

Muscle Health Awareness Day

May 28, 2010



York University

Program and Abstracts



Faculty of Health





Date: May 28, 2010

To: All Participants

From: David A. Hood, MHRC Director

David A. Hood, PhD

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Welcome to the 1st Annual

Muscle Health Awareness Day

The Muscle Health Research Centre at York University welcomes you to our first annual event, 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.

Since this represents our inaugural event, any feedback for improvement is welcome. We hope to take advantage of the strong contingent of muscle physiologists / molecular biologists in the Southern Ontario and surrounding region to continue this tradition on a yearly basis, highlighting the work of both junior and senior faculty members, and giving graduate students an opportunity to network and present their work in an informal, yet educational manner.

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 28, 2010 - CSE B, York University

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

Session 1: Metabolism (9:00-10:00)

Session Chair: Dr. David Hood

Dr. David Hood, York University

Introduction (5 mins)

Dr. Olasunkami Adegoke, York University

The nutrient sensitive kinase S6K1 and its many faces in the regulation of skeletal muscle metabolism

Dr. Graham Holloway, University of Guelph

Fatty acid transport proteins: Role in regulating skeletal muscle fatty acid oxidation

10:00 - 10:30 Break (POSTER Presenting and Viewing)

Session 2: Cardiac Muscle (10:30-12:00)

Session Chair: Dr. Robert Tsushima

Dr. Marius Locke, University of Toronto

Stress Proteins and NF-kB in Striated Muscle

Dr. Scott Heximer, University of Toronto

Identifying new GAPs in our understanding of vascular and cardiac myocyte signaling

Dr. Anthony Gramolini, University of Toronto

Mass Spectrometry Analysis in Muscle: From Discovery to Targeted Studies

*12:00 - 1:30 Lunch (Orange Snail, Stong College)
1:00 - 1:30 (POSTER Presenting and Viewing)*

Session 3: Muscle Development and Satellite Cells (1:30-3:00)

Session Chair: Dr. Mike Connor

Dr. Gianni Parise, McMaster University
Regulation of Human Muscle Satellite Cells in Exercise and Aging

Dr. John McDermott, York University
Regulation of muscle differentiation by nuclear Smad7

Dr. Anthony Scime, York University
The Rb Family member p107 Determines the Oxidative Status of Muscle

3:00 - 3:30 Break (POSTER Presenting and Viewing)

Session 4: Exercise, Metabolism and Disease (3:30-5:00)

Session Chair: Dr. Mike Riddell

Dr. Sandra Peters, Brock University
Understanding the kinetic and physiological "personalities" of the PDH kinase isoforms

Dr. Ingrid Tein, University of Toronto
Approach to muscle cramps, exercise intolerance and recurrent myoglobinuria

Dr. Greg Wells, Sick Kids Hospital
Muscle mitochondrial function is associated with physical activity and not obesity status in youth

5:15 BBQ (Michelangelo's, Atkinson College, Cash Bar)

Muscle Health Awareness Day - Poster Presentations

Poster #	First Author (last name)	Title	University affiliation
1	Abdullahi	Physiological Regulation of mRNA Translation Inhibitor PDCD4 During Myogenesis	York
2	Alli	Role and regulation of Fra-2 during skeletal muscle development	York
3	Beaudry	High circulating glucocorticoids in combination with a high fat diet induces impairments on beta-cell function	York
4	Bellamy	Angiotensin II Modulate Skeletal Muscle during Regeneration	McMaster
5	Bousette	Compensated ER stress response and α -crystallin-mediated protection from apoptosis with cardiac-specific overexpression of calcineurin.	Toronto
6	Carter	Effects of mTORC1 inhibition on mitochondrial biogenesis during differentiation and chronic contractile activity.	York
7	Chan	Quantitative Profiling of the Secretome during Skeletal Muscle Cell Differentiation	York
8	Chis	Identification of the cyto-protective mechanism of α -crystallin B in cardiac hypertrophy	Toronto
9	Chu	Fat Oxidation Rates During Exercise in Obese Early- and Late-Pubertal Boys and Girls	York
10	De Lisio	Exercise training enhances the skeletal muscle response to radiation induced oxidative stress	McMaster
11	D'souza	Glucocorticoids Combined With High Fat Diet Accelerate the Development of Insulin Resistance in the Liver	York
12	D'souza	Post-transcriptional regulation of mitochondrial transcription factor A (Tfam) varies across skeletal muscle fibre types	York
13	Duchene	Electrical Stimulation Induces AMPK-Mediated Cell Cycle Arrest in C2C12 Myoblasts	York
14	Fediuc	The acute insulin sensitizing effects of troglitazone are mediated by AMPK activation and inhibition of pyruvate dehydrogenase activity	York
15	Forn	Voluntary exercise modulates Vascular Endothelial Growth Factor / Thrombospondin ratio and prevents capillary regression in Zucker Diabetic Fatty rats	York
16	Gee	The potential role of MEF2C and ATF2 in shear stress-mediated VEGF production	York
17	Gianforcaro	Is adequate really enough? Vitamin D3 supplementation at 10x the AI blunts disease severity and enhances functional outcomes in an animal model of Lou Gehrig's disease. A Pilot Study.	York
18	Gulli	Restoration of adiponectin response is not necessary for the exercise-induced recovery of insulin response in high-fat-fed rats	Guelph

19	Haas	FOXO1 upregulation in response to hindlimb ischemia	York
20	Iqbal	Analysis of mitochondrial dynamics in C2C12 myoblasts	York
21	Jassi	Mitochondrial Oxidative Phosphorylation as an End Effector of Ischemic Preconditioning	York
22	Jeganathan	Serine phosphorylation of IRS-1 and the associated inhibition of glucose uptake are normal and reversible physiological responses to nutrition in muscle cells	York
23	Johnston	Angiotensin II signalling regulates skeletal muscle growth and myoblast chemotaxis	McMaster
24	Kishlyansky	Vasohibin-1: New Actor of Physiological and Pathological Angio-adaptation in Rat Striated Muscle	York
25	Krause	Impaired post-injury regeneration of skeletal muscle in type 1 diabetic mice	McMaster
26	Leung	Skeletal Muscle Regulatory Responses to Increased Extracellular Lactate	Guelph
27	Lindinger	Regulatory volume increase in single mouse soleus muscle fibres assessed simultaneously using intracellular fluorescence and fibre width	Guelph
28	Menzies	Resveratrol and Voluntary Running both Attenuate the Reduced Mitochondrial Function in Muscle-Specific SirT1 KO Animals	York
29	Moradi	Impaired Satellite Cell Activation in T1DM Skeletal Muscle	McMaster
30	Noronha	Identification and Characterization of Cell-Surface Associated Proteins of the Human Heart	Toronto
31	Peckett	A high fat diet combined with exogenous glucocorticoid administration influences intramyocellular lipid accumulation and accelerates the development of insulin resistance	York
32	Ramadan	Development of conditional murine knockout models of equilibrative nucleoside transporters	York
33	Ritchie	Restoration of skeletal muscle leptin response is not necessary to rescue insulin response in high fat fed rats with endurance exercise training	Guelph
34	Rodness	MitoKATP and MPTP are not end effectors of the PI3K-Akt-GSK3b pathway in myocardial ischemia preconditioning	York
35	Ryan	LC-MS Analysis to Identify Ryanodine Receptor 1 (RyR1) Protein-Protein Interactions	Toronto
36	Saleem	p53 mRNA content and stability - effect of AMP Kinase (AMPK) and contractile activity	York
37	Seisan	The intracellular abundance of the pro-obesity kinase, S6K1, is regulated by targeted proteolysis	York
38	Serino	Effects of Oral Leucine Administration on mTOR signalling in Skeletal Muscles of Different Fibre Types in Young Partial Pancreatectomized Diabetic Rats	York

39	Sharma	Endoplasmic reticulum (ER) targeting of Phospholamban: The di-arginine motif in ER retention	Toronto
40	Singh	Effect of denervation on mitochondrial protein import and machinery expression	York
41	Solomon	Neuromuscular Disease and Ethics: Considerations of Endpoint in a Mouse Model of Lou Gehrig's Disease	York
42	Syme	Muscle mitochondrial function is associated with physical activity and not obesity status in youth	Toronto
43	Toth	Satellite Cell Specific p-STAT3 Signalling in Human Muscle Following Acute Muscle Damage	McMaster
44	Trifonova	Corticosterone mediated effects on endothelial cells response to shear stress	York
45	Uchida	TIMP-1 protein but not secretion is increased by shear stress in the skeletal muscle microvasculature	York
46	Uguccioni	PGC-1 α -dependent mitochondrial adaptations in C2C12 cells: effects of contractile activity	York
47	Vainshtein	Voluntary aerobic exercise attenuates oxidative stress-induced apoptotic signalling in cardiac muscle	York
48	Wawrzyczek	Induction of the mitochondrial permeability transition pore (mtPTP) in control and denervated skeletal muscle	York
49	Zargar	The level of mRNA repressor PDCD4 and protein synthesis in skeletal muscle are inversely regulated by nutrition	York

Muscle Health Awareness Day – Abstracts

Physiological Regulation of mRNA Translation Inhibitor PDCD4 During Myogenesis

A. Abdullahi, O. Adegoke.

Department of Kinesiology and Health Science, Muscle Health Research Centre, York University, ON M3J 1P3

Defects in skeletal muscle integrity underly disease conditions such as muscular dystrophy and obesity. In individuals with these pathological conditions, their inability to maintain skeletal muscle mass often worsens their conditions. Therefore, deciphering the cellular mechanisms regulating muscle metabolism is of great interest. Studies have shown that the mammalian target of rapamycin complex one (mTORC1), through its downstream targets S6K1 and 4E-BP1, is involved in regulating mRNA translation and proteins synthesis in skeletal muscle. It does this in part by preventing the recruitment of the translation inhibitor, tumor suppressor programmed cell death 4 (PDCD4). The objective of this study was to explore the regulation of this protein, along with that of its upstream kinase S6K1, during L6 skeletal muscle cell differentiation. L6 rat skeletal myoblasts were plated in growth media and upon reaching 90% confluency, the cells were incubated in differentiation media on days 1-5. Our results indicate that total PDCD4 levels increased on Day 1 relative to Day 0(myoblast), and then decreased gradually as differentiation progressed. Furthermore, we examined through cell fractionation the level of PDCD4 in the nucleus relative to the cytoplasm during myogenesis, as previous studies have indicated that the cellular localization of PDCD4 is important for its function. Total PDCD4 accumulation in the nucleus was significantly increased in Day 1 relative to Day 0 then decreased gradually by Day 4 ($P < 0.05$). Therefore, these results are in conjunction with earlier studies that have shown that S6K-1 kinase activity increases as myoblasts form myotubes, since this kinase mediates PDCD4 degradation. Thus, our results imply that cells physiologically down-regulate PDCD4, a known protein translation inhibitor, to allow the proper translation of myofibrillar proteins required as myoblasts differentiate into the more specialized myotubes.

Role and regulation of Fra-2 during skeletal muscle development

Nezeka S. Alli¹, Eric Yang², Tetsuaki Miyake¹, Arif Aziz¹, 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 Activating Protein-1(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 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 investing how these sites affect Fra-2 regulation and its contribution to AP-1 gene regulation in skeletal myogenesis.

High circulating glucocorticoids in combination with a high fat diet induces impairments on beta-cell function

Jacqueline Beaudry, Anna D'Souza, Ashley Peckett, Jonathan Cambell, Michael Riddell
York University, Toronto, Ontario

Under normal conditions, glucose homeostasis is maintained through appropriate insulin secretion by the pancreatic beta-cells. Abnormal beta-cell activity is typically characterized by accentuated insulin resistance, termed compensation, followed by reductions in insulin secretion and eventually complete beta-cell failure, resulting in the development of T2DM. The cause(s) of beta-cell death is unclear but may be related to glucolipotoxicity such as those who have elevated blood glucose levels and are insulin resistant. Many studies have thoroughly investigated the effects of high Glucocorticoids (GCs) and high-fat (HF) feeding on beta-cell function alone, but few have examined these stressors in combination. Therefore, to address this issue, male Sprague-Dawley rats (145-155g) were administered either corticosterone pellets (CORT) (400mg subcutaneous) or wax pellets (controls) (n=6/grp) in combination with a HF diet for 14 days. After 5 days of treatment, fed blood glucose levels in CORT-HF treated rats were found to be elevated (>11 mM, p<0.05), and remained high for the duration of the study, indicating beta-cell dysfunction. Surprisingly, the administration of an oral glucose gavage (0.5mg/kg) significantly decreased insulin levels in rats given CORT and HF relative to basal insulin levels. Furthermore, there appears to be a positive trend in CORT treated animals to an overall increase in beta-cell mass. These changes may be indicative of hypertrophic mechanisms within the pancreatic islets due to the treatment of CORT and HF diet that results in these animals becoming hyperglycaemic/hyperinsulinemic. These findings suggest detrimental alterations to normal functioning pancreatic beta-cell induced by high levels of CORT and fat.

Angiotensin II Modulate Skeletal Muscle during Regeneration

Leeann M. Bellamy¹, Adam P. W. Johnston¹, Michael De Lisio¹ and Gianni Parise^{1,2}
Departments of Kinesiology¹ and Medical Physics & Applied Radiation Sciences² McMaster University, Hamilton Ontario, Canada

Angiotensin II (AngII) is a pleiotropic peptide involved in vasoactivity as well as cellular growth and proliferation. We examined whether AngII signaling is also a mediator of muscle regeneration by inducing damage in C57Bl/6 mice supplemented with captopril (an ACE inhibitor) as well as in AngII type 1a receptor knockout mice (AT1a^{-/-}). Ten days following CTX injection, inducing muscle injury, captopril treated mice demonstrated a delayed response in the myogenic program evidenced by sustained expression of Pax7 and Myf5 transcripts, and inhibited myogenin expression. Fibre CSA between captopril-treated and AT1a^{-/-} groups in

comparison to controls was consistent at early time-points representative of fibre regeneration. Thus, myofibre formation is not an AngII dependent process; however, a ~3.5 fold elevation in eMHC gene expression within fibres of captopril treated mice suggests a delay in maturation. Newly formed fibres in the captopril treated and AT1a^{-/-} mice demonstrated significantly impaired myofibre hypertrophy following regeneration. This is evidenced by increases in fibre CSA of 81% in the control group while captopril treated mice only displayed a 27% increase from days 10-21 post CTX injection. Similarly, from days 7-14 post CTX injection, control mice displayed an increase in CSA of 111% while AT1a^{-/-} only hypertrophied by 19%. These changes resulted in overall differences of ~20-25% in CSA between control and experimental groups at day 21 post injury. These results suggest that AngII is a necessary modulator of muscle stem cell activity and skeletal muscle growth, primarily signaling through the AT1 receptor.

Compensated ER stress response and α -crystallin-mediated protection from apoptosis with cardiac-specific overexpression of calcineurin.

Nicolas Boussette^{1,2}, Shaan Chugh^{1,2}, Thomas Kislinger^{3,4}, Vincent Fong^{5,6}, Ruth Isserlin^{5,6}, Liling Zhang⁷, Kyoung-Han Kim², Peter Liu⁴, Peter H. Backx², David H. MacLennan^{2,5}, Allen Volchuk^{1,4}, Andrew Emili^{2,5,6}, and Anthony Gramolini^{1,2}

¹Department of Physiology, University of Toronto; ²Heart and Stroke/Richard Lewar, Centre of Cardiovascular Excellence; ³Department of Medical Biophysics, University of Toronto and ⁴University Health Network, Toronto, Ontario, Canada. ⁵Banting and Best Department of Medical Research, ⁶Donnelly Centre for Cellular and Biomolecular Research

Rationale: Cardiac hypertrophy is a prominent characteristic of many cardiomyopathies. Therefore, delineating the proteomic profile of a model of hypertrophic cardiomyopathy may lead to the identification of novel therapeutic targets. Objective: To identify and characterize the global protein landscape of ventricular tissue from a mouse model of hypertrophic cardiomyopathy with cardiac-specific transgenic over-expression of constitutively active calcineurin (CNA). Methods and Results: We performed gel free liquid chromatography linked to shotgun tandem mass spectrometry on fractionated cardiac samples from CNA mice and their wild-type (WT) littermates. We identified 1918 proteins with high confidence, of which 290 were differentially expressed. Since characterization of the proteome demonstrated that ER stress is activated in CNA mice, we validated the occurrence of ER stress in adult CNA cardiomyocytes. We demonstrated calcineurin-mediated cyto-protection of cultured neonatal mouse cardiomyocytes (NCMs). In addition, we identified α -crystallin B (Cryab) as a potential mediator of this protective effect and showed that silencing of Cryab via LentiVector-mediated transduction of shRNAs in NCMs leads to a significant reduction in NCM viability and ablation of the calcineurin-mediated cyto-protective effect. Conclusions: Cryab plays a role in calcineurin-mediated cyto-protection. Identification of this factor as a downstream effector of calcineurin-induced cyto-protection will permit elucidation of its role in cardiac apoptosis and its potential as a therapeutic target.

Effects of mTORC1 inhibition on mitochondrial biogenesis during differentiation and chronic contractile activity.

Carter, HN and Hood, DA.

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

Mitochondrial biogenesis occurs during the course of myogenesis, and in response to chronic contractile activity (CCA) or exercise. The mammalian target of rapamycin complex 1 (mTORC1) has been implicated in the regulation of the expression of nuclear genes encoding mitochondrial proteins by facilitating an interaction between the transcription factor YY1 and the potent coactivator, PGC-1 α . The purpose of this investigation was to ascertain whether the inhibition of mTORC1 would attenuate mitochondrial biogenesis during either skeletal muscle differentiation, or CCA. C2C12 myoblasts were differentiated into myotubes over four days in the presence or absence of rapamycin, a known mTORC1 inhibitor. Cell morphology and protein markers of differentiation, mitochondrial biogenesis and mTORC1 activity were evaluated. mTORC1 inhibition blocked the expression of myosin heavy chain and prevented the formation of mature myotubes, but did not affect the differentiation-induced increase in COX I protein. COX IV and Tfam expression were also similar between the treatment conditions. In response to CCA of C2C12 myotubes, we analyzed mTORC1 signaling and protein expression of mitochondrial biogenesis and the mTORC1 complex in the presence or absence of rapamycin. Rapamycin completely inhibited mTORC1 signaling to p70S6K1, despite the induction of a 1.4-fold increase in the total amount of raptor, an integral component of the mTORC1 complex. CCA increased COX IV protein levels by 1.9-fold, and a similar fold increase was observed with rapamycin treatment. Thus, our data suggest that mTORC1 inhibition does not blunt the response of mitochondrial protein expression to CCA. However, whether mitochondrial function has been altered in the presence of mTORC1 inhibition, remains to be determined.

Quantitative Profiling of the Secretome during Skeletal Muscle Cell Differentiation

C.Y. Chan^{1,2}, O. Masui^{2,3}, O. Krakovska^{2,3}, J.C. McDermott^{1,2}, and K.W. M. Siu^{2,3}

¹Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3. ²Centre for Research in Mass Spectrometry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3. ³Department of Chemistry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

Systematic characterization of secretome (i.e. profile of secreted proteins) which regulates muscle cell differentiation, termed as myogenesis, might help to identify new therapeutic targets for various muscle disorders, such as Duchenne Muscular Dystrophy (DMD). Previously, we have successfully identified 26 secreted proteins in mouse skeletal muscle cells C2C12 (Chan, C.Y. et al., 2007). In an effort to attain a more comprehensive picture of myogenesis in extracellular milieu, quantitative profiling via Stable Isotope Labeling by Amino acids in Cell culture (SILAC) was implemented in conjunction with two parallel high throughput online reversed phase liquid chromatography tandem mass spectrometry (RPLC-MS/MS) analyses: Agilent 1100 RPLC-LTQ linear ion trap (LIT) and LC Packings RPLC-QSTAR Pulsar hybrid tandem quadrupole/ time-of-flight (QqTOF). In summary, 91 secreted proteins were quantified in total, 78 of which were shown to be differentially expressed during muscle development.

Intriguingly, some of these proteins were shown to have functional relevance with respect to muscle development and postnatal muscle regeneration. To our knowledge, this is the first systematic study on quantitative profiling of secreted proteins during myogenesis, findings of which could not only provide a platform for better understanding on orchestration of myogenesis in extracellular milieu, but also might advance the pace in exploring new therapeutic targets for various muscular dystrophies.

Identification of the cyto-protective mechanism of α -crystallin B in cardiac hypertrophy

Roxana Chis, Parveen Sharma, Nicolas Bousette, and Anthony O. Gramolini.

Department of Physiology, University of Toronto.

Background: α -Crystallin B (CryAB) is the most abundant small heat shock protein in cardiomyocytes where it has been suggested to have potent anti-apoptotic properties. Mice expressing cardiac restricted constitutively active Calcineurin A (CnA; a serine/threonine phosphatase) manifest pronounced cardiac hypertrophy. Evidence shows that calcineurin over-expression also has cardio-protective properties. In a global proteomic survey of cardiac tissue using a CnA transgenic mouse model, we identified cryAB as a likely mediator of this cyto-protection. Studies show that ex vivo perfused hearts from transgenic mice that over-express cryAB tolerate ischemia/ reperfusion better, while cryAB null mouse hearts display poorer functional recovery and a higher cell death rate following ischemia/reperfusion compared with wild-types. My study involves the characterization of the cyto-protective mechanism of crystallin in CnA hypertrophy. **Hypotheses:** 1) cryAB over-expression in cardiomyocytes will lead to decreased apoptosis by inhibiting the apoptotic pathway upstream of caspase 3 activation; and 2) this cyto-protective effect will be lost when crystallin is knocked down. **Methods and Results:** CryAB was over-expressed in neonatal mouse cardiomyocytes via Effectene transfection with plasmids encoding cryAB cDNA. The cyto-protective properties of cryAB were assessed following exposure of cardiomyocytes to 200 μ M H₂O₂ using Annexin V-based fluorescence activated cell sorting (FACS). FACS analysis showed that cryAB over-expression leads to increased cell viability under stressed conditions. CnA transgenic hearts exhibit significantly elevated cryAB levels as assessed by immunoblot analysis. To determine the cyto-protective mechanism, subcellular fractionation of WT and CNA cardiomyocytes will be performed and the localization of cryAB in stressed and un-stressed conditions and its interactions with pro- and anti-apoptotic proteins will be determined by Western Blot analysis, confocal microscopy and co-immunoprecipitation. **Conclusion:** CryAB is a regulator of cardiomyocyte apoptosis, a prominent feature of cardiomyopathies. Future work to elucidate its interaction with apoptotic proteins will provide insight into its cyto-protective mechanism.

Fat Oxidation Rates During Exercise in Obese Early- and Late-Pubertal Boys and Girls

L.Chu^{1,4,5}, J. Hamilton^{2,4,5,6}, J.E. Schneiderman^{4,5}, B. McCrindle^{3,5,6}, M.C. Riddell¹
York University¹, Divisions of Endocrinology² and Cardiology³, Physiology and Experimental Medicine⁴, the Hospital for Sick Children⁵, University of Toronto⁶, Toronto, Ontario, Canada

Background: Obesity is often associated with insulin resistance (IR) and an impaired capacity to oxidize fat. Concurrently, a transient increase in IR at the onset of puberty may influence fat

oxidation rates (FOR) in obese children. While studies have shown that maximal FOR during exercise decrease with puberty in lean and obese boys, no studies have yet evaluated this in obese girls. We hypothesized that maximal FOR during exercise will be: i) lower in late-pubertal boys and girls compared to pre- and early-pubertal boys and girls and; ii) lower in obese boys compared to obese girls at all stages of puberty. **Methods:** Obese boys and girls ages 8-18y were recruited to participate in the study. Measurements included: 1) VO₂max on a cycle ergometer; 2) FOR calculated during progressive submaximal exercise; 3) body composition via air displacement plethysmography and Tanner stage. Subjects were categorized as pre- and early-pubertal (Tanner Stage I & II) or late-pubertal (Tanner Stage III - V). For each subject, a best-fit polynomial curve was constructed using FOR (mg/kg fat free mass (FFM)/min) vs. exercise intensity (%VO₂peak) to estimate maximal FOR. **Results:** Preliminary results in 16 obese children suggest a significant interaction between gender and pubertal stage on maximal FOR during exercise (p<0.01). FOR during exercise in late-pubertal girls (4.1±0.6 mg/kg FFM/min) was higher compared to early-pubertal girls (2.3±0.6 mg/kg FFM/min) (p=0.01). In boys, FOR were lower in late-puberty (2.5±1.1 mg/kg FFM/min) compared to early-puberty (5.3±1.8 mg/kg FFM/min) (p<0.05). Late-pubertal boys had lower FOR than late-pubertal girls (p=0.02), but this relationship was not observed in early-puberty. **Conclusions:** Unlike obese boys, obese girls may have an increased capacity to oxidize fat after puberty. These findings may have relevance in determining the appropriate exercise prescription to maximize fat oxidation in obese children and adolescents.

Exercise training enhances the skeletal muscle response to radiation induced oxidative stress

Michael De Lisio¹ Jan J. Kaczor^{2,4} Nghi Phan³ Mark A. Tarnopolsky² Douglas R. Boreham³ Gianni Parise^{1,3}

Department of Kinesiology¹, Medical Physics and Applied Radiation Sciences³, and Medicine², McMaster University, Hamilton, Canada. Department of Biochemistry⁴, Academy of Physical Education and Sport, Gdansk, Poland

Introduction: Over production of reactive oxygen species (ROS) can damage cellular macromolecules leading to cellular dysfunction or death. Exercise training induces beneficial adaptations in skeletal muscle that may reduce cellular damage from ROS. **Methods:** To determine the response of exercise-conditioned muscle to acute increases in ROS, exercise trained (EX) and sedentary (SED) mice were exposed to a high dose of radiation (HDR). Antioxidant enzyme and metabolic enzyme activity were measured. **Results:** Total SOD and MnSOD activities were all increased in radiation challenged EX mice as compared to unchallenged EX mice (both p<0.05), with no such increase observed in SED mice. CS and cytochrome c oxidase activity were both elevated in radiation challenged EX mice as compared to unchallenged EX mice (both p<0.05). **Discussion:** Here, we demonstrate that preconditioning skeletal muscle with EX enhances the response of antioxidant and mitochondrial enzymes to radiation.

Glucocorticoids Combined With High Fat Diet Accelerate the Development of Insulin Resistance in the Liver

Anna D'souza¹, Jacqueline Beaudry¹, Ashley Peckett¹, and Dr. Michael Riddell¹

¹Department of Kinesiology & Health Science, York University, Toronto ON

In the liver, chronic glucocorticoid (GC) treatment disrupts the ability of insulin to regulate hepatic glucose production, resulting in hyperglycemia. High-fat (HF) feeding also alters the expression of key enzymes involved in insulin-regulated glucose production and results in hepatic lipotoxicity, both of which cause insulin resistance. No previous studies have investigated their effects combined. Therefore, the purpose of our analysis was to characterize regulation of hepatic glucose production in response to exogenous GCs and HF diet. Six-week old male Sprague Dawley rats were either administered corticosterone (CORT), (via four 100-mg pellets), or given a 60% high fat diet (HFD) or a combination of both (CORT-HFD) or neither (CNTRL). After 14 days of treatment, CORT-HFD treated animals displayed a diabetic phenotype (fasting blood glucose >11 mM) and insulin resistance, while the other three groups did not (glucose < 6 mM, $p < 0.05$). Activity of the gluconeogenic transcriptional factor, FOXO1, was three-fold higher relative to controls ($p < 0.05$). Further analysis of gluconeogenic proteins expression revealed a 25% increase relative to controls ($p < 0.05$). Surprisingly, CORT-HF mRNA expression of PEPCK was 87% lower than CNTRL. Furthermore, hepatic triglyceride content and visceral adiposity was two-fold greater relative to CNTRL ($p < 0.05$). Taken together, these findings suggest that exposure to chronic CORT and HFD have a synergistic effect in accelerating uncontrolled gluconeogenesis, and thus may result in hyperglycemia and a type 2 diabetic phenotype.

Post-transcriptional regulation of mitochondrial transcription factor A (Tfam) varies across skeletal muscle fibre types

Donna D'souza, Ruanne Lai, and David A. Hood.

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

Changes in mRNA stability may serve as a regulatory mechanism for mitochondrial biogenesis in skeletal muscle. We hypothesized that oxidative capacity would be closely associated with a greater mRNA stability of proteins vital to organelle biogenesis, prompting an enhanced mitochondrial content. Using an in vitro decay assay, the mRNA stability of mitochondrial transcription factor A (Tfam) was assessed, since it regulates the expression of mitochondrial DNA (mtDNA). The decay of Tfam mRNA was slowest in low oxidative fast-twitch white (FTW) muscle. Decay rates were 1.5- and 2.3-fold greater in fast-twitch red (FTR) and slow-twitch red (STR) fibers. Despite this, steady Tfam mRNA levels were not different between fiber types, suggesting increases in transcription in oxidative muscle which parallel mRNA decay. Degradation of mRNA in fiber types matched similar differences in the mRNA destabilizing protein AUF1, particularly the p42 isoform, with a 4.5-fold difference in protein expression between STR and FTW muscle. A more dramatic difference in the expression of the mRNA stabilizing protein HuR existed between fiber types, which may serve to restrain uncontrolled mRNA decay in highly oxidative muscle. Thus, oxidative muscle types exhibit faster rates of

Tfam mRNA turnover than low oxidative muscle, suggesting more precise control of Tfam expression, and thus mtDNA levels, in response to metabolic stress.

Electrical Stimulation Induces AMPK-Mediated Cell Cycle Arrest in C2C12 Myoblasts

Brittany Duchene and Michael K. Connor

Kinesiology & Health Science, York University, Toronto, Ontario, Canada

Skeletal muscle is phenotypically dynamic and responds to altered activity. Electrical stimulation (STIM, 3h/day) of differentiated C2C12 myotubes led to 18- and 12-fold increases in the protein levels of p27Kip1 and p21Cip1, respectively. This suggested STIM-induced cell cycle arrest in these cells. Thus, we stimulated proliferating myoblasts and examined the effects on p27 in these cells. p27 protein levels increased while cyclin E protein levels decreased from one to five days of STIM suggesting these cells underwent cell cycle arrest. Concomitant with these changes was increased phosphorylated AMPK which may underlie these STIM-induced effects. Similar effects were observed using differentiating C2C12 cells. Marked increases in T198 phosphorylated p27, a direct AMPK stabilizing phosphorylation, were correlated with increased p27 protein levels. Pharmacologic inhibition of AMPK activation completely abolished STIM-induced increases in AMPK activation, T198 p27 phosphorylation and total p27 protein levels. Thus, electrical STIM alters the activity of AMPK in skeletal muscle cells, induces cell cycle arrest and may mediate cell cycle exit prior to the initiation of differentiation.

The acute insulin sensitizing effects of troglitazone are mediated by AMPK activation and inhibition of pyruvate dehydrogenase activity

Fediuc S, Pimenta AS, Gaidhu MP, So M, Anthony NM, and Ceddia RB

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This study investigated the acute effects of troglitazone (Tro) on glucose and fatty acid (FA) partitioning in skeletal muscle. Exposure of L6 myotubes to Tro for 1h resulted in ~1.3- and ~1.6-fold increases in palmitate oxidation and CPT-1 activity, respectively. Tro inhibited basal (~25%) and insulin-stimulated (~35%) palmitate uptake but significantly increased basal (~2.2-fold) and insulin-stimulated (~2.7-fold) glucose uptake. Inhibition of AMPK prevented the effects of Tro on palmitate oxidation and glucose uptake. Although Tro exerted an insulin sensitizing effect, it reduced basal and insulin-stimulated rates of glycogen synthesis, incorporation of glucose into lipids, and glucose oxidation to values corresponding to ~30%, ~60%, and 30% of the controls, respectively. These effects were accompanied by an increase in basal and insulin-stimulated phosphorylation of AktThr308, AktSer473, and GSK3alpha/beta. Tro also powerfully suppressed pyruvate decarboxylation, which was followed by a significant increase in basal (~3.5-fold) and insulin-stimulated (~5.5-fold) rates of lactate production. In summary, we provide novel evidence that Tro exerts acute insulin sensitizing effects by increasing FA oxidation, reducing FA uptake, suppressing pyruvate dehydrogenase activity, and shifting glucose metabolism towards lactate production in muscle cells. Funding was provided by NSERC, CIHR, and CDA.

Voluntary exercise modulates Vascular Endothelial Growth Factor / Thrombospondin ratio and prevents capillary regression in Zucker Diabetic Fatty rats

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Introduction: Capillaries play an important role for muscle function by providing oxygen and nutrients to myofibers and by removing metabolic waste. Thus, the muscle vascular network has to remain well matched with myofibers' metabolic needs. This requires some vascular remodelling either by stabilization, regression or growth (angiogenesis) of capillaries. A balance between pro- and anti-angiogenic factors tightly regulates such angio-adaptive processes. Under pathological conditions such as obesity or type-2 diabetes, capillary regression is observed in skeletal muscles. Interestingly, physical activity - a powerful angiogenic stimulus - efficiently preserves muscle capillarization. The molecular mechanism underlying this beneficial effect of exercise remains unclear. **Objectives:** Our aim was to identify angio-adaptive factors involved in exercise-induced prevention of capillary regression in skeletal muscles from Zucker Diabetic Fatty (ZDF) rats. **Methods:** ZDF rats were either kept sedentary or subjected to voluntary exercise in wheel running cages for 7 weeks. Lean rats were used as healthy control. VEGF-A, VEGF-B, VEGF Receptor-2 (VEGF-R2) and Thrombospondin-1 (TSP-1) protein expression as well as muscle capillarization were measured in soleus and plantaris. **Results:** Voluntary exercise decreased protein expression of the anti-angiogenic TSP-1 and prevented capillary regression in both soleus and plantaris muscles of ZDF rats. Whereas it up-regulated the expression of pro-angiogenic factors in plantaris, it had no effect in soleus. The ratio between VEGF-A and TSP-1 proteins was however significantly increased by voluntary exercise in both muscles. **Conclusion:** Rather than analyzing angio-adaptive factors individually, measuring the balance between anti- and pro-angiogenic signals would reflect more precisely muscle angio-adaptation ability. Our results also suggest that exercise-induced up-regulation of VEGF-A / TSP-1 ratio might represent a key parameter in preserving muscle microcirculation in ZDF rats.

The potential role of MEF2C and ATF2 in shear stress-mediated VEGF production

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Increased capillary shear stress in skeletal muscle causes a form of angiogenesis termed luminal splitting. An increased number of capillaries are observed at 7 days of chronic shear stress that is reliant on p38 signaling. Others have shown that VEGF is essential for luminal splitting. We hypothesize that VEGF production is p38 dependent and is increased by p38 dependent transcription factors Creb, ATF2 and MEF2C in response to shear stress. Male Sprague-Dawley rats were administered prazosin (50 mg/L drinking water) to increase shear stress, and the extensor digitorum longus was extracted at 1, 2, 4 or 7 days. By Western blot, p38 was activated only at 2 days. Skeletal muscle endothelial cells were sheared (12 dynes/cm²) with or without 10 uM SB203580 (p38 inhibitor), then lysed and analyzed by RT-PCR or Western blot. In vitro, shear stress induced VEGF mRNA and protein elevation at 2 and 6 hours, respectively. These increases were abolished by SB203580. Shear stress increased both ATF2 phosphorylation and

MEF2C production at 2 hours, which were abrogated by SB203580. Creb phosphorylation was unchanged by shear stress. Our data show that shear stress-mediated p38 activation is required for VEGF production. ATF2 phosphorylation and MEF2C production are increased by shear stress, but further investigation is necessary to determine if they mediate p38-induced VEGF production. Supported by HSF and NSERC.

Is adequate really enough? Vitamin D3 supplementation at 10x the AI blunts disease severity and enhances functional outcomes in an animal model of Lou Gehrig's disease. A Pilot Study.

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Background: Amyotrophic lateral sclerosis (ALS) is a paralytic and fatal neurodegenerative disease caused by the death of motor neurons. In humans and animal models, vitamin D3 and its metabolites mitigate the severity of autoimmune, inflammatory and neurodegenerative diseases that share some common pathophysiology with ALS. Pilot Study Objective: To determine the effects of dietary vitamin D3 at 10x the adequate intake on functional and disease outcomes and lifespan in the transgenic G93A mouse model of ALS. Methods: Starting at age 40 d, 32 G93A mice (21 M, 11 F) were assigned to a diet with either adequate (AI; 1 IU D3/g feed) or high (HiD; 10 IU D3/g feed) vitamin D3. Since this was a pilot study, significance was set at $P \leq 0.10$. Results: HiD mice had a 17% lower clinical score over time vs. AI mice ($P = 0.088$). For motor performance, HiD mice had a 17% greater area under the curve (AUC) vs. AI mice ($P = 0.062$), and within males 23% greater AUC vs. AI males ($P = 0.049$). For paw grip endurance (PaGE), HiD mice had a 7% greater score over time ($P = 0.064$) vs. AI mice, and HiD delayed the decline in PaGE by 3 d (3%) (diet x time $P < 0.001$). Between the sexes, males reached disease onset 2.7 fold faster (HR = 2.7, 95% CI: 1.6, 8.1; $P < 0.001$) and endpoint 2.5 fold faster (HR = 2.5, 95% CI: 1.4, 6.8; $P = 0.002$) vs. females. Conclusion: Vitamin D3 supplementation at 10 fold the AI attenuates disease severity and improves functional outcomes in the transgenic G93A mouse model of ALS. However, higher vitamin D3 supplementation may be required to elicit robust improvements in this disease model. Funded by NSERC and Faculty of Health York U.

Restoration of adiponectin response is not necessary for the exercise-induced recovery of insulin response in high-fat-fed rats

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Accumulation of lipid species in non-adipose tissues following high-fat (HF) feeding has many adverse effects, including the development of insulin resistance. Leptin and adiponectin are insulin-sensitizing adipokines, which decrease lipid accumulation in tissues such as skeletal muscle, due in part to their ability to stimulate fatty acid oxidation (FAO). Within three to seven days of HF feeding, skeletal muscle responsiveness to leptin and adiponectin is lost, preceding the accumulation of lipid and the loss of response to insulin and an impaired ability to regulate blood glucose levels by four weeks. Recently, we discovered the recovery of leptin's stimulatory

effect on FAO in muscle is not required for the exercise-induced restoration of insulin response following HF feeding. In the current study we examined the effect of exercise and β -GPA (a dietary creatine analogue which depletes muscle high-energy phosphagen content similar to exercise) on adiponectin and insulin responsiveness in skeletal muscle, hypothesizing that a one-week intervention is sufficient to restore response to both hormones. Following four weeks of HF feeding, female Sprague-Dawley rats were subjected to one of six conditions: 1) continuation of the HF diet for one or two weeks (1HF, 2HF); 2) one or two weeks of HF diet combined with endurance-training (1EX, 2EX), or; 3) one or two weeks of HF feeding with β -GPA administration (1GPA, 2GPA). At each endpoint, solei were excised from rats for functional measurements of maximal adiponectin and insulin response from excised solei. As expected, insulin responsiveness was recovered in 1EX and 1GPA, as well as 2EX and 2GPA groups. Conversely, endurance training and β -GPA feeding did not restore adiponectin response. Thus, we conclude that exercise-induced recovery of insulin response while consuming a high-fat diet is not dependent on the recovery of response to the insulin-sensitizing adipokine, adiponectin, similar to our previous findings with leptin.

FOXO1 upregulation in response to hindlimb ischemia

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Skeletal muscle ischemia induces a poor angiogenic response despite local increases in growth factors. The transcription factor FoxO1 is an established mediator of cell stress responses (cell cycle arrest, oxidative stress, apoptosis), and is anti-angiogenic. We hypothesized that FoxO1 levels increase during ischemia. Rat hindlimb ischemia was induced by iliac artery ligation. Tibialis anterior and extensor digitorum longus muscles were removed after 4, 7 or 14 days for mRNA and protein analysis. FoxO1 protein increased significantly after 4 days ischemia, returning to normal by 14 days. FoxO1 mRNA was elevated at 4 days. FoxO1 target gene p27, a cell cycle inhibitor, increased after 4 days ischemia and returned to normal by 14 days. We subjected cultured endothelial cells to ischemia-mimetic conditions for 24 hrs: hypoxia (5%); metabolic stress (low serum); oxidative stress (200 μ M H₂O₂). None of these conditions altered FoxO1 protein levels. Conclusions: FoxO1 protein increases during ischemia, correlating with upregulation of target genes. Further studies will investigate FoxO1 regulation by low flow, and will determine the role of FoxO1 in limiting the angiogenic response to ischemia. Funded by Heart and Stroke Foundation of Canada.

Analysis of mitochondrial dynamics in C2C12 myoblasts

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Mitochondria are vital organelles, critical for energy supply and cell survival. Such functional versatility is paralleled by the structural complexity of the organelle. Mitochondria can compensate for alterations in energy requirements by adjusting their size and distribution within

skeletal muscle. To quantify this in living cells, we used C2C12 myoblasts transfected with fluorescently labeled pDsRED2-Mito in order to track mitochondrial dynamics. 48-hours following transfection the cells were imaged using fluorescent microscopy, at a constant temperature of 37°C. Mitochondrial dynamics were captured at 2 second intervals for a total observation time of 5 minutes using real-time imaging. Approximately 60% of the mitochondria localized in the periphery of myoblasts were punctate spheres less than 1.0µm² in area, while 13% of mitochondria were present as elongated reticular structures greater than 2.0µm² in area. These dynamic organelles also varied in their displacement, moving an average of 0.928µm from the origin. However, the total path length travelled by these organelles was approximately 15 fold greater, averaging 14.2µm within the 5 minutes time frame. Thus, mitochondrial morphology in myoblasts is under dynamic control. Understanding the changes in mitochondrial morphology will allow us to elucidate the underlying basis for mitochondrial reticular formation in skeletal muscle, and the effects of contractile activity.

Mitochondrial Oxidative Phosphorylation as an End Effector of Ischemic Preconditioning

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Ischemic preconditioning (IPC) is a potent intrinsic cellular process that confers protection to various organs from ischemia/reperfusion injury. We have shown previously that two isoforms of phosphoinositide-3-kinase, PI3K α and PI3K β , play a critical role in conferring protection to the ischemic heart. In PI3K β (-/-) mouse hearts, IPC protection is abolished while mouse hearts expressing a dominant-negative construct of PI3K β , PI3K β DN, showed improved functional recovery following ischemic-reperfusion in the absence of IPC. The mitochondria have been proposed to be critical in mediating IPC protection, with particular focus on the mitochondrial permeability transition pore (MPTP) as the key mediator in IPC. We first examined transcriptional changes in some mitochondrial and cell signalling proteins from PI3K β DN and PI3K β (-/-) mouse hearts. RT² ProfilerTM PCR Array (SABioscience) revealed only a few transcripts were differentially altered in the PI3K transgenic hearts, including *Slc25a31* (ANT4 – adenine nucleotide translocase 4) and *Ucp1* (uncoupling protein 1). ANT4 forms part of the MPTP complex, along with voltage-gated dependent anion channel (VDAC) and cyclophilin D. However, protein analysis showed no change in expression levels of any of the MPTP proteins. Secondly, MPTP kinetic analyses from isolated mitochondria from PI3K β DN and PI3K β (-/-) hearts were not different. ADP-coupled O₂ (state III) but not uncoupled respiration (state IV) was significantly diminished in mitochondria from PI3K β (-/-) hearts, which was not associated with changes in for F₁F₀ ATP synthase α -subunit levels. Our results suggest mitochondrial electron transport chain function and not the MPTP may be the critical contributor of IPC protection.

Serine phosphorylation of IRS-1 and the associated inhibition of glucose uptake are normal and reversible physiological responses to nutrition in muscle cells

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The mTORC1/S6Kinase 1 pathway has proven to be an essential regulator of cell growth and metabolism. It has also been shown that the presence of amino acids in conjunction with growth factors is enough to stimulate a negative feedback loop within this pathway via overactivation of S6 Kinase 1 (S6K1) and subsequent serine phosphorylation of IRS1 leading to inhibition of glucose uptake. However, it is not yet known whether this negative feedback loop is one that is normal and reversible, or pathological. The role of insulin in this negative reaction mechanism is also unknown. Myotubes were grown in serum-free media for 4 hours and then divided into groups based on various treatments containing either excess leucine and/or insulin. Separate cells were also incubated overnight in solution containing excess leucine solution with insulin to mimic prolonged overactivation of the S6K1 pathway. A progressive increase in phosphorylated S6K1 was found when myotubes were treated with leucine and insulin over time ($P < 0.01$). Subsequently, there was increased serine phosphorylation of IRS1 when treated with excess leucine and insulin on Day 1, with a peak at 2 hours ($P < 0.05$). Similar increases in IRS1 serine phosphorylation were seen in rats gavaged with leucine solution. When the cells were incubated overnight with excess leucine and insulin were deprived of media the next day for 4 hours and re-stimulated with amino acids and insulin, a similar trend was seen as on Day 1, indicating that the system is potentially functioning normally. To examine whether this reversibility was reflected in the regulation of glucose transport, a glucose uptake experiment was done on myotubes treated as described above. It was found that the muscle cells that had been treated with insulin and a high concentration of leucine overnight were still sensitive to insulin-dependent glucose transport, similar to cells that were not treated with amino acids. Thus, while S6K1-mediated serine phosphorylation of IRS1 and inhibition of glucose uptake occurs in response to amino acids and insulin, this is a normal, reversible physiological regulation.

Angiotensin II signalling regulates skeletal muscle growth and myoblast chemotaxis

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The regulatory role of angiotensin II (Ang II) in mediating skeletal muscle hypertrophy is largely unknown. We examined the impact of Ang II on myoblast chemotaxis using transwell assays. Exogenous treatment of Ang II significantly increased the migratory capacity of both C2C12 and primary myoblasts by 30 and 43% respectively, while Ang II receptor knockout myoblasts (AT1a^{-/-}) demonstrated a significant attenuation of basal migration (38% of control) and did not respond to exogenous Ang II treatment. Ang II appeared to increase migration by inducing MMP2 activity as gelatin zymography analysis of cell culture media and lysates revealed a ~40% increase in gelatinase activity and pharmacological inhibition of MMP2 abolished the Ang II mediated increase in myoblast migration. Ang II also appeared to signal in a paracrine fashion to induce migration as 4h of cyclic mechanical stretch of myoblasts enhanced migration of

cocultured myoblasts. To test the involvement of Ang II signaling during skeletal muscle regeneration in vivo, control and At1a^{-/-} mice were subjected to cardiotoxin injury. In comparison to controls, at 14 and 21 days following injury, At1a^{-/-} mice demonstrated 34 and 25% reductions in cross sectional area respectively. Taken together, these results demonstrate that Ang II modulates skeletal muscle fibre growth by regulating myoblast chemotaxis through MMP2.

Vasohibin-1: New Actor of Physiological and Pathological Angio-adaptation in Rat Striated Muscle

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INTRODUCTION: Vasohibin-1 (VASH-1) was recently identified as a negative feedback regulator of angiogenesis. By promoting vessel stabilization and maturation, it also contributes to vascular homeostasis. VASH-1 was found to be involved in several pathological conditions such as tumor growth and ocular neovascularization. To date, its physiological or pathological expression has never been investigated in skeletal muscle. Microcirculation is however a key determinant of muscle function. Capillaries can either grow or regress in response to various physio-pathological stimuli in order to match myofibers' metabolic needs. Such angio-adaptation is tightly regulated by pro- and anti-angiogenic signals. Whereas physical exercise represents a powerful pro-angiogenic stimulus in skeletal muscle, pathological situations such as type-2 diabetes lead to capillary regression. We have previously shown that capillary regression occurs in plantaris muscle from Zucker Diabetic Fatty (ZDF) rats. Interestingly, voluntary activity in running wheel cages prevents both capillary regression and diabetes installation in active ZDF.

OBJECTIVES: To investigate whether VASH-1 muscle expression is sensitive to exercise stimulus and whether VASH-1 represents a new actor of exercise-induced angio-adaptation in healthy and diseased skeletal muscles.

METHODS: Expression of VASH-1A and VASH-1B isoforms was analyzed by western blotting in plantaris from Sprague-Dawley rats subjected to short-term training on a treadmill for 1, 3, and 5 days (25m/min, 60min, 12% incline), as well as in plantaris from sedentary lean, sedentary ZDF (Sed-ZDF), and ZDF rats spontaneously active in running wheel cages (Act-ZDF).

RESULTS: Both VASH-1 isoforms were transiently up-regulated after one acute exercise. This response was abolished with short-term training thus favoring training-induced angiogenesis. VASH-1 levels were significantly increased in Sed-ZDF possibly accounting for capillary regression. Interestingly, voluntary activity prevented both VASH-1 increase and capillary regression in Act-ZDF plantaris.

CONCLUSION: VASH-1 is a new exercise-regulated angio-adaptive molecule with potential implications in muscle vascular diseases.

Impaired post-injury regeneration of skeletal muscle in type 1 diabetic mice

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Skeletal muscle has a remarkable capacity for regeneration following injury which may be compromised in type 1 diabetes mellitus (T1DM), a disease associated with reduced muscle mass. Thus, the purpose of this study was to i) characterize the post-injury regeneration process in diabetic skeletal muscle and ii) identify possible mechanisms underlying impaired regeneration. Ins2Akita+/- (Akita) mice become spontaneously diabetic at 4 weeks of age, mimicking adolescent-onset T1DM in humans. Akita and non-diabetic C57BL/6 (C57) mice received a cardiotoxin-induced muscle injury to the plantar (gastrocnemius/plantaris/soleus [GPS]) and dorsal (tibialis anterior [TA]) flexors of the ankle in one leg at 12 weeks of age (8 weeks of diabetes) and were assigned to 5, 10, 21, or 35 days of injury recovery. Following recovery, injured and uninjured muscles were dissected and stored for analyses. In all muscles and all time points except 5 days post injury, TA and GPS mass as a % of the uninjured contralateral muscle was reduced in Akita mice compared to C57. TA fiber area as a % of the uninjured muscle was also significantly reduced in Akita mice. To gain insight into circulating factors contributing to the impaired regenerative process, several biomarkers were analyzed in blood plasma collected at 2, 4, 6, and 8 weeks of diabetes prior to muscle injury. Of interest, insulin, leptin, and resistin were reduced, while total PAI-1 and corticosterone, were elevated in Akita compared to C57 mice. Given that insulin and leptin are associated with a positive skeletal muscle protein balance and PAI-1 and corticosterone delay muscle regeneration and increase protein catabolism, respectively, it is hypothesized that these hormonal alterations are, in part, responsible for delayed recovery of skeletal muscle mass and fiber area in the Akita mice. These data provide novel insights into mechanisms underlying reduced growth or atrophy of type 1 diabetic skeletal muscle.

Skeletal Muscle Regulatory Responses to Increased Extracellular Lactate

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Mammalian skeletal muscle cells exhibit cell shrinkage followed by regulatory volume increase (RVI) in response to increases in extracellular osmolarity caused by addition of concentrated NaCl or sucrose. We hypothesized that increased extracellular sodium lactate would similarly result in a transient loss of cell volume, followed by RVI, and that the RVI would be more rapid and pronounced than that seen with NaCl due to entry of lactate into the cell. The present study measured the time course of change single fibre width in response to varied increases in extracellular NaCl or Na-lactate. Adult mouse EDL single fibres were isolated using collagenase and incubated in DMEM prior to and during experimentation. Fibre images were obtained at 3-6 s intervals for up to 40 min. Fibre images were analyzed for width at 2 sites. Increased

osmolarity resulted in a rapid decrease in fibre width, followed by RVI. The volume recovery was faster and more complete when [lactate] 20 mmol/L. There was a linear relationship between increases extracellular [lactate], cell shrinkage, and magnitude of RVI. It appears that the inward transport of lactate into skeletal muscle contributes, together with increased NKCC activity, to the RVI in mammalian skeletal muscle.

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Regulatory volume increase in single mouse soleus muscle fibres assessed simultaneously using intracellular fluorescence and fibre width

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Mammalian skeletal muscle cells have the ability to regulate volume in response to increases or decreases in extracellular osmolarity. In the present study we measured the time course of change in single fibre intracellular calcein fluorescence (volume indicator) and width in response to varied 200 mosmol/L increase in extracellular osmolarity using NaCl or sucrose. Adult mouse EDL single fibres were isolated using collagenase and incubated in DMEM prior to and during experimentation. Fibres were loaded with calcein-AM for 30 min, and triple-rinsed with calcein-free DMEM. After obtaining baseline images NaCl or sucrose solution was added. Fibre images were obtained at 3-6 s intervals for up to 60 min. Fibre images were analyzed for intensity and width at 2-3 sites. Increased osmolarity resulted in a rapid increase in fibre fluorescence and decrease in fibre width. Both variables gradually recovered to baseline values within ~45 min. Bumetanide, an inhibitor of the sodium-potassium-2 chloride cotransporter (NKCC) impaired recovery. There was a linear relationship between increases in fibre fluorescent intensity and decreases in fibre width. It is concluded that the NKCC is involved in regulatory volume increase in skeletal muscle, and that changes in fluorescence intensity can be used as an indicator of changes in cell volume. Supported by NSERC of Canada.

Resveratrol and Voluntary Running both Attenuate the Reduced Mitochondrial Function in Muscle-Specific Sirt1 KO Animals

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Both caloric restriction and resveratrol, a natural polyphenolic compound, have been shown to activate Sirt1, which in turn extends lifespan and increases mitochondrial content and function. Interestingly, the effects of these treatments and those of exercise, rely on many of the same physiological changes. To further examine the relationship between exercise performance and Sirt1 activity, we produced a skeletal-muscle specific Sirt1-deficient mouse. These mice were generated using a myosin light chain-directed Cre-loxP targeted mutation of the Sirt1 gene. These mice showed no behavioural or phenotypic abnormalities and had similar body and muscle weights as wild-type (WT) animals. Isolated muscle mitochondria from Sirt1-deficient

mice exhibited a 45% decline in state 4 respiration along with 4-fold higher production of reactive oxygen species (ROS) in comparison to WT animals. State 3 respiration was also 70% lower, and was accompanied by a 7-fold higher ROS production. Despite these findings, over a three week period, Sirt1-deficient mice ran the same average (12 km/day) and total distances on voluntary running wheels. These data indicate that the absence of Sirt1 results in an impaired capacity for mitochondrial ATP synthesis, along with increased ROS production, which may act as a compensatory signal to maintain mitochondrial biogenesis and voluntary exercise performance.

Impaired Satellite Cell Activation in T1DM Skeletal Muscle

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Myopathy is one of many serious complications resulting from Type 1 Diabetes Mellitus (T1DM). Most individuals afflicted with T1DM develop this disease before or during adolescence, a period of significant skeletal muscle growth and development. The purpose of this on-going study is to investigate the growth and regenerative capacity of T1DM muscle and identify alterations to the resident muscle stem cell (SC) population which could affect their contribution to these processes. We hypothesize that the T1DM environment is affecting SCs ability to respond to growth and regenerative stimuli. Using Ins2Akita +/- mice, a genetic T1DM model which develops diabetes at 3-4 weeks of age, we injured tibialis anterior (TA) muscles using cardiotoxin one week after T1DM onset. Ten days later, TA muscles were harvested and fiber area assessed, as a meter of regenerative progress. Fiber areas of T1DM muscle were more than 20% smaller ($78 \pm 3\%$ of Control), indicating that even short-term T1DM can affect regeneration. To determine the effect of long-term, uncontrolled T1DM on SCs, Ins2Akita +/- muscles were harvested 8 weeks after T1DM onset. To date we have demonstrated, via immunohistochemical analysis, that SC number (% Pax7-positive of all myonuclei) is not different between Ins2Akita +/- and Control in resting muscle sections (1.6% and 1.3%, respectively). However, a 50% reduction in the capacity for activation of T1DM SCs ($52.9 \pm 9.5\%$ of Control cells) was observed using floating single fiber cultures. Preliminary data suggests that once activated, T1DM SCs exhibit similar proliferation and differentiation capacities to Control SCs while in favourable conditions, ex vivo. Based on our findings to date, we propose that although T1DM does not affect SC number, the T1DM environment impedes SC activation and could account for attenuated regeneration in T1DM muscle. Currently, we are examining alterations to the T1DM SC and extracellular environment to determine the underlying mechanisms for the impairments in SC activation.

Identification and Characterization of Cell-Surface Associated Proteins of the Human Heart

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Cardiovascular disease (CVD) is a leading cause of death in North America and the development of novel therapeutic strategies depends on a greater understanding of cardiac cell function. Many clinical drugs that treat CVD target cardiac membrane proteins, however these proteins are difficult to isolate. We have used cationic silica beads to enrich for cell-surface proteins in the human heart followed by extensive mass spectrometry (MudPIT)-based proteomics. The aim of this study is to generate an extensive database of cell-surface molecules expressed in cardiomyocytes and functionally study novel proteins of interest to gain insights into their molecular function. Human cardiomyocytes, coronary smooth muscle cells and endothelial cells were harvested and cultured. Plasma membrane proteins were cross-linked to cationic silica beads. The supernatant cytosolic fraction was separated from the plasma membrane pellet attached to the beads via centrifugation. Membranes were eluted using either 1% Triton-X-100, and 8M urea or 0.2% PPS Silent Surfactant. Samples were analyzed by liquid chromatography and mass spectrometry using an LTQ Orbitrap mass spectrometer. In total, 2274 proteins were identified with high confidence. Following extensive filtering, 969 proteins were found to be enriched preferentially in the membrane fraction, including many known plasma membrane proteins. Integrative data analysis indicated that proteins found in the cardiomyocyte membrane fraction have GO-terms of membrane and plasma membrane. A KEGG Pathway analysis showed many of the proteins were found to be involved in communication, cell adhesion and metabolism. Many unknown proteins have been identified, including proteins that have been shown to be involved in regulating calcium handling in the mouse neonatal cardiomyocyte. These results suggest that plasma membrane proteins of the human heart have been enriched and many known and novel cell-surface proteins have been elucidated. Ongoing investigations include searching for proteins of interest and functionally characterizing these protein candidates. (This study was funded in part by the Heart and Stroke Richard Lewar Centre of Excellence in Cardiovascular Research studentship).

A high fat diet combined with exogenous glucocorticoid administration influences intramyocellular lipid accumulation and accelerates the development of insulin resistance

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High fat diets and glucocorticoids (GC) may act synergistically to stimulate insulin resistance and accelerate the development of type 2 diabetes mellitus (T2DM). Our lab has shown that healthy Sprague Dawley rats administered exogenous corticosterone pellets and placed on a high fat (60% fat) diet develop severe insulin resistance within one week, while neither a high-fat diet or corticosterone administration alone display such results. We hypothesize that insulin resistance in the muscle occurs as a result of an imbalance between fatty acid transport into the cell and fat oxidation within the cell. Animals that developed insulin resistance have elevated levels of intramyocellular lipids (IMCLs) in muscle sections stained with Oil Red O, suggesting

an accumulation of lipids. Although there were no reductions in cytochrome C oxidase activity found with treatment, succinate dehydrogenase activity was reduced suggesting a decrease in oxidative capacity within the muscle fibers. Further research will investigate fatty acid uptake in these muscles through studying protein expression and activity of fatty acid transports such as FAT/CD36. An increase in uptake coupled with decreased oxidative capacity suggests a mechanism for IMCL accumulation and insulin resistance in our model of T2DM.

Development of conditional murine knockout models of equilibrative nucleoside transporters

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Background and introduction: Myocardial preconditioning is the increased tolerance of the myocardium to hypoxia or ischemia when it has been exposed to a prior brief hypoxic or ischemic episode. Adenosine has shown to be an essential component in mediating preconditioning. Levels of adenosine are regulated by membrane nucleoside transporters, known as mENT1 and mENT2 in mice. However, the role of these transporters in mediating the effects of adenosine is not well characterized. To better understand the role of nucleoside transporters in purinergic signaling in the cardiovascular system, we have used the in vitro HL-1 cardiomyocyte cell line model as well as a global mENT1 KO mouse strain. While these are useful models, we have extended our studies to use a more specific and targeted system by the development of conditional KO models. In these models, our gene of interest, mENT1 or mENT2 is present as a transgenic construct where by it is flanked on both sides by loxP sites. These sites will be excised by Cre recombinase enzyme hence generating the KO effect. Objectives: 1. Development of breeding colony for both mENT1 and mENT2. 2. Generation of Tamoxifen inducible KO model, necessary for the Cre-recombinase expression. 3. Investigate role of ENT1 and ENT2 in purinergic responses in the heart. Methodology: Heterozygous mice wt/flx (defined as having a single transgenic floxed mENT1 or 2 gene construct) have been crossed to obtain homozygous mice (flx/flx). To determine the genotypes of all mice, our lab developed a standard PCR protocol that can distinguish between wt/wt, wt/flx and flx/flx mice. We will now breed the flx/flx mice with Mer-Cre-Mer mice that express cre recombinase upon induction. I will ensure that the mENT gene is removed using RT-PCR, NBTI binding (mENT1 specific inhibitor), analysis of adenosine uptake, and western blotting. Conclusion: Development of these models represents a major advance in the field and will allow a detailed investigation into the functional and physiological roles of mENT1 and mENT2 in purinergic signaling in the cardiovascular system.

Restoration of skeletal muscle leptin response is not necessary to rescue insulin response in high fat fed rats with endurance exercise training

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Leptin administration increases FA oxidation rates and decreases lipid storage in oxidative skeletal muscle. We have previously shown high fat (HF) diets to rapidly induce skeletal muscle leptin resistance, and prior to the disruptions of normal muscle FA metabolism (increase in FA transport; accumulation of triacylglycerol, diacylglycerol, ceramide) that occur in advance of the impairment in insulin signalling and glucose transport. Thus, if the early development of leptin resistance is a contributor to HF diet induced insulin resistance, it follows that the correction of leptin resistance might also be an early event in the restoration of insulin response as induced by an intervention such as exercise training. In the current study, we sought to determine i) whether 1, 2 or 4 weeks of exercise training was sufficient to restore leptin response in skeletal muscle of rats already consuming a HF diet, and ii) whether this preceded the training-induced corrections in FA metabolism (reduction in lipid storage) and improved insulin stimulated glucose transport. In the chow fed control group, insulin stimulation increased glucose transport by 153% and leptin stimulation increased AMPK (+18%) and ACC (+19%) phosphorylation and the rate of palmitate oxidation (+73%). These responses to insulin and leptin were either severely blunted or absent following 4 weeks of HF (60% kcal) feeding. Exercise intervention decreased muscle ceramide content (-28%) and restored insulin stimulated glucose transport to control levels within one week; muscle leptin response (AMPK and ACC phosphorylation, FA oxidation) was also restored, but not until the 2-week time point. In conclusion, endurance exercise training is able to restore leptin response, but this does not appear to be a necessary precursor for the restoration of insulin response.

Mito_{KATP} and MPTP are not end effectors of the PI3K-Akt-GSK3 β pathway in myocardial ischemia preconditioning

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Phosphatidylinositol 3-kinase γ (PI3K γ) has previously been shown to play an important role in ischemic preconditioning (IPC) which leads to protection against ischemia-reperfusion injury. Although not fully characterized, there is evidence that the mitochondrial ATP-sensitive potassium channel (mitoKATP) is needed to be open prior to a prolonged (index) ischemia and for the mitochondrial permeability transition pore (MPTP) to be closed following index ischemia for IPC's protection to be enabled. Currently, a clear link between PI3K γ and the mitoKATP has not been fully described. We have subjected PI3K γ knockout (PI3K γ $-/-$) mouse hearts to the mitoKATP opening agent, diazoxide (100 μ M), to examine whether the mitoKATP is downstream of PI3K γ . Furthermore, we have subjected csGSK3 β knockout mouse hearts (which display characteristics of a pre-protected state) to the MPTP opening agent, atractyloside (20 μ M) to gain further insight into the relationship between the PI3K pathway and the MPTP. We used

functional recovery (Left Ventricular Developed Pressure [LVDP] and Left Ventricular End Diastolic Pressure [LVEDP]) as an indication of protection against cell death and tissue damage. The addition of diazoxide to PI3K γ $-/-$ mouse hearts did not increase functional recovery when compared with ischemia-reperfusion alone. We did not see a decrease in functional recovery in csGSK3 β knockout mouse hearts when treated with atractyloside when compared to ischemia-reperfusion alone. Our research suggests that the mitochondrial targets (MPTP and mitoKATP) are perhaps either upstream of the PI3K pathway or do not play a necessary role in the IPC pathway.

LC-MS Analysis to Identify Ryanodine Receptor 1 (RyR1) Protein-Protein Interactions

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Introduction: Excitation-contraction coupling is defined as the process linking action potential to contraction in striated muscle. This process depends on a large macromolecular protein complex, the central element of which is the Ca²⁺ release channel, ryanodine receptor 1 (RyR1). It has been demonstrated that protein binding partners regulate RyR1 via its cytosolic domain and mutations in RyR1 and its interacting proteins result in disease. Here, we describe the purification and LC-MS analysis of affinity-tagged RyR1 protein complexes with the overall aim of understanding calcium ion movement at a molecular level. **Methods:** Full length RyR1 rabbit cDNA and a series of five truncated RyR1 constructs were N-terminally affinity-tagged with 6x-histidine. These constructs were introduced into HEK293 cells and C2C12 mouse skeletal myocytes enabling column purification of RyR1 protein complexes under non-denaturing conditions. The purified proteins were subjected to immunoblotting experiments to verify stable expression and purification of clones. The purified complexes were analyzed using 1-dimensional gels and by gel-free LC-MS. A filtering algorithm was applied to all putative results to obtain a measure of the statistical reliability (confidence score) for each candidate identified and only proteins with confidence value $\geq 99\%$ and identified with ≥ 2 peptides were accepted. **Results:** Here, we present data using 6x-His affinity tagged proteins transfected into HEK293 and C2C12 muscle cells, followed by gel-free LC-MS to identify protein-protein interactions in skeletal muscle. Column purification of tagged proteins from transfected HEK and C2C12 shows stable expression and purification of clones by immunoblotting experiments. We show ryanodine receptor 1 (RyR1) from C2C12 myocytes, purified on a Ni-NTA column under non-denaturing conditions. LC-MS analysis of the purified protein complex showed that we have identified 5 novel RyR1 interacting proteins and we are currently validating these interactions using conventional co-expression and immunoprecipitation assays. These putative protein interacting partners were also subjected to fluorescence confocal imaging to determine co-localization with RyR1 within rat muscle tissue. This protein-protein interaction analysis provides a framework for a more detailed analysis of RyR1 function.

p53 mRNA content and stability – effect of AMP Kinase (AMPK) and contractile activity

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p53 protein is important for basal and exercise-induced mitochondrial biogenesis in skeletal muscle. As shown previously, pifithrin- α (15 μ M) a specific inhibitor of p53, reduced the AICAR-induced increase in mitochondrial content in myoblasts. This demonstrates that p53 partly mediates AMPK-induced mitochondrial adaptations. Here, we investigated whether p53 mRNA content and stability were modulated by AMPK. Two transcript variants (TV) of murine p53 have been discovered. p53 TV2 mRNA was expressed at 60% lower levels than p53 TV1 in C2C12 myoblasts. AICAR treatment (3 days) reduced p53 TV1 and TV2 mRNA levels by 28% and 13%, respectively, compared to control. To investigate whether this reduced mRNA was due to changes in mRNA stability, we generated reporter constructs containing the full length (R2) 3' untranslated region (UTR), or a shorter construct without the AU-rich region and the polyadenylation signal (R1) of p53 TV2 mRNA. Following transfection, luciferase activity of both R1 and R2 was only ~15% of control, indicating that even the shorter 3'UTR could mediate p53 mRNA decay. AICAR treatment further decreased luciferase activity of both constructs by ~35% compared to untreated cells. This suggests that AMPK regulates p53 by reducing mRNA stability and content. In contrast to this, acute (1, and 3hr) and chronic contractile activity (4 days) of C2C12 myotubes at 9Hz elicited an increase in p53 mRNA content. Our findings shed light on the complexity of p53 regulation and have significance for p53-based therapeutic interventions. (Funded by NSERC)

The intracellular abundance of the pro-obesity kinase, S6K1, is regulated by targeted proteolysis

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Obesity and its associated pathophysiological complications are on the rise in North America especially amongst children. One of the critical signaling pathways that contribute to the development of obesity and insulin resistance is the mTORC1/S6K1 pathway. Mice deficient in S6K1 are protected from diet-induced obesity, highlighting the importance of this kinase. S6K1 is a substrate of the nutrient sensor mTOR complex 1, which incorporates upstream signals such as growth factors and nutrients, and promotes protein synthesis. Activation of S6K1 which is induced under conditions of nutrient overload (especially amino acids) has been found to cause insulin resistance. This is surprising given the fact that high protein diets have been shown to promote weight loss. To examine whether reductions in S6K1 level could improve glucose uptake in L6 muscle cells, RNA interference was employed to knock down the kinase. The cells were in addition incubated in the presence of increasing concentrations of branched chain amino acids (BCAA). Co-incubation with BCAA (800 μ M) suppressed insulin stimulated glucose uptake by 30%. On the other hand, in cells in which S6K1 had been knocked down, glucose uptake was increased by 34%. Thus S6K1 abundance is important in regulating glucose metabolism. We then examined factors and mechanisms that can modulate the abundance of this

kinase. S6K1 level was reduced (25-50%, $P<0.05$) and its activity was abolished in myoblasts deprived of growth factors and amino acids, for 6-24 h; these were re-stored when previously starved cells were re-stimulated. Similar results were observed when the incubation medium lacked only leucine and serum, indicating the physiological relevance of this regulation. This suppression of S6K1 abundance was attenuated by 60% ($P<0.01$) in the presence of MG132, an inhibitor of the ubiquitin mediated proteolytic pathway. This drug in addition increased accumulation of ubiquitinated S6K1. Our results implicate S6K1 as a mediator of insulin resistance in skeletal muscle, and reveal novel mechanisms (targeted proteolysis and ubiquitination) of regulating this kinase. Nutrient formulations that can reduce the function of this kinase may represent non-pharmacological mechanisms of improving insulin sensitivity.

Effects of Oral Leucine Administration on mTOR signalling in Skeletal Muscles of Different Fibre Types in Young Partial Pancreatectomized Diabetic Rats

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Type 1 diabetes is associated with impairments in skeletal muscle growth and function. Although insulin therapy can correct these impairments, optimal treatment is difficult to maintain in young, growing children. In addition to insulin, amino acids too can stimulate pathways that increase protein synthesis in muscle. Leucine has the most potent effect. The actions of insulin and leucine are mediated by the mTOR signaling pathway, which increases protein synthesis by stimulating mRNA translation initiation. Interestingly, the effect of leucine on protein synthesis appears to be blunted in models of type 1 diabetes. Also, it has been proposed that type 1 diabetes predominately affects glycolytic muscle fibres, yet the effect of leucine on protein synthesis in different fibre types is unknown. **METHODS:** A 90% pancreatectomy (PPX) model of diabetes was used to induce a hypoinsulinemic state. Male, Sprague-Dawley rats (100-120g) were randomly assigned to one of two groups: PPX ($n=18$) and SHAM ($n=16$). At 4 weeks, overnight fasted animals either received an oral gavage of l-leucine (0.48g/kg) or water. **RESULTS:** PPX animals were hyperglycemic ~7 days post surgery ($>25\text{mM}$) and demonstrated attenuated body mass at 4 weeks (Sham: 332.0 ± 5.6 vs. PPX $245.2 \pm 9.3\text{g}$; $p<0.05$). When corrected for body weight, PPX had smaller gastrocnemius (Sham: 5.3 ± 0.2 vs. PPX: 4.5 ± 0.2 g/kg; $p<0.05$) and epitrochlearis (Sham: 155.6 ± 6.2 vs. PPX: 130.5 ± 8.2 mg/kg; $p<0.05$) muscles compared to SHAM animals, but no differences were found with soleus muscles. Corticosterone levels were elevated in diabetic animals but only after 4 weeks of chronic hyperglycemia (0800h Sham: 40.5 ± 7.9 vs. PPX: 154.7 ± 36.3 ng/mL and 2000h Sham: 234.0 ± 56.2 vs PPX 439.0 ± 59.7 ng/mL; $p<0.05$). In the gastrocnemius muscle of Sham (but not PPX) animals, oral administration of leucine increased (~2-fold) the phosphorylation of 4E-BP1 and of S6K1 (~4-fold), $p<0.05$ for both. These proteins are markers of mTOR signaling. A similar effect was found in the epitrochlearis muscles. However, in the soleus muscle, leucine stimulated similar increases in 4E-BP1 phosphorylation in both Sham and PPX groups. Ubiquitinated proteins in gastrocnemius muscle were not affected by treatments. Overall, we demonstrate that diabetes blunts the mTOR signaling response to leucine in both mixed muscles and muscles containing predominantly glycolytic fibers, whereas muscles composed of predominantly oxidative fibers appear to be less susceptible. This warrants future investigations into the underlying mechanisms that explain the fiber type specific response in the protein synthesis

signaling pathway. Understanding these processes can lead to better future therapeutic interventions for type 1 diabetes.

Endoplasmic reticulum (ER) targeting of Phospholamban: The di-arginine motif in ER retention

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Introduction: Phospholamban (PLN) is an inhibitor of the sarco(endo)plasmic reticulum Ca²⁺-ATPase. The lack of a classic ER retention signal in PLN led us to investigate the di-arginine motif in the cytoplasmic region of PLN. PLN di-arginine mutants showed that PLN retention is dependent on an intact di-arginine motif. Bioinformatic analysis determined that the di-arginine motif is found in a significant number of all ER/SR gene ontology annotated proteins. By mutating candidates identified in the bioinformatic screen we indicate that the di-arginine motif maybe an ER retention sequence. **Methods:** We performed site directed mutagenesis to generate arginine motif PLN mutants and analyzed the subcellular localisation of an N-Flag tagged wildtype and mutant PLN by confocal microscopy and by subcellular fractionation using a 20-60% linear sucrose gradient. The bioinformatic screen retrieved ER proteins with retention motifs KK and RR within the first 25 residues and ER retrieval motif XDEL in the C-terminal of proteins were from the Gene Ontology (GO) database from proteins with GO identifications as ER (GO:0005783) and SR (GO:0016529). Proteins were then analysed using Protein Centre (Proxeon, Denmark). Candidate proteins were selected from the screen and their subcellular localisation determined using the 20-60% linear sucrose gradients. **Results:** In the present study we generated arginine motif mutants and analyzed the subcellular localisation of wildtype and mutant PLN. We showed that PLN retention in the ER is dependent on its R13/R14 di-arginine motif. Co-immunoprecipitation of endogenous PLN from cardiac tissue identified the minimal machinery necessary to form retrograde transport vesicles ARF1 and COP I coat to be co-immunoprecipitated with wild type PLN but not with PLN R Δ 14. This data then implies that COP I requires the intact di-arginine motif in PLN since mutation of this motif results in COP I not binding to PLN R Δ 14 and its mislocalization from the ER. The importance of the di-arginine motif led us to bioinformatically analyse its frequency in all ER and SR gene ontology (GO) annotated proteins and found that a significant number of proteins have a di-arginine motif within the first 25 amino acids, indicating that it may be used as an ER retention sequence. We demonstrated this by mutating the di-arginine motif in two candidates identified in the bioinformatic screen by subcellular fractionation that mutation of the di-arginine motif caused mislocalization of the resident ER proteins.

Effect of denervation on mitochondrial protein import and machinery expression

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Chronic muscle disuse results in a reduction in mitochondrial content and function. However, the mechanisms regulating this reduction are not well understood. Most mitochondrial proteins are encoded within the nucleus and require an import machinery system to transport them into mitochondria. The purpose of this study was to investigate whether protein import was impaired during muscle disuse. We induced disuse by sectioning the peroneal nerve in normal rats, resulting in denervation of the tibialis anterior muscle. Subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria were isolated from denervated and control muscles after 7 days. Denervation produced a 30% reduction in muscle mass. Mitochondria from denervated muscle displayed a significant reduction in respiration rates along with elevated reactive oxygen species production. To evaluate protein import, SS and IMF mitochondria from control and denervated muscle were incubated with radiolabelled OCT precursor protein, and import was measured over time. Denervation resulted in a 28% reduction in SS mitochondrial protein import after 7 days. This was accompanied by a 50% reduction in Tim23 expression, but no change was evident in Tom20 or mtHSP70. Denervation had little effect on IMF mitochondrial protein import or machinery expression. Thus, the decline in mitochondrial content observed with chronic muscle disuse, particularly in the subsarcolemmal compartment, may be attributed to a reduced capacity to import proteins.

Neuromuscular Disease and Ethics: Considerations of Endpoint in a Mouse Model of Lou Gehrig's Disease

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Background: Research laboratories around the world use different measures of endpoint for studies involving animal models of amyotrophic lateral sclerosis (ALS), a neuromuscular disease. Examples of endpoints in the literature include paralysis in both hind legs (clinical score of 4; CS 4) in addition to 1) weight loss (wt. loss) of $\geq 20\%$ vs. peak body weight (20%Peak), 2) wt. loss of $\geq 20\%$ vs. body weight immediately prior to disease onset of CS 2 (20%CS2), 3) body condition < 2 (BC2), or 4) righting reflex (identified as CS 5; different laboratories use different time periods ranging between 10-30 sec). Rationale: Increasingly, strict standards set by research ethics boards stipulate the use of endpoints to minimize the suffering of research animals. Objective: To investigate which criteria should be used to establish a measure of endpoint that is valid and meets the highest ethical standards. Methods: Data from 3 animal studies using 120 G93A mice, a model of ALS, were analyzed to determine if differences exist between the following endpoint criteria: CS 4, CS 5 (CS 4 plus righting reflex ≥ 20 sec), and CS 4+ which combined the presence of CS 4 in addition to the earliest of the following criteria: 1) 20%Peak, 2) 20%CS2, 3) BC2, or 4) CS 5. The age (d; mean \pm SEM) at which mice reached endpoint was recorded as the unit of measurement. Results: On average, mice reached CS 4 at 126 ± 1 d, C4+ at 128 ± 1 d and CS 5 at 130 ± 1 d. Results: On average, mice reached CS 4 at 123 ± 10 d, CS 4+ at 126 ± 9 d and CS 5 at 127 ± 9 d, all significantly different from each other

($P < 0.001$). There was a significant positive correlation between CS 4 and CS 5 ($r = 0.95$, $P < 0.001$), CS 4 and CS 4+ ($r = 0.96$, $P < 0.001$), and CS 4+ and CS 5 ($r = 0.97$, $P < 0.001$). Logrank tests revealed that mice reached CS 4 43% faster than CS 5 (HR = 1.43, $P = 0.008$), however no significant differences were observed between CS 4 and CS 4+ ($P = 0.079$), and between CS 4+ and CS 5 ($P = 0.383$). Conclusion: There is a strong correlation between the different endpoints, with mice reaching CS 4 four days sooner than CS 5, and minimum bias between CS 4 and CS 5. Hence, we conclude that CS 4 is a valid measure of endpoint in the G93A mouse model of ALS, and should be adopted to lessen suffering of mice as per ethical considerations. Supported by NSERC, HHSF and Faculty of Health- York U

Muscle mitochondrial function is associated with physical activity and not obesity status in youth

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INTRODUCTION: Impaired muscle mitochondrial function has been suggested as a potential mechanism of insulin resistance, which is commonly associated with obesity. The few studies that have assessed mitochondrial function in obesity have reported conflicting results. We hypothesized that phosphocreatine (PCr) recovery in response to exercise, an index of mitochondrial function, would be slower with increasing adiposity in children. METHODS: In 21 subjects (7 overweight as a consequence of craniopharyngioma (CP), 7 overweight controls (OC) and 7 healthy weight controls (HC), matched for age and puberty status; 12 female; 9-18 years), phosphorus metabolites in the quadriceps were measured prior to and following 60 seconds of exercise in a 1.5T GE magnetic resonance (MR) scanner using MR spectroscopy. Baseline levels of PCr, inorganic phosphate (Pi), adenosine triphosphate (ATP) and cellular pH were measured, and the time constants of recovery for PCr, Pi, and their ratio (an index of ADP) were estimated by fitting exponential curves to the data. BMI z-scores, aerobic capacity (VO2 max, ml/kg/min), and self-reported physical activity (Habitual Activity Estimation Scale) were assessed. RESULTS: The groups did not differ in baseline or recovery values of phosphorus metabolites or in amount of time spent being very active (breathing/sweating hard). VO2 max was greatest in HC and lowest in CP. Time constants of PCr, Pi and Pi/PCr recovery did not correlate with BMI z-score, and tended to correlate negatively with time spent being very active ($R=0.33$, $P=0.15$; $R=0.45$, $P=0.05$; and $R=0.44$, $P=0.05$, respectively), suggesting improved mitochondrial function with increasing physical activity. CONCLUSIONS: Our preliminary data suggests that muscle metabolic function is not related to obesity status, and instead is related to physical activity. Conflicting results from previous studies may be explained by failure to match overweight and healthy controls for levels of physical activity.

Satellite Cell Specific p-STAT3 Signalling in Human Muscle Following Acute Muscle Damage

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Interleukin 6 (IL6) acting through STAT3 is a known signalling cascade active in many cell types. Whether IL6 exclusively signals in satellite cells (SC) following acute myotrauma is unknown. Twelve male subjects (21 ± 2 y; 83 ± 12 kg) preformed 300 maximal muscle-lengthening contractions of the quadriceps femoris at $180^\circ \cdot s^{-1}$ over a 55° range with muscle samples (vastus lateralis) and blood samples (antecubital vein) taken prior to exercise (PRE), 1h (T1), 3hrs (T3) and 24hrs (T24) post-exercise. Cytosolic and nuclear fractions of muscle biopsies were purified and analyzed for total and p-STAT3 using western blotting. P-STAT3 was detected only in cytosolic fractions across the time course peaking at T24 ($p < 0.01$ vs. PRE). Nuclear total and p-STAT3 was not detected at any time point. However, immunohistochemical analysis revealed a progressive increase in the proportion of SC expressing p-STAT3 with $\sim 60\%$ of all SC positive for p-STAT3 at T24 ($p < 0.001$ vs. PRE) and with cMyc, the production of which is regulated by p-STAT3, detected in SC at T24. Whole muscle mRNA analysis revealed induction of the STAT3 target genes IL6, SOCS3, cMyc (peaking at T3, $p < 0.05$), IL6R α and GP130 (peaking at T24, $p < 0.05$). Upregulation of these genes coupled with that of Myf5, (T24, $p < 0.05$) with no appreciable change in MRF4, suggests that the IL6/STAT3 axis is active in promoting SC proliferation in the early phase following muscle damage in humans.

Corticosterone mediated effects on endothelial cells response to shear stress

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Angiogenesis, the formation of new capillaries from pre-existing ones, can be promoted and inhibited by many factors. Shear stress can induce endothelial cell activation that results in luminal splitting angiogenesis. Previously, corticosterone has been identified to inhibit capillary development and abluminal sprouting angiogenesis. The purpose of this study was to evaluate the effect of corticosterone on endothelial cell responsiveness to elevated shear stress. Elevated shear stress has been shown to activate the AKT pathway, which is important in regulating cell survival. In our study we utilized rat skeletal muscle endothelial cells pre-treated with corticosterone followed by 2 hours of elevated shear stress. Our results indicate that treating endothelial cells with corticosterone has inhibitory effects on AKT phosphorylation at S473 (mediated by mTORC2) but not at T308 (mediated by PDK1) mediated by elevated shear stress. When we examined the effects on downstream targets of AKT, we found no significant changes in nitric oxide levels in corticosterone treated cells. Moreover, we found a significant decrease in cleaved caspase-3 levels, indicating decreased apoptosis, in corticosterone treated cells despite the increase in FoxO1 levels. These results provide evidence that corticosterone alters the shear stress induced phosphorylation of AKT at S473 but the overall effect on the survival pathway remains uncertain.

TIMP-1 protein but not secretion is increased by shear stress in the skeletal muscle microvasculature

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Luminal splitting is a form of angiogenesis caused by increased shear stress. A feature of luminal splitting is a lack of extracellular matrix proteolysis correlated with decreased production of matrix metalloproteinase (MMP)-2. We hypothesized that an MMP inhibitor, tissue inhibitor of matrix metalloproteinase (TIMP)-1, is upregulated by shear stress. TIMP-1 mRNA increased in the EDL of prazosin-treated rats ($p < 0.05$, $n = 4$) as measured by RT-PCR. Rat skeletal muscle microvascular endothelial cells were exposed to shear stress (12 dyne/cm²). TIMP-1 mRNA increased in cells after 2 and 24 hours of shear ($p < 0.05$, $n = 3$), and TIMP-1 protein increased at 2 and 24 but not 4 hours ($p < 0.05$, $n = 3$) as assessed by Western. TIMP-1 secretion decreased at 2 hours of shear and returned to control levels at 24 hours ($p < 0.01$, $n = 3$) as measured by reverse zymography of concentrated media. Similarly, MMP-2 activity increased in media at 2 but not 24 hours of shear ($p < 0.05$, $n = 7$) as assessed by an in vitro MMP-2 activity assay (Anaspec). 2 hours of shear caused no change in TIMP-1 surface staining (ns, $n = 3$) as assessed by confocal microscopy, but did cause an increase in intracellular TIMP-1 staining ($p < 0.05$, $n = 3$). Our results show that intracellular TIMP-1 protein is increased by shear stress, due to increased production and decreased secretion. The function of intracellular TIMP-1 is unknown. Funded by the Heart and Stroke Foundation of Canada.

PGC-1 α -dependent mitochondrial adaptations in C2C12 cells: effects of contractile activity

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PGC-1 α is a well established mediator of mitochondrial content and function in skeletal muscle. However, the essentiality of the coactivator for mitochondrial biogenesis (MB) in response to chronic contractile activity (CCA) is still in question. Thus, we depleted skeletal muscle C2C12 cells of PGC-1 α using siRNA, then stimulated the myotubes to induced MB. A 60% reduction in PGC-1 α using siRNA resulted in a compensatory increase in AMPK phosphorylation, but had no effect on the mRNA expression of PGC-1 β , PRC, NRF-1/2 or YY1, proteins that may have served to compensate for low PGC-1 α levels. In response to CCA, we found that a normal induction of PGC-1 α in response to CCA was required to observe the typical changes in MB, as revealed by attenuated increases in COX activity ($p < 0.05$) and mitochondrial immunofluorescence. Decreased levels of PGC-1 α also reduced the CCA-evoked increases in COXIV. However, PGC-1 α was not necessary for the CCA-induced changes of all mitochondrial proteins since Tfam and cytochrome c displayed normal increases, regardless of attenuated PGC-1 α levels. Thus, PGC-1 α is necessary for the normal induction of mitochondrial biogenesis, and the coactivator likely functions with parallel pathways to mediate increases in mitochondrial proteins with CCA.

Voluntary aerobic exercise attenuates oxidative stress-induced apoptotic signalling in cardiac muscle

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An increase in the production of reactive oxygen species occurs with aging, and the resulting oxidative stress has been associated with apoptosis-related cardiomyopathy. Recent research has indicated that chronic exercise is protective against mitochondrially-mediated programmed cell death. To further investigate this in the heart we compared voluntary wheel trained (T, 9 weeks) and untrained (C) animals. Training increased heart weight to body weight ratio, and produced a 54% increase in muscle COX activity. Left ventricle (LV) strips were isolated and incubated in vitro with H₂O₂ for 4 hours. LV strips were then fractionated into cytosolic and mitochondrial fractions. H₂O₂ treatment induced increases in JNK phosphorylation, Bax localization to the mitochondria, as well as cytosolic AIF by 73%, 106% and 38% in C animals respectively, all of which were attenuated in T animals. Interestingly, H₂O₂ induced a 2.5-fold increase in cofilin-2 localization to the mitochondria, as well as a 64% increase in cytochrome c release which were unaltered by training. These data suggest that regular exercise can attenuate apoptotic signalling via reduced JNK phosphorylation, Bax translocation and mitochondrial AIF release. Thus, endurance exercise training has the potential to reduce oxidative stress-induced programmed cell death in the heart.

Induction of the mitochondrial permeability transition pore (mtPTP) in control and denervated skeletal muscle

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The susceptibility of mitochondria to apoptotic stimuli is partially determined by the opening of a voltage-sensitive and Ca²⁺-dependent channel in the mitochondrial inner membrane, known as the mitochondrial permeability transition pore (mtPTP). Opening of the mtPTP is linked to mitochondrial dysfunction, since it results in the release of pro-apoptotic proteins such as cytochrome c. To investigate the optimal characteristics of the mtPTP, we incubated subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria isolated from skeletal muscle with Ca²⁺, tert-butyl hydroperoxide (ROS), and the respiratory substrates malate and ADP. In response to Ca²⁺ and ROS, SS and IMF mitochondria exhibited a dose-dependent increase in the rate of pore opening, and displayed a similar sensitivity to the triggering agents. Induction of state IV respiration with the addition of malate increased the maximal rate of pore opening (V_{max}) in SS and IMF mitochondria by 9-fold and 4-fold respectively. Moreover, the addition of malate decreased the time to V_{max} in SS and IMF mitochondria by 35-40%. The induction of state III respiration with ADP did not produce a further increase in pore opening kinetics. Incubation of SS and IMF mitochondria with malate resulted in 4.5-fold and 3.6-fold increases in released cytochrome c, respectively. The addition of ADP did not result in further cytochrome c release in either subfraction. Thus, state IV respiration appears to be more important in regulating mtPTP opening and protein release than state III respiration. Denervation of skeletal

muscle results in a drastic decrease in mass along with an overall increase in mitochondrial susceptibility to apoptosis. Incubation of the mitochondria from denervated muscle with Ca^{2+} and ROS resulted in a 40% increase in the V_{max} in IMF mitochondria, compared to mitochondria from control muscle. This suggests that denervation increases the susceptibility of the mtPTP to opening which can contribute to the muscle loss observed with denervation.

The level of mRNA repressor PDCD4 and protein synthesis in skeletal muscle are inversely regulated by nutrition

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The mass of the skeletal muscle has implication for the health of the body. Defects in muscle protein metabolism underlie or worsen metabolic diseases including type 2 diabetes. Therefore a better understanding of mechanisms regulating protein metabolism is vital for the management of these conditions. Muscle protein synthesis (PS) is regulated by nutrients (proteins and amino acids, glucose) and by insulin. At the molecular level, the kinase mTOR/S6K1 pathway integrates signals from insulin and nutrients to induce increase in mRNA translation and PS. However, the mechanisms by which this kinase pathway increases PS are unclear. Recently, the tumor suppressor programmed cell death 4 (PDCD4) was identified as a novel substrate of this pathway. Our objectives were to, i) examine the expression of this protein in skeletal muscle, and ii) to measure its regulation under a condition when PS is altered. In experiment 1, male Sprague Dawley rats (100g, n = 6-7) served as control, or were fasted for 48hrs. Fasted rats were then re-fed for 1 to 6 hrs. PS was measured by the flooding dose technique, and phosphorylation of mRNA translation regulators assessed by immunoblotting. Mixed gastrocnemius PS was suppressed in fasted animals (-50%, $P < 0.05$) and increased to a maximum by 3 h of re-feeding (+55% relative to starved group; $P < 0.05$). Phosphorylation of PDCD4 was barely detectable in the fed and re-fed groups, consistent with reduced expression of this repressor protein. Decreased activation of S6K1, the upstream kinase for PDCD4, was observed in fasted animals (-87%, $P < 0.05$), but it was re-activated in the re-fed group. To examine the specific components of the diet that mediate the observed effects, in experiment 2, rats were treated as in experiment 1, but during re-feeding, the animals were gavaged with a mixture of essential and non essential amino acids at a level equivalent to what a rat will consume in one day. Amino acids alone restored PS to the level seen in animals re-fed with chow, but the maximum stimulation was seen at 1 hr. Thus, amino acids stimulate PS in skeletal muscle in parallel with suppression of the translational inhibitor PDCD4.

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