2nd Annual
Muscle Health Awareness Day
May 27, 2011

York University

Program and Abstracts

Muscle Health Research Centre (MHRC)

Leading research on muscle adaptations.

YORK UNIVERSITÉ UNIVERSITY

Faculty of Health

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Date: May 27, 2011

To: All Participants

From: David A. Hood, MHRC Director

David A. Hood, PhD

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Welcome to the 2nd Annual

Muscle Health Awareness Day

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

Once again, we have a great line-up of speakers. The focus this year is on 1) blood flow and angiogenesis in muscle, 2) specific muscle proteins involved in muscle function, 3) muscle stem cells in muscle repair, aging and cancer, and 4) the regulation of metabolism in exercise and aging.

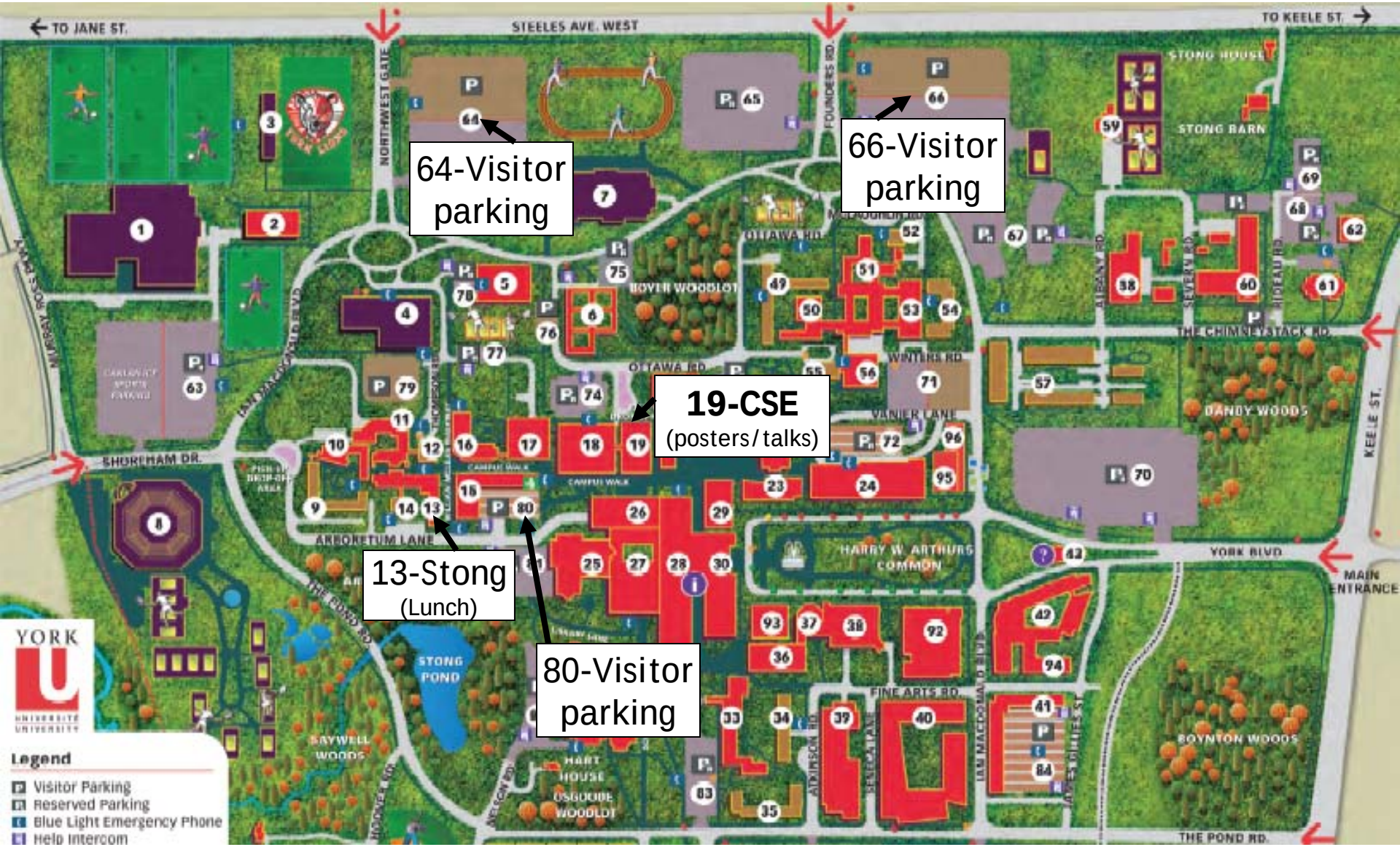
Our goal is to highlight the work of both junior and senior faculty members, and to give graduate students an opportunity to network and present their work in an informal, yet educational manner. As usual, any feedback for improvement is welcome as we continue to develop and refine the MHAD as a yearly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

Sincerely,

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

York U Campus Map



Muscle Health Awareness Day Program
May 27, 2011 – CSE B, York University

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

Session 1: Vascular Regulation of Muscle Blood Flow (9:00-10:20)

Chair: Dr. Tara Haas, York University

9:00-9:05 - Dr. David Hood, York University
Introduction (5 mins)

9:05-9:30 – Dr. Olivier Birot, York University
The role of Mdm2 in skeletal muscle angio-adaptation

9:30-9:55 – Dr. James Rush, University of Waterloo
Emerging mechanisms of endothelium-derived contracting factor control of vascular tone in hypertension

9:55-10:20 – Dr. Kyra Pyke, Queen's University
The role of endothelial function in muscle blood flow

10:20 – 11:00am Break (POSTER Presenting and Viewing)

Session 2: Muscle Proteins and Hormones (11:00-12:15)

Chair : Dr. David Hood, York University

11:00-11:25 – Dr. Celine Boudreau-Larivier, Laurentian University
Contributions of plakin crosslinking proteins to muscle health

11:25-11:50 - Dr. Russ Tupling, University of Waterloo
Phospholamban and sarcolipin are multifunctional proteins in heart and skeletal muscle

11:50-12:15 - Dr. Peter Tiidus, Wilfrid Laurier University
Estrogen and HRT influence on muscle function and repair in older females

12:15 – 1:30pm Lunch (Orange Snail, Stong College)

1:30 - 2:00pm (POSTER Presenting and Viewing)

Session 3: Stem Cells, Muscle Metabolism and Disease (2:00-4:00)

Chair: Dr. Rolando Ceddia, York University

2:00-2:25 – Dr. David Malkin, The Hospital for Sick Children
Rhabdomyosarcoma - exploring new approaches to therapy of malignant muscle tumors

2:25-2:50 – Dr. Mark Tarnopolsky, McMaster University
Is aging a mitochondrial disease and if so, what can we do about it?

2:50-3:15 – Dr. Greg Steinberg, McMaster University
Skeletal muscle AMPK beta subunits are essential for regulating exercise capacity and contraction-stimulated glucose uptake but not insulin sensitivity

3:15-3:40 – Dr. Simon Lees, Northern Ontario School of Medicine
Aging and satellite cell function

3:40- Dr. David Hood
Student Poster Award presentation, Concluding Remarks

Thank you for coming!

2nd Muscle Health Awareness Day - Poster Presentations			
Poster #	**First Author (last name)**	**Title**	**University affiliation**
1	Abdullahi	Downregulation of the mRNA translation inhibitor PDCD4 during myogenesis is mediated by the mTORC1/S6K1 pathway	York
2	Alli	Role and regulation of Fra-2 during skeletal muscle development	York
3	Baradaran	Analysis of time-dependent effects of altered dietary macronutrient composition on whole-body energy metabolism and body composition of rats	York
4	Battista	Alterations in the Function of Isolated Hearts Following the Administration of Skeletal Muscle Homogenates	Toronto
5	Bloemberg	Fiber type characterization of 10 rat and mouse muscles using a relatively rapid and simple immunofluorescence staining procedure	Waterloo
6	Boyd	Changes in PGC-1a and SIRT1 mRNA following 10 weeks of exercise training in skeletal muscle from healthy controls and Type 1 Diabetic rats	Queen's
7	Carter	Chronic contractile activity, mTORC1 inhibition and mitochondrial biogenesis in skeletal muscle	York
8	Caterini	The potentiation of dynamic function in mouse fast muscle is speed dependent	Brock
9	Charles	Characterizing an Animal Model of Anorexia Nervosa	Toronto
10	Chis	Elucidation of the Protective Mechanism of α-Crystallin B in Cardiomyocytes	Toronto
11	Darling	Protein turnover and regulation in rat skeletal muscle during osmotic stress	Brock
12	Edgett	Effect of acute endurance exercise on gene expression of mTOR pathway intermediates in rat skeletal muscle	Queen's
13	Fajardo	Sarcolemmal membrane lipid composition from mechanically skinned skeletal muscle fibres	Brock
14	Gillen	Short-Term High-Intensity Interval Training Reduces Hyperglycemia in Type 2 Diabetics	McMaster

15	Gittings	The effect of skeletal myosin light chain kinase gene ablation on the fatigability of mouse fast muscle	Brock
16	Herbst	The Role of Pyruvate Dehydrogenase Kinase-4 in Post-Exercise Glycogen Recovery	Brock
17	Jassi	Investigations into mitochondrial oxidative phosphorylation as an end effector of ischemic preconditioning	York
18	Jeganathan	Impaired insulin sensitivity caused by leucine is reversible in skeletal muscle of healthy rats	York
19	Jenkins	Differences in Isometric and Dynamic Strength in Pre- and Late-Pubertal Boys	Brock
20	Kerr	Effects of phosphatidylinositol 3-kinase isoform inhibitors on myocardial ischemia-reperfusion recovery	York
21	King	Comparing the dynamic response characteristics of flow-mediated dilation in the brachial and radial arteries	Queen's
22	Leung	Skeletal muscle regulatory volume response to increased extracellular lactate via monocarboxylate transporters	Guelph
23	MacPherson	Lipid droplets, ADRP, and OXPAT subcellular localization and co-localization at rest and after skeletal muscle contraction	Brock
24	Marcinko	Disrupting AMPK phosphorylation of acetyl coA carboxylase induces hepatic insulin resistance and liver fibrosis	McMaster
25	Martin	Baseline assessment of pulse wave velocity and carotid distensibility in adolescents with cerebral palsy: A pilot study	McMaster
26	Masilamani	Differential Expression Of RNA Binding Motif Protein RBM5 During Skeletal Muscle Differentiation	Laurentian
27	McMeekin	Influence of high fat diet on subsarcolemmal and intermyofibrillar mitochondrial membrane phospholipid fatty acid composition in rat skeletal muscle	Brock
28	McMillan	Examination of apoptotic protein expression, proteolytic enzyme activity, and DNA fragmentation across muscles and fiber types	Waterloo
29	Mitchell	Examination of skeletal muscle apoptotic protein expression and morphology in apoptosis repressor with caspase recruitment domain (ARC)-deficient mice	Waterloo
30	Oczak	SNARE protein involvement in ANF secretion from cardiomyocytes	York

31	O'Neill	ACC2 Ser 212Ala knock-in mice reveal that AMPK-independent pathways are important for controlling fatty acid oxidation in response to metabolic stress	McMaster
32	Osborn	Integrin-Linked Kinase (ILK) as a therapeutic target in the treatment of rhabdomyosarcoma	Toronto
33	Ostojic	Effects of Chronic Muscle Use and Disuse on Cardiolipin Metabolism	York
34	Pagiatakis	A novel myocyte enhancer factor 2 dependent signalling pathway regulating vascular smooth muscle cell gene expression	York
35	Polidovitch	Role of the mTORC2 pathway in mediating cardioprotection in PI3K transgenic hearts subjected to ischemia and reperfusion	York
36	Ramadan	Role of the equilibrative nucleoside transporter ENT1, in cardioprotective effects of ethanol	York
37	Sharples	Modulation of cortical excitability and interhemispheric inhibition prior to a voluntary unimanual contraction	Wilfrid Laurier
38	Sikkema	High-intensity interval training improves insulin sensitivity independent of adipose tissue inflammation	McMaster
39	Smith	In skeletal muscle, electron transfer flavoprotein (ETF) plays a minor role in fatty acid supported state 3 respiration	Guelph
40	Smith	Fibre type specific distribution of SERCA1a, SERCA2a and Phospholamban in human vastus lateralis	Waterloo
41	Tricarico	Metabolic response of human skeletal muscle to continuous low volume high-intensity exercise training	McMaster
42	Vainshtein	Contractile activity-induced alterations in autophagy in striated muscle	York
43	Zhang	Does p53 affect mitochondrial protein import in skeletal muscle?	York

Downregulation of the mRNA translation inhibitor PDCD4 during myogenesis is mediated by the mTORC1/S6K1 pathway

Abdikarim Abdullahi, Olasunkanmi Adegoke.
School of Kinesiology and Health Science and Muscle Health Research Centre, York University, Toronto, N3J 1P3

Defects in skeletal muscle integrity underly diseases such as muscular dystrophy and obesity. In affected individuals, the inability to maintain skeletal muscle mass often worsens the diseases. The mammalian target of rapamycin complex one (mTORC1), through its downstream target S6K1, is involved in regulating mRNA translation, protein synthesis and skeletal muscle mass. It does this in part by preventing the recruitment of the mRNA translation inhibitor, the tumor suppressor programmed cell death 4 (PDCD4). Because it inhibits protein synthesis and cell cycle progression, PDCD4 may regulate myogenesis. To examine this, L6 myoblasts were plated in a growth medium and upon reaching 90% confluency, they were switched to a differentiation medium. Differentiation was followed for the next 5 days. We found that total PDCD4 level and its nuclear accumulation increased approximately 7.2 folds on Day 1, and then decreased as differentiation progressed ($p<0.05$). We hypothesized that the regulation of PDCD4 abundance during differentiation likely involved S6K1. In testing this, we showed that siRNA-mediated knockdown of S6K1 reversed the decrease in PDCD4 expression during differentiation. In these cells, we observed a 10-fold decrease in myosin heavy chain one (MHC 1), a contractile protein that is used as a marker of differentiation. This indicated that PDCD4 regulation was vital for myogenesis. To specifically examine this, myoblasts were depleted of this protein by siRNA. Cells with depleted PDCD4 level showed delayed myoblast fusion and abnormal myotube length relative to control cells. In line with this, on Day 3 of differentiation, MHC 1 was detected in the non-transfected cells but not in cells depleted of PDCD4. By Day 5 of differentiation, however, no significant difference was found either in morphology or in MHC 1 abundance, indicating the cells were able to recover from the defects associated with PDCD4 depletion. In summary, our results unravel a link between PDCD4 and skeletal myogenesis, and identify mTORC1/S6K1/PDCD4 as a pathway that can be manipulated to promote muscle regeneration.

Role and regulation of Fra-2 during skeletal muscle development

Nezeka S. Alli1, Eric Yang2, Tetsuaki Miyake1, Arif Aziz1, and John C. McDermott1.
1Department of Biology, York University, Toronto, ON, Canada. 2Proteomics Core Facility, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre and University of Toronto, Toronto, ON, Canada.

The AP-1 transcription factor complex has been classically associated with tumourgenesis but some AP-1 subunits have also been implicated in certain developmental processes such as osteogenesis and cartilage formation. Our investigations on AP-1 suggest it may also have a role in gene regulation during skeletal muscle development. Previously, we determined that AP-1 proteins were expressed at significant levels in proliferating myoblast cells. We also observed that Fra-2 is the primary subunit in AP-1 complexes that bind DNA during muscle differentiation and have postulated that its regulation may be controlled by phosphorylation. Fra-2 is a known downstream target of the ERK 1/2 MAPK pathway which is activated by variety of extracellular signalling molecules including Cardiotrophin-1 and TGF-β. These molecules are known to potently alter the differentiation program in C2C12 cells and we have demonstrated that AP-1 proteins are targeted. Therefore, our aim is to investigate the downstream signalling events and posttranslational modifications induced by Cardiotrophin-1 and TGF-beta which affect AP-1 in skeletal muscle formation during development. Currently, we have implicated ERK1/2 MAPK as an upstream effector of Fra-2 and have identified five ERK 1/2 specific phosphorylation sites on Fra-2 by LC-MS. We are now investigating how these sites affect Fra-2 regulation and its contribution to AP-1 gene regulation in skeletal myogenesis.

Analysis of time-dependent effects of altered dietary macronutrient composition on whole-body energy metabolism and body composition of rats

Sanaz Baradaran, Fawad Noor and Rolando Ceddia
Muscle Health Research Center, School of Kinesiology and Health Sciences, York University

Objective – To determine the effects of altered macronutrient composition on whole-body energy metabolism and body composition.
Design – Male Wistar rats were fed ad libitum for 8 weeks with the following diets: 1) standard chow (SC), 2) high-carbohydrate sucrose-enriched diet (HC), 3) high-fat diet (HF) and 4) low-carbohydrate high-fat/high-protein diet (HP). Food intake and body weight were measured on daily basis. Weekly blood samples were collected to measure plasma concentrations of insulin, leptin, adiponectin, ghrelin, TNF-α and IL-6. At the end of the study, oxygen consumption (VO2), respiratory exchange ratio (RER), spontaneous physical activity and energy expenditure (EE) were assessed. After 8 weeks of dietary intervention, the rats were dissected to determine changes in body composition.
Results – Within 4-5 days, food consumption was adjusted to precisely match the energy intake of SC rats, indicating that alterations in the energy density of the diet were rapidly detected and food intake was regulated accordingly. This was associated with a significant reduction in circulating ghrelin levels in rats fed HC, HF and HP diets. They also demonstrated a significant increase in body weight which was associated with enhanced adiposity. VO2 and EE were also significantly higher relative to SC rats. Spontaneous physical activity was not different between groups. The RER value was ~0.95 in SC and HC groups, and ~0.8 in the HF and HP groups. The plasma concentrations of TNF-α, IL-6, leptin and insulin were also increased. Conversely, adiponectin concentrations were significantly reduced.
Conclusion – Despite increased energy expenditure, rats fed HC, HF and HP diets demonstrated a significant increase in adiposity which was associated with an unfavourable adipokine and insulin profile. These adaptations were specifically driven by the nutrient composition of the diet since all animals elicited isoenergetic intake.

Alterations in the Function of Isolated Hearts Following the Administration of Skeletal Muscle Homogenates

Alex Di Battista and Marius Locke.
Faculty of Physical Education and Health, University of Toronto. Toronto, Ontario. M5S 2W6.

Skeletal muscle damage can lead to the release of various intracellular molecules, including heat shock proteins (HSPs). To investigate whether HSPs or other molecules specific to fast and slow skeletal muscles can influence myocardial function, hearts from Sprague-Dawley rats were placed on the Langendorff isolated heart apparatus and paced at 320 bpm. Hearts were perfused for 75 min with a Krebs-Henseleit buffer supplemented with 1 □g/ml of muscle homogenate from either: 1) soleus, 2) white gastrocnemius (WG), 3) heat stressed white gastrocnemius (HSWG) or 4) no homogenate (control). At the end of the 75 minutes, hearts were removed, frozen and later assessed for NF-kB and AP-1 activation by EMSA. A decrease in left ventricular developed pressure, +dP/dt and –dP/dt were observed in hearts treated with muscle homogenates when compared to untreated (control) hearts. In addition, hearts treated with HSWG homogenates showed a greater decrease in cardiac function early in perfusion while hearts treated with soleus muscle homogenates showed a decrease in cardiac function later in perfusion. No significant alterations in NF-kB or AP-1 activation were detected. These data suggest that when certain molecules in skeletal muscles are released into the circulation they may be capable of altering contractile function. Furthermore, given that a greater decrease in cardiac function was observed when hearts were treated with the contents of muscles with an elevated HSP content, it also suggests that HSPs may play a role in the diminished myocardial response.

Fiber type characterization of 10 rat and mouse muscles using a relatively rapid and simple immunofluorescence staining procedure

D. Bloemberg and J. Quadrilatero
Department of Kinesiology, University of Waterloo, Waterloo ON, Canada, N2L 3G1.

Rodent skeletal muscle is composed of fibers containing four myosin heavy chains. Monoclonal antibodies enable histological identification of all fiber types with more ease and accuracy than traditional myosin ATPase procedures. In addition, the use of fluoropore-labelled antibodies simplifies the procedure by allowing classification of all fibers in a single section, as well as enabling clearer identification of hybrid fibers. The purpose of this study was to compare fiber type composition data obtained with our immunofluorescence procedure to previous reports, as well as to provide a comprehensive analysis of fiber type, size, oxidative potential, and glycolytic potential in 10 muscles of rat (Sprague-Dawley) and mouse (C57BL/6). We performed analyses on entire muscle cross-sections, which resulted in counting an average of 11552 total fibers per rat, and 6733 total fibers per mouse. In general, fiber type distribution was in agreement with previously published findings. Additionally, hybrid fibers were found to make up a significant proportion of total fibers in some muscles. For example, rat mixed gastrocnemius had a fiber type composition as follows: Type I: 4.5%, I/IIA: 0.0%, IIA: 14.9%, IIA/IIX: 6.6%, IIX: 42.5%, IIX/IIB: 5.6%, and IIB: 25.8%. Similarly, mouse plantaris fiber type composition was found to be: Type I: 0.0%, I/IIA: 0.0%, IIA: 19.4%, IIA/IIX: 8.3%, IIX: 22.4%, IIX/IIB: 3.0%, and IIB: 46.9%. In conclusion, this study not only provides a quick and reliable procedure for performing fiber type analysis on rat and mouse muscle, but also serves as an important resource for researchers studying muscle biology.

Changes in PGC-1a and SIRT1 mRNA following 10 weeks of exercise training in skeletal muscle from healthy controls and Type 1 Diabetic rats

J.C. Boyd1, M. Fortner1, C.W. Melling2, & B.J. Gurd1
1School of Kinesiology and Health Studies, Queen's University, 2School of Kinesiology, The University of Western Ontario

PURPOSE: Type 1 diabetes mellitus is known to be accompanied by impaired skeletal muscle function, but the precise mechanism underlying this change is unknown. In an effort to determine a potential cause, we investigated whether the mechanisms regulating mitochondrial biogenesis are impaired in Type 1 diabetic muscle. METHODS: Male Sprague-Dawley rats were separated into a diabetic (STZ) or non-diabetic (control) condition, then assigned to either a sedentary or exercise group within that condition. Rats in the diabetic groups (n = 13) were injected with streptozotocin (STZ) (20 mg/kg) for 5 consecutive days. Subsequently, rats either exercised on a treadmill one hour each day, 5 days per week for 10 weeks at a progressively increasing speed (control, n = 5; STZ, n = 8), or performed no exercise (control, n = 4; STZ, n = 5). After the 10-week study period the red (RG) and white (WG) gastrocnemius muscles were harvested. Reverse-transcription polymerase chain reaction analysis (RT-PCR) was performed on the muscle samples to quantify mRNA content. RESULTS: Exercise training decreased PGC-1a mRNA ($p < 0.05$) in the RG (-40%) and WG (-25%) of control rats. No change was observed in PGC-1a mRNA following training in either the RG or WG of STZ rats. PGC-1a mRNA was lower in the RG from the non-exercised STZ rats (-35%) compared to non-exercised controls. ALAS mRNA was also decreased ($p < 0.05$) in the RG (-34%) and WG (-29%) of the control animals while no effect of exercise training was observed within the STZ group. SIRT1 mRNA was decreased in both the RG (-38%) and WG (-33%) of control rats but only in the RG (-17%) of STZ rats. CONCLUSIONS: These results suggest that the changes in PGC-1a and SIRT1 mRNA observed following exercise training in healthy rats are altered in skeletal muscle from Type 1 diabetic rats. PGC-1a and SIRT1 mRNA were also decreased as compared to control in non-exercised diabetic muscle. It is not yet clear if these results are associated with decreases in transcriptional or mitochondrial protein.

Chronic contractile activity, mTORC1 inhibition and mitochondrial biogenesis in skeletal muscle

H.N. Carter and D.A. Hood
Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, ON., M3J 1P3

Chronic contractile activity (CCA) enriches the mitochondrial content of skeletal muscle, and this adaptation requires an increased expression of nuclear genes encoding mitochondrial proteins (NUGEMPs). The mammalian target of rapamycin complex 1 (mTORC1) has been implicated in regulating the expression of NUGEMPs by linking the co-activator PGC-1α to the transcription factor YY1. We wished to ascertain if mTORC1 was required for CCA-induced mitochondrial biogenesis. C2C12 myotubes were subjected to CCA (3 hours/day, 4 days) in the presence of 1nM rapamycin, an mTORC1 inhibitor, or DMSO. CCA increased mitochondrial content, as reflected by cytochrome oxidase (COX) activity. This adaptation was also evident in the presence of rapamycin. Indeed, mTORC1 inhibition led to an increase in COX activity in both the absence (28%) and presence (20%) of CCA. Surprisingly, the mTORC1 component raptor, as well as YY1, were both significantly elevated due to mTORC1 inhibition. However, CCA had no effect on raptor levels, but increased YY1 expression by 1.8-fold. State III respiration declined in rapamycin-treated cells, but this was not observed in the presence of CCA, suggesting that CCA recovered mitochondrial function. These data suggest that mTORC1 may be a negative regulator of NUGEMPs, and that the inhibition of mTORC1 does not blunt CCA-induced mitochondrial biogenesis.

The potentiation of dynamic function in mouse fast muscle is speed dependent

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It is well known that prior contractile activity enhances or "potentiates" isometric twitch force of mouse fast twitch muscle. The influence of potentiation on dynamic muscle function is less well studied, however. The purpose of this study was to examine the influence of potentiation on dynamic force, work and power in mouse (C57BL) extensor digitorum longus (EDL) muscle (in vitro, 25° C). Sinusoidal length changes were applied to the motor arm to which one tendon of the muscle was attached. The resultant work cycle of muscle shortening and lengthening, centered around optimal muscle length (Lo), had a total amplitude equal to 5 % Lo. Work cycle frequency was varied from 1.5 to 3.3 and 6.9 Hz, in which muscle stimulations were carefully timed so that peak twitch force occurred synonymous to Lo. A brief, high frequency potentiating stimulus (PS) that augmented isometric twitch force to 1.19 ± 0.01 of pre-stimulus control values was utilized (all data n=9; $p < 0.05$). Concentric forces obtained ~ 20 sec after (experimental) cessation of the PS demonstrated a relative increase of 1.19 ± 0.01 (1.5 Hz), 1.25 ± 0.01 (3.3 Hz) and 1.30 ± 0.01 (6.9 Hz) from control values obtained ~ 1 min pre-PS. The potentiation influence on relative concentric work and power was only observed at the fastest cycle frequency however (i.e. 0.84 ± 0.01, 1.05 ± 0.01 and 1.39 ± 0.01 of control values at 1.5, 3.3 and 6.9 Hz, respectively). Contrary to concentric findings, the CS failed to significantly potentiate eccentric twitch force (1.06 ± 0.006, 1.02 ± 0.006 and 0.95 ± 0.009), or work and power (0.88 ± 0.02, 0.92 ± 0.04 and 0.98 ± 0.01) relative to control values at 1.5, 3.3 and 6.9 Hz, respectively. These results thus suggest that the potentiation of concentric force is greater than that for isometric force; however moderate to fast shortening is required for this difference to manifest. Conversely, the potentiating effect appears to be either negligible, or absent during muscle lengthening. This suggests that potentiation of dynamic function during cyclic length changes mimicking locomotion in vivo is highly speed, and direction dependent. Research funded by NSERC.

Characterizing an Animal Model of Anorexia Nervosa

Sarah Charles, Catherine Amara.
University of Toronto

Anorexia Nervosa is a condition of severe under-nutrition characterized by alterations in multiple neuroendocrine axes. An animal model has yet to characterize alterations in: body composition, wheel running activity, food intake, and neuroendocrine parameters, over a sustained period of food restriction. PURPOSE: To investigate the temporal changes in body composition and neuroendocrine hormones in food restricted rats, with and without access to running wheels, over a four week period. METHODS: Sixteen, 12 wk old, female Wistar rats were randomly divided into four groups: Control (C), Voluntary Running (VR), Food Restricted (FR), and Food Restricted/Voluntary Running (FR/VR). C animals were fed ad libitum, while FR animals were food restricted with the goal of achieving 75% of body mass within the first two weeks with a maintenance phase for the final two weeks. Wheel running activity and food intake were measured constantly through-out the study, and IGF-1, leptin (ELISA) and body composition (DXA, GE Lunar Prodigy) were measured at baseline and once a week for 4 weeks (baseline (B), T1, T2, T3, T4). RESULTS: FR/VR animals demonstrated a rapid loss of body fat (BF) (B = 43.4±5.3g, T4 = 6.6±5.0g) mimicked by significant decreases in both leptin (B = 12.5±1.48ng/mL, T4 = 1.8±0.12ng/mL) and IGF-1 (B = 184±13.2ng/mL, T4 = 44±5.8ng/mL). FR animals demonstrated similar reductions in BF (B = 37.8g±3.8, T4 = 5.9g±2.4), leptin (B = 7.5±1.3ng/mL, T4 = 2.0±0.3ng/mL) and IGF-1 (B = 196±20.3ng/mL, T4 = 67±7.9ng/mL). C and VR animals grew to roughly 110%-115% of their original body mass over the study and demonstrated similar increases in IGF-1 (C: B = 181.5±22.9ng/mL, T4 = 244±14.4ng/mL; VR: B = 180±13.2ng/mL, T4 = 246±21.6ng/mL). However, despite an increase in body mass, wheel running resulted in a reduction in BF (CWR: B = 42.4±6.6g, T4 = 33.3±3.9g) while C animals gained BF (B = 38.2±9.83g, T4 = 46.9±10.1g). In both cases, leptin changed in parallel with BF (VR: B = 16.4±1.6ng/mL±, T4 = 10.3±0.9ng/mL; C: B = 5.37±0.3ng/mL, T4 = 14.3±3.2ng/mL. CONCLUSION: These data demonstrate that changes in leptin occur very rapidly upon initiation of food restriction and in parallel with losses in BF, while adaptations in IGF-1 parallel changes in lean tissue. Furthermore, a tight biological tie between body mass and voluntary wheel activity was identified in the food restricted population, as well as a slight preservation of lean tissue and BMC in VR animals. This illustrates that voluntary physical activity was of no further detriment to food restricted animals, and that with adequate nutritional support there could be some potentially protective or beneficial side effects of wheel running in this population. An investigation into a refeeding program for food restricted animals that includes exercise is warranted and might not be counter-productive as previously thought.

Elucidation of the Protective Mechanism of α-Crystallin B in Cardiomyocytes

Roxana Chis, Nicolas Bousette, Parveen Sharma, Tetsuaki Miyake and Anthony O. Gramolini.
Department of Physiology, University of Toronto, Toronto, ON.

Background: α-Crystallin B (cryAB) is the most abundant small heat shock protein in cardiomyocytes (CMs) where it has been shown to have potent anti-apoptotic properties. Studies show that ex vivo perfused hearts from transgenic mice that over-express cryAB tolerate ischemia/ reperfusion (I/R) better, while cryAB null mouse hearts display poorer functional recovery and a higher cell death rate following I/R compared with wild-types. The mechanism by which cryAB prevents apoptosis has not been fully characterized. Therefore, the objective of my project is to elucidate the protective mechanism of cryAB in CMs, specifically validating its protective effects by knockdown and over-expression, identifying its sub-cellular localization and identifying its binding interactors under H2O2- induced stress. Methods: Plasmids expressing shRNA targeting mouse cryAB mRNA or cryAB cDNA were used for Lentivirus transduction of neonatal cardiomyocytes (NCMs) to knockdown and over-express cryAB, respectively. The protective properties of cryAB were assessed following exposure of CMs to H2O2 using Annexin V-

based fluorescence activated cell sorting (FACS), dissipation of mitochondrial membrane potential, cytochrome c release and TUNEL assay. To characterize the protective mechanism of cryAB, its localization was determined using sub-cellular fractionation and its interactions with apoptotic proteins were determined using co-immunoprecipitation. Results: A significant decrease in viability was observed in CMs when cryAB was silenced (cryAB KD) when compared with WT CMs following exposure to 200 μM H2O2. Sub-cellular fractionation of CMs showed that cryAB is found in the cytosol in control conditions and that it translocates to the mitochondria following H2O2 exposure. Caspase 3 activation was significantly higher in KD cells when compared to control cells following exposure to 200 μM H2O2 as demonstrated by immunoblot analysis. Co-immunoprecipitation revealed an apparent increased level of interaction of cryAB with caspase 3 in stressed cells when compared to controls. The interaction between cryAB and cytochrome c and caspase 12 was similar in control and stressed cells. Conclusions: The protective effects of cryAB in CMs were validated by showing a significant decrease in CM viability in the absence of cryAB following its knockdown. The localization results suggest that the protective effects of cryAB are mediated by its translocation from the cytosol to the mitochondria under conditions of stress. The co-immunoprecipitation results suggest that cryAB interactions with cytochrome c, caspase 9 and caspase 12 do not contribute to its protective effects. Its interaction with caspase 3, however, may be part of the cryAB protective mechanism following exposure to H2O2 in CMs. This work was funded by the Ontario Graduate Scholarship (RC), Heart and Stroke Foundation of Ontario (AOG) and a Heart and Stroke/Lewar Centre Fellowship (NB).

Protein turnover and regulation in rat skeletal muscle during osmotic stress

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The cellular hydration state plays an important regulatory role in hepatocyte metabolism, demonstrating anabolic effects during cell swelling and catabolic effects during cell shrinkage. It has been shown in skeletal muscle that extracellular osmolality influences glucose metabolism, but the research on protein turnover is limited. The purpose of the current investigation was to examine the effects of hyper and hypo-osmotic extracellular stress on protein metabolism in resting rat extensor digitorum longus (EDL) muscle in vitro. We hypothesized that protein incorporation and degradation would be favouring a net gain during hypo-osmotic stress while hyper-osmotic stress would result in a negative protein balance. 8 EDL muscles from male Long Evans rats were pre-incubated in hyper-osmotic (400±10 Osm/kg), iso-osmotic (290±10 Osm/kg) or hypo-osmotic (190±10 Osm/kg) custom modified tyrosine and phenol red-free medium 199 (Gibco) (95% O2, 5% CO2, pH 7.4, 30±2 °C) for 60 min. This was then immediately followed by a 75 min incubation with 5mM L-[14C]-U-phenylalanine and 5mM cycloheximide to quantify protein synthesis and degradation, respectively. Protein degradation was estimated through tyrosine appearance in the media. Intramuscular ATP & PCr concentrations were also measured to confirm metabolic viability. Phenylalanine uptake increased 99% in HYPO compared to ISO, while protein synthesis increased 69% as compared to ISO. Protein incorporation in the HYPER condition decreased 41% from ISO while non-incorporated amino acid increased 22% from ISO conditions. Protein degradation in the HYPER condition increased 32% while no significant differences were found between the HYPO and ISO condition. PCr and ATP in the HYPER condition decreased 52% and 6% compared to the ISO control, respectively. In conclusion, extracellular osmolality can significantly alter protein metabolism in isolated rat skeletal muscle. More specifically, hyper-osmotic stress creates a catabolic state, while hypo-osmotic stress creates an anabolic state within muscle. Future research should be directed at establishing what signalling mechanisms are responsible for these observations.

Effect of acute endurance exercise on gene expression of mTOR pathway intermediates in rat skeletal muscle

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PURPOSE: While the literature is abundant when it comes to the regulation of transcription following endurance exercise, very little is known about the regulation of protein translation. The current study examined changes in the expression of mRNA for proteins involved in the regulation of translation, specifically in the mTOR-dependant signalling pathways in rat skeletal muscle in response to an acute bout of endurance exercise. METHODS: Female Sprague-Dawley rats ran for 2 hours at 15 m/min followed by an increase in speed of 5 m/min every 5 minutes until volitional cessation of exercise. The red gastrocnemius muscle was harvested from non-exercised animals (control), immediately following cessation of exercise (0 hr) and after 3 hours of recovery from exercise (3 hr). Gene expression of mammalian target of rapamycin (mTOR), ribosomal protein S6 (rpS6), S6 kinase (S6K), eukaryotic initiation factor 2B subunits δ (eIF2Bδ) and ε (eIF2Bε), and eukaryotic initiation factor 2α (eIF2α) were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR). RESULTS: Compared to control, rpS6 mRNA was elevated ($p<0.05$) at both 0 hr (+32%) and 3 hr (+47%). eIF2Bδ was also elevated ($p<0.05$) at 0 hr (+40%) and 3 hr (+64%). Both eIF2Bε (+67%) and mTOR (+44%) were higher than control ($p<0.05$) at 3 hr. The increase in mTOR mRNA at 3 hr was also increased relative to 0 hr ($p < 0.05$). No changes were observed in the mRNA levels of S6K or eIF2α. CONCLUSIONS: These results are the first to suggest that an acute bout of endurance exercise is effective in increasing expression levels of proteins involved in the mTOR-dependent signalling pathway. This implies that expression of these genes might play a role in the ability to undergo protein synthesis with successive bouts of endurance exercise.

Sarcolemmal membrane lipid composition from mechanically skinned skeletal muscle fibres

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The sarcolemmal membrane is integral to skeletal muscle function and health. Many important structural and metabolic processes are associated with this membrane. Membrane lipid composition, which includes phospholipid (PL) headgroup, fatty acid (FA) saturation and cholesterol, has been shown to affect membrane proteins, which in turn may impact function. Past research has relied on crude membrane isolation procedures that may not be representative and do not allow for fibre-type specific analyses. Thus, to overcome these limitations, this study assessed a method of individually skinned skeletal muscle fibres as an alternative to analyze sarcolemmal membrane lipid composition. Single muscle fibre segments (~3 – 5 mm in length, ~ 40 – 60 μm in diameter) were mechanically skinned from rat extensor digitorum longus muscle for lipid analysis (n=9). Lipids were extracted, PL and cholesterol were separated by high-performance 2-dimensional thin-layer chromatography, fatty acid composition of each PL was determined by gas chromatography and cholesterol content was measured using a fluorometric assay. PL percent relative abundance indicated 27 % phosphatidylcholine (PC), 24 % sphingomyelin (SM), 22 % phosphatidylethanolamine, 15 % phosphatidylinositol, and 12 % phosphatidylserine (n=6). These values are similar to those previously reported with the exception of SM demonstrating higher (~ 10 - 20%) and PC lower (~ 5 – 25%) relative abundance compared to past studies. Total lipid saturation was similar to those previously reported (60 % saturated FA, 26 % monounsaturated FA, 4% n3 polyunsaturated FA, 9% n6 polyunsaturated FA, and an unsaturation index of 67, n=6). Cholesterol content (0.30 ng of lipid / ng of protein; n=9) was 2-fold higher than a previous study examining sarcolemmal cholesterol. Thus, these results suggest that the skinning fibre technique may be a viable

option to quantify sarcolemmal membrane lipid composition. Furthermore, the high SM and low PC percent relative abundance in conjunction with a higher cholesterol content seen in the present study may reveal an important consequence of previous sarcolemmal isolation procedures relying on membrane disrupting protocols that may segregate detergent-resistant membrane microdomains. Future studies could use the skinning technique to assess the influence of disease/disorder (eg. muscular dystrophy, type 2 diabetes) and environmental perturbations (eg. exercise, diet) on SL lipid composition.

Short-Term High-Intensity Interval Training Reduces Hyperglycemia in Type 2 Diabetics

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Glycemic regulation is impaired in individuals with type 2 diabetes (T2D). Current guidelines recommend that people with T2D should participate in a cumulative total of 150 minutes of continuous, moderate-intensity aerobic exercise each week, or at least 90 minutes of vigorous intensity exercise. While effective for improving glycemic control, most individuals with T2D do not perform sufficient activity to achieve health benefits, often citing "lack of time" as a key barrier to regular exercise participation. Low-volume high-intensity interval training (HIT) is a potent stimulus for inducing metabolic adaptations typically associated with traditional high-volume endurance training. However, the effects of low-volume HIT on glycemic control in people with T2D is currently unknown. PURPOSE: To determine the utility of HIT to reduce hyperglycemia in patients with T2D in a practical, time-efficient manner. METHODS: 7 subjects with T2D (age: 62 ± 3 yr, BMI: 33 ± 3.8 kg/m2, Wmax: 125 ± 18 W) performed 6 training sessions over 2 wk. Each session consisted of 10 x 1 min cycling efforts at ~90% Wmax elicited during a ramp VO2peak test, interspersed with 1 min of rest (113 ± 11 W). Continuous Glucose Monitoring (CGM) technology (Medtronic CGMS® iPro Recorder) was employed under standard dietary conditions for a 24 hr period on a pre-training control day and ~48-72 hr following the final training session. RESULTS: CGM data revealed lower mean 24 hr blood glucose concentration following HIT (7.6 ± 0.5 mmol/L vs. 6.6 ± 0.3 mmol/L, $p < 0.05$). The sum of the 3 hr postprandial area under the glucose curve for breakfast, lunch and dinner was also reduced following training (689 ± 196 mmol/L x 9hr vs. 991 ± 214 mmol/L x 9hr, $p < 0.05$). CONCLUSION: These preliminary data shed light on the potential for low-volume HIT to represent a unique, time-efficient exercise strategy to improve glycemic regulation in people with T2D.
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The effect of skeletal myosin light chain kinase gene ablation on the fatigability of mouse fast muscle

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Contraction-induced activation of Ca2+ and calmodulin-dependent myosin light chain kinase (skMLCK) catalyzes phosphorylation of the myosin regulatory light chain (RLC), a reaction that modulates myosin motor behavior and potentiates low frequency force. The influence of this molecular mechanism on the contractile performance of fatigued skeletal muscle remains unclear. To this end, extensor digitorum longus (EDL) muscles from wildtype (WT) and skMLCK knockout (KO) mice (n=10-12) were studied in vitro at 25°C during a fatigue protocol consisting of 5 minutes of repetitive high-frequency stimulation. Isometric twitch (Pt) and tetanic (Po) forces and unloaded shortening velocity (Vo) were assessed before, during, and following fatiguing stimulation. The progressive decline of Po and Vo (i.e. fatigue) was similar in both genotypes despite a significant difference in RLC phosphate content at all time points

(P<0.01). Pt was intially potentiated in both WT and KO muscles (to 1.37 ± 0.05 and 1.14 ± 0.02 of control values, respectively) although the magnitude and duration of the effect was significantly greater in WT muscles (persisting until Po had declined by 60% of control)(P<0.05). Decreased half-relaxation time (½RT) was displayed in skMLCK devoid muscles compared to WT muscles during all tetanic (150Hz) contractions despite no relative change in peak force production (i.e. fatigue). Conversely, muscle twitch ½RT was similar in both genotypes despite significant differences in concomitant twitch force potentiation. Our results provide evidence that skMLCK-catalyzed RLC phosphorylation contributes to the maintenance of low-frequency contactile performance during moderate levels of fatigue, and is an important modulator of force development and relaxation kinetics during repetitive use.

The Role of Pyruvate Dehydrogenase Kinase-4 in Post-Exercise Glycogen Recovery

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The pyruvate dehydrogenase (PDH) complex regulates the oxidation of carbohydrates in mammalian tissues. Decreased activation of PDH following exhaustive exercise may aid the resynthesis of glycogen through increased activity of PDH kinase-4 (PDK4), one of four kinases that down-regulate and inactivate the PDH complex. The purpose of this study was therefore to examine the role of PDK4 in post-exercise glycogen resynthesis. Wild-type (WT) and PDK4-knockout (PDK4-KO mice) were exercised to exhaustion and were sampled at rest (Rest), at exercise exhaustion (Exh), and after two-hours of recovery (Rec) following exercise. Differences in feeding post-exercise led to the addition of a PDK4-KO group, pair-fed (PF) with WT mice. In muscle, glycogen fully recovered in the Rec WT and PF groups and supercompensated in PDK4-KO mice. Liver glycogen resynthesised fully at Rec in WT and PDK4-KO mice, but remained low in the PF group. Concentrations of lactate in the blood and muscle and alanine in muscle were depressed in the PF group. Results suggest that PDK4 is involved in post-exercise glycogen resynthesis, and important with limited caloric availability.

Investigations into mitochondrial oxidative phosphorylation as an end effector of ischemic preconditioning

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Myocardial ischemic preconditioning (IPC) is an effective adaptation to ischemic stress that protects tissue from injury during prolonged periods of ischemia. Our previous work has shown that two isoforms of phosphoinositide-3-kinase (PI3K), PI3Kα and PI3Kγ, are integral in conferring protection to the heart. The PI3KαDN strain has been shown to be inherently protected from ischemia, while the PI3Kγ (-/-) mice show a lack of IPC protection. The mitochondria have been proposed to be critical in mediating IPC protection. Therefore, we explored the changes in mitochondrial transcripts and function to determine the mechanisms associated with the differentially response of the PI3K transgenic mice to ischemic injury. Initially, we measured transcriptional changes in genes involved in mitochondrial energetics using the RT² Profiler™ PCR Array (SABioscience). The PCR array revealed that both PI3Kγ (-/-) and PI3KαDN hearts had increased gene expression of subcomplexes of complex I (NADH-coenzyme Q reductase) of the electron transport chain. However, we did not identify any differential regulation of mitochondrial transcripts between these 2 mouse strains. Next, we isolated intermyofibrillar mitochondrial fractions from the transgenic PI3K hearts that were exposed first to various treatments of IPC or ischemia/reperfusion (IR). Basal (State IV) and active (State III) mitochondrial respiration rates revealed

no differences between wildtype and the PI3K transgenic hearts. The respiratory control ratio (RCR) represents the tightness of oxygen consumption in the presence and absence of ADP. Wildtype mitochondria had an RCR of 7.7 ± 3.6 (n=3), while hearts subjected to IR had a reduced RCR of 6.9 ± 1.8 (n=5). IPC restored the RCR to 7.9 ± 2.6 (n=3). Importantly, PI3KαDN hearts subjected to IR had a higher RCR (10.9 ± 3.1, n=3), while the RCR of PI3Kγ hearts that underwent IPC was decreased to 4.8 ± 1.0 (n=5). Although the respiration rates were not different amongst the various treatment groups, we suggest the protection conferred by IPC and observed in PI3KαDN hearts may be due to improved mitochondrial respiratory coupling.

Impaired insulin sensitivity caused by leucine is reversible in skeletal muscle of healthy rats

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High protein diets are known to promote weight loss and increase lean body mass in obese individuals. The mTORC1/S6K1 pathway is vital to this process as it is responsible for nutrient sensing and protein synthesis. However, excess amino acids and insulin may regulate a negative feedback loop within this pathway, causing insulin resistance. S6K1, a substrate of mTORC1, has been implicated in this. We hypothesized that amino-acid induced insulin resistance in skeletal muscle is reversible, but this may become compromised under pathological conditions. To test this, myotubes were starved in serum-free medium for 4 hours and then re-incubated with leucine (800uM) and/or insulin (100nM). A time-dependent increase in S6K1 (Thr 389) and IRS1 (serine residues) phosphorylation was found when myotubes were treated with leucine and insulin ($P<0.01$); incubation in this condition also decreased insulin-stimulated glucose transport by 65% ($P<0.05$). To examine the reversibility of this regulation, cells were incubated overnight with leucine, starved in serum-free media (washout) and then re-incubated with leucine and/or insulin. Following refeeding, the cells responded (as measured by S6K1 Thr389, IRS1 serine phosphorylation and insulin stimulated glucose transport) as if they had not been previously exposed to leucine, indicating that these effects are reversible. The pattern of S6K1 and IRS1 phosphorylation observed in myotubes was recaptured in skeletal muscle of healthy rats gavaged with leucine (0.048g/kg, n=5/treatment). To examine the implication of these results on whole body insulin sensitivity, an insulin tolerance test (ITT) was conducted on healthy rats (n=5). Rats were gavaged with leucine or water, and 30minutes later, the ITT began. Following insulin injection (2U/kg), rats gavaged with leucine had higher levels of blood glucose compared to the control at 15, 60 and 120 minutes post-injection (by 30%, 41% and 17% increases respectively, $P<0.05$). Rats gavaged with leucine and starved overnight showed similar changes in blood glucose following insulin injection as rats that had not been previously gavaged with leucine. Thus, insulin resistance attributed to high protein diets is likely a normal reversible event in the muscle of healthy rats.

Differences in Isometric and Dynamic Strength in Pre- and Late-Pubertal Boys

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Children's muscle strength is lower than adults', even when body size is accounted for. Most research has focused on isometric strength but there is little data on dynamic strength differences. The purpose of this study was to investigate changes taking place during the pubertal years in both isometric and dynamic (isokinetic) peak torque. As part of an ongoing study, 8 pre-pubertal (PP, 8-9 years old, pubertal stage 1) and 6 late-pubertal (LP, 12-13 years old, pubertal stages 4 & 5) were examined. Following habituation, the dominant leg of each participant was tested for knee-extension peak torque on an isokinetic dynamometer (Biodex III) during isometric, and isokinetic contractions at 60 & 240□/s in a

counterbalanced order. EMG was used to evaluate the agonist muscle's rate of activation (Q30) and the degree of antagonist coactivation. As expected, the older, more mature boys were stronger in both isometric and dynamic (60 & 240□/s) contractions ($p<.001$ for all). These differences persisted after normalizing for body mass (e.g., 240□/s: 1.00±0.19 & 1.25±0.19 N•m/Kg for PP & LP, respectively), or for quadriceps muscle cross-sectional area (e.g., 240□/s: 0.38±0.08 & 0.50±0.07 N•m/cm2 for PP & LP, respectively). Q30 and coactivation were similar across groups and contraction velocities. These data indicate that factors other than body size, inter-muscle coordination and rate of activation contribute to the increasing quadriceps strength during the pubertal years.

Effects of phosphatidylinositol 3-kinase isoform inhibitors on myocardial ischemia-reperfusion recovery

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Ischemic preconditioning (IPC) is a potent protective mechanism shown to reduce myocardial damage after prolonged ischemia. We have previously shown that the deletion of phosphatidylinositol 3-kinase γ [PI3Kγ)] in mouse hearts abolishes IPC protection. In contrast, suppression of PI3Kα activity (PI3KαDN) in murine hearts resulted in significantly enhanced recovery following ischemia-reperfusion. Therefore, we explored whether pharmacological inhibition of the different PI3K isoforms, PI3Kα (Compound 15e, 1 μM) PI3Kβ (TGX-221, 10 μM) and PI3Kγ (AS605240, 1 μM), would mimic the differential response of the PI3K transgenic mouse hearts subjected to ischemia and reperfusion. We examined the functional recovery of isolated perfused mouse hearts subjected to either 30 min of ischemia followed by 40 min of reperfusion (IR), or 4 cycles is 5 min of ischemia and 5 min of reperfusion (IPC) prior to the 30 min index ischemia. Wildtype hearts subjected to IR resulted in a reduction of left ventricular developed pressure (LVDP) from 118.0 ± 12.3 mmHg to 16.8 mmHg ± 5.4 mmHg at 40 min of reperfusion. In stark contrast, IPC resulted in markedly improved LVDP at the end of 40 min of reperfusion (65.9 ± 4.6 mmHg). As observed with PI3Kγ (-/-) hearts, AS605240 blocked IPC recovery in wildtype hearts (18.4 ± 3.9 mmHg). The PI3Kβ inhibitor, TGX-221, also abolished IPC protection (17.8 ± 7.7 mmHg). In contrast to our previous observations with PI3KαDN hearts, the PI3Kα inhibitor, Compound 15e, failed to protect wildtype hearts from IR (21.5 ± 5.9 mmHg). Moreover, Compound 15e exacerbated IPC recovery (6.2 ± 1.9 mmHg). Our results suggest that pharmacological inhibition of either PI3Kα, β and γ in the heart abolishes IPC protection. The lack of beneficial effects of the PI3K□ inhibitor suggests that alternative signaling pathway(s) in the PI3KαDN hearts may be responsible for the enhanced contractile function seen in these hearts after IR.

Comparing the dynamic response characteristics of flow-mediated dilation in the brachial and radial arteries.

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Recent research indicates that smaller arteries tend to dilate more when exposed to shear stress (flow mediated dilation (FMD)) vs. larger arteries. However, the dynamic characteristics of FMD have not been compared in differently sized vessels. PURPOSE: To compare the Phase 1 (initial dilation) dynamics of the FMD response in the radial artery (RA) and the brachial artery (BA). METHODS: N = 15 healthy male subjects. BA and RA diameter and mean blood flow velocity (MBV) were measured by echo and Doppler ultrasound respectively. Rhythmic handgrip exercise was used to create a shear rate (shear rate (SR) estimate of shear stress = vessel diameter/MBV) of 60 s-1 for 6 minutes in the BA and RA. Subjects performed 3 exercise trials at the 60 s-1 SR target in each artery over three visits. These

trials were averaged to provide one mean RA and one mean BA response. Curve fitting allowed determination of the phase 1 response dynamics (time delay (TD1) and time constant (tau 1)). Data are reported as means ± SD. RESULTS: RA diameter was 0.215 ± 0.0445 mm; BA diameter was 0.368 ± 0.0664 mm (P=0.002). The shear rate achieved in the RA (56.027 ± 3.935 s-1) and BA (57.939 ± 5.850 s-1) was not significantly different (P = 0.120). The Phase 1 %FMD and %FMD at the end of the 6 min exercise bout were significantly greater in the RA (RA phase 1 = 6.25 ± 3.42%; 6 min 9.84 ± 3.99 %; BA 2.59 ± 1.69%; 6min 2.526 ± 1.96 %)(phase 1 P=0.005; 6min P=<0.001)). The RA had a significantly shorter TD1 (9.60 ±15.42 s) than BA (31.80 ± 26.15 s) (P = 0.017). However there was no significant difference in the speed of the phase 1 response (tau1) between the BA (51.30 ± 43.26 s) and RA (65.75 ± 38.40s) (P = 0.461). TD1 was modestly correlated with percent FMD in the RA (r2 = 0.146, P = 0.039) but not in the BA (r2 = .205, P = 0.104). Tau1 was not correlated with FMD in either the RA (P = 0.583) or the BA (P = 0.104). CONCLUSION: When experiencing a SR of 60 s-1, the RA began to dilate sooner (shorter TD1) and had a greater phase 1 %FMD magnitude than the BA. However, no differences could be detected in the speed of the phase 1 response (tau1). FMD response dynamics were largely unrelated to FMD magnitude. The distinct phase I FMD magnitudes and time delays in response to the same shear rate indicate vessel specific differences in the initiation of the FMD response. Supported by NSERC and CFI.

Skeletal muscle regulatory volume response to increased extracellular lactate via monocarboxylate transporters

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Mammalian skeletal muscle cells regulate volume in response to fluctuations in extracellular osmolarity. As a result of increased osmolarity, the rate of regulatory volume increase (RVI) is hypothesized to occur more rapidly in the presence of lactate- anions, as opposed to Cl- anion, because the inward transport of osmotically active lactate via monocarboxylate transporters (MCTs) should increase the rate at which intracellular osmolarity equilibrates with extracellular osmolarity. The purpose of the present work was to investigate the potential role of lactate- in the contribution of RVI and to verify the involvement of the MCT in RVI response. Adult mouse EDL and peroneous muscle single fibres were isolated using collagenase and incubated in DMEM. Width measurements were obtained and analyzed at 2 separate sites per fibre, every 5-10 sec for 30 min, in response to an increase in extracellular osmolarity via NaCl or NaLactate treatment. The extracellular changes in osmolarity, which were separately tested and verified, were conducted in the presence or absence of NKCC (bumetanide) and/or MCT (pCMBS and phloretin) inhibitors. Increasing extracellular osmolarity resulted in rapid cell shrinkage followed by a subsequent recovery back up towards baseline volume. The extent of volume loss was lessened in response to Na-Lactate in comparison to NaCl treatments of the same step increase in extracellular osmolarity. Inhibition of the MCT by pCMBS and phloretin demonstrated a more pronounced volume reduction in comparison to control, however the magnitude of decrease was greater in contrast to NKCC inhibition alone. Combining inhibition of the NKCC and MCT however showed a much reduced volume loss, not observed in any other treatment condition, suggesting that an alternate means of re-establishing volume may also be present. In summary, these results suggest that the MCT may play a notable role in facilitating RVI by way of lactate transport in the optimal response of skeletal muscle cells to undergo volume recovery.

Lipid droplets, ADRP, and OXPAT subcellular localization and co-localization at rest and after skeletal muscle contraction

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Skeletal muscle lipid droplet proteins are thought to regulate lipolysis through proteinprotein interactions on the lipid droplet surface. In adipocytes, ADRP is found only on lipid droplets, while OXPAT (expressed only in oxidative tissues) is found both on and off the lipid droplet, and may be recruited to lipid droplet membranes when needed. Our purpose was to determine if OXPAT is recruited to lipid droplets with contraction, and to investigate the myocellular location and co-localization of lipid droplets, ADRP, and OXPAT. Rat solei were isolated and assigned to one of two groups: 1) 30min resting incubation; 2) 30min stimulation (n=10 each). Immunofluorescence microscopy was used to determine subcellular content, distribution, and co-localization of lipid droplets, ADRP, and OXPAT. There was a main effect for lower lipid and ADRP content in stimulated compared to rested muscles ($p<0.05$). Lipid droplet distribution declined exponentially from the sarcolemma to the fibre centre in the rested muscles ($p=0.001$, $r^2=0.99$) and a linearly in stimulated muscles (slope=-0.0023±0.0006, $p<0.001$, $r^2=0.93$). ADRP distribution declined exponentially from the sarcolemma to the fibre centre in both rested and stimulated muscles ($p<0.0001$, $r^2=0.99$ rest; $p=0.0004$, $r^2=0.98$ stimulated) while OXPAT distribution declined linearly (slope=-0.0085±0.0009, $p<0.0001$, $r^2=0.94$ rest; slope=-0.0078±0.0010, $p=0.0003$, $r^2=0.91$ stimulated). OXPAT-lipid droplets colocalized at rest with no difference post stimulation ($p=0.47$; rest $r^2=0.55$±0.02, stimulated $r^2=0.58$±0.03). ADRP-lipid droplets co-localized at rest with no difference post stimulation ($p=0.48$; rest $r^2=0.66$±0.02, stimulated $r^2=0.65$±0.02). Contrary to our hypothesis these results show that OXPAT is not recruited to lipid droplets with contraction in isolated skeletal muscle.

Disrupting AMPK phosphorylation of acetyl coA carboxylase induces hepatic insulin resistance and liver fibrosis

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In obesity, non-alcoholic fatty liver disease (NAFLD) has been associated with the development of hepatic insulin resistance; however, the molecular mechanisms underlying this relationship are unclear. Acetyl coA carboxylase (ACC), which exists as two separate isoforms (ACC1 and ACC2), is an important metabolic enzyme which controls the production of the metabolic intermediate malonyl-CoA. Malonyl-CoA production is the first committed step in fatty acid synthesis and an allosteric inhibitor of carnitine palmitolyl-transferase-1 (CPT-1), and therefore, fatty acid oxidation. AMP-activated protein kinase (AMPK) has been shown to be important for regulating lipid metabolism and has been shown to inhibit ACC activity by phosphoryating ACC1 at Ser79 and ACC2 at Ser 221; however, the importance of this phosphorylation in controlling lipid metabolism in vivo has not been investigated. Therefore, the objectives of this thesis were to determine the physiological importance of AMPK phosphorylation of ACC as it relates to the development of NAFLD and insulin resistance. To address the above objectives we examined the metabolic phenotype of C57Bl6 mice with a targeted ACC1 Ser79 to Ala and ACC2 Ser221 to Ala double knock-in mutation (ACCDKI) or their wildtype (WT) littermates. Body composition, energy expenditure, and feeding behaviour were evaluated. Glucose and insulin sensitivity were measured by: intraperitoneal tolerance tests, serum analysis, hyperinsulinemic-euglycemic clamp and tissue-specific glucose uptake. The mRNA expression of gluconeogenic and mitochondrial genes in the liver were measured. Finally, the degree of liver steatosis and fibrosis was assessed by histological analysis. ACC DKIs had reduced food intake but surprisingly body mass and energy expenditure were not different compared to WT. Despite similar whole-body adiposity there was a dramatic increase in visceral

adipose tissue stores in ACCDKI mice. As anticipated liver ACC activity and malonyl coA were higher in ACCDKI mice. Assessment of liver profile showed that ACCDKIs displayed greater aggregation of neutrophils in the liver and increased tissue fibrosis compared to WTs. ACCDKI mice had elevated fasting blood glucose and insulin indicating whole-body insulin resistance. Hyperinsulinemic-euglycaemic clamps revealed that ACCDKIs had liver insulin resistance as indicated by a reduced suppression of hepatic glucose output during the clamp and reduced Akt phosphorylation. Insulin-stimulated glucose disposal rate was also reduced and was associated with impaired glucose uptake into visceral adipose tissue but not skeletal muscle. In summary we have shown that the phosphorylation of ACC1 Ser79 and ACC2 Ser221 is critical for maintaining ACC activity and malonyl-CoA levels in the liver. The dysregulation of this pathway results in liver fibrosis and the development of insulin resistance. These studies demonstrate that AMPK phosphorylation of ACC is essential for maintaining metabolic homeostasis. Future studies examining the role of this pathway in regulating liver fibrosis, appetite and adipogenesis may be important for understanding the importance of this pathway in controlling whole-body insulin sensitivity.

Baseline assessment of pulse wave velocity and carotid distensibility in adolescents with cerebral palsy: A pilot study

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Impairments in mobility place youth with cerebral palsy (CP) at a higher risk of physical inactivity, a major risk factor for cardiovascular disease. The consequences of reduced levels of physical activity on vascular health in adolescents with CP have not been evaluated. GMFCS levels I to III were included, with each subject being ambulatory or ambulatory with assistive devices. Baseline arterial stiffness was assessed using pulse wave velocity (PWV) and distensibility in eight 10 to 18 year old patients with cerebral palsy (6 males; age 13 ± 2.4 yrs) and compared to nine 10 to 16 year old controls (7 males; age 11.3 ± 3.1 yrs). Data for the control population were previously collected in our laboratory (Proudfoot, 2010). Baseline measurements of whole body PWV were taken using electrocardiography and photoplethysmography. PWV was calculated using the distance (sternal notch to the dorsalis pedis artery) and time delay between ventricular depolarization and the foot of the dorsalis pedis waveform. Carotid distensibility, a direct measure of central artery stiffness, was assessed using a combination of B-mode ultrasound imaging and applanation tonometry. Brachial artery blood pressures were determined using an automated sphygmomanometer. There were no significant differences in whole body PWV between the CP (4.1 ± 0.3 m/s) and control (4.0 ± 0.7 m/s) groups ($p=0.726$). However, carotid distensibility was significantly lower in the CP group (0.0066 ± 0.0013 $mmHg^{-1}$) than the control group (0.0101 ± 0.0033 $mmHg^{-1}$) ($p<0.05$) with systolic blood pressures of 110.7 ± 11.3 $mmHg^{-1}$ and 104.7 ± 6.4 $mmHg^{-1}$ and accordingly. The findings indicate that youth with CP have increased central artery stiffness at rest. This decline in vascular health at a young age may increase risk of cardiovascular disease into adulthood. Future research will further evaluate conduit artery structure and function in adolescents with CP. By identifying potential compromises to vascular health in this clinical population, appropriate interventions and precautionary measures can be taken at the earliest possible stage.

Differential Expression Of RNA Binding Motif Protein RBM5 During Skeletal Muscle Differentiation

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RNA binding motif proteins are involved in a variety of functions ranging from splicing to apoptosis. Our lab focuses on two RNA binding motif proteins, RBM5 and RBM10. In cancer cells, RBM5 is involved in apoptosis, cell cycle regulation and tumour suppression. RBM5 is temporally and tissue-specifically regulated, being highly expressed in both heart and skeletal muscle. This drove us to investigate the potential role of RBM5 during development. We hypothesized that RBM5 is involved in the differentiation of myoblast to myotube and the alternative splicing of muscle-specific mRNAs. To validate our hypothesis we are characterizing the expression levels of RBM5 in the murine C2C12 skeletal muscle differentiation model. We have observed differential RBM5 mRNA expression levels in the differentiated cells analysed by qPCR. Verification of this expression changes at the protein level is being done. We also have knocked down RBM5 using shRNA in the C2C12 myoblasts, which will enable us to identify the potential role of RBM5 in skeletal muscle during differentiation. Determination of the role of RBM5 as an important splicing regulator and apoptotic modulator in normal muscle development may provide a better understanding of some of the mechanisms underlying various disease states such as muscular dystrophy.

Influence of high fat diet on subsarcolemmal and intermyofibrillar mitochondrial membrane phospholipid fatty acid composition in rat skeletal muscle

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Membranes are important structures that are vital for proper cellular function. Membrane structure is, in part, dependent on phospholipid (PL) fatty acid composition. Specifically, the degree of fatty acid saturation is positively correlated with membrane rigidity, which may influence integral membrane protein function. Perturbations, such as high fat diets, have been shown to influence membrane structure. However, these observations have been made on whole mixed skeletal muscle, with little information on subcellular membranes, specifically the two subpopulations of mitochondria found in the subsarcolemmal (SS) and intermyofibrillar (IMF) regions of muscle. Thus, the purpose of the study was to examine the influence of high fat diets of differing quality (saturates and polyunsaturates) on skeletal muscle SS and IMF mitochondrial membrane phospholipid fatty acid composition. Forty-day-old male Sprague Dawley rats were fed high fat (20% by weight) diets consisting of coconut (SFA), flaxseed (n-3 PUFA), or safflower (n-6 PUFA) oil for 65 days, and compared to a control group that was fed a chow diet (5.7% by weight soybean oil). SS and IMF mitochondria were isolated from mixed hind limb muscle, lipids were extracted, PLs were separated by thin layer chromatography, and fatty acid composition of each PL was quantified using gas chromatography. Overall, each high fat diet did not have an effect on their respective fatty acid subclasses found in the mitochondrial membranes (e.g. high SFA diet did not result in high SFA in mitochondria compared to control). In addition, there was no significant difference between SS and IMF mitochondrial membranes composition within their respective dietary manipulations. This demonstrates that both SS and IMF mitochondrial membranes may not be altered by high fat diets, possibly, in part, to preserve their important metabolic function of energy production. Future studies will utilize different high fat feeding regimes to confirm if mitochondrial membrane structure continues to be

preserved post intervention and possibly examine the effect of altered membrane structure on mitochondrial function. Supported by NSERC.

Examination of apoptotic protein expression, proteolytic enzyme activity, and DNA fragmentation across muscles and fiber types

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Skeletal muscle is a complex tissue comprised of several fiber types with unique properties. Increased skeletal muscle proteolytic activity caused by apoptotic events is associated with a number of conditions including aging, disuse, and cardiovascular disease. To date, no report has specifically examined the apoptotic differences across muscles or fiber types. Therefore, the purpose of this study was to investigate several apoptotic factors across healthy rat red (RG) and white gastrocnemius (WG) muscle, as well as examine the expression of key apoptotic proteins across fiber types in a mixed muscle (mixed gastrocnemius). The protein content of apoptosis repressor with caspase recruitment domain (ARC) apoptosis-inducing factor (AIF), cytochrome c, Bax, Bcl-2, heat shock protein 70 (Hsp70), and second mitochondria-derived activator of caspases (Smac) are significantly ($P < 0.05$) higher in RG vs. WG muscle. Cytosolic AIF, Smac, cytochrome c, as well as nuclear AIF are also significantly ($P < 0.05$) higher in RG compared with WG muscle. In addition, ARC protein expression is related to muscle fiber type and found to be greatest ($P < 0.001$) in type I fibers. AIF protein expression is also differentially expressed across fiber types; however, AIF is correlated to oxidative potential ($P < 0.001$). Hallmarks of apoptosis such as caspase-3, -8, and -9 activity, calpain activity, and DNA fragmentation are also significantly higher ($P < 0.05$) in RG compared with WG muscle. Furthermore, total muscle reactive oxygen species generation, as well as Ca(2+)-induced permeability transition pore opening and loss of membrane potential in isolated mitochondria are greater in RG muscle. These data illustrate a differential expression and activity of a number of apoptosis-related indices across muscles and fiber types. In conclusion, muscle and fiber-type differences in apoptotic protein expression, signaling, and susceptibility should be considered when studying cell death events in skeletal muscle

Examination of skeletal muscle apoptotic protein expression and morphology in apoptosis repressor with caspase recruitment domain (ARC)-deficient mice

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Altered apoptotic signaling has been observed in a number of disease states. Apoptosis repressor with caspase recruitment domain (ARC) is a potent anti-apoptotic protein that is able to inhibit apoptosis mediated by both the death-receptor and mitochondrial pathways. In addition, ARC has a unique distribution pattern and is highly expressed in terminally differentiated tissue such as skeletal muscle. To characterize the role of ARC in skeletal muscle morphology and apoptosis, soleus and plantaris muscles of 18 week-old ARC-deficient mice were excised and compared to those of age-matched wild-type littermates. Western blot analysis confirmed the absence of ARC protein in both the soleus and plantaris. There were also differences in the expression of several apoptotic proteins between wild-type and ARC-deficient animals. For example, AIF, Bax, Smac and p53 protein content was significantly increased in soleus. While there was no change in Bcl-2 and XIAP protein content, Hsp-70 protein was significantly decreased in ARC-deficient animals. In plantaris, there were no significant changes in any of the apoptotic proteins we measured. Isolated mitochondria from ARC-deficient animals were more susceptible to calcium induced swelling, as well as membrane potential loss compared to controls. Interestingly, preliminary data also demonstrated a shift in fiber type distribution in both the soleus and

plantaris of ARC-deficient mice. In soleus, there was a decrease in the proportion of type I fibers with a corresponding increase in the proportion of type IIA and type IIA/IIX fibers. Similarly, there was a decrease in the proportion of type IIA fibers along with an increase in the proportion of type IIB fibers in plantaris muscle. Additional studies will be performed to examine several apoptotic signaling pathways as well as muscle size and function. Although preliminary, these results suggest that in skeletal muscle of ARC-deficient mice there may be an altered apoptotic environment as well as morphological changes.

SNARE protein involvement in ANF secretion from cardiomyocytes

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Atrial natriuretic factor (ANF), the hallmark hormone of the endocrine heart, is involved in the physiological maintenance of blood pressure and volume. As with all secretory cells, a subset of soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins is involved in hormone exocytosis. Syntaxin 1A (STX1A), synaptosomal associated protein 25 (SNAP25) and vesicle associated membrane protein 2 (VAMP2) were first discovered in neuronal cells, and their functional isoforms, syntaxin 4 (STX4) and SNAP 23 have been implicated in skeletal muscle, and newly discovered in cardiac muscle tissue. We demonstrate that ventricular neonatal cardiomyocytes (NCM) express STX4, SNAP23 and VAMP2 at consistently high levels, with STX1A expression increasing with postnatal development, while SNAP25 levels remain undetectably low. According to our immunocytochemistry data, the majority of ANF is localized around the nucleus (perinuclear), and sparse at the sarcolemma. Electroporation of NCMs with siRNA constructs against STX4 or SNAP23, or with tetanus toxin (TeTX) which cleaves VAMP2 demonstrated marked reduction of the target SNARE protein levels. To determine SNARE protein involvement in both basal and evoked (endothelin-1 stimulated) ANF secretion, we transfected our NCMs with siRNA or TeTX and observed a marked reduction in evoked ANF secretion. We further demonstrate that NCMs can be positively transfected with STX1A and SNAP25, which are expressed in adult cardiac myocytes. We are currently determining whether heterologous expressed STX1A and SNAP25 can rescue ANF secretion following siRNA/TeTX treatment. Our research supports the novel finding that STX4, SNAP23 and VAMP2 represent the cardiac specific SNAREs involved in ANF secretion, and that different SNARE isoforms could potentially function as analogs.

ACC2 Ser 212Ala knock-in mice reveal that AMPK-independent pathways are important for controlling fatty acid oxidation in response to metabolic stress

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AMP-activated protein kinase (AMPK) is an evolutionarily conserved regulator of energy homeostasis effects which are believed to be mediated through phosphorylation of substrates controlling multiple metabolic pathways. One of the first substrates identified for AMPK was the enzyme acetyl-CoA carboxylase (ACC), which exists as two distinct isoforms, ACC1 and ACC2, which are important for regulating fatty acid metabolism. In vitro studies have shown that AMPK phosphorylates and inhibits ACC1 at Ser79, which is the equivalent phosphorylation site of Ser212 on ACC2. The activation of AMPK in skeletal muscle by exercise increases ACC2 Ser212 phosphorylation and fatty acid oxidation, and results in improvements in insulin sensitivity; however, genetic evidence supporting this association does not exist. In the current study, we generated ACC2 Ser212 Ala knock-in (ACC2 KI) and find that despite multiple phosphorylation sites ACC2 Ser 212 is the primary site critical for regulating ACC2

activity, malonyl-CoA production and insulin sensitivity in skeletal muscle in resting chow-fed mice. However, during metabolic stress-induced by either high-fat feeding or exercise, ACC2 Ser 212 phosphorylation is not essential for the regulation of fatty acid oxidation or insulin sensitivity. These data suggest that physiological redundant pathways are important for controlling fatty acid oxidation during metabolic stress.

Integrin-Linked Kinase (ILK) as a therapeutic target in the treatment of rhabdomyosarcoma.

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Rhabdomyosarcoma is the most common soft tissue malignancy in children, and the two most common subtypes are embryonal and alveolar subtypes. Previous work in our lab has demonstrated that integrin-linked kinase (ILK) has roles in both promoting and suppressing growth of these tumors, depending on the type of rhabdomyosarcoma in question. In embryonal rhabdomyosarcoma ILK acts as a tumor suppressor, and in alveolar rhabdomyosarcoma ILK acts as a growth promoter. We have examined the candidacy of ILK as a therapeutic target by examining the susceptibility of rhabdomyosarcoma cell lines to chemotherapeutic agents in the setting of targeted inhibition of ILK. siRNA-mediated knockdown of ILK, as well as the use of a chemical ILK inhibitor, causes cells of the alveolar rhabdomyosarcoma subtype to become more susceptible to chemotherapy. Cells of the embryonal subtype do not appear to be affected. Although the mechanism that accounts for the differential activity of ILK in the two rhabdomyosarcoma subtypes is not known, our preliminary immunohistochemical investigation into the prevalence of various potential ILK interactors in the two subtypes suggests that the m-TOR pathway may be involved.

Effects of Chronic Muscle Use and Disuse on Cardiolipin Metabolism

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Mitochondrial membranes have a diverse structure composed of numerous phospholipids, including cardiolipin (CL). CL helps to maintain the integrity of both mitochondrial membranes and is essential for the proper functioning of multiple membrane-embedded enzymes. Several dynamic processes are required in order to synthesize and remodel the CL molecule. Alterations in these processes have been shown to lead to mitochondrial dysfunction. We have previously shown that CL levels increase with chronic stimulation-induced contractile activity (CCA; 10 Hz, 3h/day, 7 days), a model of muscle use, and decrease with denervation (7 days), a model of muscle disuse. We were interested in examining the mRNA expression of enzymes involved in CL metabolism in order to shed light on the mechanisms underlying these effects. With CCA, we found that mRNA levels encoding cardiolipin synthase, an enzyme involved in CL synthesis on the inner mitochondrial membrane, significantly increased (1.9-fold). CCA also elicited a 1.7-fold increase in the transcript encoding mitoPLD, which is involved in CL production on the outer mitochondrial membrane, but had no effect on tafazzin, a CL remodelling enzyme. These alterations in gene expression coincided with a significant 26% increase in CL content in young (6 mo old) animals. Interestingly, CL levels were not affected by CCA in older (35 mo old) animals. In response to denervation, cardiolipin synthase increased 1.8-fold, while mitoPLD and tafazzin did not change. This occurred despite evidence for large (50%) decreases in CL content with denervation. Our data support a relationship between CL content and the mRNA levels of enzymes involved in CL synthesis and remodelling under conditions of chronic muscle use. However, a disruption of this

relationship appears to occur with two forms of chronic muscle disuse, denervation and aging, the mechanisms of which remain to be determined.

A novel myocyte enhancer factor 2 dependent signalling pathway regulating vascular smooth muscle cell gene expression.

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BACKGROUND: Contraction of vascular smooth muscle cells (VSMC) is triggered by an increase in cellular calcium. Once elevated, VSMCs regulate their calcium sensitivity through the RhoA signaling pathway. Interestingly, this RhoA signaling cascade has also been shown to regulate the production of smooth muscle contractile proteins. In addition, mice that habour a genetic mutation in the myocyte enhancer factor 2C (MEF2C) gene fail to form a proper vasculature and have decreased expression of smooth muscle contractile proteins. Therefore, we hypothesized that the RhoA pathway might regulate the activity of MEF2 proteins. METHODS AND RESULTS: To evaluate calcium-mediated signaling in VSMCs, we used a cell culture model treated with high levels of potassium chloride (KCl) to promote calcium entry, along with gene transfection techniques, and common pharmacological inhibitors that block specific signaling pathways. KCl treatment of VSMCs increased the protein of level of the MEF2-target genes myocardin and c-Jun. Analysis of the gene regulatory regions for c-Jun and myocardin revealed that KCl induction requires the MEF2 binding site for increased expression. Interestingly, increased expression of myocardin was prevented by pharmacological inhibition of the RhoA and p38 signaling pathways; whereas, increased c-Jun expression was inhibited by blockade of the calcium/calmodulin pathway. We have previously identified protein phoshatase 1 alpha (PP1) as a potent repressor of MEF2 activation that is regulated by p38 signaling to MEF2 proteins. In VSMCs, treatment with a PP1 inhibitor resulted in increased expression of myocardin. Consistent with our pharmacological findings, forced expression of PP1 could inhibit myocardin expression, and the RhoA-regulated PP1 inhibitor, CPI-17, could rescue PP1's repressive effects. CONCLUSION: These data provide evidence of a novel signaling pathway that links RhoA-mediated calcium sensitivity to MEF2-dependent myocardin expression in VSMC through a mechanism involving p38 and PP1 alpha regulation of MEF2 proteins. This knowledge could have important implications for both vascular disease and birth defects associated with heart's outflow tract.

Role of the mTORC2 pathway in mediating cardioprotection in PI3K transgenic hearts subjected to ischemia and reperfusion

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Myocardial infarction (MI) resulting from interrupted coronary blood supply (ischemia) is defined by irreversible necrosis of heart muscle. We have previously demonstrated that phosphatidylinositol 3-kinase γ (PI3Kγ) is a key mediator of cardioprotection following ischemia through the PI3Kγ-Akt-GSK3β pathway. We have further demonstrated that cardiac-specific inhibition of PI3Kα activity with a dominant-negative kinase-dead PI3Kα (PI3KαDN) construct resulted in cardioprotection following ischemia-reperfusion. We hypothesized the latter to be due to enhanced signalling through thePI3Kγ pathway. In order to explore this hypothesis further, we have generated double PI3K transgenic (dPI3K Tg) mice by crossing PI3Kγ (-/-) mice with the PI3KαDN mice. Hearts from the wildtype, PI3KαDN and dPI3K Tg mice were subjected to 30 minutes of ischemia and 40 min of reperfusion using an *ex vivo* isolated heart perfusion system. dPI3K Tg hearts exhibited significantly greater functional recovery in comparison to hearts isolated from wild type mice (64.7 ± 18.0% vs 20.7 ± 12.8%, respectively; $p < 0.05$)

measured at the end of the 40 min reperfusion period. This was similar to the level of recovery observed in the PI3K□DN hearts (67.7 ± 12.1%). Akt is a downstream target of the PI3K signalling pathway. We observed no differences in either the phosphorylation levels of Akt (Ser473) or total Akt levels between the wildtype, PI3KαDN, dPI3K Tg hearts. Thus, we propose that there may be an alternative cellular signalling pathway that is active and has cardioprotective potential in the PI3KαDN and dPI3K Tg hearts. We examined the possible role of the mammalian target of rapamycin complex 2 (mTORC2) pathway. Accordingly, we treated PI3KαDN and dPI3K Tg mice hearts with the selective mTOR inhibitor, Ku-0063794 (1 μM), for 5 min prior to 30 min of ischemia followed by reperfusion. There was a significant reduction in functional recovery in PI3KαDN (43.5 ± 5.2%, $p < 0.05$) and dPI3K Tg hearts (39.0 ± 3.0%, $p < 0.05$) at the end of the reperfusion period. Our research suggests the mTORC2 pathway may play a significant role in activation of cardioprotection in the PI3KαDN and dPI3K Tg hearts subjected to ischemia-reperfusion.

Role of the equilibrative nucleoside transporter ENT1, in cardioprotective effects of ethanol

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A number of epidemiological studies have shown that light-to-moderate alcohol consumption carries certain health benefits including a 25–30% lower risk in developing cardiovascular disease (CVD) and ischemic stroke. This well-established decrease in risk and incidence of developing CVD suggests that ethanol renders the heart cardioprotected, a phenomenon that has been described as "ethanol preconditioning". While the mechanisms of ethanol preconditioning are unclear, one critically important signalling pathway has been identified as involving adenosine. Moreover, the adenosine transporter, ENT1, has been shown to be inhibited by ethanol in neural and bronchial cells, leading to alterations in adenosine signaling, suggesting it is part of the mechanism underlying ethanol preconditioning. Therefore our aim in this study is to 1) investigate the nature of ethanol sensitivity of ENT 1 in cardiomyocytes 2) examine the regulation of ethanol sensitivity of ENT1 by phosphorylation via PKC and PKA. We found that ethanol consistently and significantly inhibited adenosine uptake in HL-1 cells (9.27± 0.33 pmole/mg $p<0.001$), compared to control cells (12 ± 0.37 pmole/mg). Overall uptake was reduced by 24% ($p<0.001$), confirming that adenosine uptake in cardiomyocytes is sensitive to inhibition by ethanol. Ethanol sensitivity in neural cells has been previously correlated with PKA activation and we found that incubation of HL-1 cells with the specific PKA activator, Sp-cAMPs prior to ethanol treatment resulted in enhanced inhibition of uptake compared to control cells treated with ethanol only. Ethanol inhibited adenosine uptake by 37% in Sp-cAMPs treated cells compared to untreated cells (24%, $p<0.01$). These results suggest that ethanol sensitivity of ENT 1 transport is affected by PKA activity. We also investigated the role of PKCε in regulation of ethanol sensitivity of adenosine uptake via using PKCε null mice primary cardiomyocytes treated with varying concentrations level of ethanol. Our data shows that primary cardiomyocytes, treated with ethanol displayed ≥ 50% inhibition of adenosine uptake compared to control PKCε cardiomyocytes. These data indicate that loss of PKCε results in a highly sensitive mENT1 transporter and hence increased inhibition of adenosine uptake implying that PKCε is a key element in determining ethanol sensitivity of ENT1. Overall our preliminary data suggest that adenosine uptake via ENT1 is partially inhibited by exposure to ethanol in cardiomyocytes which we propose leads to enhanced adenosinergic signaling and cardioprotection. Furthermore, signaling pathways involving PKC and PKA modulate ethanol sensitivity of ENT1.

Modulation of cortical excitability and interhemispheric inhibition prior to a voluntary unimanual contraction

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The supplementary motor area (SMA) is a premotor structure that plays a role in unimanual movement. Given the strong structural connectivity between hemispheres of the SMA and the primary motor cortex, we hypothesized that the SMA controls unimanual movement through the modulation of intracortical and interhemispheric inhibitory circuits within the primary motor cortex. Transcranial magnetic stimulation (TMS) was used to assess short interval intracortical (SICI) and interhemispheric (IHI) inhibition 500-ms prior to planned contractions of the right first dorsal interosseous in 10 participants (aged 21.4±1.9 years). These measures of inhibition were made in three different states: 1) with the hand at complete rest (with no plan to contract), 2) with the hand at rest just prior to a planned contraction, and 3) during low level contractions. In a second experiment (n = 10, aged 23.4±5.6 years) pre-contraction SICI and IHI were assessed before and after repetitive TMS (rTMS) to the SMA . Subthreshold rTMS was delivered at 5Hz with the intent of increasing SMA activity and at 1Hz to decrease SMA activity. Cortical excitability was enhanced prior to a contraction and during a contraction compared to at rest. IHI was also increased prior to a contraction compared to at rest and during a contraction while SICI was only reduced during a contraction. While these findings are consistent with premotor regulation of primary motor cortex excitability, there was no effect of subthreshold rTMS to the SMA on measures of cortical excitability and inhibition following either 5Hz or 1Hz stimulation.

High-intensity interval training improves insulin sensitivity independent of adipose tissue inflammation

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Abstract: Obesity is associated with a state of chronic, low-grade inflammation that contributes to the development of insulin resistance. Exercise is known to improve insulin resistance, and emerging evidence suggests that exercise also reduces adipose tissue inflammation. However, the relationship between exercise and inflammation has not been separated from the confounding effect of weight loss. The objectives of this study are 1) to determine whether exercise improves insulin sensitivity in obese mice independent of weight loss and 2) to assess the effect of exercise on the relationship between adipose tissue inflammation and insulin sensitivity.

C57Bl6 mice were assigned to one of three groups: a control, chow diet (Chow), 12 weeks of high-fat diet with no exercise (HFD Sed), or 6 weeks of high-fat diet feeding followed by an additional 6 weeks of high-intensity interval training (HFD Ex). Body mass, adiposity, insulin sensitivity, and adipose tissue inflammation were assessed following 6 weeks of exercise.

In HFD-induced obese mice, HIT had no effect on body mass, epididymal fat mass, adiposity, or adipocyte size, despite increased food intake compared to sedentary controls. Nevertheless, exercised mice had improved fasting insulin levels, HOMA-IR, and insulin sensitivity, as measured by both insulin tolerance test and hyperinsulinemic-euglycemic clamp. Together, these findings conclude that HIT improves insulin sensitivity independent of changes in body mass.

However, adipose tissue inflammation, macrophage infiltration, and adipose tissue macrophage polarization were unaffected by exercise training. In contrast to previous studies, this data suggests that exercise training does not improve adipose tissue inflammation associated with obesity.

Taken together, this study concludes that HIT improves insulin sensitivity in HFD-induced obese mice independent of both body mass and adipose tissue inflammation. The benefits of exercise in obese individuals are obvious; however, the mechanisms underlying the improvements in insulin sensitivity observed following chronic, HIT remain to be elucidated.

In skeletal muscle, electron transfer flavoprotein (ETF) plays a minor role in fatty acid supported state 3 respiration

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INTRODUCTION. Reducing equivalents derived from fatty acid catabolism enter the electron transport chain at three different sites; complex I (CI), complex II (CII) and the less characterized site known as the electron transfer flavoprotein (ETF). The relative contribution of each of these entry points to maximal state 3 fatty acid supported respiration is currently unknown.
METHODS. Permeabilized skeletal muscle fibres were utilized and the kinetic characteristics for various fatty acid species (ie. palmitate, palmitoyl-CoA and palmitoyl carnitine) were determined to ensure that the contribution of each entry point was examined under maximal state 3 fatty acid supported respiration. Under these conditions, specific inhibitors of CI (rotenone) and CII (malonate) were used to determine the relative importance of these entry points. The remaining respiration following the addition of both rotenone and malonate estimated the contribution of the ETF.
RESULTS. The VMax of palmitate (272±24 pmol O2/mg dw) was similar to P-CoA (253±22), but both were significantly higher than PC (207±15). Of note, P-CoA and PC inhibited respiration at concentrations above the observed VMax. The Km values for palmitate (~92μM) and P-CoA (~81μM) were similar, while the Km for PC (~21μM) was significantly lower than both. Given that palmitate generated the highest VMax, we utilized this substrate to determine the relative contribution of CI, CII and the ETF to fatty acid supported respiration. Inhibition of CI with rotenone decreased respiration by 70%, inhibition of CII with malonate decreased respiration by 15%, while the ETF also accounted for 15% of the respiration.
CONCLUSION. In conclusion, the non-physiological substrates P-CoA and PC decrease respiration at high concentrations consequently limiting VMax. As a result, the more physiological substrate palmitate has the highest VMax. In addition, we conclude that CI is the main entry point for reducing equivalents during fatty acid supported respiration, while CII and the ETF play minor roles.

Fibre type specific distribution of SERCA1a, SERCA2a and Phospholamban in human vastus lateralis

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Previous studies in rodents have shown that the expression patterns of sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA) isoforms and myosin heavy chain (MHC) isoforms in skeletal muscle are closely matched. Specifically, fast-twitch skeletal muscles contain SERCA1a and MHCIIb/IIx/IIa whereas slow-twitch muscles contain SERCA2a and MHCI. Phospholamban (PLN), a known regulator of the SERCA pumps, has been shown to be coexpressed almost exclusively with SERCA2a in rodent skeletal muscle. The purpose of this study was to examine the fibre type specific expression of SERCA1a, SERCA2a and PLN in human skeletal muscle. Muscle biopsies from the vastus lateralis were extracted from five healthy university males and serial cross sections were immunohistochemically stained for SERCA1a (A52), SERCA2a (2A7-A1) and PLN (2D12). Immunofluorescence analysis of MHC expression was also performed with primary antibodies against MHCI (BA-F8), MHCIIa (SC-71) and MHCIIx (6H1). Although the general fibre type distribution of SERCA and MHC isoforms, which is well-defined for rodent skeletal muscle, also clearly exists in human vastus lateralis, we found that SERCA1a was expressed in 58.2±7.5% of muscle fibres containing MHCI and SERCA2a was expressed in 17.7±9.5% of muscle fibres containing MHCIIa. Fibres containing MHCIIx expressed the SERCA1a isoform exclusively. PLN expression was found in all fibres containing MHCI and SERCA2a and in a large population of fibres containing MHCIIa and SERCA1a suggesting that PLN may interact with both SERCA2a and SERCA1a. These data suggest differences in the regulation of genes encoding muscle

contractile and sarcoplasmic reticulum proteins between rodents and humans and underscore the importance of applying caution when translating findings from animal models to humans.
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Metabolic response of human skeletal muscle to continuous low volume high-intensity exercise training

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A continuous 3-5 min bout of "all-out" exercise appears capable of sufficiently activating signalling molecules linked to PGC-1 α mRNA expression and mitochondrial biogenesis, and could therefore potentially stimulate significant metabolic and performance enhancements in skeletal muscle. PURPOSE: To investigate the metabolic and performance improvements induced by 6 wk of continuous low volume high-intensity exercise training in human skeletal muscle. METHODS: Six healthy men (84+27 kg, 24+3 y, 46+8 mL/kg/min) and four healthy women (68+8kg, 21 y, 42+4 mL/kg/min) reported to the laboratory to perform 3 supervised cycle ergometer-training sessions per week for 6 wk (~18 sessions). During each training session, subjects completed designated workloads as quickly as possible in one continuous effort. 1 kJ/kg BM was assigned during each training session for the first 2 wk, and 1.25 kJ/kg BM was completed during each session for the last 4 wk of training. RESULTS: Training decreased time required to complete 250 kJ time trials by 9.9% ($P < 0.05$). 4-8 min of exercise was completed during each training session. Training completion times, mean power outputs and peak power outputs improved over the 6 wk training period, while heart rates and ratings of perceived exertion remained constant. CONCLUSION: 4-8 min of continuous low volume "all-out" exercise, performed 3 d/wk for 6 wk, significantly improves exercise performance. This improvement seems less pronounced than those observed in previous HIT studies, perhaps because the work volume is not increased to compensate for the lower exercise intensity relative to HIT. Physiological and biochemical analyses may provide further insight into the nature and benefits of this stimulus.

Contractile activity-induced alterations in autophagy in striated muscle.

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Autophagy is a proteolytic pathway that functions within cells to degrade damaged or dysfunctional organelles, and to remove harmful protein aggregates. Recently, this intracellular signalling pathway was shown to be involved in the regulation of muscle mass, and it is known that muscle fiber types appear to atrophy at different rates. To examine whether this is related to oxidative capacity, we investigated the levels of autophagic proteins in various fiber types (soleus, plantaris, and heart), in response to a 9 week voluntary wheel training protocol, or to unilateral chronic muscle stimulation (CCA, 10Hz, 3h/day, 7 days). Beclin-1 and LC3II protein expression was highest in muscles possessing a high oxidative capacity (heart) and lowest in the least oxidative plantaris muscle. In response to training and CCA, mitochondrial content increased by 35-40%. The autophagic proteins ATG7, ULK1, LC3II and Beclin1 were elevated 2- to 3-fold following CCA, but were not significantly elevated following 9 weeks of training. Therefore, these data demonstrate a relationship between muscle oxidative capacity and autophagic protein expression under steady state conditions, but also indicate that autophagy may be an early event in the muscle remodeling that occurs with exercise.

Does p53 affect mitochondrial protein import in skeletal muscle?

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Mitochondrial biogenesis requires the coordinated interplay of the nuclear and mitochondrial genomes. Nuclear-encoded mitochondrial proteins are imported into the organelle by the protein import machinery (PIM). Thus, we sought to elucidate whether the reduced mitochondrial content in p53 deficient animals could be attributed to defects in the import pathway. Subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria were isolated from muscle of p53 WT and KO mice. The PIM proteins Tom20 and Tim23 were decreased by 28% and 36% ($p<0.05$) respectively in SS mitochondria of KO mice. The mitochondrial heat shock protein70 (mtHSP70), crucial for import of proteins into the mitochondria, was reduced by ~51% in SS mitochondria from KO mice, compared to WT counterparts. In contrast, other mitochondrial chaperones such as mtHSP60 and cpn10 were not different between the two genotypes. To evaluate protein import, isolated SS and IMF mitochondria from p53 WT and KO mice were incubated with radiolabelled, matrix-destined precursor protein. No changes were observed in the rate of protein import between the two genotypes. These data suggest that absence of p53 results in lower levels of important PIM components, however this was not reflected by a functional deficit as measured by matrix protein import. Whether import into other compartments, such as the outer or inner membranes, is defective, remains to be determined.

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