaptation should be minimized. Fifteen paired motor unit recordings were made from the soleus during ramp contractions of plantarflexors with three different rates of rise and durations. ΔF estimates of PIC increased with decreased rates of ramp rise ($p=0.026$) and increased ramp durations ($p=0.035$), most likely due to spike

frequency adaptation. A correlation ($r=0.652$; $p<0.001$) between ΔF and reciprocal inhibition provides evidence that PIC is the primary contributor to ΔF in shorter ramps with faster rates of rise.

ChIP-exo analysis reveals novel and conserved MEF2 target genes in C2C12 and cardiomyocytes

York University: S Wales, S Hashemi, J C McDermott; University of Ottawa: A Blais.

Myocyte Enhancer Factor-2 (MEF2) is a member of the MADS-box family of transcription factors that is necessary for skeletal and cardiac muscle development. In vertebrates there are four MEF2 isoforms (A-D) that may dimerize within A/T rich regions of the promoter element of genes. Binding of MEF2 to target genes is regulated by post-translational modifications and cofactor interactions in a tissue dependent manner.

The goal of this exploratory study was to identify novel MEF2 target genes in skeletal and cardiac muscle using chromatin immunoprecipitation (ChIP) coupled to high-throughput sequencing. This method has been used extensively to identify target genes of transcription factors, however, one limitation of this method is that the precise location of protein:DNA interaction can be difficult to define as sequenced reads can be several hundred nucleotides long. To address this issue we used ChIP-exo to identify MEF2 target genes. This is a novel approach to ChIP-seq that exploits exonuclease activity to digest unprotected DNA, and thereby provides refined sequencing data.

Using a skeletal muscle cell line (C2C12 at 48 hr DM) and primary cardiomyocytes, 3,619 and 2,660 MEF2 target genes were identified, respectively. Centdist analysis identified that common motifs within these binding locations contained MEF2 consensus sequences. Gene Ontology terms corresponded to muscle differentiation and metabolic processes. Density mapping of histone modifications within MEF2 target genes in C2C12 revealed that at 48 hr DM MEF2 is mainly associated with genes that are transcriptionally active in myoblasts but inactive in myotubes.

Approximately 60 common MEF2 target genes were identified in C2C12 and cardiomyocytes. One particular gene, DUSP6, an ERK1/2 specific phosphatase, was selected for further study. A conventional MEF2 binding site was not found within the ChIP-exo peak, however there is a well characterized ETS binding motif. Using reporter gene analysis, MEF2 was shown to synergize on DUSP6 with PEA3, an ETS family member known to interact with MEF2. Further analysis in C2C12 showed that MEF2 may have a dual role in DUSP6 expression as both a positive and negative regulator of transcription. Intriguingly, both DUSP6 and PEA3 are upregulated in activated satellite cells. These results may provide a MEF2 dependent explanation between PEA3 and DUSP6 expression in satellite cell mediated muscle regeneration.

Neurotrophic Growth Factor Response to Lower Body Resistance Training in Older Adults

Walsh, J.J., Scribbans, T., Bentley, R.F., Kellawan, J.M., Gurd, B., and Tschakovsky, M.E.
Queen's University

Multimodal cognitive interventions (resistance + cognitive training; RT + CT) may synergistically improve cognitive function in seniors. RT stimulates the release of neurotrophic growth factors (NGF) which orchestrate functional and structural plasticity in the aging brain, while CT increases regional cerebral blood flow. Timing when CT occurs following a bout of RT, when NGF levels are at their highest, may maximize the blood-borne dose of NGF delivered to active brain tissue, thereby optimizing the growth signal to age-vulnerable cognitive processes. Currently, post-RT changes in blood-borne NGFs are poorly understood. The purpose of this work was to characterize the timing and magnitude of insulin like growth factor-1 (IGF-1) and brain derived neurotrophic factor (BDNF) levels in venous blood for 2 hours following an acute bout of RT. Methods: 10 older adults (ages 60 – 77) performed 1 hour of lower-body RT and rested for 2 hours post-exercise. Blood was taken before and for 2 hours after RT at set time points. This procedure was repeated after 8-weeks of regular RT. Results: BDNF increased

immediately post-exercise then returned to resting levels before and after 8 weeks of RT. IGF-1 levels did not change. Conclusions: these data are the first to show an increase in BDNF immediately following RT in older adults and this response is unaffected by 8 weeks of training. Further, the immediate increase in BDNF post-RT may represent the optimal time to prescribe CT in a multi-modal cognitive intervention.

ERp44 deficiency in mice, zebrafish and mouse embryonic stem cell derived cardiomyocytes display aberrant Ca2+ homeostasis, ER stress-induced apoptosis, and cardiomyopathy

Ding-Yan Wang1, Cynthia Abbasi1, Suzan El-Rass1.3, Jamie Yuanjun Li1, Fayez Dawood2,
Kotaro Naito2, Parveen Sharma1, Aaron Wilson1, Shunichi Yokoe1, Nic Bousette1,
Xiao-Yan Wen1.3, Peter Liu1,2, and Anthony O. Gramolini1,2
1. Department of Physiology, University of Toronto, 2. University Health Network, 3. St Michael Hospital

BACKGROUND Endoplasmic reticulum (ER) proteins and ER stress-associated pathways are the novel unfolded protein response (UPR)-targeted therapies for cardiovascular diseases. Endoplasmic reticulum resident protein 44 (ERp44) is a novel UPR-induced ER protein of the thioredoxin family. In addition, ERp44 has been shown to be involved in multiple cellular functions including the inhibition of Ca2+ release by inactivating inositol 1,4,5-trisphosphate receptor1 (IP3R1), and by supporting disulfide bond formation through reinforcing the of ER oxidoreductin 1 (Ero1-α)/oxidoreductase (OX) system. ERp44 protein is widely expressed in a variety of secretory tissue cells, including cardiomyocytes, and can be upregulated upon ER stress. Previous proteomic results have shown an eight-fold increase of ERp44 in various models of heart disease, but the precise physiological roles in the heart are unclear. METHOD/RESULTS: ERp44 lacZ knockin/out mice, aortic banded mouse model, morpholino knockdown zebrafish were generated and utilized as in vivo models. Embryonic stem cell (ESC) derived cardiomyocytes (ESCC), neonatal cardiomyocytes (NCM) and lentiviral expression were utilized as in vitro models. ERp44 deficiency in mice, zebrafish and mouse embryonic stem cell derived cardiomyocytes disrupted Ca2+ signaling, increased ER stress-induced apoptosis, and induced cardiomyopathy. ERp44 inhibits the activity of Ca2+ release channels, including IP3R, Ryanodine receptor 2 (RyR2) and Na+/Ca2+ exchanger 1 (NCX1). Moreover, ERp44 is also involved in regulation of mitochondrial apoptosis pathway. CONCLUSION: ERp44 plays an important role in regulation of Ca2+ signaling and protection of cardiomyocytes from ER stress-induced apoptosis through regulation of IP3R, RyR2 and NCX1 pathway as well as mitochondrial apoptosis pathway.

A comparison of inflammatory and functional effects of different cold-water immersion protocols for recovery from high-intensity sprint exercise

Gillian E. White1, Greg D. Wells 2,3
1Graduate Department of Exercise Sciences, The University of Toronto, Toronto, Canada
2 Faculty of Kinesiology and Physical Education, The University of Toronto, Toronto, Canada
3 Physiology and Experimental Medicine, The Hospital for Sick Children, Toronto, Canada

Cold-water immersion and other forms of cold therapy are commonly used following exercise to reduce delayed onset muscle soreness and speed the return of muscle performance. It is thought that the therapeutic and/or recovery effects of cold to this end is due to its anti-inflammatory action. Although it is commonly used by athletes of all types and by exercise and rehabilitation scientists alike, no protocol (duration of immersion/application or temperature) has been identified nor has the anti-inflammatory mechanistic theory been validated. We are conducting a comparison of four different cold-water immersion protocols of different duration and temperature for their efficacy to reduce plasma markers of inflammation and speed recovery of decreased performance induced by high-intensity sprint exercise. In a randomized cross-over study, we compared four different cold-water immersion protocols and passive

recovery for their efficacy to reduce plasma markers of inflammation and speed recovery of decreased performance induced by high-intensity sprint exercise. n=8 recreationally active male subjects performed 12x120m sprints on an indoor track. Immediately following exercise, subjects were randomly assigned to one of four cold-water immersion protocols (10deg for 10min, 20deg for 10min, 10deg for 30min, 20deg for 30min) or passive seated rest. Performance on squat jumps (SJ) and drop jumps (DJ), subjective measures of soreness and perceived recovery, and plasma levels of pro-inflammatory markers (MPO, GM-CSF, IFN-y, IL-1B, IL2, IL-6, IL-8, IL-10, IL-12p70, and TNF-a) were measure at time-points pre-exercise, post-exercise, 1h, 2h, 24h, and 48h post-exercise. Results have not yet been analyzed and assays for plasma pro-inflammatory markers are also currently being analyzed. We expect that measures for soreness will positively correlate with pro-inflammatory markers and DJ performance, SJ performance, and ratings of perceived recovery will negatively correlate with pro-inflammatory markers.

Exploring the process of psychosocial development and glycemic control in youth with type 1 diabetes mellitus (T1DM) attending a unique diabetes sports camp.

Dessi P. Zaharieva*, Theresa Beesley*, Jessica L. Fraser-Thomas, Michael C. Riddell

Current research has identified the benefits of attending a youth "diabetes-focused" camp as improvements in diabetes knowledge, coping skills and decreases in trait anxiety about diabetes. Less is known about the process involved in the development of psychosocial skills. Furthermore, there is limited investigation on the effects of attending a sports camp on diabetes self-management. The present study explored positive youth development (PYD) and glycemic control of youth living with T1DM attending a sports camp. Participants (n=65) aged 9-16 engaged in soccer, tennis, track and field or basketball for a one- or two-week diabetes sports camp at York University. Capillary blood glucose measurements were collected and continuous glucose monitor (CGM) technology was used on a subsample of participants (n=29). On the last day of camp, youth participated in a 30-minute focus group that explored their experiences at camp with an emphasis on psychosocial development. The findings revealed that youth developed an increased self-efficacy for physical activity and self-management of diabetes and enhanced their social skills. Counselors with diabetes were the main influential factor in facilitating PYD. The camp structure of integrating youth living with and without T1DM in a sports context further enhanced their opportunity for greater PYD. Youth improved fasting and pre-meal blood glucose levels (both $p<0.05$) and increased the percentage of time spent in euglycemia as measured by CGM. This study highlights the important role of "diabetes-aware" counselors and an integrative sports camp environment for facilitating PYD and improved short-term glycemic control in this vulnerable patient population.

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