

ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

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APSIPE.5

Clinical Relevance and Underlying Mechanisms of Exercise-Induced Browning of White Adipose Tissue

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Adipose is a dynamic and vibrant tissue that responds robustly to exercise training. Similar to skeletal muscle, exercise training in rodents leads to increases in indices of mitochondrial content and oxidative capacity. More recent studies have expanded upon this and have demonstrated a “browning” of white adipose tissue that is characterized by increases in the expression of Uncoupling Protein 1 (UCP1) and the appearance of multilocular fat cells. A shift in the metabolic profile of white adipose tissue to more closely resemble brown adipose, a tissue which generates heat and burns calories, has led some to suggest that this could be a mechanism through which exercise facilitates improvements in metabolic health. Given this, there has been a concerted effort to identify mechanisms mediating the effects of exercise on white adipose tissue. To date multiple players have been suggested to play a role including both classical neuroendocrine factors and muscle and liver derived signalling molecules. While work in rodents has consistently demonstrated that exercise increases indices of oxidative metabolism in white adipose tissue this has not been a repeatable finding in the vast majority of studies in human participants. There are a number of potential explanations for these discrepancies including differences in the metabolic phenotypes of adipose tissue depots that are typically sampled in human and rodent based investigations and the temperature which animals are housed.

APSIPE.7

Regulation of extracellular matrix tendon tissue in response to resistance training

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Tendons are made up of an extracellular matrix (ECM) containing primarily type I collagen, as well as other collagens, elastin, and various proteoglycans. Tendon ECM is arranged in a hierarchical manner, with densely packed collagen fibers wrapped by layers of basement membrane. Tenocytes, or tendon fibroblasts, are the main cell type in tendons and are thought to be responsible for the production, organization, and maintenance of the tendon ECM. The organization of the ECM allows the tendon to properly transmit forces from muscle to bone and allow for locomotion, and to respond to mechanical stimuli. Mechanical loading can increase tendon cross sectional area and modify mechanical properties of the tendon matrix. The purpose of this talk will be to discuss the cellular and molecular factors that underlie the response of tendon tissue to mechanical loading.

APSIPE.9

Massage in the form of cyclic compressive loading enhances remodeling of skeletal muscle extracellular matrix in old rats recovering from disuse atrophy.

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Background: Skeletal muscle from older adults displays more and stiffer extracellular matrix (ECM) which may inhibit the response to external mechanical stimuli and therefore lead to the observed impairment in recovery from atrophy. ECM remodeling is an important process during the regrowth of muscle after disuse atrophy and interventions targeting ECM remodeling may lead to better regrowth responses. Purpose: The purpose of this study was to use massage in the form of cyclic compressive loading (CCL) to restore ECM remodeling in old rats undergoing muscle regrowth following atrophy to levels observed in adult. Methods: adult (10 month; n =8) and old (30 month; n =8) male F344/BN rats were used. All rats

underwent hind limb suspension for 14 days to induce disuse atrophy. Following the suspension period rats ambulated in weight bearing conditions for 7 days, with or without the massage intervention. Gastrocnemius muscles underwent CCL for 30 minutes every other day for a total of 4 bouts using a custom-built robotic CCL device at a load of 4.5N and a frequency of 0.5Hz. At the end of the reloading period, rats were euthanized and gastrocnemius muscles were immediately harvested and frozen for immunohistochemistry (wheat germ agglutinin (WGA) and collagen hybridizing peptide (CHP)) and collagen content assessment (hydroxyproline). WGA was used to assess total area occupied by ECM and CHP is used as a marker for denatured collagen. Results: There was a main effect for age in total area of the ECM ($p=0.008$) indicating elevated levels in aged. Hydroxyproline was 2.4-fold higher in muscle from old reloaded rats compared to adult ($p=0.03$). Total area of ECM was lower in muscles from old reloaded rats receiving massage compared to those without massage ($p=0.03$). Lastly, CHP was elevated in muscles of reloaded old rats compared to adult, which was lowered by massage application ($p=0.01$). Conclusion: Results from this study indicate that massage in the form of CCL is a useful intervention to restore ECM remodeling in old rats undergoing regrowth of muscles after disuse atrophy. The project described was supported by a TL1 predoctoral fellowship to ZRH through NIH National Center for Advancing Translational Sciences through grant number UL1TR001998 and NIH grants RO1AT009268 and R21AG042699 to TAB, BFM and EEDV.

APSIPE.10

Racial Differences in Endothelial Dysfunction: Does PP2A Play a Role?

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Background: African Americans (AA) exhibit a higher prevalence of endothelial dysfunction compared to Caucasian Americans (CA). Endothelial dysfunction is characterized by a reduction in endothelial nitric oxide synthase (eNOS) activity and ultimately lower nitric oxide production. Protein phosphatase 2A (PP2A) reduces eNOS activity by reducing phosphorylation of eNOS at the Serine 1177 residue (eNOSS1177). However, it is unclear if there are racial differences in PP2A expression, which may contribute to racial differences in endothelial dysfunction. Moreover, high laminar shear stress (HiLSS) is an exercise mimetic that has been shown to improve endothelial function. Therefore, the purpose of this study was to investigate whether there is a racial difference in PP2A expression in human umbilical vein endothelial cells (HUVECs) from AA and CA donors, and the ability of HiLSS to mitigate the potential racial difference. Methods: We cultured HUVECs ($n=6$ cell lines; 3 CA & 3 AA) under two conditions: control (static) or HiLSS (20 dyne/cm², 24 hours). Western blots were used to measure protein expression of p-eNOSS1177 and PP2A. Furthermore, phosphorylation of PP2A at the Tyrosine residue (p-PP2AY307) was measured for protein expression, as it has

been shown to decrease PP2A activity. Racial differences were examined in the proteins of interest using the Man-Whitney test. Results: Compared to CA, HUVECs from AA exhibited lower expression of p-eNOSS1177 at baseline ($p=0.05$; Fig. 1A). While no racial differences in PP2A (Fig. 1B) levels were found, p-PP2AY307 ($p=0.01$ Fig. 1C) was significantly lower in HUVECs from AA compared to CA at baseline. After 24 hours of HiLSS, p-PP2AY307 was similar in HUVECs from AA and CA individuals (Fig. 2B).

Conclusion: Racial difference in the phosphorylation of PP2A appears to be attenuated by HiLSS. Therefore, PP2A activity may mediate, in part, the beneficial effects of shear stress on nitric oxide production and consequently endothelial function. Acknowledgements: Funded partially by AU PGOP Fellowship and UAB CCTS TL1 Fellowship (UL1TR003096) to DHG.

APSIPE.15

Arterial Architecture is Unaltered by Acute Formaldehyde Exposure in Female Adults

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ABSTRACT Introduction: Short-term exposure to formaldehyde (FA) can cause irritation to the eyes, nose, and throat with long-term exposure leading to disorders of the skin, cancer, and cardiovascular disease. The latter is associated with vascular alterations, yet little work has investigated the impact of FA exposure on arterial architecture. Purpose: The purpose of this study was to investigate the impact of acute 90-minute FA exposure (259 ± 95 ppb) on arterial architecture as determined by central pressures, augmentation index (AIx), and carotid-femoral pulse wave velocity (cfPWV) ($n = 13F$, 24 ± 1 yr) as well as carotid stiffness ($n = 9F$, 23 ± 1 yr) prior to and immediately following exposure to FA in human cadaver dissection laboratories. Results: Central pressures, cfPWV (Pre-FA: 5.2 ± 0.8 m·s⁻¹, Post-FA: 5.2 ± 1.1 m·s⁻¹), and PWA were unchanged by acute FA exposure ($p > 0.05$). Carotid stiffness parameters and distension (Pre-FA: 0.6 ± 0.4 , Post-FA: 0.6 ± 0.3 mm) were unchanged by acute FA exposure ($p > 0.05$). Individual Pre- to Post-FA changes in these markers of arterial architecture did not correlate with levels of FA exposure ([FA]: 20-473 ppb) ($p > 0.05$). Conclusion: Acute FA exposure typically experienced in human cadaver laboratories does not appear to induce changes in arterial architecture of adult females.

APSIPE.17

Interindividual variability in skeletal muscle responses to moderate intensity continuous training

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The standard deviation of individual response (SDIR) method estimates true interindividual variability by subtracting the SD of responses to a no-exercise control group from the SD of responses to exercise training. The novel purpose of the present study was to estimate the magnitude of interindividual variability in human skeletal muscle responses to exercise training. Twenty-nine healthy, recreationally-active males (age: 21 ± 2 years; BMI: 24.45 ± 2.9 ; $\text{VO}_{2\text{peak}}$: 45 ± 7 mL/kg/min) completed four weeks of moderate-intensity continuous training (MICT; $n = 14$) or a no-exercise control period (CTRL; $n = 15$). MICT involved 30-minutes of cycling at 65% of peak work rate 3d/wk. Maximal enzyme activities (citrate synthase and β -HAD), capillary density, fibre type composition, fibre-specific SDH activity and substrate storage (IMTG and glycogen), and markers of mitophagy (BNIP3, NIX, PRKN, and PINK1) were measured in vastus lateralis samples collected before and after the four-week intervention. MICT increased glycogen content in both type I and IIA fibres (group-time interactions: $p < 0.05$), and appeared to increase citrate synthase maximal activity (near significant group-time interaction: $p < 0.10$). No other main or interaction effects were observed. Positive SDIR values, indicating true interindividual variability, were observed for citrate synthase and β -HAD maximal activity, capillary density, all fibre-specific outcomes, and BNIP3 protein content. However, the negative SDIR values for the remaining outcomes indicates a large degree of measurement error and/or within-subject variability. Given, that true interindividual variability was not observed for all outcomes, our findings suggest that appraising the variability in observed changes to MICT alone is an inappropriate method for estimating true interindividual variability in skeletal muscle responses.

APSIPE.18

The role of muscle in adult neurogenesis and memory function

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Most neurons in the adult central nervous system are terminally differentiated and cannot be replaced when they die. However, small populations of new neurons are generated in the mature olfactory bulb and the hippocampus. In the adult hippocampus, newly born neurons originate from putative stem cells that exist in the subgranular zone of the dentate gyrus. The production, survival and functional integration of newborn hippocampal cells is strongly upregulated by voluntary wheel running in

rodents. Enhanced adult hippocampal neurogenesis is correlated with changes in synaptic plasticity, new neuron networks, spatial navigation and pattern separation in rodents. Another aspect of our work is to investigate the peripheral triggers that may mediate exercise induced changes in the brain. Using proteomic analyses, we identified lysosomal enzyme Cathepsin B (Ctsb) as a novel myokine that influences brain function. Analyses across species in mice, monkeys and humans showed that this factor upregulated in plasma with exercise. In humans, these changes correlated with improved fitness and hippocampus-dependent memory function. A recent focus of our work is to determine whether myokine levels may be upregulated in aging subjects following an exercise intervention. Overall, our research evaluating the relationship between myokines, adult hippocampal neurogenesis, neurotrophin levels and memory function aims to further our understanding of effects of exercise on the brain.

APSIPE.19

Vascular Dysfunction and Oxidative Stress Caused by Acute Formaldehyde Exposure in Female Adults

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Introduction: Formaldehyde (FA) is a common, volatile organic compound used in organic preservation with known health effects of eye, nose, and throat irritation linked to oxidative stress and inflammation. Indeed, long-term FA exposure may provoke skin disorders, cancer, and cardiovascular disease. However, the effects of short-term FA exposure on the vasculature have yet to be investigated. Purpose: We sought to investigate the impact of an acute FA exposure on: 1) macrovascular function in the arm (brachial artery flow-mediated dilation, FMD), 2) microvascular function in the arm (brachial artery reactive hyperemia, RH) and leg (common femoral artery, supine passive limb movement, PLM), and 3) circulating markers of oxidative stress (xanthine oxidase, XO; protein carbonyl, PC; and malondialdehyde, MDA) and inflammation (C-reactive protein, CRP). Methods: Ten ($n=10$) healthy females (23 ± 1 y) were studied before and immediately after a 90-minute FA exposure ([FA]: 197 ± 79 ppb) in cadaver dissection laboratories. Results: Brachial artery FMD% decreased following FA exposure (Pre-FA Exp: $9.41 \pm 4.21\%$, Post-FA Exp: $6.74 \pm 2.57\%$, $p=0.043$), and FMD/Shear decreased following FA exposure (Pre-FA Exp: 0.13 ± 0.07 AU, Post-FA Exp: 0.07 ± 0.03 AU, $p=0.016$). The area under the curve for brachial artery RH (Pre-FA Exp: 481 ± 191 ml, Post-FA Exp: 499 ± 165 ml) and common femoral artery PLM (Pre-FA Exp: 139 ± 95 ml, Post-FA Exp: 129 ± 64 ml) were unchanged by FA exposure ($p > 0.05$). Circulating MDA increased (Pre-FA Exp: $4.8 \pm 1.3 \mu\text{M}$, Post-FA Exp: $6.3 \pm 2.2 \mu\text{M}$, $p=0.047$) while XO, PC, and CRP were unchanged by FA exposure ($p > 0.05$). Conclusion: These initial data suggest a

short FA exposure can adversely alter vascular function and oxidative stress, influencing cardiovascular health.

APSIPE.20

High-intensity interval training increased aerobic capacity but did not improve peripheral insulin sensitivity, in either insulin resistant or insulin sensitive obese adults

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High-intensity interval training (HIIT) promotes important metabolic health benefits in adults with obesity. However, it remains unclear whether metabolic adaptations to HIIT differ in individuals with discordant metabolic health status. The purpose of this study was to determine the impact of a 12-week HIIT program on insulin sensitivity and other key clinical and sub-clinical markers related to metabolic health in a cohort of insulin resistant ("IR"; BMI 33±4 kg·m⁻², n=7) and a well-matched cohort of insulin sensitive ("IS"; BMI 33±3 kg·m⁻², n=7) adults. Group assignments (IR vs IS) were determined from baseline peripheral insulin sensitivity measurements of glucose rate of disappearance (Rd; isotope dilution methods) during a hyperinsulinemic-euglycemic clamp (IR: < 9 mg glucose·kg fat-free mass (FFM)·1·μU/ml insulin·1·min⁻¹; IS: > 11.5 mg·FFM·1·μU/ml·1·min⁻¹). All subjects participated in a 12-week HIIT program (4 d·wk⁻¹), with each session involving 10x1 min @ 90% HRmax. Importantly, body weight was strictly maintained throughout the training period to determine the direct effects of HIIT, independently of changes in body weight or fat mass. Hyperinsulinemic-euglycemic clamps and skeletal muscle biopsies were performed before training, and again 4 days after the last training session. As designed, 12 weeks HIIT did not change body weight (IR: 96±15 vs 97±16 kg; IS: 96±10 vs 96±11 kg) or fat mass (IR: 43±10 vs 43±10 kg; IS: 40±5 vs 40±6 kg) in either group. HIIT increased peak oxygen consumption (VO₂peak) similarly in both groups (14±7% and 8±4% in IR and IS, respectively; P<0.01 compared with baseline in both groups; P=0.17 for IR vs IS). Consistent with the increase in VO₂peak, abundance of skeletal muscle mitochondrial protein complexes I, II, III, and IV increased significantly after HIIT (all P<0.05), with no differences between IR and IS. Despite the increase in aerobic capacity, HIIT did not improve peripheral insulin sensitivity in either IR (5.9±2.6 vs 7.6±2.2 mg·FFM·1·μU/ml·1·min⁻¹) or IS (13.1±2.2 vs 12.4±3.4 mg·FFM·1·μU/ml·1·min⁻¹), which is consistent with findings from many studies investigating effects of moderate-intensity steady-state exercise training when weight loss and the acute effects of the last exercise session were controlled. Interestingly, HIIT significantly increased hepatic insulin sensitivity (P=0.01), and reduced plasma triglyceride concentration (P=0.01, Group x Time) in IR only. In conclusion, 12 weeks of HIIT increased aerobic capacity similarly in both IR and IS, but this did not translate to improvements in peripheral insulin sensitivity, regardless of baseline insulin sensitivity. However, HIIT may modify mechanisms leading to improved hepatic insulin sensitivity and plasma triglyceride regulation in those with insulin resistance, thereby

enhancing some important aspects of metabolic health in insulin resistant adults with obesity. Supported by NIH grants: R01 DK077966, P30DK089503, F32DK117522, U24DK097153, UL1TR002240, Canadian Institutes of Health Research DFS146190.

APSIPE.21

Effects of Neuromuscular Electrical Stimulation on Insulin Sensitivity and Glycemic Control: A Systematic Review and Meta-Analysis

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Background Physical inactivity increases the risk for metabolic diseases such as insulin resistance, obesity, and type 2 diabetes. Neuromuscular electrical stimulation (NMES) is an effective method to induce involuntary muscle contraction, particularly for populations that are more prone to physical incapacities and metabolic diseases. However, the effectiveness of improving glycemic control and insulin sensitivity with NMES is not well known. Purpose To systematically evaluate existing evidence to determine the effectiveness of NMES on glycemic control and insulin sensitivity. Methods Electronic search consisted of MEDLINE (PubMed), EMBASE, Cochrane Library, Google Scholar, and Web of Science to identify existing original research studies that investigated the effects of NMES on glycemic control and insulin sensitivity in humans. Studies that met inclusion criteria for the systematic review were then considered for meta-analysis if the studies were designed as randomized controlled trials. Risk of bias and quality assessment were performed for all studies included in the meta-analysis. Effect sizes were calculated as the standardized mean difference and meta-analyses were completed using a random-effects model. Results 31 studies met the inclusion criteria for systematic review and of those, 10 longitudinal studies qualified for the meta-analysis. Existing evidence suggest that NMES can effectively improve glycemic control (acute effect) and insulin sensitivity (chronic effect) in middle-aged and elderly men and women with type 2 diabetes, obesity, and spinal cord injury. The meta-analysis comprised of 189 subjects which reported NMES resulted in an improvement in insulin sensitivity (MD: 0.41; 95% CI, 0.09 to 0.72; p=0.01; I²=11%). The methods used to assess insulin sensitivity varied among studies, and different markers of insulin sensitivity was reported. Therefore, additional analysis was performed to determine the effects of NMES on fasting glucose, which was reported in all the included studies except one (n=9). Similarly, there was a significant effect of NMES on lowering fasting blood glucose (MD: 0.48; 95% CI: 0.17 to 0.78; p=0.002; I²=0%). Conclusions NMES could be considered as an alternate therapeutic strategy to improve

glycemic control and insulin sensitivity in populations that are faced with physical incapacities and metabolic disease.

APSIPE.22

Disorganized Capillary Regeneration Coincides with Impaired Myofiber Reinnervation Following Skeletal Muscle Injury

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Acute injury of skeletal muscle damages microvessels and motor nerves along with myofibers. Regeneration of myofibers is well-characterized but little is known of concurrent events in microvessels and motor nerves. In the context of development, growth and patterning are coordinated between innervation and vascularization; therefore, we tested the hypothesis that disrupting capillary regeneration impairs myofiber reinnervation in the adult. To impair angiogenesis, Cdh5-CreERT2 x EfnB2fl/fl male mice (age, 3-6 mo; n=5-8/group) were injected with tamoxifen to delete ephrin-B2 in endothelial cells (ECs) (EfnB2CKO; confirmed with RT PCR); non-injected mice of the same genotype (EfnB2WT) were studied for reference. The gluteus maximus (GM) or tibialis anterior (TA) muscle was injured by local injection of 1.2% BaCl₂ to study capillary morphology (GM) and evaluate neuromuscular junction (NMJ) morphology and neuromuscular transmission (TA). Muscles were evaluated without injury and at 10 days post injury (dpi), when myofibers had regenerated. Intravascular injection of fluorescent wheat germ agglutinin (to identify perfused vessels) or immunolabeling for CD31 (stains all ECs) at 10 dpi revealed capillaries in the GM of EfnB2CKO to be vacuolated with disorganized ECs vs. intact capillary networks with aligned ECs in EfnB2WT and uninjured mice. Isometric force produced by the TA was evaluated using indirect stimulation (IS force) of NMJs through the sciatic nerve and with direct stimulation (DS force) of myofibers with intramuscular electrodes. Data are reported as mean $\pm$ standard error of the mean. During stimulation at 120 Hz, IS force was 35.5 $\pm$ 1.54 N/cm² and DS force was 22.3 $\pm$ 1.62 N/cm² in uninjured EfnB2WT. At 10 dpi, mean IS force was 18.1 $\pm$ 2.20 N/cm² and DS force was 24.5 $\pm$ 1.66 N/cm² in EfnB2CKO vs. 20.8 $\pm$ 4.08 N/cm² and 19.6 $\pm$ 2.24 N/cm² in EfnB2WT. Thus, disorganized capillary regeneration in the GM coincided with diminished neuromuscular transmission in the TA. Immunostaining for presynaptic neurofilament-heavy (NF-H) in combination with α -bungarotoxin staining of postsynaptic acetylcholine receptors indicated that EfnB2CKO alone had no effect on NMJ morphology. Coverage of NMJs by NF-H in EfnB2WT was 89 $\pm$ 4.38% in uninjured TA, 50 $\pm$ 12.2% in 10 dpi EfnB2CKO and 71.5 $\pm$ 7.12% in 10 dpi EfnB2WT with no difference in postsynaptic receptor morphology. We suggest that the absence of ephrin-B2 in ECs during regeneration adversely affects microvascular regeneration in association with impaired neuromuscular transmission and reduced presynaptic

coverage of NMJs. Support: APS Postdoctoral Fellowship (ABM), R01AR067450 (DDWC), R37HL041026 (SSS)

APSIPE.23

Regulation of renal and muscle sympathetic nerve activity during stress in humans

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The sympathetic nervous system is critical for the regulation of arterial blood pressure (BP). However, muscle sympathetic nerve activity (MSNA), exhibits large inter-individual variability at rest and during stress and is commonly unrelated to BP. Given the ability of the sympathetic nervous system to be functionally regulated in an organ dependent manner, variability in MSNA responses could be paralleled by variability in sympathetic outflow directed to other organs that contribute to BP regulation (i.e. kidneys). Therefore, we tested the hypothesis that changes in MSNA would be negatively associated with renal vasoconstrictor responses during stress in humans. All study procedures were approved by the University of Guelph Research Ethics Board (REB 19-17-028) in accordance with the Declaration of Helsinki. Beat-to-beat heart rate (electrocardiography), BP (photoplethysmography), MSNA (microneurography) and renal blood velocity (RBV; Doppler ultrasound) were measured in 11 healthy young participants (four women) at rest and during isometric handgrip exercise (IHG; 2 minutes at 30% of maximum voluntary contraction) with subsequent post-exercise circulatory occlusion (PECO) to isolate the muscle metaboreflex. Following a 15-minute rest, a cold pressor test (CPT) was employed as a non-exercise sympathoexcitatory stimulus by submerging the left hand in ice water (~4°C) for 2 minutes. Renal vascular resistance (RVR), an index of sympathetically mediated renal vasoconstriction, was calculated as mean BP/RBV. Resting RVR was unrelated to MSNA burst frequency (r=0.26, P=0.44) or total MSNA (r=0.14, P=0.68). As expected, heart rate (620 $\pm$ 11 bpm, P<0.001), mean BP (620 $\pm$ 9 mmHg, P<0.001), MSNA burst frequency (621 $\pm$ 14 bursts/min, P<0.001), total MSNA (61460 $\pm$ 1115 a.u., P=0.001) and RVR (60.46 $\pm$ 0.30 mmHg/cm/s, P<0.01) increased during IHG and remained elevated during PECO, with exception of heart rate which returned to baseline values. During CPT, heart rate (69 $\pm$ 8 beats/min, P<0.01), mean BP (619 $\pm$ 7 mmHg, P<0.001), MSNA burst frequency (621 $\pm$ 6 bursts/min, P<0.001), total MSNA (61622 $\pm$ 773 a.u., P<0.001) and RVR (60.44 $\pm$ 0.48 mmHg/cm/s, P=0.01) were similarly increased. Despite neuro-cardiovascular variables being increased during all stressors, there were no relationships between changes in RVR and MSNA burst frequency (IHG: r=-0.30, P=0.37; PECO: r=0.05, P=0.88 and CPT: r=0.32, P=0.34) or total MSNA (IHG: r=-0.27, P=0.42; PECO: r=-0.19, P=0.58 and CPT: r=0.32, P=0.34). In a sub-analysis, we compared 5 high MSNA responders and 5 low MSNA responders (total MSNA response to exercise: 62398 $\pm$ 969 vs. 6589 $\pm$ 307 AU, P<0.01). High MSNA responders exhibited a similar increase in RVR (60.4 $\pm$ 0.1 vs. 60.5 $\pm$ 0.4 mmHg/cm/s,

P=0.50) and a trend towards a larger increase in mean BP (624 ± 10 vs. 616 ± 5 mmHg, $P=0.13$) during IHG. During CPT, MSNA responders exhibited similar increases in RVR (80.5 ± 0.6 vs. 60.4 ± 0.5 mmHg/cm/s, $P=0.79$) and mean BP (621 ± 7 vs. 618 ± 9 mmHg, $P=0.60$) than MSNA non-responders. Despite the absence of a negative relationship, these findings support the concept of differential regulation of muscle and renal sympathetic outflow during stress in young healthy humans. Funding: Natural Science and Engineering Research Council of Canada; Canada Foundation for Innovation; Ontario Ministry of Research, Innovation and Science.

APSIPE.24

Nutrition as Fuel for Space Exploration

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Two decades of continuous human presence off the planet has helped us gain a better understanding of some of the challenges we will face as we look to our next destinations – the moon and Mars. Space flight is challenging to human physiology, from environmental stressors to adaptations affecting virtually all body systems. The search for countermeasures to the negative effects of spaceflight started more than a half century ago. As a cross-cutting discipline, nutrition represents an obvious area for a countermeasure in and of itself, along with enabling and even optimizing others, like exercise. Musculoskeletal losses have long been a concern for astronauts, and exercise has been the obvious countermeasure. Deployment of the Advanced Resistive Exercise Device (ARED) to the International Space Station (ISS) in 2008 allowed for sufficient loading to affect bone. Astronauts who consumed enough calories, had optimal vitamin D status, and exercised with the ARED managed to maintain bone mineral density at preflight levels. Questions remain about the architecture of the resulting bone, but there is no doubt this is a remarkable improvement. Studies to evaluate time saving exercise protocols have recently been conducted, with results starting to appear in publications. Vitamin D has been a concern for astronauts, where a lack of UV light exposure and limited food options had led to lower vitamin D status after early ISS missions, prompting supplementation with 800-1000 IU vitamin D/d. Vitamin D has been documented to have actions well beyond the prevention of rickets. In our research, stress and vitamin D status were implicated in viral reactivation. These findings, along with others, have helped posit that vitamin D status and physical activity before exposure are keys in mitigating the severity of COVID-19 infection. The food system on ISS is limited and repetitive, but offers advantages that a Mars mission will not, including occasional resupply with fresh fruits and vegetables, some food selection based on preference, and shorter shelf life requirements. Despite this, the food system is limited and far from optimal. Intake of fruits and vegetables on ISS is lower than recommended, and intakes correlate with inflammatory and oxidative stress outcomes. Recently identified ocular

pathologies affect some astronauts, and while this is widely hypothesized to be cardiovascular in nature, resistive exercise is a debated potential confounding factor. We identified evidence that one-carbon biochemistry (e.g., homocysteine) is altered before flight in astronauts who experienced ophthalmic changes. Genetic effects on B-vitamin status and on coupling of endothelial nitric oxide synthase might affect vascular and retinal biochemistry and provide the link to which astronauts are affected. While often characterized independently, nutrition and exercise provide the underpinning of optimal human health – on Earth and in space. By uniting these fields, we can develop countermeasures that will serve to counteract the deconditioning of space flight. Work remains to define an optimal space food system, but this will be required for successful, safe, journeys to the moon, Mars, and beyond. This work was funded by the NASA Human Research Program's Human Health Countermeasures Element.

APSIPE.25

Role of extracellular vesicles in tissue cross talk during exercise

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Almost 50 years ago Goldstein proposed the hypothesis that muscle cells possess a “humoral” component that contributes to the maintenance of glucose homeostasis during exercise¹. Approximately 15 years ago, we identified skeletal muscle as a cytokine-producing organ, demonstrating that the metabolic and physiological effects of exercise may be mediated by muscle derived humoral factors (for review see^{2,3,4}). The number of myokines discovered by us and others continues to grow, but these have been discovered largely by serendipity. Inspired by the growing appreciation that many of these myokines might be proteins packaged in extracellular vesicles (EV), we have carried out a deep quantitative proteomic analysis of 1160 proteins contained in the EV fraction of plasma in 11 male participants carrying out 1h of cycling. 325 proteins were differentially regulated by exercise with a notable upregulation of several classes of proteins that compose the canonical ~40-100nm exosome. Pathway analysis revealed significant enrichments in a multitude of biological processes and signalling pathways⁵. These data provide a novel mechanism by which newly released exosomes can influence tissue cross-talk and reveal an intriguing pathway by which exercise can exert multiple biological effects and possibly affect disease risk in the setting of obesity. 1. Goldstein MS. Humoral nature of hypoglycemia in muscular activity. *Am J Physiol* 200 :67 –70, 19612. Pedersen BK, Febbraio MA. Muscle, exercise and obesity: skeletal muscle as a secretory organ. *Nature Rev. Endocrinol.* 8: 457-465, 20123. Whitham M, Febbraio MA. The ever expanding myokinome: discovery challenges and therapeutic implications. *Nature Rev. Drug Discov.* 15: 719-729, 2016.4. Whitham M, Febbraio MA. Redefining Tissue Crosstalk via Shotgun Proteomic Analyses of Plasma Extracellular Vesicles. *Proteomics* 19: e1800154, 2019. 5. Whitham et al.

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APSIPE.26

Female excellence in rock climbing likely has an evolutionary origin

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The human body is exceptional for many reasons, not the least of which is the wide variety of movements it is capable of executing. Because our species is able to execute so many discrete activities, researchers often disagree on which were the movements most essential to the evolution of our species. This paper continues a recently introduced analysis, that the performance gap between female and male athletes narrows in sports which most reflect movements humans evolved to do. Here, I examine the performance gap in rock climbing. Because rock climbing is so similar to tree climbing, which bountiful evidence suggests has been key to the origin and proliferation of our species, we would expect to see a narrow performance gap between men and women in the sport. Indeed, this is the case. Female climbers are some of the best in the world irrespective of gender, a trend that is not found in any other major sport. I conclude that exceptional ability of female climbers is further evidence of the existence of sex-blind musculoskeletal adaptations, which developed over the course of human evolution to facilitate essential movements. These adaptations abate the general physical sexual dimorphism which exists in humans. This paper provides more evidence that the performance gap in sport can be used as a measure of human evolution.

APSIPE.27

The effects of repeated sprinting in the heat on key signaling pathways in human skeletal muscle

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Training in heat condition induces physiological adaptations that improve thermoregulation of body and aerobic performance, but it is not clear how skeletal muscle responds to exercise with heat stress. This study aimed to uncover the effect of repeated sprint exercise in heat condition on key signaling pathways in human skeletal muscle. Twenty-six healthy men (age: 24.4 ± 4.6 years, BMI: 24.9 ± 2.2 kg/m², maximal oxygen uptake 51.2 ± 4.9 mL/kg·min) were randomly assigned to heat (40°C, n=13) or control (20°C, n=13) condition. All participants completed the repeated sprint training, which included 5 training sessions every other day during 9 days. Graded exercise test (GXT) and YO-YO intermittent recovery test level 1 (YO-YO test) were conducted before and after the training. Muscle biopsies were taken before, immediately and 3 hours after the first exercise session, as well as 48 hours after the last training session. Muscle samples were analyzed for gene expression, protein content and citrate

synthase (CS) activity. Phosphorylation of heat shock protein 27 (p-HSP27), Calmodulin-dependent protein kinase II (CaMKII), Phosphorylation of extracellular regulated kinases (p-ERK1/2), were significantly higher after acute sprint exercise in both conditions. mRNA content of total peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), peroxisome proliferator-activated receptor delta (PPARδ) and pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) were significantly higher 3 hours post exercise in both conditions, while mRNA content of PGC-1α isoform 4 (PGC-1α4), Peroxisome proliferator-activated receptor alpha (PPARα) and vascular endothelial growth factor A (VEGF) were only increased significantly in the heat condition. Furthermore, YO-YO test performance increased significantly after 5 training sessions in both training conditions, but 5 sessions of sprint training did not increase mitochondrial content.

APSIPE.28

Graded exercise testing underestimates maximal oxygen uptake in hypertensive older adults

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Purpose: While it has been almost a century since A. V. Hill first described the concept of maximal oxygen uptake (VO₂max), methodological challenges related to its assessment remain. Therefore, the present cross-sectional study investigated whether a VO₂max verification phase (VER) improves the accuracy of a traditional graded exercise test (GXT) to assess individual VO₂max in hypertensive individuals. Methods: Thirty-three older adults with hypertension (67.1 ± 5.1 years; 77.3 ± 13.8 kg; 1.55 ± 0.09 m; 24 females) taking part in the HAEL Study (NCT03264443) were recruited. Briefly, after performing a treadmill GXT to exhaustion, participants rested for 10 min and underwent a multistage VER. All tests were supervised by a physician and participants were familiarized with the equipment and safety procedures prior to the experimental session (IRB approval: 62427616.0.2001.5313). Individual VO₂max, respiratory exchange ratio (RER), maximal heart

rate (HRmax), and rating of perceived exertion (RPE) were measured during both GXT and VER tests. Mean values were compared between bouts using paired sample t-tests and, considering that the comparison between mean values may conceal between-trials variability, comparisons between GXT and VER VO2max results were also conducted individually. The VO2max during GXT was considered “verified” when the difference between VER and GXT VO2max was $\leq 3\%$, as previously proposed. The agreement between GXT and VER VO2max measures was assessed using Bland-Altman plots and reliability was measured using coefficient of variation (CV). GXT test results were also confirmed based on the presence of a VO2 plateau (change ≤ 150 ml.min⁻¹ with an increase in workload); or when at least two secondary criteria were met (i.e. ± 10 bpm of age-predicted HR (≥ 220 -age), RER > 1.10 or RPE ≥ 18). The level of significance was set at $\alpha=0.05$. Results: Testing was well-tolerated by all participants and no adverse events were registered. Both absolute ($p=0.011$) and relative ($p=0.014$) VO2max values were higher in VER than in GXT. RER ($p<0.001$) and RPE ($p=0.002$) were lower in VER, whereas HRmax ($p=0.286$) was not different between the two trials. VO2max CV was 8.1% (CI95%: 6.1 to 10.1%) between GXT and VER. Bland-Altman analyses indicated no agreement between GXT and VER for either absolute ($p=0.011$) or relative ($p=0.014$) VO2max, and there was no evidence of proportional bias ($p=0.300$, $p=0.189$, respectively). Individual VO2max comparisons between GXT and VER revealed that 54.6% of participants (18/33) achieved a VO2max value that was $\geq 3\%$ higher during VER (mean: 13.5%, range: +3% to +22%, ES=0.062), whereas 87.9% (29/33) of the tests would have been considered as valid if the classic criteria were employed (i.e. VO2 plateau or at least two secondary criteria). Further, nearly all (94.4%, 17/18) of the tests in which VER yielded a $\geq 3\%$ higher value than GXT met classical criteria for validity. Conclusion: GXT to exhaustion underestimated VO2max in more than half of tested participants, even when established criteria were used to confirm whether a maximal effort was attained. Because many exercise regimens are based on intensity relative to VO2max, it is critical to measure this outcome with accuracy, thus ensuring an appropriate training stimulus for individuals with chronic conditions such as hypertension. Employing VER after GXT is a quick approach to assist with the verification of an individual’s VO2max. CNPQ grant number 429849/2016-8.

APSIPE.29

Effects of High-Intensity Interval Exercise and Moderate-Intensity Continuous Exercise on Executive Function of Healthy Young Males

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Purpose: Exercise induced psycho-physiological responses attribute to the improvement of executive function (EF). Although high-intensity interval exercise (HIIE) was suggested to be more effective than moderate-intensity

continuous exercise (MICE) in affecting EF, the results were not consistent in previous studies. Additionally, various exercise modalities with the same intensity elicited different psycho-physiological responses which may also influence EF. Therefore, the present study aimed to investigate the acute effects of HIIE and MICE with two different exercise modalities on EF. Methods: In a randomized cross-over design, 16 male participants completed 4 main trials, i.e., 40 minutes of moderate-intensity continuous running (MICR) or cycling (MICC) with 60% maximal oxygen consumption (VO2max), 33 minutes of high-intensity interval running (HIIR) or cycling (HIIC). For HIIR or HIIC trials, the exercise intensity was 60% for the first 5 minute, and followed by four 4-minute bouts of exercise at 90% VO2max, separated by 3-minute active recovery at 60% VO2max. The Eriksen Flanker test (EFT) was performed to assess EF before (T0), immediately after (T1), as well as 10 minutes after exercise (T2). The concentrations of blood glucose and lactate, heart rate, and rate of perceived exertion were also measured in each main trial. Results: (1) no differences were found in reaction time and accuracy in EFT among four trials; (2) for response time, compared with T0 (415.15 $\pm$ 8.08 ms), participants responded faster in T2 (398.23 $\pm$ 9.34 ms, $P = .01$) but not T1 (403.59 $\pm$ 7.7 ms, $P = .06$); (3) significant interactive effects were found in blood lactate [$F(3, 45) = 13.02$; $P < .01$] and RPE value [$F(4, 60) = 35.07$; $P < .01$; partial $\eta^2 = .7$]. Conclusion: The two different kinds of HIIE and MICE adopted in the present study resulted in similar effects on EF. Improved response time was observed immediately and 10 minutes after exercise, whereas the improvement of accuracy occurred only 10 minutes after exercise. The present study was supported by Internal Research Grant (RG 91/2017-18R), Education University of Hong Kong.

APSIPE.30

Exercise to mitigate multisystem deconditioning: the NASA 70-day bed rest study

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BACKGROUND: Over the past sixty years, NASA developed a state-of-the-art countermeasures program with two major objectives: (1) comprehensive characterization of spaceflight multisystem physiological toxicity and (2) toxicity management with exercise therapy-based intervention before, during, and after spaceflight. Standard-of-care exercise on early International Space Station (ISS) missions (2001–2009; mission length: 91–215 days) consisted of combinational aerobic and strength training implemented using a treadmill, cycle ergometer, and resistance device with high-volume (~80 min/day), moderate-intensity (55%–75% of CRF or repetition maximum) using a linear schedule (all sessions performed at same duration and intensity). Despite these comprehensive efforts, persistent decrements in muscle strength, bone density, and VO2peak were still observed. Accordingly, we performed a randomized controlled trial to evaluate an integrated exercise program (SPRINT) using exercise timing, schedules, and equipment similar to that

aboard the ISS with the spaceflight analog of head down tilt bed rest. Bed rest, particularly in the head down tilt configuration, is used as an analog of spaceflight and offers advantages with respect to the ability to manipulate conditions and research design, better control of confounding factors and risk, and afford considerably faster throughput of subjects. The objective of this presentation is to: (1) provide a comprehensive overview of the tightly controlled NASA 70 day bed rest study, (2) outline the development of new physiological characterization tools, (3) summarize efficacy of SPRINT on multisystem deconditioning, and (4) discuss translation of findings to spaceflight. FUNDING: This study was supported by the Human Research Program of the National Aeronautics and Space Administration (NASA) and National Space Biomedical Research Institute (NSBRI NNJ11ZSA002N). JMS is supported by research grants from AKTIV Against Cancer, National Cancer Institute, and the Memorial Sloan Kettering Cancer Center Support Grant/Core Grant (P30 CA008748).

APSIPE.31

Exercise training protects myocardium against ischemia injury: A role of skeletal muscle Nrf2

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While it has been well-acknowledged that exercise training (ExT) protects myocardium against ischemia injury, the underlying mechanism(s) remain to be elucidated. Here we addressed a hypothesis that skeletal muscle Nrf2 contributes to this beneficial effect. Experiment was carried out on muscle-specific Nrf2 deficient mice (MS-Nrf2KO) and GFP reporter mice (MS-mG). Mice received 4 wks of ExT or being sedentary (Sed), followed by a coronary artery ligation surgery. Four weeks later, cardiac hemodynamics and infarct size were analyzed. We found that, compared with WT-Sed, WT-ExT mice displayed a significantly lower left ventricular end-diastolic pressure (LVEDP; 14.8 ± 3.1 vs 28.5 ± 4.4 mmHg; $P < 0.01$) and higher left ventricular systolic pressure and dP/dt. These mice also had a smaller infarct size (42.4 ± 7.1 vs $81.8 \pm 10.6\%$; $P < 0.01$). However, the ExT-evoked cardioprotective effects were attenuated when skeletal muscle Nrf2 was deleted (LVEDP, 20.8 ± 2.9 in MS-Nrf2KO-ExT vs 14.8 ± 3.1 mmHg in WT-ExT; $P < 0.05$; Infarct Size; 61.6 ± 10.6 in MS-Nrf2KO-ExT vs $42.4 \pm 7.1\%$ in WT-ExT; $P < 0.05$). We further found that, plasma extracellular vesicles (EVs) of WT-ExT mice contained more NQO1 and SOD2 than WT-Sed and MS-Nrf2KO-ExT mice, suggesting that skeletal muscle Nrf2 contributes to the ExT-induced increase in circulating antioxidant EVs. In addition, we demonstrated that ExT promoted skeletal muscle and non-muscle tissues to release EVs with a remarkable effect on muscle. These data suggest that skeletal muscle Nrf2 plays a critical role in ExT-induced cardioprotection via circulating EVs-mediated antioxidant defense.

APSIPE.34

Insulin sensitizes the response to capsaicin in small dorsal root ganglion neurons and thin fiber muscle afferents

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[Background] Insulin activates the central sympathetic nervous system, although the mechanism underlying insulin-induced sympathoexcitation remains unknown. We previously demonstrated that insulin augments the response to mechanical stimuli of dorsal root ganglion (DRG) neurons and thin-fiber afferents innervating skeletal muscle, which transmit sensory information to cardiovascular centers in the brainstem. However, little is known about the effect of insulin on DRG neuron and thin fiber afferent responses to chemical stimuli. [Purpose] The transient receptor potential cation channel subfamily V member 1 (TRPV1) is a known chemically-sensitive metaboreceptor widely expressed in skeletal muscle afferents. Capsaicin (CAP) is a known TRPV1 agonist. As such, the purpose of this investigation was to determine the effects of insulin on CAP-activated currents in small DRG neurons and CAP-induced action potentials in thin-fiber muscle afferents of normal healthy rodents. [Methods] We obtained whole cell patch-clamp recordings from cultured mice DRG and recorded CAP-activated currents before and after the application of insulin or vehicle (control) solution. We obtained single-fiber afferent recordings from the rat common peroneal nerve-extensor digitorum longus muscle preparation ex vivo. Before and after the injection of insulin or vehicle solution, CAP-induced action potentials were compared. At the end of each experiment, an insulin receptor blocker, GSK1838705 (GSK), was administered and the responses re-tested. [Results] 40 small DRG neurons and 64 group-IV fibers in total were studied. The fold change in CAP-activated current from pre- to post-application of insulin ($n=13$) was significantly higher compared to control ($n=14$) in patch-clamp experiments ($P<0.05$). Likewise, the fold change in the magnitude of CAP-induced action potentials from pre- to post-injection of insulin ($n=9$) was significantly higher than control ($n=9$) in single-fiber recording experiments ($P<0.05$). Importantly, GSK significantly suppressed the insulin-induced augmentation of CAP-activated currents ($n=13$) as well as the response magnitude of CAP-induced action potentials ($n=9$). In 15 fibers originally CAP-insensitive, 8 responded to CAP after the injection of insulin. Similar insensitivity was not reversed by administration of the control vehicle or combined insulin and GSK administration. [Conclusion] The present study demonstrated that insulin augments the chemical responsiveness of DRG neurons and sensitizes group IV

afferents to chemical stimuli at the muscle tissue level, possibly contributing to insulin-induced sympathoexcitation. [Funding] This study was supported by JSPS KAKENHI (JP17K01769), the Lawson & Rogers Lacy Research Fund in Cardiovascular Disease, and the National Heart, Lung, and Blood Institute (R01HL-151632).

APSIPE.35

Skeletal muscle AMPK is indispensable for overload-induced muscle glucose uptake and glycogenesis but dispensable for inducing hypertrophy in mice

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PURPOSE: Chronic muscle loading (overload) induces the skeletal muscles to undergo hypertrophy and an increased glucose uptake. AMPK reportedly serves as a negative regulator of hypertrophy and a positive regulator of glucose uptake. However, the role of AMPK in overload-induced skeletal muscle hypertrophy as well as glucose uptake is not clear. This study aimed to determine whether AMPK regulates overload-induced hypertrophy and glucose uptake in the skeletal muscles. **METHODS:** The skeletal muscle overload was induced through unilateral synergist ablations in the wild type (WT) and transgenic mice, expressing the dominant-negative mutation of AMPK (AMPK-DN). After two weeks, the parameters, such as muscle weight, glycogen, and in vivo [3H]-2-deoxy-D-glucose uptake, were assessed. **RESULTS:** There was no significant difference in the body weight and blood glucose level between the WT and AMPK-DN mice. The two-week muscle overload activated the AMPK pathway in the skeletal muscle of the WT mice; however, the response was impaired in the AMPK DN mice. Despite a normal gain in muscle mass (WT 12.6 ±1.5 vs. AMPK-DN 11.9 ±1.5 mg), the AMPK-DN mice demonstrated a significant impairment of overload-induced muscle glucose uptake (WT 2.5 ±0.5 vs. AMPK-DN 1.5 ±0.1 ng glu/mg tissue/min) and glycogenesis (WT 6.4 ±1.5 vs. AMPK-DN 0.9 ±1.8 μmol/g tissue), compared to the WT mice. Overload-induced the change in GLUT4 and HKII expression levels were lowered in the AMPK-DN mice, compared to those in the WT mice. **CONCLUSIONS:** This study demonstrated that AMPK activation is indispensable for overload-induced muscle glucose uptake and glycogenesis but dispensable for inducing hypertrophy in the AMPK-DN mice. It also suggested that the AMPK/GLUT4 and HKII axes may regulate overload-induced muscle glucose uptake and glycogenesis. This work was supported by the Japan Society for the Promotion of Science, Grants-in-Aid for Scientific Research (no. 18J01392 and 19K20007 to K.K., no. 18H03148 and 19K22806 to T.E., no. 19K11520 to T.H.).

APSIPE.36

Acute flywheel exercise does not impair the brachial artery vasodilation capacity in healthy men of varying cardiorespiratory fitness

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This cross-sectional, comparative study examined the effects of high-intensity flywheel exercise on the central hemodynamics and brachial artery vasodilation capacity in normotensive men. Nineteen men (age range from 20 to 57 years) provided written informed consent to participate in the present study, conducted in agreement with the Declaration of Helsinki. All participants completed three laboratory visits, including detailed medical screening, a ramp exercise test to determine their maximal oxygen uptake (VO₂ max.), and squat exercise intervention on a flywheel ergometer set at 0.075 kg·m² moment of inertia. According to the median of their VO₂ max. readings, all participants were allocated into two groups - aerobically untrained (VO₂ max.= 38.5 ±5.8 mL·kg⁻¹·min⁻¹, n=10) and trained (VO₂ max.= 50.6 ±6.5 mL·kg⁻¹·min⁻¹, n=9) individuals. Throughout the flywheel exercise, the mean arterial pressure (MAP) and heart rate (HR) were constantly monitored via Finometer (Finapres Medical Systems, Arnhem, the Netherlands), while a pre/post exercise flow-mediated dilation (FMD) assessment was performed using ultrasound imaging¹. There were no differences observed between the groups in their anthropometrics, age, or resting brachial artery diameter, while the VO₂ max. was ~15% higher (p=.001) in the aerobically trained compared to the untrained group. The central hemodynamics response to the flywheel exercise were similar between the groups, with peak MAP and HR readings reaching ~160 mmHg and ~140 bpm, respectively. The flywheel exercise did not impair the FMD (%) response, which was comparable between the groups (p=.256). A significant main effect of time was observed for mean brachial artery blood flow and shear rate (peak values of ~600 - 800 mL·min⁻¹ and ~190 - 240 s⁻¹, respectively) immediately after the cuff release during both pre and post-exercise assessments. When data from primary outcome variables were pooled, the regression analysis showed an inverse relationship among FMD (%), age (β= -.936, p=.001), and V̇O₂ max. (β= -.359; p=.045). Taken together, these findings suggest that aerobic fitness alone does not directly explain the FMD response to flywheel exercise. However, aerobically untrained individuals, as they get older tend to have lower brachial artery FMD, irrespective of the baseline diameter. Taken together, a positive influence of a higher aerobic fitness on vascular health should not be overlooked, since a reduced FMD capacity is a strong predictor of adverse cardiac events². **References:** Obad A, Palada I, Valic Z, et al. The effects of acute oral antioxidants on diving-induced alterations in human cardiovascular function. *J Physiol.* 2007; 578(3), 859-870. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. *Clin Sci (London).* 2011;120(9):357–75.

APSIPE.37

The effect of glycation stress on strength-enhancing effect of resistance training in young male

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[Aims] Glycation stress, which is caused by glycation and includes the formation of advanced glycation end products (AGEs) and a subsequent dysfunction of proteins and/or cellular signaling, is considered related with the progress of muscle dysfunctions. However, there are no observations examining the effect of glycation stress on resistance training. In this study, we aimed to clarify the effect of glycation stress on the strength-enhancing effect of resistance training. [Methods] The subjects were 20 healthy young male (19.0 ±0.2 years old; 171.7 ±1.0 cm; 65.1 ±2.0 kg, mean ±SE), and after measuring the subcutaneous glycation state with an AGEs sensor (RQ-AGO1J, Sharp Life Sciences, Hyogo, Japan), they were classified into two groups, a low value group (L) and a high value group (H). Both groups were subjected to three sets of leg extension and leg press exercises (10 times / set) three times a week for 12 weeks at a load of 80% of the maximum lift. The glycation state and the maximum lift weight were measured before and after the training. The entire human study was conducted in accordance with the Helsinki Declaration. [Results] The maximum lift weight of leg extension and leg press before training was significantly higher in the L group than in the H group (leg extension: L group, 99.5 ±5.7 kg, H group, 108.3 ±6.1 kg, $p = 0.02$; leg press: L group, 272.5 ±19.8 kg, H group, 266.0 ±9.6 kg, $p = 0.003$). There were no significant differences between the two groups in the maximum lifting weight after the exercise (leg extension: L group, 64.9 ±6.0 kg, H group, 50.8 ±3.7 kg, $p = 0.02$; leg press: L group, 212.1 ±15.3 kg, H group, 179.7 ±8.2 kg). The glycation state was significantly decreased by training in both groups (L group, 0.44 ±0.06 → 0.36 ±0.06 a.u., $p < 0.001$; H group, 0.53 ±0.02 → 0.40 ±0.07 a.u., $p < 0.001$). [Conclusions] Negative relationships were found between the glycation state of the body and muscle strength when muscle training was not performed, and resistance training improves body glycation status in young men. On the other hand, it was found that the glycation state of the body does not affect the strength-enhancing effect of resistance training. In conclusion, glycation stress influences muscle strength at non-exercise state in young male, but does not interfere with the strength-enhancing effect during muscle resistance training.

APSIPE.38

Greater pressor response to static forearm exercise but not isolated metaboreflex activation with age in normotensive men

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INTRODUCTION: An increase in heart rate, cardiac output and muscle sympathetic nerve activity (MSNA) contribute to the progressive increase in blood pressure during static forearm exercise. Notably, aortic stiffness contributes to the pressor response during cold stress mediated increases in cardiac output; whereby higher aortic stiffness with age is associated with a greater pressor response. Whether this effect is present during static forearm exercise is unknown. METHODOLOGY: We studied the heart rate (electrocardiography), cardiac output (echocardiography), MSNA total activity (fibular microneurography; burst frequency x burst amplitude) and pressor (finger photoplethysmography) responses to static handgrip exercise (SHG) and isolated metaboreflex activation in 23 young (age, 23±3 years; VO2Peak, 50.1±14.6 mL·kg⁻¹·min⁻¹) and 22 middle-aged (age, 55±4; VO2Peak, 43.8±10.9 mL·kg⁻¹·min⁻¹) healthy men. Baseline aortic stiffness (carotid-femoral pulse wave velocity) was also measured. Participants performed SHG at 35% of maximal voluntary contraction until task failure, before 2 minutes of isolated metaboreflex activation via post-exercise muscle ischaemia (PEMI; forearm cuff occlusion at 220 mmHg). These two stressors were utilised as cardiac output increases from baseline during SHG but decreases during PEMI. Separate linear mixed models were used to determine main effects of age and time and the age*time interaction in response to SHG and PEMI. Pearson correlation coefficients were generated to determine the relationship between resting aortic stiffness and the delta blood pressure responses during SHG and PEMI. RESULTS: Resting brachial systolic blood pressure (SBP; 114±10 vs 119±8 mmHg, $P=0.136$) was not different between groups; whereas, diastolic blood pressure (DBP) was higher in MA (68±7 vs 75±6 mmHg, $P=0.001$). Aortic stiffness was higher in middle-aged compared to young men (6.1±1.0 vs 7.8±1.0 m·s⁻¹, $P<0.001$). In response to SHG, heart rate, cardiac output, MSNA and blood pressure increased, whereas stroke volume decreased (main effect of time, all, $P<0.05$). Middle-aged men displayed a greater increase in MSNA (Young: 736±421 to 2123±1497 vs Middle-aged: 1535±614 to 4499±2555 %·min⁻¹, age*time interaction $P=0.016$) and SBP (Young: 124±9 to 133±10 vs Middle-aged: 127±11 to 158±17 mmHg, age*time interaction $P<0.001$). There were no effects of age on the response of DBP ($P=0.053$) or MAP ($P=0.319$) with SHG. In response to PEMI, MSNA and blood pressure increased, whilst stroke volume and cardiac output decreased (main effect of time, $P<0.05$). There were

no effects of age on the responses to PEMI. Notably, in young and middle-aged men ($n=45$), the delta pressor responses were positively related to baseline aortic stiffness during SHG (SBP, $r=0.527$, $P<0.001$; DBP, $r=0.343$, $P=0.022$; MAP, $r=0.454$, $P=0.002$) but not during PEMI (SBP, $r=0.157$, $P=0.301$; DBP, $r=-0.014$, $P=0.926$; MAP, $r=0.083$, $P=0.589$). CONCLUSION: These data suggest that the age-related increase in aortic stiffness contributes to a greater pressor response during static forearm exercise mediated increases in cardiac output. Future studies should examine the relationship between resting aortic stiffness and the pressor response to whole body exercise.

APSIPE.39

In Vitro HUVEC model of chronic exercise protection of vascular endothelial toxicity of FEC-T chemotherapy treatment for breast cancer using ex vivo serum preconditioning

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Background: Chemotherapy treatments for breast cancer are toxic to the cardiovascular system and can lead to the development of cardiovascular disease. As the first point of contact with chemotherapy drugs, it is important to consider the role of the vascular endothelium in the development of cardiovascular toxicity and to identify potential therapeutics to protect against this toxicity. Exercise has been recognised as a possible adjunct therapy to counteract the detrimental effects of chemotherapy on the endothelium. Therefore, the aim of this study is to determine if there are protective effects of chronic exercise training on chemotherapy-induced vascular toxicity. Methods: Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial cell growth medium supplemented with 5% serum from healthy trained ($n=3$) and untrained ($n=3$) females. After 24-hours of serum preconditioning, HUVECs were exposed to physiological concentrations of FEC-T (5-fluorouracil, epirubicin, cyclophosphamide, and docetaxel) drugs. At appropriate time-points (0-, 3-, 4-, 6-, 12-, 24-, and 48-hours), the effects of exercise and chemotherapy on HUVEC viability, apoptosis and eNOS activity were measured using intracellular flow cytometry. Results: All FEC-T drugs increased cell death, caspase-3 activity, and annexin-V expression, and reduced eNOS and Phospho-eNOS expression, in a dose-dependent manner. Preconditioning HUVECs with serum from exercise trained females showed a reduction in cell death, caspase-3, and annexin-V expression, when compared to untrained serum, reaching significance in 5-FU (viable HUVECs for trained: $73.08\pm6.96\%$ vs untrained: $53.52\pm2.64\%$, $p=0.03$; caspase-3 expression for trained: $27.23\pm6.35\%$ vs untrained: $59.77\pm8.95\%$, $p=0.04$) and epirubicin (caspase-3 expression for trained: $34.10\pm5.44\%$ vs untrained $51.20\pm1.31\%$, $p=0.04$; annexin V expression for trained: $13.03\pm0.79\%$ vs untrained: $17.73\pm1.11\%$, $p=0.03$). There was no between-group difference for eNOS and Phospho-eNOS expression. Conclusions: Our results confirm that FEC-T drugs commonly used in breast cancer treatment cause death and dysfunction of endothelial cells in a dose-dependent

manner. For the first time, we have shown that this endothelial cell damage may be affected by training status.

APSIPE.40

Introduction to the NASA SPRINT exercise studies

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The SPRINT exercise program is an experimental exercise prescription that was developed to mitigate the myriad of spaceflight-induced health consequences, particularly cardiovascular, neuro and neuromuscular, bone, and psychological health. This introduction will provide an overview and the physiologic rationale for the development of the experimental exercise prescription. SPRINT differs from the standard-of-care exercise primarily with its novel periodization, the dependence on high intensities, and the control and variation of the modes of exercise. The SPRINT program was evaluated in 9 astronauts during long duration missions aboard the International Space Station as well as in 27 ground based subjects during 70 days of bed rest. Several novel techniques were developed or implemented to monitor the training adaptations during flight and bed rest. Today's speakers will provide detailed results from some aspects of these studies. Dr. Jessica Scott will focus on the bed rest study results and the efficacy of SPRINT on multisystem deconditioning. Dr. Scott Smith will discuss on how nutritional and exercise countermeasures can be used together to support astronaut health. Finally, COL Michael Fossum, who was the first person to perform the SPRINT exercise program in space will provide his insights on spaceflight exercise physiology research.

APSIPE.41

High volume and high load resistance training causes fragmented mitochondria and decreases mitochondrial volume

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Purpose: Resistance training is known to increase skeletal muscle strength and hypertrophy. However, there is currently inconclusive evidence to its effects on mitochondrial physiology. The purpose of this study was to investigate possible changes in mitochondrial volume and dynamics with high volume (HV) versus high load (HL) resistance training. Methods: Fifteen trained college-age males (age: 23 ± 3 years old; training experience: 7 ± 3 years) performed unilateral lower body training (leg press and leg extension exercises), with one leg randomly assigned to HV and the other to HL training for six weeks (3 d/week). HV utilized 60% one repetition maximum (1RM) with ten repetitions per set, where sets per exercise were increased

from 5/week to 10/week by the end of training. HL utilized 82.5 – 95% 1RM and five repetitions per set, where sets remained 9/week throughout training. Muscle biopsies from the vastus lateralis were taken prior to training (PRE) and 72 hours following the last bout of training (POST). Western blots were run on mitochondrial fission (Drp1), mitochondrial fusion (Mfn2), and mitophagy (PINK1 & Parkin) markers. Further, citrate synthase activity assays were performed, and data was used as a surrogate of mitochondrial volume. Results: Mitochondrial fission marker Drp1 increased after the training intervention regardless of group, albeit non-significantly ($p=0.058$). No significant differences were observed for mitochondrial fusion marker Mfn2. Interestingly, mitophagy marker PINK1 increased over time, regardless of training intervention ($p=0.009$). No significant changes were observed for the mitophagy marker Parkin. Lastly, citrate synthase activity was significantly lower after training ($p=0.019$) regardless of training style. Conclusion: High volume and high load resistance training both resulted in an increase in markers of mitochondrial fission and mitophagy, and training decreased mitochondrial volume. These data further support the hypothesis that resistance training results in a mitochondrial dilution effect.

APSIPE.42

Motor unit discharge properties following concentric and eccentric exercise-induced fatigue are dependent upon contraction type.

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Increases in force production are partly mediated by adjustments in motor unit (MU) firing properties. Muscle fatigue is characterised by a reduction in force output, however, conflicting research has found MU firing rate to both increase and decrease following fatiguing exercise (1). This disparity may be influenced by contraction type, as concentric and eccentric contractions are known to require different levels of neural input and result in different responses to fatigue (2). The aims of this study were to determine the effects of fatigue following concentric (CON) and eccentric (ECC) exercise on MU discharge properties of the vastus lateralis (VL) muscle. High-density surface electromyography (HD-EMG) was used to identify individual MU potentials (MUPs) from the VLs of 8 young volunteers (4 females; 21 ± 0.5 years). All MUPs were recorded during 4 sustained isometric contractions held at 25% and 40% of the participants maximum voluntary contraction (MVC) before and after completing CON and ECC fatiguing exercise (CON: 'stepping up' with one leg, ECC: 'stepping down' on the other leg, wearing a weighted vest +25-40% body weight) (3). A total of 733 MUs were identified, with a mean of 46 ± 23 per person per leg from which MU discharge properties of firing rate (FR) and variability were

assessed. Multilevel mixed effects linear regression models were performed on each group to account for within-subject variability and to determine effects of fatigue on these parameters. Significance was assumed when $p < 0.05$. MVC decreased in both CON and ECC legs post exercise (-10.6% and -19.2% respectively; $p < 0.001$). FR decreased following CON (-7.7%; $p = 0.015$) but increased with ECC (+8.0%; $p = 0.003$). FR variability was greater after ECC only (+11.4%; $p = 0.002$). These data demonstrate that MU firing properties following exercise-induced fatigue differ according to exercise modality. These differences may be as a result of acute muscle damage observed after ECC or variation in muscle afferent feedback between modalities. The possibility of differential MU recruitment strategies compensating for discharge characteristics requires further investigation as this may have translational relevance in neuromuscular conditions and for post-exercise fatigue recovery. 1. Contessa P, De Luca CJ, Kline JC. The compensatory interaction between motor unit firing behavior and muscle force during fatigue. *J Neurophysiol.* 2016;116(4):1579–85. 2. Kay D, St Clair Gibson A, Mitchell MJ, Lambert MI, Noakes TD. Different neuromuscular recruitment patterns during eccentric, concentric and isometric contractions. *Journal of Electromyography and Kinesiology.* 2000. p.425–31. 3. Kostek MC, Chen YW, Cuthbertson DJ, Shi R, Fedele MJ, Esser KA, et al. Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: Major changes in CSRP3, MUSTN1, SIX1, and FBXO32. *Physiol Genomics.* 2007;31(1):42–52.

APSIPE.43

The influence of menstrual phase and oral contraceptive use on group III/IV afferent control of ventilation during exercise

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Estrogen can reduce sympathetic activity, but its effects on ventilatory responses to group III/IV afferent activation remain unclear. Therefore, the purpose of this study was to determine the influence of menstrual cycle phase and oral contraceptives (OC) on the ventilatory response during submaximal lower-extremity exercise with group III/IV afferent activation (ergoreflex). METHODS: Participants who did use OCs and who did not (N-OC) completed two identical study visits, one in the follicular/placebo pill phase and one in the ovulatory/active pill phase. Participants performed three minutes of baseline steady-state cycle ergometry (BL) at 60 watts and 65-70 RPM. To activate group III/IV afferents during exercise, proximal thigh cuffs were inflated to pressures of 20, 60, and 100mmHg in randomized order for two minutes (ergoreflex), with five

minutes of steady-state exercise between each occlusion. Ventilation was measured continuously throughout with rating of perceived exertion (RPE) recorded at the end of each stage. Mixed-model ANOVA tests were used to compare resting and baseline exercise between groups and menstrual phases, and to evaluate the effects of group and phase on ventilatory variables and RPE with sub-systolic occlusion. RESULTS: Nineteen females (N-OC: n = 9; OC: n = 10) completed the study (age: 24 ±3 years; BMI: 23 ±2 kg/m²). In the N-OC group, resting and BL VT were higher during the follicular/placebo pill phase than the ovulatory/active pill phase (phase effect: p = 0.02). Neither menstrual cycle phase nor OC use influenced (phase and group effects: p > 0.05) the progressive increase in ventilation, respiratory rate, and VT (condition effect: p < 0.05) with ergoreflex activation. Rating of perceived exertion rose with increasing occlusion pressure (condition effect: p < 0.05) and was unaffected by OC use or menstrual phase (group and phase effects: p > 0.05). CONCLUSIONS: While menstrual phase influences VT in eumenorrheic females, neither phase nor OC use affects ventilatory responses to ergoreflex activation. Thus, eumenorrheic females may experience menstrual-phase-related alterations in resting and exercise VT, but the influence that skeletal muscle afferents have on ventilatory control in young females does not depend on menstrual phase and is unaffected by OC use.

APSIPE.44

High-Intensity Interval Training Effect on Monocyte Transcriptome in Older Women with Type 2 Diabetes: A Pilot Study

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Type 2 diabetes (T2D) is associated with higher risk of cardiovascular disease (CVD), lowering the health-related quality of life and increasing mortality rates of affected individuals. Circulating monocytes are cells that are tightly involved in the atherosclerosis process leading to CVD, and their phenotype can be modified by physical activity. Among the different types of aerobic exercise, high-intensity interval training (HIIT) is promising, since it provides similar or even greater cardiovascular benefits when compared with moderate intensity continuous training, while requiring a lesser time commitment. The objective of this pilot study was therefore to assess, for the first time, the chronic effects of HIIT on the transcriptome of circulating monocytes of individuals at high CVD risk. Methods: Next generation RNA sequencing (RNAseq) analyses were conducted on CD14⁺ cells isolated from the blood of 7 T2D women aged 60 and over who completed a supervised 12-week HIIT intervention (3 sessions per week) on treadmill. The research protocol was approved by the Research ethics review boards of the CIUSSS de l'Estrie-CHUS and all patients provided their written informed

consent upon recruitment. Results: Following the intervention, 56 transcripts were significantly downregulated; most of them involved in immune cell activation or to pathologies with a major immune component. Namely, the regulated genes were associated with monocyte cellular adhesion, migration, cytokine production and their differentiation into an "atherosclerosis-specific" phenotype. Conclusion: The downregulation of these genes globally suggests a favorable cardiovascular impact of HIIT in elderly women with T2D. Shedding light on mechanisms underlying HIIT effects on cells involved in CVDs is of critical importance to develop efficient non-pharmacologic prevention strategies. This pilot study was supported by the Quebec Network for Research on Aging, and the University of Sherbrooke Center for excellence in Diabetes, Obesity and Cardiovascular Complications.

APSIPE.45

Exercise and the resolution of joint inflammation: Is there a role for intra-articular knee fat lipolysis?

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PURPOSE: Exercise therapy is strongly recommended for long-term management of joint pain and function in osteoarthritis (OA) patients. However, the molecular mechanisms by which exercise helps OA patients remain elusive. We found that wheel running exercise in healthy adult mice induced acute inflammation in knee infrapatellar fat and synovium, which resolved by day 14 of running. Here, we describe our ongoing work to understand the metabolic effects of exercise on knee joint tissues and how these may be coupled to the resolution of inflammation. METHODS: 12-week-old male C57BL/6J mice were individually housed with running wheels for 0, 1, 3, or 14 days following an approved IACUC protocol. Combined synovium-infrapatellar fat samples were collected at each timepoint for gene expression analysis. Knees were collected at 0, 3, and 14 days for histomorphometry and immunofluorescence analysis of oxidized phospholipids (Ox-PLs). Synovial fluid (SF) was collected at 0 and 1 day of running for metabolomic profiling using an Agilent 1290 UPLC and 6538 Q-TOF mass spectrometer. Enrichment analysis of differentially expressed metabolite features was performed using the MS Peaks to Pathways mummichog application in MetaboAnalyst. Statistical analyses were performed in MetaboAnalyst and MATLAB. We compared median metabolite distributions using the Kolmogorov-Smirnov (KS) test (a priori pks<0.05). RESULTS: 1 night of running upregulated the expression of several genes associated with lipid metabolism, including Pparg, Lipe, and Slc2a4. After 14 days of running, Adipoq, Lep, and Pparg expression were reduced relative to baseline. Although the overall cross-sectional area of the infrapatellar fat pad did

not change with running, the area of individual adipocytes was reduced at day 3 ($p=0.038$). Running also induced a transient increase in Ox-PLs staining at day 3 ($p<0.05$). 1 night of running significantly altered the global intensity of SF metabolite distributions based on 2812 metabolite features ($p<0.01$). Mechanosensitive metabolites included coenzyme A and prostaglandin derivatives, phospholipid species, tryptophan, methionine, vitamin D3, fatty acids, and thiocholesterol. Enrichment analyses identified changes in citrulline-nitric oxide cycle, catecholamine biosynthesis, ubiquinol biosynthesis, amino acid and phospholipid metabolism. **CONCLUSIONS:** Our findings show that exercise induces acute changes in SF metabolites, gene expression, and adipocyte size associated with transient infrapatellar fat pad lipolysis. Our working hypothesis is that joint inflammation resolves with sustained exercise due to a temporal coupling of IFP lipolysis with macrophage lipid uptake and fatty acid metabolism to drive alternative activation. We are currently testing this hypothesis using mouse genetic and pharmacologic approaches to selectively inhibit or stimulate intra-articular lipolysis in two models of joint inflammation: resolving (i.e., running) and non-resolving (i.e., ACL injury). **FUNDING SOURCE:** NIH R03AR066828 and VA I01BX004666.

APSIPE.46

The impact of habitual physical activity, exercise training and sleep on vascular remodeling in older adults: a review.

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Aging is associated with increased risk of cardiovascular and cerebrovascular events, which are preceded by early, negative remodeling of the vasculature. Low physical activity is a well-established risk factor associated with the incidence and development of disease. However, recent physical activity literature indicates the importance of considering the 24-hour movement spectrum as a holistic tool for examining activity of older adults. Therefore, the purpose of this review is to examine the impact of the 24-hour movement spectrum, specifically habitual physical activity, exercise training (aerobic and resistance training), and sleep, on cardiovascular and cerebrovascular outcomes in older adults. Primary outcomes examined include endothelial function, smooth muscle function, arterial stiffness, and cerebrovascular function, with a focus on recent evidence (<10 years) and sex and/or gender considerations. The review indicates that both habitual physical activity and aerobic exercise training are associated with improvements in endothelial function, arterial stiffness, and cerebrovascular function, but not smooth muscle function. Additionally, there is evidence of sex-differences in endothelial function: a blunted improvement with physical activity or aerobic exercise training in postmenopausal women compared to men.

Minimal research has been conducted in older adults examining resistance exercise, however, resistance exercise training does not appear to influence arterial stiffness. Poor sleep quantity (duration) or quality are associated with impaired endothelial function and increased arterial stiffness; however, further research is needed exploring the impact of sleep on these outcomes in older adults. Finally, the review highlights key mechanistic pathways involved in the regulation of vascular and cerebrovascular function – specifically the balance between pro- and anti-atherogenic factors, such as oxidative stress and inflammation, which mediate the relationship between activity, sleep and vascular outcomes. This review also proposes areas for future research to address several identified knowledge gaps, including examining the role of duration and intensity of exercise training, combining aerobic and resistance training, and further exploration to sex-based differences in cardiovascular and cerebrovascular outcomes. Given the growing number of aging adults, it is imperative to develop and evaluate effective strategies for mitigating the age-associated decline in vascular health.

APSIPE.48

SERCA2a Function and Level in the Right Atrium of the Diseased and Type 2 Diabetic Human Heart

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Sarco(endo)plasmic reticulum calcium (Ca²⁺) ATPase 2a (SERCA2a) regulates cardiac function by transporting Ca²⁺ from the cytosol of cardiomyocytes back into the sarcoplasmic reticulum. This lowers the cytosolic Ca²⁺ concentration within cardiomyocytes, facilitating cardiac relaxation and diastole. The diseased heart is characterized by abnormal Ca²⁺ handling in cardiomyocytes, resulting from alterations to myocardial Ca²⁺ transport proteins. The objective of this study was to assess the changes to SERCA2a function and level within the right atrium (RA) of the diseased and type 2 diabetic (T2D) human heart. To accomplish this, human RA tissue samples were obtained from 61 patients undergoing cardiac surgery. Tissue collected was categorized based on type of heart disease, level of cardiac dysfunction determined by left ventricular ejection fraction (LVEF), and by T2D status. The study included patients undergoing a valve procedure (VP) in whom LVEF was preserved within the normal range; patients undergoing coronary artery bypass grafting surgery, in the absence of a VP, in whom LVEF was preserved within the normal range; two additional groups similar to those described, but in whom LVEF was abnormal; four additional groups similar to those described, but who also had T2D. SERCA2a function and level were measured using a spectrophotometric assay and Western blotting, respectively. Maximal SERCA activity (V_{max}) in the

RA of patients with coronary artery disease (CAD) was 32% greater compared to patients with heart valve disease (HVD) ($P = 0.001$). SERCA Vmax in the RA of patients with systolic dysfunction (SD) was 22% greater compared to patients without SD ($P = 0.044$). The Hill coefficient was 29% greater in patients with T2D compared to patients without T2D ($P = 0.021$). RA SERCA2a level was reduced by 36% in patients with T2D compared to patients without T2D ($P = 0.008$). RA SERCA function was altered by type of heart disease, level of cardiac dysfunction, and T2D status, and T2D was found to diminish RA SERCA2a level. This study was supported by an operating grant from the Heart and Stroke Foundation of Canada.

APSIPE.49

Testosterone supplementation, energy deficit and performance

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Many military personnel frequently endure sustained periods of strenuous physical activity, sleep deprivation, and semi-starvation during training or combat operations, often in environmentally challenging conditions, the effects of which can degrade muscle mass and impair service member health and performance. The extent to which muscle mass declines and performance is impaired during multi-stressor military operations may be partly attributable to suppression of the hypothalamic pituitary gonadal (HPG) axis, resulting in clinical hypogonadal testosterone concentrations and associated blunting of muscle anabolic response to dietary protein. Pharmacologic restoration of testosterone to normal physiological range may be a biomedical countermeasure to offset these detrimental effects of multi-stressor military operations. Recent evidence demonstrates that during 28 days of severe exercise- and diet-induced energy deficit, providing healthy young males with supplemental testosterone promotes lean mass gain and attenuates total body mass loss. However, supplemental testosterone did not affect muscle fiber cross-sectional area, isotopic-derived estimates of muscle protein mass, and lower-body functional deterioration, raising concerns over the necessity of testosterone restorative therapy as a viable and ethical performance enhancement strategy during military operations. This presentation will provide a fundamental review of the HPG axis, muscle, and physical performance responses to multi-stressor environments that elicit energy deficit, muscle loss, and functional decline in service members. Further, the mechanistic basis for leveraging testosterone restoration therapy will be reviewed and recent findings will be presented demonstrating the effects, or lack thereof, of supplemental testosterone on physiological status, physical performance, body composition, androgen-mediated cell signaling, muscle protein kinetics and morphological adaptations to severe energy deficit. Disclaimer: The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views

of the U.S. Department of Army, U.S. Department of Defense, or the U.S. Government.

APSIPE.50

Physical Activity Changes During the Spring 2020 COVID-19 Shutdown in the United States

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Background: State and local shutdowns in the U.S. due to the COVID-19 pandemic limited access and time for some individuals to participate in physical activity (PA) during the Spring of 2020. Others found more flexibility in their lives to participate in more or different types of PA. Objective: The purpose of this study was to examine how PA changed from early March 2020 (pre- pandemic) to June 2020 (during the pandemic). Methods: In June 2020, study participants were asked to complete a short electronic survey in which they described their PA habits before the pandemic (early March 2020) and during the spring shutdown (mid-March to June 2020). Participants responded with average days of exercise, average minutes per session, average session intensity (on a 0 to 5 scale), and a prediction of how likely they were to maintain the PA habits they adopted during the pandemic for one year. Participants were recruited from across the U.S. through social media. T-tests, one-way ANOVA, and descriptive statistics were used to analyze the data with an alpha value of 0.05. Results: 873 individuals completed the survey from 48 states/territories (85.3% female, 38.7 ± 12.9 years, 89.3% Non-Hispanic white, 89.9% income greater than \$45,000, 87.6% earned a bachelor's degree or higher). 52.7% of participants reported meeting or exceeding the guidelines of 150 minutes of moderate to vigorous PA per week. The percentage of participants not meeting the PA guidelines decreased by 7.8% from pre to during the shutdown. The percentage of participants exceeding the PA guidelines increased by 11.3%. Overall, days per week of PA significantly increased from pre to during shutdown (3.75 ± 1.9 to 4.27 ± 2.1 , $p < 0.001$) while minutes of activity per session remained relatively the same (1.87 ± 0.95 to 1.93 ± 0.98 , $p = 0.10$; 1= 1-30 mins, 2= 31-60 mins). Average intensity of exercise sessions significantly decreased from pre to during the shutdown (2.81 ± 1.16 to 2.62 ± 1.14 ; $p < 0.001$). Open ended responses indicate the lack of access to fitness facilities and exercise classes as well as more aerobic walking outdoors were related to the decrease in exercise intensity. Overall, 73.4% of participants indicated they were likely or very likely to maintain the PA habits they adopted during the shutdown. 93.8% of those who increased their PA during shutdown reported they were likely or very likely to maintain their habit over the next year. Participants who did not change (1.75 ± 0.82 ; $p < .01$) or increased PA (1.77 ± 0.63 ; $p < .01$) during shutdown were significantly more likely to report they would maintain PA levels for one year compared to those who decreased PA levels during the shutdown (2.6 ± 0.67). Conclusions: Overall, minutes of PA per week increased during the spring 2020 shutdown; however, average intensity of

exercise sessions decreased in part as a result of limited access to fitness facilities and exercise classes. Those exceeding the PA guidelines before the pandemic maintained PA levels during the shutdown, whereas those not meeting the PA guidelines prior to the shutdown increased PA and were more likely to report they would maintain their increased PA level for one year.

APSIP.E.51

Skeletal Muscle Metabolic Biomarker Adaptations to High Load Versus High Volume Unilateral Resistance Training

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Purpose: Resistance training is known to enhance skeletal muscle strength and hypertrophy. However, there is currently inconclusive evidence to its effects on bioenergetic pathways within skeletal muscle. Therefore, the purpose of this study was to investigate changes in markers of mitochondrial biogenesis, nutrient transporters and glycolytic enzymes with high volume (HV) versus high load (HL) resistance training. **Methods:** Previously-trained college age males (n=15; age: 23±3 years old; training experience: 7±3 years) performed unilateral lower body training (leg press and leg extension exercises), with one leg randomly assigned to HV and the other to HL training for six weeks (3 d/week). The HV training condition performed 5 sets of 10 repetitions at 60% of est. one repetition maximum (1RM) per exercise at week one – increasing sets weekly – concluding at 10 sets of 10 repetitions at 60% of est. 1RM per exercise at week 6. The HL condition performed 3 sets of 5 repetitions at 82.5% est. 1RM per exercise at week one – increasing intensity weekly – concluding with 3 sets of 5 repetitions at 95% est. 1RM per exercise at week 6. Vastus lateralis (VL) muscle biopsies were taken prior to training (PRE), 72 hours following the last training bout (POST), and following ten days of passive recovery (DL). Western blots were performed to measure relative protein abundance of mitochondrial biogenesis markers (PGC-1α, TFAM and NRF1), nutrient transporters (LAT1, GLUT4), and markers reflective of the ATP-PCR and glycolytic pathways (PYGM, CKM, PFK, and LDHA). **Results:** A condition x time interaction was observed for mitochondrial biogenesis marker NRF1 (p=0.016). An LSD Post-hoc analysis revealed a significant increase over time with HV from PRE to DL compared to HL training (p = 0.017). No significant condition x time interactions were observed for PGC-1α and TFAM. Furthermore, no condition x time interactions were observed for measured glycolytic enzymes. Interestingly, a main effect of condition was observed for CKM (p = 0.019, HL>HV), PYGM (p = 0.005, HV>HL), and PFK (p = 0.018, HV>HL). No significant main effect of time was observed for LDHA. Lastly, no significant condition x time interaction was

observed for nutrient transport proteins. An effect of time was observed for LAT1, whereas LAT1 protein expression increased over time regardless of training condition (p = 0.009). **Conclusion:** HV and HL resistance training both altered markers of mitochondrial biogenesis, nutrient transporters and glycolytic enzymes. However, there were no appreciable differences between conditions over time. We speculate the lack of differences between HV and HL resistance training for these metabolic markers was likely due to either the high training status of subjects and/or the lack of appreciable total training volume differences between legs. Therefore, more research is needed examining the effects of training volume and load on metabolic markers in skeletal muscle.

APSIP.E.52

Locomotor-Respiratory Coupling and Attentional Strategies in Healthy, but Untrained, Individuals

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Background: Locomotor-respiratory coupling (LRC) is a subconscious synchronization of locomotor and breathing patterns with proposed beneficial effects on energetic and perceptual responses to exercise. It is unknown if attentional focus, which independently can impact each of the locomotor and ventilatory rhythms, alters LRC. **Purpose:** The purpose of this study is to examine the role of attentional focus on LRC, exercise economy and perceptual responses during exercise in untrained individuals. **Methods:** Eight subjects (7 M, 23.6 ±4 yr, 179.4 ±9.8 cm, 76.3 ±11.0 kg, V_{O2peak}: 46.6 ±3.4 ml kg⁻¹ min⁻¹) ran on a motorized treadmill at three predetermined submaximal speeds (2.08 m·s⁻¹ to 2.98 m·s⁻¹) under associative (ASSOC) and dissociative (DISSOC) attentional focusing conditions. During ASSOC, subjects listened to prompts to focus on their breathing and leg movements; during DISSOC, subjects watched a lighthearted sitcom. Light-weight, plastic event switches were placed on the bottom of each foot to measure time of foot-ground contact. Inspiratory and expiratory flows were continuously measured. The degree of LRC was then calculated as the percentage of breaths occurring during the same decile of the step cycle. Running economy at each speed was calculated as the oxygen consumption per distance traveled. Ratings of perceived exertion and dyspnea were assessed at each speed. A repeated measures analysis of variance was performed to examine potential differences. **Results:** LRC was greater during ASSOC compared with during DISSOC (37 ±14% vs 31 ±9%, p=0.04). Running economy was not different between ASSOC and DISSOC (ASSOC: 228.7 ±10.7 ml·kg⁻¹·km⁻¹; DISSOC: 227.3 ±8.9 ml·kg⁻¹·km⁻¹; p=0.58). Ventilation (VE) was not different between the two slowest speeds but increased at the fastest speed during ASSOC, whereas VE differed between all speeds during DISSOC (condition x speed interaction; p=0.046). Breathing frequency (ASSOC: 30.9 ±6.9 br·min⁻¹; DISSOC: 33.6 ±7.1 br·min⁻¹; p=0.08) and tidal volume (ASSOC: 2.55 ±0.73 L; DISSOC: 2.36 ±0.67 L; p=0.09) did

not differ between ASSOC and DISSOC. Additionally, perceptual responses for breathing and effort did not differ between the two conditions. Conclusion: Altering attentional focus can influence LRC and VE in untrained individuals. However, the difference in LRC does not result in any benefits to the economy of movement or perception of effort during exercise.

APSIPE.53

Molecular Transducers of Human Skeletal Muscle Remodelling Under Different Loading States

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Loading and unloading skeletal muscle induce heterogeneous inter-individual adaptation, the molecular regulators of which are unclear. The lack of understanding in this regard is due to, amongst other factors, an averaging of molecular responses across a relatively small number of individuals exhibiting divergent adaptations. We hypothesized that a within-subjects' design that reduced physiological heterogeneity would identify a reproducible gene signature important for muscle growth. Twelve young men participated in 10wk of unilateral lower-limb resistance exercise training (RT) and 2wk of contralateral immobilization (IMB), which permitted a more consistent differential physiological response to alterations in muscle loading. Gene expression was quantified at the transcript level using HTA 2.0 microarrays (Affymetrix) from muscle samples taken following RT and IMB. We employed a heuristic that enabled the unique characterization of the full-length mRNA and the untranslated region (UTR) of the same gene. Correlational analysis was conducted to assess which differentially regulated genes scaled with the degree of leg lean mass gains from three independent exercise cohorts from our laboratory (total n=100). Greater than 2000 of the 11,628 genes that passed quality control had at least one full-length transcript regulated (FDR<5%, FC>1.2; 1435 up-regulated and 649 downregulated). Of these genes, 141 correlated with lean muscle mass gains consistently across all independent datasets (either at the full-length transcript, 5'UTR or 3'UTR). Several genes in this growth signature had well-defined roles in muscle plasticity, (i.e. the apelin receptor and FOXO3); however, our analysis also revealed novel regulatory molecules that are not detected using traditional analytical approaches. For instance, the 3'UTR signals of BCAT2 and FKBP1A – but not their full-length transcripts – were correlated with increased muscle size. RNAi-mediated knockdown of these genes in primary human myotubes attenuated IGF-1-

stimulated increases in protein synthesis. Our data are the first to demonstrate that UTR regulatory events covary with a lean mass transition in humans and underscore the importance of probing regulation at specific gene regions in addition to the full-length gene transcript.

APSIPE.55

Habitual side-specific loading leads to structural, mechanical and compositional changes in the patellar tendon of young and elderly life-long athletes

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Effects of life-long physical activity on tendon function have been investigated in cross-sectional studies, but these are at risk of "survivorship" bias. Habitual side-specific loading offers a strong model to overcome this issue by using one leg as an internal control. Here, we investigate if life-long side-specific loading is associated with greater cross-sectional area (CSA), mechanical properties, cell density (DNA content) and collagen cross-link composition of the human patellar tendon (PT), in vivo. Nine elderly and six young male life-long elite badminton players and fencers were included. CSA of the PT obtained by 3-tesla MRI, and ultrasonography-based bilateral PT mechanics were assessed. Collagen fibril characteristics, enzymatic cross-links, non-enzymatic glycation and DNA content were measured biochemically in PT biopsies. The elite athletes had a ≥15% side-to-side difference in maximal knee extensor strength, reflecting chronic unilateral sport-specific loading patterns. The patellar tendon CSA was greater on the lead extremity compared with the non-lead extremity (17 %, p=0.0001). Furthermore, greater tendon stiffness (18 %, p=0.0404) together with lower tendon stress (22 %, p=0.0005) and tendon strain (18 %, p=0.0433) were observed on the lead extremity. Moreover, tendon fibril density was greater (28 %, p=0.0013) and mean fibril area was lower (23 %, p=0.0306) on the lead extremity compared with the non-lead extremity. These data demonstrate for the first time that life-long side-specific loading yields altered tendon collagen fibril morphology that may lead to the greater structural and mechanical properties of the human PT.

APSIPE.56

Atrophy associated transcription factor FOXO1 suppresses transcriptional coactivator PGC-1 β gene expression in skeletal muscles

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Background/Aims: Skeletal muscle is the largest organ in the human body, accounting for approximately 40% of body weight, plays important roles in exercise and energy expenditure. Lack of exercise causes muscle atrophy and reduces energy expenditure. FOXO1 is a forkhead-type transcription factor, whose expression is markedly upregulated in skeletal muscles during atrophy, i.e., under conditions such as starvation, unloading (plaster cast), and denervation. Transgenic (Tg) overexpression of FOXO1 in skeletal muscles causes muscle atrophy, with increased expression of atrophy-related genes, including cathepsin L, lysosomal proteinase. Peroxisome proliferator-activated receptor- γ coactivator-1 β (PGC-1 β) is a transcriptional coactivator and is highly expressed in skeletal muscles. PGC-1 β is known to increase the mitochondrial content in cells. PGC-1 β activates nuclear receptors, such as the estrogen-related receptor, and activates target genes (i.e., medium-chain acyl CoA dehydrogenase, MCAD) in skeletal muscles. Indeed, the overexpression of PGC-1 β in skeletal muscles in mice led to increased energy expenditure and an anti-obesity phenotype. However, how PGC-1 β is regulated remains largely unclear. In this study, we attempted to analyze the possible regulation of FOXO1-mediated PGC-1 β gene expression, as the level of PGC-1 β mRNA was decreased in the skeletal muscles of FOXO1-overexpressing Tg mice. **Methods/Results:** We used a skeletal muscle sample of FOXO1 overexpressing Tg (FOXO1-Tg) mice and examined the gene expression using real-time qPCR. In the skeletal muscles of FOXO1-Tg mice, PGC-1 β gene expression was decreased. We also analyzed the expression of the PGC-1 β gene under other conditions with increased endogenous FOXO1 expression in C57BL/6J mice. Denervation or plaster cast-based unloading, as well as fasting, increased endogenous FOXO1 expression in skeletal muscles, with decreased PGC-1 β expression. In addition, mitochondrially encoded cytochrome c oxidase subunit 2 (Cox2) DNA levels were slightly decreased and citrate synthase activity was also significantly decreased in FOXO1-Tg mice. Denervation or fasting for 24 h also caused decreased mitochondrial DNA level and decreased citrate synthase activity. In the skeletal muscles of FOXO1-knockout mice, the decrease in PGC-1 β expression caused by fasting was attenuated. Moreover, we used a tamoxifen-inducible FOXO1 activation system in C2C12 myoblasts. Interestingly, in the presence of tamoxifen (FOXO1 activation), there was a marked decrease of PGC-1 β expression. Thus, PGC-1 β gene expression was negatively regulated by FOXO1 in C2C12 myoblasts. **Conclusions:** These findings together reveal that FOXO1 activation suppresses PGC-1 β expression. During atrophy with FOXO1 activation, decreased PGC-

1 β may decrease energy expenditure and avoid wasting energy in order to prevent a greater decrease of muscle mass.

APSIPE.57

Gene regulation in skeletal muscle atrophy by a transcription factor FOXO1

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Objective and Methods: Skeletal muscle is the largest organ in the human body, accounting for approximately 40% of body weight. Skeletal muscle atrophy, or wasting, occurs in various catabolic conditions, such as aging, inactivity, and undernutrition, leading to the decrease of physical activity and exercise capacity. Maintaining the skeletal muscle mass and function is necessary for promoting health and inhibiting the decline in the quality of life as well as improving physical activity and exercise capacity. Hence, it is required to precisely understand the molecular mechanism underlying skeletal muscle atrophy. Forkhead box O1 (FOXO1) is a transcription factor that induces the expression of atrophy-related genes during energy deprivation and inactivity. In a previous study, we developed transgenic mice with skeletal muscle-specific overexpressed FOXO1 (FOXO1-Tg mice) that markedly showed skeletal muscle atrophy, indicating that FOXO1 is the essential factor of muscle atrophy. In this study, we developed mice with skeletal muscle-specific deletion of the FOXO1, FOXO3a, and FOXO4 (FOXO1,3a,4-/- mice). The objective of this study was to identify the novel FOXO1 target genes in in vivo skeletal muscle of fasted mice by comprehensively analyzing the gene expression profile in skeletal muscle derived from both FOXO1-Tg and FOXO1,3a,4-/- mice using microarray analysis. **Results and Discussion:** In FOXO1,3a,4-/- mice, fasting-induced skeletal muscle atrophy was suppressed. As the results of microarray analysis and qRT-PCR, authentic FOXO1 target genes (Cathepsin L, Gabarapl1, Gadd45a, Glul, 4EBP1, and PDK4 etc.) were significantly upregulated in the skeletal muscle of FOXO1-Tg and fasted WT mice (fasting: FOXO1 activated). However, the increase of these atrophy-related gene expression was completely suppressed in FOXO1,3a,4-/- mice. In addition, several novel FOXO1 target genes were identified. Moreover, tamoxifen-inducible activation of FOXO1 in C2C12 myoblasts caused a marked increase in the expression of these novel FOXO1 target genes. Among these genes, we focused on CCAAT/enhancer-binding protein δ (C/EBP δ), the muscle atrophy-inducible transcription factor, because the association between FOXO1 and C/EBP δ has been unclear so far. In C2C12 myoblasts, knockdown of C/EBP δ using siRNA markedly suppressed FOXO1-induced increased expression of muscle atrophy-related genes such as Atrogin1 and MuRF1 (FOXO1 target genes). Furthermore, an analysis of the muscle cells with stably overexpressed C/EBP δ revealed that several FOXO1-dependent induced genes identified in this study were upregulated by C/EBP δ , indicating the potential roles of C/EBP δ to cooperatively regulate the expression of FOXO1 target genes in skeletal

muscle. Conclusion: In this study, we identified novel FOXO1 target genes in fasted skeletal muscle using both FOXO1-Tg mice that showed marked muscle atrophy and FOXO1,3a,4-/- mice that suppressed fasting-induced muscle atrophy. Moreover, we found that the transcription factor C/EBP δ functions as a key factor that links FOXO1 and muscle atrophy-related genes. Due to its importance of FOXO1 in skeletal muscle physiology, the data obtained from this study sheds light on the novel molecular mechanism underlying muscle atrophy.

APSIPE.59

Background inactivity blunts metabolic adaptations to intense short-term training

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The objective of this study was to determine if the level of background physical activity (steps/d), influences the acute and short-term adaptations to intense aerobic training. Methods: Sixteen initially untrained participants (24 $\pm$ 2 y) completed intense (80-90% VO₂peak) short-term training (5 bouts of exercise over 9 days) while taking either 4,767 $\pm$ 377 steps/d (n=8; Low Step) or 16,048 $\pm$ 725 steps/d (n=8; High Step). At baseline and following one day of acute exercise and then following the short-term training, metabolic responses to a high fat meal (i.e. plasma triglyceride concentration and fat oxidation) were assessed during a 6-h high fat tolerance test (HFTT). Additionally, responses during submaximal exercise were recorded during 15-min of cycling (~79% of baseline VO₂peak), including heart rate, blood lactate, and deoxygenated muscle hemoglobin both before and after training. Results: During the HFTT, High Step displayed a reduced incremental area under the curve (AUCI) for plasma triglyceride concentrations by 31% after acute exercise and by 27% after short-term training, compared to baseline (p<0.05). This was accompanied by increased whole-body fat oxidation (p<0.05). Furthermore, muscle stress during submaximal exercise, as reflected by heart rate, blood lactate and deoxygenated hemoglobin, were all reduced in High Step (p<0.05). Despite completing the same training regimen, Low Step taking <5,000 steps/day showed no significant improvements in fat metabolism or markers of stress during submaximal exercise after training (p>0.05). However, the two groups showed a similar 7% increase in VO₂peak (p<0.05). Conclusion: When completing an intense 5-bout exercise training program, decreasing daily background steps from 16,000 to approximately 5,000 steps/day blunts some of the classic adaptations to training. The blunting might be more pronounced regarding metabolic factors (i.e.; fat oxidation and blood lactate concentration) compared to cardiovascular factors (i.e.; VO₂peak).

APSIPE.60

The Associations Between Testosterone and Bone Mineral Density in Male Collegiate Athletes

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Background: Testosterone (T) deficiency and low bone mineral density (BMD) are common symptoms among those experiencing relative energy deficiency in sport. However, a lack of research exists on the associations between T concentration and BMD in various male athletes. Purpose: To assess the associations between T concentration and low BMD in male collegiate athletes. Methods: Male collegiate cross-country (CC) runners, club rugby (R) athletes, and collegiate track and field (TF) sprinters/jumpers, completed one lab visit at the end of their sports off-season. Participants arrived fasted (8 hours), abstained from exercise (12 hours), and within 60 minutes of waking. Testing included completing an eating disorder risk questionnaire, a 24-hour dietary food recall, 3 dual-energy x-ray absorptiometry (DXA) scans (whole-body, left femur, and lumbar spine), and collection of a saliva sample. Descriptive statistics were performed on dependent variables using measures of central tendency, variability, and frequencies. The statistical hypotheses were examined using analysis of variance (ANOVA), in order to compare the three athletic groups. A post hoc tukey test was then used to determine where the significant differences occurred. Pearson correlations were utilized to assess the associations between T and BMD at whole-body, left femur, left femoral neck, and lumbar spine among all the male athletes and for each sport. Significance was accepted at p $\leq$ 0.05. Results: Seven of the ten R athletes were considered to have a high risk of eating disorders because of their responses to the extreme weight control behavioral questions. CC had a higher caloric intake than R but not TF (CC: 3,813 $\pm$ 1,239 kcal; R: 2,402 $\pm$ 589 kcal; p=0.005). CC had lower whole-body BMD when compared to R but not TF (CC: 1.21 $\pm$ 0.03g/cm²; R: 1.32 $\pm$ 0.07g/cm²; TF: 1.28 $\pm$ 0.09g/cm², p=0.007, p=0.365, respectively). There were no significant differences in T concentration between groups. There were no significant associations between T concentration and BMD in CC and TF, but R had significant associations between T concentration and whole-body (r=0.635, p=0.049), left femur (r=0.671, p=0.034), and left femoral neck BMD (r=0.686, p=0.028). Conclusion: Our findings support previous research findings demonstrating low BMD in endurance runners compared to other athletes. However, no associations were observed between T and BMD except for in R athletes. T may not be a great predictor of BMD in male collegiate athletes.

APSIPE.61

Metformin-leucine treatment during disuse improves muscle force production, increases satellite cells, and reduces fibrosis in aged mice.

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The hospitalization of older adults is predicted to increase within the next 15 years. The aging population is growing, and the majority will deal with prolonged and incomplete muscle and strength recovery. An inability to fully regain lost muscle after inactivity drives the diminished response to rehabilitation and is accompanied by muscle fibrosis. **PURPOSE:** Thus, the purpose of this study was to investigate how combination therapy of metformin and leucine (MET+LEU) affects muscle recovery following disuse in aged mice. **METHODS:** Aged mice (22-24 mo) underwent 14 days of hindlimb unloading (HU) then 7 or 14 days of recovery. Age and weight-matched mice did not undergo HU and were used as ambulatory comparators (AMB). Mice assigned to each time point received either standard drinking water (control), water with metformin (MET), leucine (LEU), or water with metformin and leucine (MET+LEU) throughout the HU and recovery period. Hindlimb muscles were dissected, snap frozen, sectioned, and stained for histological analysis or used fresh for ex-vivo isometric force assessments. **RESULTS:** HU decreased gastrocnemius and soleus muscle mass and fiber cross-sectional area in all groups vs. AMB ($P < 0.05$). After 7 or 14 days of recovery, gastrocnemius muscle mass was not recovered compared to AMB in any groups ($P < 0.05$). Immediately after HU, MET+LEU mice were the only group that exhibited enhanced specific isometric force (mN/mm²) in soleus compared to control mice ($P < 0.05$). Following HU, MET+LEU mice had increased Pax7⁺ cells in gastrocnemius vs. control ($P = 0.05$); after 7 days of recovery in soleus, there were also increased Pax7⁺ cells ($P = 0.019$) compared to control. Additionally, after 7 days of recovery gastrocnemius of MET+LEU mice had increased central nuclei compared to control ($P = 0.015$). Lastly, after HU and throughout the recovery period, MET+LEU mice had increased collagen turnover (main effect $P < 0.001$) and decreased fibrosis vs. control (Tukey's post-hoc $P = 0.033$) indicated by collagen IV content. Sirius red staining displayed a trend for decreased fibrosis with MET+LEU following HU ($P = 0.07$). **CONCLUSION:** MET+LEU treatment improves recovery in aged mice following muscle disuse by promoting muscle force generation, regenerative cells, and decreasing fibrosis but does not affect fiber size. Support provided by the University of Utah Sports Medicine & Science Grant

APSIPE.62

Surrogate biomarkers for estimation of energy intake levels in male athletes

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[Background] It is important for athletes to follow a diet that provides sufficient energy intake (EI) proportional to the amount of physical activity performed. However, a physiological method for determining the status of energy intake has not yet been established. If EI can be screened using blood markers, it will be useful for athletes' conditioning and nutritional education in various sports field. [Purpose] The purpose of this study was to identify surrogate blood markers to estimate the status of EI in male athletes. [Method] The participants were 27 male athletes who joined the sports clubs of the university. The sports played by the athletes were track and field (throw/deathlon), handball, and basketball. The participants were subjected to measurements of height, weight, and body composition using a dual-energy X-ray absorption (DXA) method and fasting blood tests early in the morning. For the dietary survey, athletes were requested to self-record their food consumption for three consecutive days (two days with and one day without training) and provide evidence with photographs. The basal metabolic rate (BMR) was estimated from the lean body mass obtained by DXA according to the BMR estimation formula (Japan Institute of Sports Sciences formula), and the daily EI/BMR ratio was used as an index for determining the energy intake status. A formula for estimating energy intake status by multiple regression analysis (stepwise method) was created from 36 different blood biomarkers. This study was conducted with the approval of the ethics committee of the Graduate School of Sport and Health Sciences, Juntendo University. [Results] From the results of the multiple regression analysis, free T3, white blood cell count, insulin-like growth factor 1, uric acid, aspartate aminotransferase, high-density lipoprotein cholesterol, and urea nitrogen were selected as explanatory variables and used to create an EI/BMR estimation formula, which determined EI/BMR with high accuracy ($R^2 = 0.862$, adjusted $R^2 = 0.812$). No systematic error was observed in the estimated value. [Conclusion] The results suggest that male athletes' energy intake status could be calculated using an estimation formula consisting of multiple blood markers, as a surrogate biomarkers.

APSIPE.63

Myokine: musclin is relate to prevention of atherosclerosis by aerobic exercise training

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Introduction: Atherosclerosis causes cardiovascular events. Habitual aerobic exercise could prevent atherosclerosis. A novel myokine: musclin secretion has been reported to associate with deterioration of blood lipid profiles and consequently affect the increase in cardiovascular disease risk. However, whether alterations in muscle-derived musclin affect prevention of atherosclerosis by habitual aerobic exercise remains unclear. **Purpose:** This study aimed to examine whether aerobic exercise training-induced changes in muscle-derived musclin affect prevention of atherosclerosis and blood lipid profiles. **Methods:** Male 8-week-old apolipoprotein E knockout (ApoE KO) mice were randomly divided into sedentary control (ApoE KO-SED) or aerobic exercise training (ApoE KO-EX) groups. Additionally, male 8-week-old C57BL/6 wild type mice were used as a sedentary healthy control group (WT). The mice in both ApoE KO-SED and ApoE KO-EX groups were fed high fat diet (41% fat) for 16 weeks from 8-week-old. ApoE KO-EX group conducted voluntary running on a wheel for 16 weeks. The area of lipid accumulation in the aorta was measured by oil red-O staining as an index of the atherosclerotic lesion area in the aorta. As the blood lipid profiles, circulating levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol, and triglyceride (TG) were measured. Furthermore, muscle musclin protein level was measured by western blot. **Results:** In the ApoE KO-SED group, body weight and body fat were significantly increased, and the atherosclerotic lesions were significantly deteriorated as compared to the WT group (each $p < 0.05$). Furthermore, circulating levels of LDL, HDL, and TG were significantly elevated in the ApoE KO-SED group as compared to the WT group (each $p < 0.05$). In the ApoE KO-EX group, muscle citrate synthase activity, an index of exercise training effect, were significantly increased, the atherosclerotic lesions were significantly mitigated, and circulating levels of LDL and TG were significantly reduced, as compared to the ApoE KO-SED group (each $p < 0.05$), but did not change in circulating HDL level. Interestingly, the muscle musclin protein expression in the ApoE KO-EX group was significantly reduced, as compared to the ApoE KO-SED group ($p < 0.05$). **Conclusions:** These results suggest that habitual aerobic exercise-induced decrease in muscle-derived musclin may be related to mitigation of atherosclerotic progression via prevention of blood lipid profiles. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (#17H02182, #19K22828, M. Iemitsu).

APSIPE.64

Muscle fiber type differences in nitrate and nitrite storage and nitric oxide signaling in rats.

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Recent studies have emphasized the importance of the nitric oxide synthase (NOS)-independent, nitrate (NO₃-) → nitrite (NO₂-) → nitric oxide (NO) pathway in skeletal muscle. In particular, it has been hypothesized that this pathway is especially active in type II, or fast-twitch, muscle fibers, necessitating greater NO₃- and NO₂- storage. We therefore measured NO₃- and NO₂- concentrations in the predominantly fast-twitch vastus lateralis (n=9-10) and predominantly slow-twitch soleus (n=9-10) muscles of male Sprague-Dawley rats via high-performance liquid chromatography. Muscle cyclic guanosine monophosphate (cGMP) content, a known NO second messenger and mediator of vasodilation in skeletal muscle was measured using an enzyme-linked immunosorbent assay. Results are presented as Mean (SE). Contrary to the above hypothesis, we found that NO₃- and NO₂- concentrations were 3.4-fold (222±44 vs. 65±8 μmol/kg) and 1.8 fold higher (1.58±0.14 vs. 0.88±0.11 μmol/kg), respectively, in the soleus vs. vastus lateralis ($p < 0.01$). On the other hand, NO signaling (i.e., cGMP level) was comparable in the two muscles ($p = 0.49$). Additionally, NO₃-, NO₂- and cGMP were not correlated with indicators of skeletal muscle performance including grip strength, voluntary running speed/distance or treadmill endurance time. Although the physiological significance of these observations remains to be determined, we speculate that NO production via the NO₃- → NO₂- → NO pathway is normally higher in slow-twitch muscles, thus helping compensate for their inherently lower NOS activity.

APSIPE.67

Diminished metabolic rate following traumatic orthopaedic injury may predispose chronic disease development

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The objective of this project was to examine the impact of volumetric muscle loss (VML) injury on physical activity and whole-body metabolism, and to evaluate how restricted activity affects these physiologic aspects. VML is the traumatic loss of skeletal muscle fibers and function, after which patients likely undergo periods of inactivity that may contribute to long-term muscle dysfunction. We hypothesized that 24-hr ambulation, RER, and metabolic rate would decrease following VML injury and in a restricted activity state. First, adult male C57BL/6 mice (n=7) underwent a full-thickness multi-muscle VML injury to the gastrocnemius, soleus, and plantaris muscles (~15%; 20.6mg). Twenty-four hour whole-body physical and

metabolic activity were measured at baseline (i.e., prior to VML) and 6 weeks post-VML. Second, the effects of housing mice in restricted cages to model clinical conditions (i.e., bedrest) on physical and metabolic activity outcomes were investigated. A cross-over design was implemented in which adult male C57BL/6 mice (n=8) were assigned to standard or restricted (12.5x8.5x6.3cm) housing for one week in each condition. Total daily activity and whole-body metabolism were evaluated over a 24-hr period and divided into 12-hr active and inactive periods, based on the light cycle. First, prior to VML, 24-hr total ambulation, RER, and metabolic rate were ~1.3km/day, 0.91, and 18.7kcal/kg/hr, respectively. Six weeks post-VML, mice displayed a significantly decreased 24-hr RER and metabolic rate of 0.88 and 16.9kcal/kg/hr ($p \leq 0.036$), respectively, with a similar significant decline in RER and metabolic rate specifically during the active period ($p \leq 0.047$). Second, in the restricted cage, mice demonstrated ~50% lower total ambulation, which was reflected in the ~23% reduction in 24-hr metabolic rate ($p \leq 0.002$). In contrast, mice demonstrated an ~4% greater 24-hr RER in the restricted cage compared to the standard cage ($p < 0.001$). Observations herein suggest that physical and metabolic activity outcomes are negatively impacted following traumatic VML injury, similar to a restricted physical activity state. In the clinical setting, these findings support a potential increased risk for VML-afflicted patients to develop various chronic diseases, such as metabolic disorder, in part due to restricted physical activity levels following injury. Future studies are needed to evaluate chronic comorbidities associated with VML injury and to examine how these comorbidities may be attenuated or prevented long-term. Second, the higher 24-hr RER in the restricted activity state suggests greater carbohydrate utilization, similar to observations reported in patients restricted to bedrest following traumatic injury, such as burn. In future investigations, it is possible that modeling VML injury with restricted activity will recapitulate the clinical condition. Furthermore, the restricted activity cages may prove useful in other physiologic models of disease. Supported by W81XWH-18-1-0710 & T32AR050938

APSIPE.68

The changes in the responses of autophagy-related factors with repeated bouts of resistance exercise

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Resistance exercise training is effective for skeletal muscle hypertrophy. On the other hand, continuous resistance training gradually attenuates muscle hypertrophic responses (Ogasawara et al., 2013). Inactivation of mammalian target of rapamycin complex 1 (mTORC1) signal after resistance exercise is considered to be involved in the process due to its role in the muscle anabolic responses (Takegaki et al., 2020). Meanwhile, mTORC1 also plays a role in the control of muscle catabolic systems, especially in autophagy. However, the influence on the autophagy system by repeated bouts of resistance exercise remains

unclear. The present study aimed to investigate the bouts dependent changes in the autophagy-related factors. Male Sprague-Dawley rats were resistance-exercised 10 bouts with interval of 48 hr between bouts. The resistance exercise consisted of 3 s X 10 times X 5 sets (with 3 min rest between sets) of maximal isometric contractions, which were elicited by transcutaneous electrical stimulation on the right gastrocnemius muscle under anesthesia. The left gastrocnemius muscle was used as the internal control. Muscle samples were collected immediately (0h-post RE) and 3h (3h-post RE) after the 1st (1B) and 10th (10B) exercise bouts. Protein expression levels of phosphor-UNC-551-like kinase 1 (ULK-1, Ser757), phosphor-ULK1 (Ser317), and microtubule-associated protein 1 light chain 3 (LC3) -II were analyzed by western blotting. This study was approved by the Ethics Committee for Animal Experiments at Ritsumeikan University (BKC2018-037) and all Experiments were performed according to the APS "Guiding Principles in the care and Use of Animals." At 0h-post RE, as compared with that of internal control, resistance exercise increased the phosphorylated protein expression of ULK1 (Ser757) in 10B ($P < 0.001$), but not in 1B. However, at 3h-post RE, the phosphorylated protein expression of ULK1 (Ser757) was increased in exercised legs in both groups (main effect of exercise, $P < 0.05$). On the other hand, the phosphorylated protein expression of ULK1 (Ser317) was increased in exercised legs in both groups at 0h-post RE (main effect of exercise, $P < 0.0001$), but not at 3h-post RE. The protein expression of LC3-II did not change either group at 0h-post RE, but decreased in exercised legs in both groups at 3h-post RE (main effect of exercise, $P < 0.01$). The present results suggest that the blunting of mTORC1 activation with repeated bouts of resistance exercise does not negatively affect upstream factor of autophagy (involved in the initial step of autophagosome formation). Furthermore, repetition of resistance exercise may not affect the autophagosome formation.

APSIPE.70

Role of DNA methyltransferase 3a (Dnmt3a) in skeletal muscle of mice

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Introduction: Muscle atrophy is associated with aging, malnutrition and chronic unloading as well as nerve injury. During atrophy, the muscle regeneration capacity after injury (e.g., falling, trauma, or extreme exercise) is known to be decreased. However, the mechanism involved is largely unknown. DNA methylation is an epigenetic mechanism regulating gene expression. It has been reported that DNA methylation of several genes change after exercise. Genomic DNA methylation patterns are established by the actions of DNA methyltransferases (Dnmts). One of the five Dnmts reported, Dnmt3a is known to have a high

expression in the skeletal muscle and its expression decreases during atrophy. However, its functions are still largely unknown. Therefore we have created two types of gene modified mice to understand the role of Dnmt3a in skeletal muscle, one with skeletal muscle specifically knocked out Dnmt3a mice (Dnmt3a-KO), and another with skeletal muscle specifically overexpressed Dnmt3a mice (Dnmt3a-Tg). Results and Conclusion: In Dnmt3a-KO mice, diminished mRNA and protein expression of Dnmt3a were observed in skeletal muscles as well as in satellite cells, which are important for muscle regeneration. Differentiated Dnmt3a-KO satellite cells showed smaller in size (length/area), suggesting suppressed myotube differentiation. Microarray analysis of satellite cells showed expression of growth differentiation factor 5 (Gdf5) mRNA was markedly increased in Dnmt3a-KO mice. The DNA methylation level of the Gdf5 promoter was markedly decreased in Dnmt3a-KO satellite cells. In addition, DNA methylation inhibitor azacytidine treatment increased Gdf5 expression in satellite cells of wild type mice, suggesting Gdf5 expression is regulated by DNA methylation. Also, we observed increased Id (Inhibitor of differentiation, a target of Gdf5) mRNA expression in Dnmt3a-KO satellite cells. Thus, Dnmt3a appears to regulate satellite cell differentiation via DNA methylation of Gdf5. (FASEB J 32:1452-1467, 2018.) We have recently created Dnmt3a-Tg mice to further elucidate the role of Dnmt3a in skeletal muscle. From the results of microarray analysis, immunohistochemistry and indirect calorimetry, we observed a phenotype of increased rate of red muscle in Dnmt3a-Tg mice. Interestingly, in satellite cells of Dnmt3a-KO mice, the expression of mature red muscle marker protein myosin heavy chain 7 (Myh7) decreased, supporting the idea that Dnmt3a may have the role of changing muscle fiber type. In conclusion, Dnmt3a functions to regulate satellite cell differentiation and also may change muscle type composition from white to red muscles.

APSIPE.72

Dose-response effect of a concurrent exercise intervention on the inflammatory profile of young adults: a randomized controlled trial

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Background: The prevalence of obesity continues raising worldwide, together with its related comorbidities. All these conditions seem to be partially explained by a derangement in the interplay between metabolic and immune processes. Among other strategies, it has been suggested that physical exercise might be an efficient strategy to modulate the immunological profile, although research in inconclusive in healthy adults. We aimed to examine the dose-response effect of a concurrent exercise intervention of 6 months on the inflammatory profile of young healthy and sedentary adults. Methods: A total of 76 participants (68.4% women, 22±2 years old, body mass index: 24.3±4 kg/m²) were included in this randomized,

parallel-group, controlled trial. Eligible participants were randomly assigned to 3 different groups: control (non-exercise), and moderate and vigorous-intensity exercise groups. Exercise groups underwent a 6-months supervised intervention, combining both, aerobic and resistance training, and had to train 3-4 days/week, approximately 1 to 1.5 h/ day. In the moderate-intensity group, participants constantly worked at 60% reserve heart rate (rHR) (aerobic training) and at 50% of repetition maximum (RM, resistance training). Participants from the vigorous-intensity group, progressed from 60 to 80% rHR in the aerobic training, and from 50 to 70% RM, in the resistance training. Before and after the exercise intervention, blood extractions were performed in fasting state. Then a set of inflammatory markers - including C-reactive protein, interleukins (IL)-2, IL-4, IL-6, IL-8, IL-10, IL-17a, interferon gamma, tumor necrosis factor alpha, several components of the innate immune system (C3, C4, and B2-microglobulin), and adiponectin and leptin - were assessed using immunoturbidimetric assay and MILLIPLEX map technologies for cytokines and adipokines. Body composition was assessed by means of dual-x ray energy absorptiometry. Results: This exercise intervention did not have any effect ($P \geq 0.05$) on any of the interleukins measured. However, we found a significant effect for time ($P=0.02$) and for the interaction time*group ($P=0.05$) on C-reactive protein. In addition, the exercise program had a significant effect for time on C3 and β 2-microglobulin concentrations ($P=0.05$; $P=0.004$; respectively), but not for the group or interaction time*group ($P \geq 0.05$). Adiponectin and leptin levels neither changed ($P=0.09$). When the change in the previous outcomes was adjusted for sex, or for sex and BMI, all results were non-significant ($P \geq 0.05$). Conclusions: Despite the potential role of physical exercise to modulate the immunometabolic profile, a 6-month concurrent exercise intervention does not appear to have any effect on systemic inflammatory markers, in young healthy adults. This may be explained by the relatively healthy status of our sample. Further research is warranted.

APSIPE.73

Muscle-specific microRNAs are increased in cultured muscle cells derived from patients with Idiopathic Inflammatory Myopathy

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Aim: Idiopathic inflammatory myopathies (IIM) are systemic autoimmune diseases characterized by proximal muscle damage and weakness. Metabolic and mitochondrial dysfunction of skeletal muscle can contribute to disease progression. Regular physical activity improves muscle

performance and clinical state of patients with IIM. The levels of muscle specific microRNAs (myomiRs) are altered by muscle inflammation and degeneration, and can be modulated by exercise training. Methods: Samples of m. vastus lateralis were obtained by Bergström needle biopsy under local anesthesia from IIM patients before and after 6-month intense supervised training intervention (IIM patients n=7/7) and sedentary age/gender-matched healthy controls (n=7). Primary muscle cell cultures were established. Differentiating cells were exposed to palmitate (100 μM/3-days), harvested and used for the assessment of target miRNA/mRNA/mitochondrial DNA (mtDNA) (qPCR), protein content of mitochondrial oxidative phosphorylation/OXPHOS complexes (immunoblotting), fat oxidation/FOX (radiometric assay) and accumulation of lipids (thin layer chromatography). Results: Levels of miR-133a, -133b, -1, -206 were substantially upregulated (2.6, 2.1, 3.3, 0.5-fold) in primary muscle cell cultures from IIM patients compared to matched healthy controls. There was no significant effect of exercise training intervention on myomiR levels. In palmitate treated cells, we found negative correlations of miR-1, -133a, -133b levels with the rate of incomplete FOX, represented by accumulation of intermediate metabolites – acid soluble products (ASPs; n=21, R=-0.607, p=0.004; R=-0.577, p=0.006; R=-0.597, p=0.004). Complete FOX (CO₂) positively correlated/tended to correlate with expression of miR-1, -133a, -133b (n=21, R=0.458, p=0.037; R=0.308, p=0.089; R=0.401, p=0.071). MiR-1, -133a, -133b also positively correlated with mtDNA-content (n=21, R=0.446, p=0.043; R=0.564, p=0.008; R=0.527, p=0.014), protein levels of OxPHOS complex-I (n=21, R=0.479, p=0.028; R=0.438, p=0.047; R=0.547, p=0.010) and miR-133a and -133b negatively with OxPHOS complex-V (R=-0.577, p=0.006; R=-0.480, p=0.028). MiR-1 & miR-133b correlated negatively with accumulation of diacylglycerols (n=21, R=-0.434, p=0.049; R=-0.501, p=0.021) and miR-133a & -133b with TAGs (n=21, R=-0.492, p=0.023; R=-0.526, p=0.014). Conclusion: Upregulation of specific myomiRs involved in myogenesis and muscle regeneration found in cultured myotubes from IIM patients compared to healthy controls indicates activation of compensatory epigenetic mechanisms which could contribute to the amelioration of muscle degeneration in IIM. Support: MZCR 16-33574A, SAS-MOST Joint Research Cooperation grant 2018/10, VEGA 2/0107/18, Slovak Research and Developmental Agency APVV-15-0253, VEGA 2/0091/19, SAS-MOST JRP 2016/4

APSIPE.74

Comprehensive analysis on blood of skeletal muscle-specific PGC-1α overexpressing mice

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Background and Aim: In the skeletal muscle, peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) is considered to be involved in mitochondria

function, alteration of muscle fiber type, and fatty acid oxidation, also its level increases during exercise.

Transgenic mice overexpressing PGC-1α in the skeletal muscle (PGC-1α-Tg mice, a mouse model for exercise) have been developed previously. Recently, myokines, physiologically active substances secreted by the skeletal muscle during exercise have been the center of attraction. Irisin and β-aminoisobutyric acid (BAIBA) are known as PGC-1α-dependent myokines and contribute to browning of white adipocytes. From previous reports, considering that PGC-1α changes metabolites in skeletal muscle (Y Hatazawa. et al. PLoS ONE. 10: e0129084. 2015), metabolites secreted in the blood are expected to change. In this study, we comprehensively investigated changes in blood metabolites of PGC-1α-Tg mice (search for the new myokines related to the expression of PGC-1α). Methods and Results: We investigated the changes in blood metabolites related to the expression of PGC-1α in the skeletal muscle using CE-TOFMS. We also examined the gene expression in the skeletal muscle using qRT-PCR. In this study, we observed that the level of several metabolic products changed in the blood of PGC-1α-Tg mice. Therefore, we also discussed metabolites changes in the blood of PGC-1α-Tg mice by combining metabolomic data in the skeletal muscle of PGC-1α-Tg mice. The level of homovanillic acid (dopamine metabolite, 1.8-fold) in the blood increased in PGC-1α-Tg mice. Alternatively, the level of homovanillic acid (2.2-fold) and the gene expression of dopamine metabolic enzymes (catechol-O-methyltransferase and monoamine oxidase B) increased in the skeletal muscle of PGC-1α-Tg mice. These data suggest that the homovanillic acid produced in the skeletal muscle by PGC-1α was secreted into the blood, which caused its increase in the blood. Interestingly, in independent human studies, the level of homovanillic acid in the blood increased with increased physical activity (E Anggård. et al. Eur J Pharmacol. 24: 37-42. 1973, K S Kendler. et al. Psychiatry Res. 8: 215-223. 1983). On the other hand, the level of branched-chain α-keto acids (α-ketoisovaleric acid, 0.5-fold; α-ketoisocaproic acid, 0.4-fold; α-keto-β-methylvaleric acid, 0.4-fold) and β-alanine (0.5-fold) decreased in the blood of PGC-1α-Tg mice. We previously reported that PGC-1α in skeletal muscles is considered to significantly contribute to BCAA metabolism and β-alanine metabolism. These metabolites in the skeletal muscle of PGC-1α-Tg mice were present in low concentration. These data suggested that the level of branched-chain α-keto acids and β-alanine in skeletal muscles decreased, and level of these metabolites secreted into the blood decreased. Conclusion: In this study, we suggest that homovanillic acid may be a myokine that is secreted from skeletal muscles during exercise. Furthermore, our data indicate that branched-chain α-keto acids and β-alanine decreased in the blood of PGC-1α-Tg mice, possibly because of increased metabolism in the skeletal muscle.

APSIPE.75

Exercise as “medicine” to enhance insulin sensitivity and metabolism in type 2 diabetes: Inspired by John O. Holloszy

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Type 2 diabetes is a life-threatening metabolic disease reaching epidemic proportions, with disease risk scaling linearly due to obesity and inactivity. Disturbed circadian rhythms can cause metabolic dysfunction, highlighting a role of this circuit in type 2 diabetes pathogenesis. Nevertheless, mechanisms underlying disrupted circadian rhythmicity of the intrinsic molecular-clock in type 2 diabetes are unknown. In this lecture, I will cover some of my latest work related to mechanisms by which the timing of food intake or exercise (energetic stressors) interact with peripheral clocks to control metabolic homeostasis. My overarching hypothesis is that synchronizing energetic stressors such as diet and exercise to the molecular circadian clock may maximize the health promoting benefits on glucose and energy metabolism. My work is inspired by Professor John O. Holloszy, M.D., one of the great applied physiologists of our time and “father” of modern exercise biochemistry. I had the privilege of working in Dr. Holloszy’s laboratory between 1986-1989, where I first developed my interest in understanding how exercise can be used as a “medicine” to enhance insulin sensitivity and metabolism in people with type 2 diabetes. The goal of my current work is to elucidate the mechanisms by which physiological processes are modulated by the clock machinery in a diurnal manner including, glucose control, systemic metabolism, and mitochondrial respiration, and how dysregulation of these processes contribute to type 2 diabetes. Molecular mechanisms underpinning the link between peripheral circadian clocks, energy sensing pathways, and the control of glucose homeostasis may one day form the basis of a new class of therapies to prevent insulin resistance.

APSIPE.76

Wall shear stress patterns during aerobic interval and continuous exercise training in aging and the resultant effects upon vascular health

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Introduction: The exact mechanisms of age-related declines in vascular structure and function in humans remain unknown. Wall shear stress (WSS) can regulate vascular health. Laminar WSS, comprised mainly of antegrade WSS, promotes nitric oxide production and vasodilation. Conversely, oscillatory WSS (thus greater volumes of retrograde WSS) promotes inflammatory factors, oxidative enzymes, and vasoconstriction. Older adults have greater volumes of oscillatory WSS at rest (1).

Exercise training preserves and improves vascular health independent of traditional cardiovascular disease risk factors, likely through exercise induced WSS. However, few studies have assessed the relationship of in-exercise WSS, during exercise training, with aging and its potential impact on vascular health. Methods: 35 healthy males and females with a mean age of 25 (range 20-35 years (8 m; 9 f)) or 53 (range 45-60 years (5 m; 13 f)) were randomly assigned to 4 weeks of cycling: AIT: 4min at 85-90% heart rate peak (HRpeak) with 4min active recovery at 60-65% HRpeak repeated 4 times; or 32min CON: 65-70% HRpeak. Doppler ultrasound was used to characterise both in exercise brachial artery antegrade and retrograde WSS and pre and post exercise endothelial function (FMD) during the first and last training sessions. Ultrasound was also used to assess carotid artery stiffness and wall thickness pre and post training. Linear mixed models were used to analyse group (age and exercise protocol) and time effects. Results: Antegrade and retrograde WSS patterns mirrored the work rate (WR) profile of the exercise, consistent with HR and VO₂ patterns. In-exercise mean and total volume of antegrade WSS did not differ with training, age or exercise protocol (P>0.05). Mean and total volume of retrograde WSS were 50% and 48% higher in AIT versus CON exercise (P<0.05) and did not change with training or age (P>0.05). Prior to training, FMD assessed pre and post the first exercise session increased (6.0±4.4 to 11.0±5.3 %; P<0.05). Following training, the acute impact of exercise upon FMD was blunted (8.9±4.6 to 10.3±4.7 %; P>0.05). Endothelial function assessed prior to the first and final exercise training sessions increased by 53% (P<0.05) but was unaffected by age or exercise protocol. Carotid artery stiffness (older 7.41±2.42 v. younger 4.24±1.16 a.u.) and thickness (older 1.09±0.19 v. younger 0.77±0.15 mm) were higher in older versus younger adults (P<0.05) and were unchanged with training (P>0.05). Conclusions: HR, VO₂ and WR requirements drive the WSS response. Oscillatory WSS seen at rest in older adults is abolished during exercise (1), explaining why no difference in WSS during exercise was observed between age groups in the current study. Both AIT and CON exercise training promoted acute and chronic improvements in endothelial function, although acute FMD following training was blunted likely due to a ceiling effect. Perhaps unexpectedly, FMD was comparable between young and older adults suggesting no impairment of endothelial function in the older population, despite signs of arterial stiffening. CON and AIT exercise improve aspects of vascular health in young and older adults. 1. Padilla J. et al. Impact of Aging on Conduit Artery Retrograde and Oscillatory Shear at Rest and During Exercise. NO, 2011.

APSIPE.77

Exercise induced inflammation in tendinopathy

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Exercise overuse and inflammation are important factors contributing to the onset and progression of tendon disease. Single cell RNA sequencing has identified human tendons are comprised of functionally distinct fibroblast,

immune and endothelial cell subsets¹, advancing understanding of the phenotypes and function of these cell types in tendon health and disease. The following pathological features have been identified in biopsy samples collected from patients with chronic tendinopathy, classified as established disease greater than 3 months duration: 1) Macrophages show complex activation states, which change with disease stage 2,3 2) Tendon cells show an activated pro-inflammatory phenotype and capacity for 'inflammation memory'^{3,4} 3) Tendon cells from patients with chronic tendinopathy show dysregulated resolution responses⁵⁻⁷ Collectively, these discoveries have advanced understanding of the cellular basis of chronic tendinopathy, informing exciting new approaches to address the pathogenic stromal microenvironment in tendon disease 8. However, there are significant challenges associated with the successful treatment of chronic tendon disease. This is largely attributable to the formation of permanent scar tissue during tendon healing. Therefore, strategies to therapeutically target exercise-induced tendinopathy during earlier stage disease, prior to the development of permanent changes within the tendon are likely to be more fruitful. Less is known about inflammation in the context of exercise-induced early tendinopathy, where patients have had symptoms for weeks instead of many months. Tran et al. reported increased angiogenesis and anabolic signalling were features of early stage tendinopathy in samples collected from patients with disease less than 3 months duration⁹. We recently studied the histological features of tendon biopsy samples collected from patients with early patellar tendinopathy (<3 months duration). These tissues showed mildly increased cellularity and vascularity and moderate localised expansion of the tendon interfascicular matrix (IFM) compared to biopsies from healthy patellar tendon donors. We also identified markers of fibroblast and macrophage activation were localised to these expanded IFM regions, suggestive of an inflammatory phenotype in early stage disease. Improved understanding of how exercise influences tendon inflammation at the cellular level is critical to develop new therapeutic strategies to target pathogenic cells active in early stage disease. This knowledge will in turn inform the development of physiotherapy regimes to optimise early tendon healing and reduce scar tissue formation. References 1 Kendal, A. R. et al. Multi-omic single cell analysis resolves novel stromal cell populations in healthy and diseased human tendon. *Sci Rep* 10, 13939, doi:10.1038/s41598-020-70786-5 (2020). 2 Dakin, S. G. et al. Inflammation activation and resolution in human tendon disease. *Science translational medicine* 7, 311ra173, doi:10.1126/scitranslmed.aac4269 (2015). 3 Dakin, S. G. et al. Chronic inflammation is a feature of Achilles tendinopathy and rupture. *Br J Sports Med* 52, 359-367, doi:10.1136/bjsports-2017-098161 (2018). 4 Dakin, S. G. et al. Persistent stromal fibroblast activation is present in chronic tendinopathy. *Arthritis Res Ther* Jan 25 (2017). 5 Dakin, S. G. et al. Increased 15-PGDH expression leads to dysregulated resolution responses in stromal cells from patients with chronic tendinopathy. *Sci Rep* 7, 11009, doi:10.1038/s41598-017-11188-y (2017). 6 Dakin, S. G. et al. 15-epi-LXA4 and MaR1 counter inflammation in stromal cells from patients with Achilles tendinopathy and rupture.

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APSIPE.79

Female Rats Selected for High Intrinsic Aerobic Capacity Switch Inflammatory Cascade to Maintain Vascular Physiology

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Sedentary behavior often leads to chronic inflammation, a triggering factor of metabolic syndrome (MetS). Conversely, exercise enhances resolution of inflammation. Two primary markers essential for vascular inflammation are cyclooxygenase (COX) and lipoxygenase (LOX); however, it is unknown how this inflammatory cascade is affected in MetS. To study this, we used rats selected for their low (LCR) and high (HCR) intrinsic aerobic capacity. LCR are models for MetS and have increased risk of vascular dysfunction, while HCR are models for a genetically untrained "exercise" state and show decreased risk factors. Therefore, we questioned how differences in the inflammatory cascade were affecting vascular physiology, leading to divergent disease and health states in these models. As obese females present with increased risk of cardiovascular disease compared to males, female LCR were used as the model for metabolic syndrome. Thus, we hypothesized that female LCR would present increased COX and LOX-dependent vascular function, while female HCR would show decreased COX-dependent vascular function and increased resolution of inflammation. We used 18-28 week old female LCR, HCR, and high-response trained rats (HRT) as control. HRT have a trained aerobic capacity higher than LCR but lower than HCR. Statistics: one-way ANOVA: $p < 0.05$: *vs. control (HRT); #vs. HCR; &vs. absence of indomethacin (Indo), %vs. absence of CDC. As expected, LCR showed increased periovarian fat pad [HRT: 0.95 ± 0.1 (n=7) vs. LCR: 1.80 ± 0.1 * (n=7) vs. HCR: 1.18 ± 0.1 (n=7) (g)]. Mesenteric resistance arteries (MRA) were mounted onto a wire myograph. LCR MRA displayed an impaired phenylephrine (PE)-induced maximum contractile response (Emax) [PE: Emax: HRT: 106 ± 5 (n=9); LCR: 80 ± 9 * (n=13); HCR: 119 ± 10 (n=11)]. Incubation with a nonspecific COX inhibitor [Indo, 10 μ M] abolished PE-induced contraction in LCR MRA, but interestingly, had little effect on HCR MRA [PE+Indo: Emax: HRT: 17 ± 13 #& (n=8); LCR: 4 ± 2 #& (n=8); HCR: 91 ± 1 * (n=7)]. Similarly, HRT and LCR MRA incubated with a 5/12 LOX inhibitor [CDC, 1 μ M] displayed

impaired Emax to PE [PE: Emax: HRT: $11 \pm 6\%$ (n=6); LCR: $23 \pm 13\%$ (n=5); HCR: $83 \pm 7^*$ (n=5)]. No differences in ACh-induced maximum relaxation (Emax) were seen between models; however, in presence of Indo, LCR MRA showed impaired ACh-induced Emax [ACh: Emax: HRT control - 90 ± 3 vs. Indo - 85 ± 7 (n=7); LCR control - 98 ± 0.3 vs. Indo - 74 ± 12 (n=7); HCR control - 91 ± 3 vs. Indo - 95 ± 3 (n=7)]. Conversely, no differences were seen in ACh-induced Emax in the presence of CDC. Overall, these data reveal a novel mechanism for vascular inflammation in LCR and HCR models. LCR display chronic inflammation through COX-dependent vascular contraction and relaxation, and 5/12 LOX-dependent vascular contraction. In contrast, HCR suggest a swap between COX and 5/12 LOX-dependent vascular physiology in the presence of a COX and 5/12 LOX inhibitor, perhaps switching to a higher resolution state to maintain vascular contractility. This study constitutes a new foundation for the discovery of alternative mechanisms for vascular inflammation in low and high aerobic capacity.

APSIPE.80

Effect of acute bout of resistance exercise in combination with leucine-enriched essential amino acids intake on the protein expression of vitamin D receptor in human skeletal muscle

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Skeletal muscle mass and function are important for physical performance and activity of daily living. Skeletal muscle-specific and systemic vitamin D receptor (VDR) knockout mice showed decreased muscle mass and function (Girgis et al., 2019), suggesting that vitamin D metabolism is critical for maintaining skeletal muscle mass and function. On the contrary, a single bout of resistance exercise has been shown to increase not only mTORC1 activation which is responsible for muscle protein synthesis (Terzis G. et al., 2008), but also the protein expressions of VDR and CYP27B1, an enzyme which converts inactive 25(OH)D into 1,25(OH)2D (Makanae et al., 2015). Additionally, previous study demonstrated that leucine-enriched essential amino acids (LEAA) intake stimulated muscle protein synthesis (Syed S. I. Bukhari et al., 2014). In our laboratory, it has been demonstrated that adding LEAA further augmented resistance exercise-induced mTORC1 activation after a single bout of resistance exercise (Takegaki et al., 2020). However, combined acute effects of resistance exercise and LEAA intake on vitamin D metabolism in human skeletal muscle has not been clarified. Therefore, the purpose of this study was to investigate the effect of acute bout of resistance exercise in combination of LEAA ingestion on the protein expression of VDR in human skeletal muscle. Twenty healthy young men (21.6 ± 1.4) were recruited and overnight fasted before the study. In the morning of the study, they ingested placebo or LEAA before and after the knee extension and knee flexion exercises. Each exercise was performed 10

reps at 70% of the 1-RM, for a total of 3 sets. Muscle biopsies were taken from the vastus lateralis muscle before and 90 min after resistance exercise. Protein expression levels of VDR and CYP27B1 were analyzed by Western Blotting. All procedures were approved by the Ethics Committee for Human Experiments at Ritsumeikan University (BKC-IRB-2018-058) and the institutional review board of Ajinomoto Co., Inc. (No. 2018-016) and was conducted in accordance with the Declaration of Helsinki. As results, single bout of resistance exercise significantly increased the protein expression level of VDR in the placebo group ($p = 0.040$), but not in the LEAA group. The increase of VDR protein expression in the LEAA group tended to be lower than that in the placebo group (38% and 2% for LEAA and placebo, respectively; $p = 0.058$). However, no significant change and difference in/between either group was observed for CYP27B1 protein expressions. In conclusion, current study indicated that ingestion of LEAA pre and post exercise attenuates the resistance exercise-induced increase in VDR protein expression in young men during fasted state. On the contrary, intake of LEAA does not lead any change in the CYP27B1 protein expression.

APSIPE.81

Exercise training differentially affects androgen control of adipocyte fatty acid uptake by sex and adipose tissue depot

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White adipose tissue (WAT) secretes factors to modulate whole body metabolism and insulin sensitivity. In rodents, aerobic exercise decreases adipocyte size and lipid content and promotes beige-ing of WAT. Androgens also modulate WAT function and peripheral insulin sensitivity. Low androgen levels in men and elevated androgen levels in women cause accumulation of visceral WAT (vWAT) and development of insulin resistance. Yet, little research has examined exercise as a therapy for diseases of androgen imbalance. We hypothesized that aerobic exercise would decrease vWAT adipocyte size and baseline and androgen-induced fatty acid uptake in female, but not male, pigs. Male and female Yucatan pigs were exercised (n=4 female; n=2 male) or sedentary (n=3 female; n=2 male) for 14 weeks. The exercise group completed a progressive exercise regimen (treadmill run; five days/week up to ~75 min per day), while sedentary pigs were limited to pen activity. Both sedentary and exercised pigs were fed 2,500 kCal per day with ad libitum access to water. Pigs were humanely euthanized and vWAT and subcutaneous abdominal WAT (scWAT) were collected for assessment of basal (0nM) and ins-stim (10nM) fatty acid uptake with no steroid or 1nM of dihydrotestosterone (DHT), flutamide (FLUT), DHT + FLUT, fulvestrant (FULV), DHT+FULV, or DHT+FLUT+FULV. Duplicate vWAT and scWAT explants

were incubated 45 min with steroids $\pm$ insulin as well as BODIPY-C12 (fatty acid), calcein red-orange (live stain), and DAPI (nuclear stain). Explants were rinsed, fixed, and imaged with confocal microscopy. Total BODIPY-C12 uptake and Feret diameter per adipocyte per WAT stack were assessed with ImageJ (NIH). Normality of the data was checked prior to analysis with PROC MIXED (SAS, Inc, Cary, NC). Components in the model were: sex, exercise, tissue, insulin, and steroid. Sex, tissue, steroid, and sex*tissue were significantly different ($p < 0.01$). Exercise had a sexually dimorphic effect on vWAT, decreasing adipocyte size in males ($p < 0.0001$) and increasing ins-stim fatty acid uptake in females ($p < 0.01$). Contrary to our expectation, exercise did not affect vWAT adipocyte size in females ($p > 0.05$). DHT decreased basal ($p = 0.04$) and ins-stim ($p = 0.05$) fatty acid uptake in exercised female vWAT. Degrading estrogen receptor alpha (ER α) with FULV increased ins-stim fatty acid uptake in scWAT of exercised females ($p = 0.05$) and decreased ins-stim fatty acid uptake in exercised male vWAT ($p = 0.05$). Antagonizing androgen receptor (AR) with FLUT decreased ins-stim fatty acid uptake in exercised male vWAT ($p = 0.04$). Exercise has opposite lipogenic effects in vWAT of males and females. Interestingly, exercise increases the sensitivity of vWAT to the anti-lipogenic effects of androgens in females. Lastly, ER α is anti-lipogenic in exercised female scWAT but pro-lipogenic in exercised male vWAT. Our findings suggest that exercise regulates fatty acid uptake in WAT in response to androgens via ER α and AR. This research was funded by NIH grants K01-OD011177 (ANF) and R01-HL139903 (CLH).

APSIPE.82

Association between vascular parameters and blood pressure increase during isometric handgrip in hypertensive men.

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OBJECTIVES: Blood pressure increase during isometric exercise can be expressive in hypertensives being important to detect physiologic parameters that may be associated with the greater responses. Thus, this study aimed to analyze whether the increase in systolic blood pressure (DSBP) during isometric handgrip is associated with vascular parameters in hypertensive men. **METHODS:** Thirty-five middle-aged hypertensive men receiving regular anti-hypertensive medication (53 ± 9 years; systolic/diastolic blood pressure: $130 \pm 11/87 \pm 9$ mmHg) executed 1 set of 2-min isometric handgrip contraction at 30% of MVC. Beat-by-beat blood pressure (photoplethysmography) was continuously measured for 3-min before and during all the exercise execution. δ SBP was calculated by the difference between peak and pre-exercise (average of 3 min) values. In another visit, at least 48h apart, brachial artery (arterial diameter, blood flow, peak shear stress rate and flow mediated dilation) and carotid (diameter, intima-media

thickness and stiffness) vascular parameters were assessed by ultrasonography and an automatic detection software. Correlations between vascular parameters and δ SBP during isometric handgrip were tested by Pearson's correlations. **RESULTS:** No significant correlation was observed between any vascular parameter and δ SBP during isometric handgrip. Brachial parameters: arterial diameter ($r = +0.162$ $p = 0.369$), blood flow ($r = +0.063$ $p = 0.724$), shear rate ($r = -0.048$ $p = 0.790$) and flow-mediated dilatation ($r = +0.206$ $p = 0.243$). Carotid parameters: diameter ($r = -0.140$ $p = 0.568$), intima-media thickness ($r = 0.321$ $p = 0.131$) and stiffness ($r = -0.077$ $p = 0.753$). **CONCLUSION** In medicated hypertensive men, δ SBP during isometric handgrip is not associated with carotid nor brachial vascular structure or function, suggesting that greater responses may not be attributed to vascular abnormalities in this vessels. **FUNDING:** FAPESP (process number: 2018/23653-3, 2018/12390-1, 2018/12390-1, 2019/02649-0)

APSIPE.83

Late-In-life treadmill-Training Improves Autophagy, Protein clearance, and Contractile Function in Mouse Hearts

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Evidence exists that a progressive decline of the cellular quality control mechanism called macroautophagy occurs during the process of primary aging. This leads to the accumulation of protein aggregates (i.e., proteotoxicity) that would otherwise be transported to and degraded in the lysosome. Post-mitotic cells like cardiomyocytes rely heavily on macroautophagy because of their inability to clear defective proteins via cell division. While voluntary wheel-running improves cardiac autophagy, lessens cardiac proteotoxicity, and attenuates myocardial dysfunction in adult mice with desmin-related cardiomyopathy (Bhuiyan et al., JCI, 2013), the ability for a physiological intervention to improve age-associated cardiac proteotoxicity is unknown. We hypothesized that late-in-life exercise training improves autophagy, protein aggregate clearance, and function that is otherwise dysregulated in hearts from old vs adult mice. As expected, 24-month old male C57Bl6 mice (old) exhibited repressed autophagosome formation in the heart, myocardial protein aggregate accumulation, systolic and diastolic dysfunction, and reduced exercise capacity vs. 6-month old (adult) mice (all $p < 0.05$; $n = 10$ per group). Separate cohorts of 21 month old mice completed a 12-week progressive resistance and duration treadmill-running program (old-ETR) that improved (all $p < 0.05$): (i) body composition; (ii) maximal workload capacity; and (iii) soleus muscle citrate synthase activity vs.

age-matched sedentary mice (old-SED). Of note, (iv) mRNA and protein expression of autophagy markers indicated trafficking of the autophagosome to the lysosome increased, (v) protein aggregate clearance improved, and (vi) contractile function was enhanced (all $p < 0.05$), in hearts from old-ETR vs. old-SED mice. Dietary maneuvers (e.g., caloric restriction, nutraceutical supplementation) and pharmacological interventions (e.g., rapamycin) are reported to elevate myocardial autophagy and mitigate or reverse age-associated cardiac dysfunction. Here we show the first evidence that a physiological intervention i.e., late-in-life exercise training, improves autophagic flux, protein aggregate clearance, and contractile function in hearts from aged mice. Support or Funding Information JMC (AHA 20PRE35110066, UU Research Fellowship), KL [APS STRIDE, UU Undergraduate Research Program (UROP)], SKP (AHA 17POST33670663), CR (APS UGRF, UROP), LT (UROP), RG (UU College of Health Seed Grant); SB (NIDDK R01-DK-098646-01A1, R01-DK-099110, AHA 16GRNT30990018), JDS (AHA16GRNT31050004, NIH RO3AGO52848, NIHRO1HL141540).

APSIPE.84

Left ventricular myofilament calcium sensitivity increased by treadmill running in a diabetic rat model

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The cardiovascular benefits of regular exercise are unequivocal, yet patients with type-II diabetes respond poorly to exercise due to a reduced cardiac reserve. The contractile response of diabetic cardiomyocytes to beta-adrenergic stimulation is attenuated, which may result in altered myofilament calcium sensitivity and post-translational modifications of cardiac troponin I (cTnI). Treadmill running increases myofilament calcium sensitivity in non-diabetic rats, however diabetes alters myofilament protein structure and, consequently, function. Additionally, it remains unclear if the cellular mechanisms behind reduced cardiac reserve can be reversed in the diabetic heart after training. Thus, we hypothesized that endurance training would increase calcium sensitivity of diabetic cardiomyocytes. We also hypothesized that alterations in site-specific phosphorylation of cTnI, a key regulator of contraction, would correspond with an increase in calcium sensitivity in a rat model of diabetes. Calcium sensitivity, or pCa50, was measured in Zucker Diabetic Fatty (ZDF) rat hearts, non-diabetic (nDM) and diabetic (DM), after 8 weeks of either a sedentary (SED) or progressive treadmill running (TR) intervention. Skinned cardiomyocytes were connected to a capacitance-gauge transducer and a torque motor to measure force as a function of pCa ($-\log[Ca^{2+}]$). Specific phospho-sites on cTnI were quantified by immunoblot. A two-way ANOVA was used to determine disease, intervention or interaction effects. DM rats were hyperglycaemic compared to nDM rats, but training significantly reduced fasted blood glucose concentrations in DM rats (30 vs. 23.5 mmol/L, DM SED vs. DM TR respectively; $p = 0.008$). DM cardiomyocytes had greater

pCa50 than nDM cardiomyocytes regardless of the intervention ($p = 0.013$). Additionally, training increased pCa50 in DM and nDM cardiomyocytes ($p = 0.009$). There was a reduction in the phosphorylation of cTnI amino acid residues Ser23/24, a crucial protein kinase A site, and Thr143, a protein kinase C site, in DM TR hearts compared to DM SED hearts, but no difference in site-specific phosphorylation between nDM and DM SED hearts. These findings suggest regular endurance training improves, but does not normalize, the function of diabetic cardiomyocytes. However, it is unclear whether training-induced alterations in cTnI directly affect these changes. Future works should test if the alterations in cTnI phosphorylation seen in this study are responsible for the increase in pCa50 in the trained diabetic heart.

APSIPE.85

Late-in-life exercise training improves composition of the intestinal microbiome.

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An increased abundance of microbial taxa associated with intestinal dysbiosis exists in aged vs. adult C57Bl6 male mice that concurrently display myocardial dysfunction. Here we tested the hypothesis that late-in-life exercise-training rejuvenates the composition of intestinal microbes to an extent that associates positively with indexes of systolic and diastolic function. Exercise capacity, systolic function (ejection fraction; EF) and diastolic function [velocity during early diastolic filling / velocity during late diastolic filling (E/A ratio)], and microbial community profiling (16S rRNA gene amplicon sequencing of cecal contents), were evaluated in 7-month (adult) and 24-month old male mice that did not train (older), and older mice that completed a treadmill-running program from 21-24 months of age (older-trained; $n=10$ per group). Compared to adult mice, older animals displayed: (i) reduced exercise capacity; (ii) lower soleus muscle citrate (CS) synthase activity; (iii) depressed systolic and diastolic function, (iv) decreased abundance of commensal microbes *Paraprevotella* and *Rikenella*; and (v) increased abundance of microbes *Anaerotruncus* and *Ruminococcus* (all $p < 0.05$). These results substantiate age-associated myocardial dysfunction and intestinal microbial dysbiosis of older vs. adult mice. Compared to older mice, older trained mice exhibited: (i) greater maximal exercise capacity and soleus muscle CS activity; (ii) increased systolic and diastolic

function; (iii) elevated commensals *Paraprevotella*, *Rikenella*, *Parabacteroides*, and *Bifidobacterium* (all $p < 0.05$); but (iv) no suppression of age-associated elevations of the microbes *Anaerotruncus* and *Ruminococcus*. Of note, heightened abundance of commensals *Parabacteroides* and *Dorea* in older-trained mice correlated positively ($p < 0.05$) with improved FS and E/A. On balance, these findings indicate late-in-life exercise training supports intestinal symbiosis by improving commensal microbes. Support or Funding Information JMC (AHA 20PRE35110066, UU Research Fellowship), KL [APS STRIDE, UU Undergraduate Research Opportunities Program (UROP)], CR (APS UGRF, UROP), LT (UROP), SKP (AHA 17POST33670663), UDW (USDA-ARS Project 6026-51000-012-06S), SB (NIDDK R01-DK-098646-01A1, R01-DK-099110, AHA 16GRNT30990018), PVAB (5R01AT010247, NIFA-2019-67017-29253); JDS (AHA16GRNT31050004, NIH RO3AGO52848, NIHRO1HL141540).

APSIPE.86

The Effects of Exercise on Mitochondrial Enzyme Expression in High-Calorie Fed Rats

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Introduction Weight gain and obesity have a direct relationship with a sedentary lifestyle and easy access to high-calorie foods. However, fat-rich diet negatively affects muscle mitochondrial functions, leading to mitochondrial damage and apoptosis. Citrate synthase (CS) is one of the most important biomarkers of mitochondrial content and muscle oxidative capacity. On the other hand, carnitine palmitoyltransferase-I (CPT-I) and carnitine palmitoyltransferase-II (CPT-II) have a crucial role during the transport of long-chain fatty acids into mitochondria. This study aims to evaluate the effect of high-calorie feeding and regular aerobic exercise on the skeletal and cardiac muscle CS, CPT-I and CPT-II enzyme expressions in rats. **Material-Method** Wistar-Albino male rats were used in this study. Ethics committee approval (25.06.2015/5/6) was obtained from the Local Ethics Committee of Cukurova University in accordance with the Declaration of Helsinki. Rats were fed with normal calorie (NC) and high calorie (HC, 10% higher calories than NC) chow. Rats divided into four groups: NC sedentary (NC-S, n=15), NC exercising (NC-E, n=15), HC sedentary (HC-S, n=15) and HC exercising (HC-E, n=15) animals. The animals were separated to their groups at the age of 5th week, and at the age of 12th week began to moderate treadmill exercise training 5 days/week for 8 weeks. Rats were sacrificed at the end of the training period, and gastrocnemius-soleus muscle complex (GC-S) and hearts were excised. CS, CPT-I and CPT-II mRNA expression levels were analysed by Real-Time PCR method. The change in gene expression was calculated by the formula $2^{-\Delta\Delta Ct}$. Changes in mRNA expression levels were expressed as multiple increases. Normally distributed data were evaluated using one-way ANOVA with Bonferroni test for post hoc analyses. Non-normally

distributed data were evaluated using the Mann Whitney U test. Significance was set at $p < 0.05$. **Results** Final body weight of HC fed rats was significantly higher than NC fed rats. Besides, GC-S weight was significantly higher in all groups than NC-S rats. In NC-E group CS mRNA expression was increased by approximately 1.4 fold compared to the NC-S group ($p < 0.05$). In the HC-E group, there was a 1.32 fold increase at CS mRNA expressions observed compared to the HC-S group ($p > 0.05$). CPT-I enzyme expression in skeletal muscle did not change significantly for all groups. However, the CPT-II expression of skeletal muscle in the NC-E group increased by 41% compared to the sedentary group ($p > 0.05$), exercise did not make any significant difference in HC-E group. Calorie intake and exercise did not change left ventricular (LV) CS, CPT-I and CPT-II enzyme expression significantly. **Discussion** Even 10% of calorie-rich food consumption was sufficient for weight gain in non-obese rats. Exercise intervention without calorie restriction did not cause weight loss. Significant increase of skeletal muscle CS expression in NC group is important to show that low-to-moderate exercise intensities triggered adaptive changes, which was not observed in the HC group. CPT-I and CPT-II enzyme expressions did not change significantly in either group. In addition to that, exercise and HC diet did not affect LV CS, CPT-I and CPT-II enzyme expressions significantly. Further studies are required to determine the effect of various feeding strategies and exercise intensities to evaluate the mitochondrial function and fat metabolism.

APSIPE.87

Exercise Attenuates Tumor Growth in an Animal Model of Castration-Resistant Prostate Cancer

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Prostate cancer is the most common solid-tumor cancer among men in the United States. Although most patients respond well to androgen-deprivation therapy in the initial stage, cancer relapses and progresses to castration-resistant prostate cancer (CRPC). Exercise has been recognized as beneficial in prostate cancer by reducing the risk of advanced-stage prostate cancer and mortality. However, it remains largely unknown about the impact exercise has on CRPC and its molecular mechanisms. **Purpose:** The purpose of this study is to determine the effects of voluntary wheel running on tumor growth and intertumoral molecular pathways in a mouse xenograft model of CRPC. **Methods:** Male immunodeficient mice were surgically castrated and then injected subcutaneously with human prostate cancer cells (CWR22-RV1 cells, 1×10^6) in suspension. Twenty-four hours after tumor transplantation, mice were randomly assigned to exercise (n=6) or non-intervention control (n=6) group. The exercise group was given voluntary access to a running wheel for the duration of the study, while the control group remained sedentary. Bodyweight, food consumption, and tumor volume were monitored throughout the study. Running wheels were

removed from the cage on day 21 of the intervention, and tissues were collected 24 hours after the running wheel removal. RNA was isolated from tumor samples, and gene expressions were determined using RT-PCR. RNA sequencing was performed on tumor samples using an Illumina HiSeq 2500. Differentially regulated genes were identified as $\text{Log}_2(\text{FC}) \geq 0.58$ and $P < 0.05$, and gene set enrichment and gene ontology were used to determine pathway modulations with $\text{FDR} < 0.25$ and $P < 0.05$. Results: There were no differences in body weight or food consumption between groups. Exercise attenuated tumor growth throughout the study ($P = 0.037$). Tumor volume in the exercise group had a trend that it was smaller than the control group at the endpoint (-31.2% , $P = 0.06$). Prostate cancer diagnosis marker prostate-specific androgen (PSA) gene expression was significantly reduced in CRPC tumors from the exercise group compared to the control group (-26.7% , $P = 0.004$). Furthermore, exercise differentially expressed 287 genes in the CRPC tumors by upregulating 157 genes and downregulating 120 genes in the exercise group compared to the controls. The gene enrichment analysis further revealed that epithelial tissue mesenchymal transition and angiogenesis were downregulated in the exercise group compared to the controls. Lastly, gene ontology analysis indicated that voluntary wheel running downregulated pathways relating to angiogenesis, blood vessel development, extracellular matrix organization, endothelial cell proliferation, and migration while increasing pathways relating to response to hypoxia. Conclusion: Our data demonstrate that voluntary exercise effectively attenuates tumor growth in a mouse model of CRPC, possibly via the reduction in angiogenesis and endothelial cell proliferation in CRPC.

APSIPE.88

The effects of high intensity exercise training on cardiac morphology and function in a preclinical Hypertrophic Cardiomyopathy (HCM) mouse model

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Concern for worsening of cardiac remodeling and dysfunction, as well as the risk of sudden cardiac death, have led to clinical recommendations for patients with HCM to not participate in intense physical activity or exercise. Retrospective data, however, has suggested that patients with HCM who participate in lifetime vigorous exercise can incur favorable cardiac remodeling resembling that in healthy athletes. Further, our recent clinical trial determined improved cardiorespiratory capacity in patients with HCM following moderate intensity exercise absent of adverse events. Cardiac hypertrophy is highly variable in HCM patients and may mask physiologic hypertrophy induced by exercise. PURPOSE: Evaluate the effects of high intensity interval training (HIIT) on select cardiac structural and

functional parameters known to be altered by exercise in a transgenic cardiac troponin T delta160E (TG) HCM mouse model. METHODS: Female and male C57BL/6J non-transgenic (nTG) ($n = 12$) and TG ($n = 6$) mice (13-16 mos) underwent a translationally parallel cardiac rehabilitation HIIT protocol. One treadmill training bout included 4-4 minute high intensity intervals ($\sim 80\%$ preVO₂ max speed) interspersed by 5-3 minute recovery intervals ($\sim 50\%$ preVO₂ max speed) for 31 total minutes. Bouts were repeated 3 times/wk for 6 wks. Pre and post HIIT murine echocardiography (ECHO) was measured and analyzed by a blinded technician. Paired t-tests were used for data analysis. RESULTS: Comparing pre- and post-HIIT, left ventricular (LV) mass was significantly greater in both nTG (mean difference $\pm$ SEM: $0.55\text{g} \pm 0.205$; $p = 0.0219$) and TG (mean difference $\pm$ SEM: $0.490\text{g} \pm 0.0924$; $p = 0.032$) mice following exercise training. LV volume also increased following HIIT in nTG mice (mean difference $\pm$ SEM: $7.068\text{uL} \pm 2.59$; $p = 0.0232$) but was not observed in TG mice (mean difference $\pm$ SEM: $0.0755\text{uL} \pm 2.357$; $p = 0.9757$). Left atrial (LA) volumes, cardiac output (CO), stroke volume (SV) and ejection fraction (EF) all remained unchanged in both nTG and TG post HIIT. CONCLUSION: In a preclinical HCM mouse model that doesn't demonstrate pathologic cardiac hypertrophy, HIIT training resulted in LV hypertrophy similar to what was observed in nTG wildtype mice. Our data provides initial evidence that high intensity exercise training may result in physiologic hypertrophy in HCM mice. Biochemical analyses are underway to elucidate the underlying type of cardiac remodeling that resulted from exercise training.

APSIPE.89

A Single Bout of Exercise Increases Vascular Insulin Sensitivity in Adults with Obesity

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Purpose: Obesity increases type 2 diabetes and cardiovascular disease risk, in part, through decreased insulin sensitivity. While a single session of exercise enhances metabolic insulin sensitivity in adults with obesity, the effect on vascular insulin sensitivity is unknown. Thus, we assessed the impact of a single bout of exercise on insulin-stimulated responses in conduit arteries and capillaries. Methods: Twelve sedentary adults (6F/6M, Age: 49.5 ± 2.3 yrs; VO₂max: 23.7 ± 1.6 kg/mg/min) with obesity (BMI: 34.5 ± 1.2 kg/m²) completed a control and acute exercise bout (70% VO₂max to expend 400 kJ between 4-7pm) condition. After an overnight fast, participants underwent a 2-hr euglycemic-hyperinsulinemic clamp (90 mg/dL; 40 mU/m²/min) to determine vascular and metabolic (glucose infusion rate, GIR) insulin sensitivity. Endothelial function was measured by brachial arterial flow-mediated dilation (FMD) while capillary blood flow (MBF = microvascular blood volume * microvascular flow velocity) was assessed using contrast enhanced ultrasound in the

forearm. Fat (Fox) and carbohydrate oxidation (CHOox) were also measured to determine substrate utilization and non-oxidative glucose disposal (NOGD). All measures were collected at 0 and near 2-hr of the clamp. Statistical significance was accepted as $P \leq 0.05$. Results: Exercise increased insulin-stimulated pre-occlusion diameter (Δ : 0.15 ± 0.20 vs -0.02 ± 0.26 mm, $P=0.01$), yet had no further effect on allometrically scaled FMD ($P=0.57$). Exercise also raised MBF versus control (condition effect: 0.05 ± 0.03 vs 0.02 ± 0.02 Al/sec, $P=0.03$), despite no effect on fasting MBF. This suggests that that exercise elevated microvascular insulin sensitivity. Prior exercise increased GIR (3.51 ± 0.55 vs 2.95 ± 0.47 mg/kg/min, $P<0.05$), which correlated with the improvements in MBF after exercise ($r=0.58$, $P<0.05$). Exercise did not affect fasting Fox ($P=0.07$) or insulin-stimulated CHOox ($P=0.08$), but it increased NOGD compared to control (2.56 ± 0.51 vs 1.89 ± 0.39 mg/kg/min, $P<0.05$). Conclusion: A single bout of exercise increases muscle vascular insulin sensitivity to support glucose metabolism in adults with obesity. Further work is needed to unravel the mechanism(s) for these insulin responses and to assess the impact of exercise dose on optimizing chronic disease risk reduction. Funding: ACSM Foundation Doctoral Student Research Grant (EMH) and NIH RO1-HL130296 (SKM).

APSIPE.90

The Effect of Notch Manipulations on Skeletal Muscle

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Notch is a complicated signaling pathway that has opposing roles in skeletal muscle. There are conflicting reports on the role of Notch in skeletal muscle repair. The purpose of this study was to compare various models of Notch manipulation on indices within skeletal muscle. METHODS: Using either C2C12 cell culture myoblasts or myotubes) or mice exposed to exercise, the Arthur laboratory utilized Notch inhibition models (gamma secretase inhibitor or short hairpin loop RNA [shRNA]) as well as Notch activation modles (Delta like 1 in suspension or adhesion) to measure the effects of Notch on myogenesis, collagen, protein synthesis and mTOR signaling. All animal protocols were approved through the Institutional Animal Care and Use Committee and the APS Guiding Principles in Care and Use of Animals. RESULTS: Inhibition of Notch with shRNA in mice exposed to injurious exercise result in increased MyoD ($p \leq 0.05$) and Myogenin ($p \leq 0.05$) expressions, protein synthesis ($p \leq 0.05$) and collagen levels ($p = 0.04$). Force activating Notch may not affect mTOR signaling in proliferating C2C12 cells. CONCLUSION: Notch inhibition accelerates muscle repair but may also promotes the expression of other cell types including collagen. There may be a ceiling of the ability of Notch to influence mTOR expression in proliferating C2C12s.

APSIPE.91

Arterial Blood Gases profiles during Breath-Hold Diving.

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Background: adaptations during voluntary breath-hold diving have been increasingly investigated since these athletes are exposed to critical hypoxemia during the ascent. However, only a limited amount of literature explored the pathophysiological mechanisms underlying this phenomenon with direct measurements. With this study we measured arterial blood gases before, at depth, and after a breath-hold dive in real conditions, to explore the variations of oxygen (O₂) and carbon dioxide (CO₂) developed by these athletes. Methods: Six well-trained breath-hold divers were enrolled for the experiments held at the "Y-40 THE DEEP JOY" pool (Montegrotto Terme, Padova, Italy). Before the experiments, an arterial cannula was inserted in the radial artery of the non-dominant limb. All divers were involved in two experimental sessions. In the first, arterial blood samples were obtained: at rest before submersion; at depth during a sled-assisted dive at -42 m; and 2 minutes after the end of the dives. In the second session, the divers performed: a breath-hold while moving at the surface using a sea-bob; a sled-assisted breath-hold dive to 42 m; and a breath-hold dive to 42 m with fins. Arterial blood samples were obtained at rest and at the end of each breath-hold, before the resumption of normal breathing. Results: No diving-related complications were observed, and the dives had similar durations. The reduction of arterial partial pressure of oxygen (PaO₂) from 96.2 ± 7.0 mmHg (mean $\pm$ SD) at rest to 64.5 ± 4.7 mmHg after breath-hold at surface (attached to the sea-bob) reflected oxygen consumption by basal metabolism and a minimal energy expenditure needed to remain in contact with the instructor/sea-bob system. Interestingly, PaO₂ dropped after the sled-assisted dive (39.8 ± 8.7 mmHg) and especially after the dive with fins (31.6 ± 17.0 mmHg). At the bottom of the sled-assisted dive, PaO₂ reached a mean value of 197.8 ± 103.7 mmHg. The high SD in this sample is related to extremely low values found in two subjects, in which a paradoxical hypoxemia at depth was noted (respectively: 75 and 61 mmHg). Overall, such changes in PaO₂ could be explained by the ventilation/perfusion mismatch and right-to-left intrapulmonary shunt caused by atelectasis at depth, in association with oxygen consumption by normal metabolism, and exacerbated by physical effort. Of note, one subject reached a PaO₂ of 18 mmHg at the end of the dive with fins but without symptoms, thus reflecting intense adaptations of trained breath-hold divers to extreme hypoxemia. The arterial partial pressure of CO₂ varied somewhat, without reaching extreme values. Mean values at depth showed a slight increase but within normal ranges (42.7 ± 12.3 mmHg) also after each step (38.2 ± 3.0 mmHg at rest; 31.4 ± 3.7 mmHg after the sled-assisted dive; 36.1 ± 5.3 after the dive with fins). Conclusion: We confirmed that the arterial partial pressure of oxygen reaches hazardously low values at the end of breath-hold, especially after the dive performed with voluntary effort, and that some subjects

show a paradoxical hypoxemia at depth probably related to pulmonary atelectasis. The implications of our experiments shed light on clinical unanswered questions regarding hypoxic syncope and the risk of sudden death in breath-hold divers.

APSIPE.92

Mechanoreflex-induced chronotropic and ventilatory responses are sensitized by metabolites accumulation

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BACKGROUND: The exercise pressor reflex plays an important role in the cardiovascular and ventilatory adjustments to exercise. Even though animal studies suggest that the previous activation of the metabolically sensitive pathway (i.e. metabolites accumulation) may sensitize the mechanoreflex-induced response, this interaction in humans is still equivocal. **PURPOSE:** The aim of the study was to assess whether the magnitude of the hemodynamic and ventilatory responses evoked by isolated stimulation of the mechanoreceptors (i.e. no central command) are influenced by the metabolic conditions within the muscle. **METHODS:** Twelve healthy young subjects performed two exercise bouts consisting of 5 min of intermittent (60% duty cycle, 3s on/2s off) isometric knee-extensions performed 10% above the previously determined critical torque. In one bout, a suprasystolic cuff occlusion was applied on the proximal part of the exercised quadriceps at the end of the exercise bout (PECO trial). In the control trial (CON), the subject was allowed to recover without any occlusion. At the end of the 5 min recovery period, 1 min of passive leg movement (PLM) was performed both during PECO and CON. The order of the trials was counterbalanced. Central hemodynamics, ventilatory data and electromyography (EMG) from the vastus lateralis of the exercising/passively moved leg were recorded throughout the trial. Ventilatory data were linearly interpolated to 1-s intervals and together with hemodynamic data were smoothed using 3-s rolling averages. The last 30s of recovery were averaged and considered as baseline while the highest value from the 3-s rolling averages during the 1-min PLM was considered as peak response. For the purpose of this study, heart rate (HR) and minute ventilation (VE) were chosen as main outcomes. Data were statistically analyzed using a paired samples t-test and presented as mean $\pm$ SD. Statistical significance was considered for $p \leq 0.05$. **RESULTS:** δ HR response was higher in PECO compared to CON (6 ± 2 vs 3 ± 3 bpm, $p=0.05$). Also, δ V?E was higher in PECO than CON (5.1 ± 2.4 L/min vs 2.9 ± 1.9 L/min, $p=0.03$). No difference was found between baseline values in HR (71 ± 9 vs 69 ± 8 bpm, $p=0.35$) or V?E (11.7 ± 2.9 vs 12.0 ± 1.9 L/min, $p=0.72$). No difference was found in all the other measured variables between conditions ($p>0.05$). Importantly, EMG data showed no difference from baseline values during the PLM ($p>0.05$) nor between conditions, indicating that no active contractions were present during PLM in both trials. Even

though the response appears to be transient, there was a trend towards significance in the area under the curve between PECO and CON in both HR ($p=0.13$) and V?E ($p=0.09$). **CONCLUSION:** These findings suggest that mechanoreflex-mediated hemodynamic and ventilatory adjustments are sensitized by metabolites accumulation. **Funding:** No funding was received for this study.

APSIPE.93

The effects of structured physical activity on biomarkers of cellular senescence in older adults

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Background: Cellular senescence has emerged as a significant and potentially tractable mechanism of aging and multiple aging-related conditions. Biomarkers of senescent cell burden, including molecular signals in circulating blood cells and the abundance of circulating senescence-related proteins, have been associated with chronological age and/or parameters of biological age in humans. The extent to which these biomarkers are affected by interventions that enhance health and function has not yet been examined. **Methods:** We studied the effects of a 12-week structured exercise program on markers of senescence in circulating CD3+ T cells and circulating concentrations of senescence-related proteins in older adults. Further, we examined the relationship between the changes in these biomarkers and changes in measures of muscle performance, physical function, and self-reported health. **Results:** Thirty-three participants [mean (SD) age 66.4 (3.6) years; 88% female] completed the study. Exercise drove significant improvements in muscle strength, lower extremity mobility, body composition and self-reported health (all $p < 0.05$). Impressively, the expression of key markers of the senescence program, including p16Ink4a, p21, cGAS, and IFN γ , were significantly lowered in CD3+ T cells in response to the intervention (all $p < 0.05$), as were the circulating concentrations of multiple senescence-related proteins. Correlation analyses revealed significant associations between exercise-induced reductions in the abundance of senescence biomarkers and improvements in measures of muscle performance, physical function, and well-being. **Conclusion:** Our study provides first-in-human evidence that biomarkers of senescent cell burden in older adults are significantly lowered by a structured exercise program in concert with improvements in measures of health and function. The degree to which a reduction in senescent cells mediates the health benefits of exercise is worthy of further investigation.

APSIPE.95

Association between vascular parameters and blood pressure increase during dynamic resistance exercise in hypertensive men

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OBJECTIVE Blood pressure (BP) increase during dynamic resistance exercise (DRE) can be noticeable in hypertensives, being important to detect physiologic parameters that may be associated with the greater responses. Thus, this study aimed to verify whether carotid and brachial vascular parameters are associated with systolic blood pressure increase (Δ SBP) during DRE in hypertensive men. **METHODS** 35 treated hypertensive men (52.9 ± 8.7 years; baseline systolic/diastolic BP: $130 \pm 11/87 \pm 9$ mmHg) executed one set of unilateral leg extension at 50% of 1RM until concentric failure. BP was measured by photoplethysmography for 3 min before and throughout the exercise set. Δ SBP was calculated by the difference between their peak and pre-exercise values (mean of 3 min). In another visit, at least 48h apart, vascular parameters were obtained with ultrasonography. Common carotid artery diameter, arterial stiffness and intima-media thickness were assessed in 19 subjects, and brachial artery diameter, blood flow, conductance and response to hyperaemic stress (shear rate and flow mediated dilation – FMD) were assessed in 35 subjects. Associations between the vascular parameters and Δ SBP were evaluated by Pearson's correlation, considering $P < 0.05$ as significant. **RESULTS** Carotid arterial diameter showed a significant and negative correlation with Δ SBP increase during DRE ($r = -0.546$; $p = 0.016$), while all other carotid parameters presented no significant correlations (all $p > 0.05$). Considering the brachial vascular parameters, none of them presented any significant correlation with the Δ SBP increase during DRE (all $p \geq 0.05$). **CONCLUSION** In medicated hypertensive men, only carotid arterial diameter was associated with BP increase during DRE, suggesting that hypertensive men with smaller carotid diameter may present greater increases in SBP during DRE. **FUNDING:** FAPESP (process number: 2018/23653-3, 2018/12390-1, 2018/19151-2 and 2019/02649-0)

APSIPE.96

The influence of diet-induced obesity and exercise on bone marrow extracellular vesicles in an irradiated mouse model

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The purpose of this project was to determine the extent to which obesity and exercise influence the regenerative potential of bone marrow-extracellular vesicles (EVs) following radiation. Mice were randomly divided into

control ($n=20$; CON) or high fat diet ($n=20$; HF) groups, then further subdivided into exercise-trained (EX, $n=10$) or sedentary (SED, $n=10$). Mice underwent whole-body exposure to a 3 Gy dose of gamma-radiation at age 13 weeks followed by bone marrow collection at 20 weeks of age. EVs were subsequently isolated from the bone marrow by ExoQuick and ultracentrifugation. A non-irradiated, sedentary, control group ($n=10$) was used to determine the effects of radiation alone. The concentration of EVs in irradiated mice was significantly lower compared to EVs from non-irradiated mice ($p < 0.01$). Radiation, obesity, exercise, or their combination had no significant effect on hematopoietic stem and progenitor cells content in co-culture assays. Colony-forming unit (CFU) assays demonstrated that EVs from irradiated mice significantly increased the number of CFU

Granulocyte/Erythroid/Macrophage/Megakaryocyte progenitors, CFU- Granulocyte, and the total number of colonies compared to EVs from nonirradiated mice ($p < 0.01$). Our findings demonstrate that irradiation can deplete the concentration of bone marrow-EVs and that irradiated bone marrow-EVs can increase the total number of myeloid colonies in vitro. These findings suggest radiation induces myelopoiesis via a mechanism that includes EVs; however, exercise and obesity induce their effects via a different mechanism. This work was supported by the American Institute for Cancer Research, and Natural Sciences and Engineering Research Council.

APSIPE.97

Endurance exercise reverses the molecular and mechanical effects of metabolic syndrome on fast and slow muscles in sucrose-fed rats

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Introduction Skeletal muscle accounts for 40% of total body mass and for 22% of the basal metabolic rate. It is composed by fast and slow fibers that differ in their metabolic and contractile characteristics. The Soleus and the Extensor digitorum longus (EDL) muscles are composed by slow oxidative, and fast glycolytic fibers, respectively. Metabolic syndrome (MetS) seems to induce a shift from slow to fast phenotype of the Soleus, with minor changes in the EDL; it can also alter their contractile performance¹. Endurance exercise reverses the MetS effects on skeletal muscle by increasing oxidative capacity and inducing fiber type transition towards the slow phenotype. However, the molecular mechanisms responsible for these metabolic and mechanical changes are unclear; therefore the aim of this work is to study the mechanisms by which endurance exercise restores the mechanical and molecular properties of skeletal muscle during MetS. **Methodology** MetS was induced in 250 g male Wistar rats by adding 30% sucrose in drinking water. Sex and weight-matched rats under standard diet, were used as controls. After 14 weeks of MetS induction they were divided into two groups: one that exercised 5 days a week with an intensity higher than 70% of their VO₂ max, and other sedentary. After 9 weeks of training, the MetS

parameters were determined and then they were euthanized. Soleus and EDL were extracted from one leg and contractile performance tests were made to determine maximal force, fatigue resistance (FatR) and force recovery (FoR). The muscles from the other leg were used to determine myosin heavy chain isoforms (MyHC1) expression by qPCR. Results Exercise was able to mildly reduce the insulin resistance and the triacylglycerides in MetS animals, it was also able to significantly reduce the adiposity to control levels, and also, exercised MetS animals gained 50% less weight than sedentary control animals. MetS reduced the EDL mass by 10% and exercise was not capable of restoring it. MetS mitigated the mass gain of soleus induced by exercise. MetS induced a 25% increase in maximal force only in EDL of about 25% and exercise reverted this effect. EDL from exercised-MetS animals presented an increase of around 30% in the FatR. Exercise increased the FatR of soleus in control and MetS animals similarly. EDL and Soleus from sedentary MetS rats had a 30% increase in FoR. MetS induced more mechanical changes in the EDL than in the soleus, but exercise reverted the majority of them. Regarding fiber type composition, MetS induced a shift into a slower EDL phenotype increasing by 5-fold the levels of slow MyHC1, but exercise reverted this effect. No changes were seen in the Soleus. The shift into a slower phenotype could explain some, but not all the observed mechanical results, implicating that the other regulatory factors involved in fiber type transition such as PGC1 α , AMPK and NFAT and of key energetic metabolism enzymes, such as Hexokinase (HK), Phosphofructokinase 1 (PFK1) and Citrate synthase (CS) could be involved as well. Conclusions Exercise reverses the fiber type transition and contractile changes seen in EDL induced by MetS. The MetS did not induce a transition into a faster phenotype of the soleus. Interaction of other factors such as PGC1 α , AMPK, NFAT, HK, PFK1 and CS could explain these phenotypes. Financial support: INP 021/2017; CONACYT 619938. 1) Jason Tallis, Rob S James, Frank Seebacher. The effects of obesity on skeletal muscle contractile function. *J Exp Biol.* 2018 Jul 6;221(Pt 13).

APSIPE.98

Cellular and molecular responses to exercise and inactivity in human tendon loading

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The overall turnover of the tendon and cartilage in humans seems to be taking place primarily within the first 13-17 years of life, indicating that the major basic structure remains relatively unchanged through adult life. Nevertheless, mechanical loading of adult human tendon results in tendon cell responses by producing anabolic growth factors and loading-induced increase in tendon collagen synthesis. Comparing tissue turnover in different tissues simultaneously suggests that a combination of a more basic structure that remains relatively unchanged through adult life, and a smaller pool of collagen that is more quickly turned over and can be influenced by mechanical loading. Conversely, physical inactivity down

regulates collagen synthesis and phenotypic tendon characteristics. Adjustment of the tendon mechanical properties in the form of increased stiffness and modulus after strength training, and the reverse after period of immobilization occurs relatively faster than macroscopic morphological changes of tendon. Age related changes in tendon connective tissue with reduced stiffness is largely explained by a reduction in overall physical activity and can thus be at least partly counteracted by regular training.

APSIPE.99

Variable Stimulation of Norepinephrine Release and Increased Heart Rate During Simulated Exercise

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The benefits of exercise include physiological, biochemical and psychological changes. Exercise acutely improves health and lowers the long-term probability of cardiovascular diseases, diabetes mellitus, obesity, cancer (colon and breast), osteoporosis, hypertension and lipid abnormalities. Hence, finding a therapeutic method to provide the benefits of exercise to sedentary humans would be of substantial benefit to the healthcare industry. Evidence from animal studies indicates that exercise affects dopaminergic, noradrenergic and serotonergic systems. We previously found that octopamine, a *Drosophila* equivalent of norepinephrine, is critical for exercise adaptation in flies. Interestingly, octopamine feeding and intermittent activation of octopaminergic neurons are sufficient to provide a full suite of beneficial exercise adaptations in sedentary *Drosophila*, equaling the benefits experienced by siblings that performed chronic exercise training. We wondered if the same principle could be used in humans by using virtual reality simulated exercise to stimulate periodic norepinephrine release and heart rate increase. As a proof of principle, this study evaluated the ability of virtual reality-simulated exercise to increase heart rate and stimulate norepinephrine release. In this study, 24 college students (15 males and 9 females) were randomly assigned to one of the two 15-minute experimental or control group. The experimental group (11 males and 6 females) underwent three sessions, each on a separate day – exercise alone, virtual reality without exercise, virtual reality with exercise. Each participant's response to virtual reality was compared internally to his/her response to actual cycling. The control group (4 males and 3 females) underwent a single virtual reality session without prior exposure to exercise. Heart rate and norepinephrine were measured before and after each session. ECG was measured continuously during each session and was used to assess heart rate variability. VR simulation did not consistently increase heart rate and norepinephrine levels across all participants. However, when participants were grouped based on the intensity of

their response to the prescribed exercise protocol (moderate intensity vs high intensity), virtual reality-simulated exercisers experienced heart rate variability similar to that seen in actual exercise. This trend was not seen in participants that underwent high-intensity exercise. These results suggest that virtual reality exercise has the potential to mimic effects of moderate-intensity exercise. Further work examining the effects of long term exposure to virtual reality simulated exercise, and in more diverse participant groups, will be needed to better evaluate the potential for virtual reality as a therapy in enforced sedentary populations.

APSIPE.100

August Krogh, Nobel Laureate: 100 Years Anniversary Celebration

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Professor August Krogh was a unique scientist who won the Nobel Prize in Physiology and Medicine in 1920 for identifying the phenomenon of capillary recruitment in skeletal muscle during exercise. He inspired both the field of physiology itself, and contributed largely to the basis of exercise physiology as well as the development of clinical research within diabetes. After being a student of Prof Christian Bohr, he published papers demonstrating the diffusion (rather than active transport) of oxygen in the lungs, and later he collaborated with Prof Johannes Lindhard M.D. which was considered the birth of human integrative physiology or exercise physiology in Copenhagen. Studies focused on neural control of circulation and respiration, as well as substrate metabolism during exercise. The coupling of the physiologist Krogh and the medical doctor Lindhard was a fruitful symbiosis between natural and medical science, and they initiated the tradition for integrative and invasive exercise research as well as for metabolic research in Denmark. Finally, August Krogh also was the primus motor in bringing insulin to Denmark and he stimulated the development of pharmacological industry within treatment of diabetes in Denmark

APSIPE.102

Astaxanthin Supplementation Enhances Metabolic Adaptation with Aerobic Training in Elderly

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Background: Aerobic training (ET) is widely recommended for the elderly for its benefits on metabolic health, improved aerobic capacity, and reduced risk of frailty. However, it can lead to an exaggerated inflammatory response and oxidative stress that may contribute to suboptimal adaptations. The natural anti-oxidant and anti-inflammatory dietary supplement astaxanthin (AX) has been

found to increase fat oxidation (FATox) and endurance performance in mice and young athletes, but limited investigations have focused on the elderly. Purpose: We tested a formulation of AX in combination with ET in health subjects age (65-82 yrs) to determine if AX facilitates improvement in endurance and FATox with ET. Method: We conducted a randomized, double-blind, placebo-controlled study, forty-two subjects supplemented with a daily oral dose of AX or placebo (PL) during 3 months of ET. Specific muscle endurance was measured as number of contractions in ankle dorsiflexion. Whole body exercise endurance and FATox was assessed by a graded exercise test (GXT) in conjunction with indirect calorimetry. Results: Changes in fasting blood triglycerides were significantly elevated in AX after ET compared to PL (PL -5.5±4.2% vs. AX 14.0±6.0%). ET led to improved specific muscle endurance only in the AX group (Pre 353±26 vs. Post 472±41 contractions), and submaximal treadmill time improved in both groups (PL 40.8±9.1% and AX 41.1±6.3%). The increase in total FATox was greater in the AX (PL 0.6±0.23g vs. AX 1.33±0.27g) and was associated with reduced CHOox at early stages (PL -2.5±0.56g -4.5±0.82g) Conclusion: This is the first study to examine AX combined with ET on metabolic profile in elderly. AX promoted a shift toward increased FATox, which led to carbohydrate sparing effect at lower intensity. This work was supported by Astavita, Inc.; National Institutes of Health grants (T32 AG000057, T32 HL007028-42, P01 AG001751).

APSIPE.103

Exercise of a high intensity ameliorates hepatic inflammation and the progression of steatohepatitis

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The prevalence of nonalcoholic fatty liver disease (NAFLD) is estimated at 25% worldwide and is one of the leading causes of abnormal liver function. NAFLD covers a wide spectrum of liver pathology ranging from fatty liver to non-alcoholic steatohepatitis (NASH), which is characterized by steatosis, inflammation, hepatocellular injury, and fibrosis. Notably, immune cell-driven inflammation is a significant mechanism in the transition from fatty liver to the more serious NASH. Although exercise training is effective at ameliorating obesity-related diseases, the underlying mechanisms of these beneficial effects of exercise remain unclear. Whether there is an optimal modality and intensity of exercise to treat NAFLD is unknown. The objective of this study was to determine whether high intensity interval training or continuous moderate intensity training is more effective at ameliorating the progression of NASH. We hypothesized that exercise of high intensity is superior in dampening the inflammatory process of NASH, compared with an iso-caloric, moderate-intensity exercise modality. We fed 5-week-old wildtype mice a high-fat high-carbohydrate (HFHC) diet for 6 weeks to induce NASH. Then, mice were assigned to one of three training

regimens for 14 weeks: sedentary, moderate intensity training (MIT), or high intensity interval training (HIIT). The HIIT and MIT mice were pair-fed to ensure that caloric intake was similar between these groups. Compared with sedentary mice, both HIIT and MIT improved whole-body metabolic parameters and ameliorated the progression of NASH including decreased body weight, adiposity, hepatic triglycerides, inflammation, and fibrosis. Remarkably, HIIT was superior to MIT in ameliorating NASH progression as evidenced by improved hepatic inflammation, metabolism, and adiposity. Specifically, in comparison to MIT, HIIT resulted in an improved glucose and insulin tolerance, a reduced hepatic steatosis and fibrosis, a mild decrease in body fat percentage, and a lower frequency of infiltrating monocyte-derived macrophages. In addition, bulk mRNA sequencing of liver tissue showed a pattern of gene expression confirming that HIIT was more effective than MIT in improving hepatic metabolic parameters. Notably, an acute bout of MIT or HIIT exercise had no effect on the immune cell driven progression of NASH. Our data suggests that exercise reduces hepatic immune cell driven inflammation via a reduction in infiltrating macrophages and that high intensity interval training is superior to moderate intensity training in ameliorating NASH.

APSIPE.104

Testosterone and Health: Medical Need or Anti-Aging Fad? Clinical Use of Testosterone in Men.

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Testosterone is used clinically for the treatment of hypogonadism in men, and less commonly for gender-affirming therapy in transmen (female-to-male transgender). Hypogonadism is defined as the presence of clinical manifestations of testosterone deficiency and consistently low circulating testosterone concentrations, not as testosterone deficiency symptoms or low testosterone levels in isolation. The diagnosis of hypogonadism is challenging because: the symptoms and signs of testosterone deficiency are largely nonspecific; testosterone levels are highly variable (due both to biological variability, e.g., due to day-to-day, diurnal and food intake variation, and assay-to-assay variability); changes in total but not free testosterone levels due to alterations in sex hormone-binding globulin (e.g., due to obesity or age) occur commonly; and testosterone levels may be suppressed transiently, e.g., due to recovery from recent illness or use of certain medications (such as opioids and glucocorticoids). Organic hypogonadism (due to a pathological structural, infiltrative or congenital defect in the hypothalamus, pituitary or testes) is the only approved indication for testosterone treatment and is usually associated with severe, unequivocal clinical and biochemical hypogonadism. Clinical manifestations of severe hypogonadism include poor sexual development, reduced male hair, sexual dysfunction, low energy and mood, mild hematocrit (in the female range), low bone density, infertility, breast enlargement (gynecomastia) as

well as reduced muscle mass and strength and physical performance. Testosterone treatment that increases serum testosterone concentrations from below to within the normal adult male reference range improves muscle mass and strength but results in more modest and less consistent improvements physical performance (depending on the measure assessed) in men with organic hypogonadism, and to a lesser extent in older men with age-related hypogonadism. The therapeutic goal of testosterone treatment of hypogonadism is to restore normal functional performance, not to enhance athletic performance. In contrast to organic causes of hypogonadism that are uncommon and irreversible, functional hypogonadism due to suppression of gonadotropin stimulation of the testes by extrinsic conditions is common and potentially reversible with treatment of the underlying etiology or discontinuation of an offending medication. Uncommonly, athletes might develop organic hypogonadism (e.g. hypopituitarism due to traumatic brain injury) that could require testosterone treatment. More commonly, they might experience functional hypogonadism e.g., due to relative energy deficit (e.g., secondary to excessive training/energy expenditure relative to inadequate energy intake) or current or recent anabolic steroid, opioid or glucocorticoid use, that could be managed without testosterone therapy. However, upon request from athletes, some physicians who are not aware of the potential reversibility of functional causes for low testosterone might inappropriately initiate testosterone therapy and a therapeutic use exemption for testosterone use.

APSIPE.105

Sestrin is required for exercise adaptations of flies and mice

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Sestrin is an evolutionary conserved protein family that are induced upon environmental stresses and regulate metabolic homeostasis. Sestrins are highly expressed in skeletal muscle; however, its role in muscle tissue was understudied. Using *Drosophila* and mouse model, we showed that Sestrins are genetically essential for maintaining exercise capacity by upregulating mitochondrial biogenesis and oxidative respiration. In *Drosophila*, Sestrin deficiency nullified exercise benefit in extending running endurance, while Sestrin overexpression was sufficient to increase running endurance without exercise intervention. Also in mice, partial (*Sesn1*-KO) or complete (*Sesn1/2/3*-TKO) removal of Sestrins abolished exercise effects and impaired running endurance. This endurance phenotype was associated with strong defects in respiratory metabolism, associated with decreased maximal aerobic capacity (VO₂ max) and increased respiratory exchange ratio (RER). Using cultured myotubes, we investigated molecular mechanisms underlying Sestrin-dependent regulation of exercise

metabolism. We showed that Sestrin1, the muscle-specific Sestrin homolog, was strongly induced during muscle differentiation. In this process, Sestrin induction was essential for maximum AMPK activation, PGC1 upregulation and subsequent mitochondrial biogenesis. Sestrin-deficient myofibers showed relatively normal mitochondrial metabolism in resting condition; however, their maximum oxidative capacity was dramatically reduced. Also in *Drosophila*, AMPK-PGC1 axis is also important for endurance-extending effects of Sestrin and exercise. These results explain how Sestrins-deficient mice are metabolically normal but specifically impaired in exercise metabolism.

APSIPE.106

Nocturnal Blood Pressure Dipping Relates to Metabolic Insulin Sensitivity but not Vascular Function in Metabolic Syndrome

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Blunted dips in nocturnal systolic blood pressure (sBP) are independently related to cardiovascular disease. However, the role of metabolic and/or vascular insulin sensitivity in explaining nocturnal sBP regulation is unclear. **PURPOSE:** To test the hypothesis that nocturnal sBP dipping relates to metabolic insulin sensitivity as well as endothelial function. **METHODS:** Twenty-eight adults with metabolic syndrome (MetS; 53.2 ± 6.5 y; 35.8 ± 4.5 kg/m²) according to ATP III criteria were categorized as “dippers” (>10% change in sBP; n=11; 6F) or “non-dippers” (<10%; n=18; 13F). Twenty-four-hour ambulatory blood pressure monitoring was recorded to assess percent sBP dipping status. A 2-hr euglycemic-hyperinsulinemic clamp (40 mU/m²/min, 90 mg/dl) was performed to test metabolic (glucose infusion rate/insulin) and vascular (brachial artery FMD) insulin sensitivity. Augmentation index corrected to 75 bpm heart rate (Aix; arterial waveforms), VO₂max (indirect calorimetry) and body composition (DEXA) were also measured. **RESULTS:** Dippers had a significantly higher drop in sBP than non-dippers (17.82 ± 5.25 vs. 1.78 ± 6.17 %, $P < .001$). There were no significant differences in ATP III criteria, age, or body composition between dippers and non-dippers, but VO₂max tended to be higher in dippers (24.23 ± 4.44 vs. 21.17 ± 3.52 mL/kg/min, $P = 0.059$). Fasted FMD (6.85 ± 0.94 vs. 7.39 ± 1.05 %, $P = 0.28$), insulin-stimulated FMD 2-hr (6.94 ± 0.85 vs. 6.76 ± 0.66 %, $P = 0.63$), Aix fasted (26.7 ± 8.0 vs. 26.3 ± 8.6 %, $P = 0.90$), and Aix 2-hr (21.4 ± 10.8 vs. 21.5 ± 9.5 %, $P = 0.97$) did not differ between groups. Non-dippers though had higher metabolic insulin sensitivity (0.035 ± 0.017 vs. 0.020 ± 0.008 mg/kg/min/uU/ml, $P = 0.04$) and LDL concentrations (146.39 ± 28.56 vs. 110.20 ± 21.11 mg/dL, $P = 0.002$) than dippers. sBP dipping correlated with lean body mass ($r = 0.44$, $P < 0.001$), LDL ($r = -0.59$, $P = 0.001$), fasting insulin levels ($r = 0.57$, $P = 0.01$), and metabolic insulin sensitivity ($r = -0.49$, $P = 0.04$). **CONCLUSION:** Metabolic insulin sensitivity, but not vascular function, differ between dippers and non-dippers in MetS. Further, lean body mass

and LDL appear to be important factors contributing to nocturnal sBP regulation. Funding: NIH RO1-HL130296.

APSIPE.107

A multi-omics approach reveals an intricate network of exercise training-induced mitochondrial adaptations in human skeletal muscle

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Mitochondria are the powerhouses of the cell and play a critical role in many biological processes. Consequently, mitochondrial health and dysfunction is implicated in multiple diseases, ageing, and endurance performance. Many studies have shown that exercise induces mitochondrial biogenesis and improves mitochondrial function; however, questions remain over the intricate molecular pathways regulating mitochondrial adaptations. To answer these questions, we utilized a multi-omics approach integrated with classic biological mitochondrial techniques. Ten men (22 ± 5 y, 47 ± 8 mL min⁻¹ kg⁻¹) performed 3 sequential training phases of high-intensity interval training (HIIT): normal (NVT; 2-w, 6 sessions), high (HVT; 3-w, 40 sessions) and reduced (RVT; 1-w, 6 sessions) volume training. Vastus lateralis muscle biopsies were taken before and after each phase. Mitochondrial content, as assessed by mitochondrial protein yield, CS activity, and protein content of OXPHOS subunits (by WB) mirrored increases in training volume, and was maintained following RVT. The enzyme activity of ETC complexes and mitochondrial respiration in permeabilized fibers showed a similar pattern, as did supercomplex formation (by BN-PAGE). However, following normalization by CS activity, a marker of mitochondrial content, the majority of the above changes were no longer significant, suggesting that these were driven by the increase in mitochondrial content. It has been proposed that during mitochondrial biogenesis there is a fixed stoichiometry between changes in mitochondrial content and individual mitochondrial proteins. To verify this we performed proteomics analysis and identified 498 mitochondrial proteins (out of 1029 total); of these, 186 were differentially expressed by exercise. An in-depth cluster analysis of the differentially expressed mitochondrial proteins identified distinct patterns of change within the 3 training phases. Initially (post-NVT), mitochondria prioritized increases in proteins involved in fatty acid oxidation (FAO) and tricarboxylic acid (TCA) cycle pathways, and OXPHOS assembly factors, whereas OXPHOS and mitochondrial contact site and cristae organising system (MICOS) proteins were decreased. These divergent adaptations continued post-HVT (TCA and OXPHOS pathways increased; FAO, assembly factors, and

MICOS decreased) and post-RVT (MICOS increased, all other pathways decreased). Transcriptomics (RNA-seq) showed the expected increases in gene transcripts encoding OXPHOS subunits, but also revealed greater complexity in the transcriptional responses to training. Finally, lipidomics showed diverse and complex changes including increases in cardiolipins (CLs), the main lipids of mitochondrial membranes, and decreases in acylcarnitines, with training. Moreover, CLs exhibited internal remodeling with decreased 18:2 (dietary form of CL) and increased 18:1 (CL synthesized by the body) CL species. Our study highlights an extremely varied and intricate network of exercise-training induced mitochondrial

APSIPE.108

Exercise inter-arm systolic blood pressure differences in children and adolescents

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An inter-arm difference (IAD) in systolic blood pressure (SBP) ≥ 10 mmHg (IAD+) is clinically linked with hypertension, peripheral vascular disease, and premature mortality in adults. IAD+ is also present in ~10% of apparently healthy adults and the magnitude of IAD is known to increase with exercise.^{2,3} However, the prevalence of IAD+ and the magnitude of IAD in children under either resting or exercise conditions is unknown. The aim of this study was therefore to quantify the frequency of IAD+ and magnitude of IAD at rest and under exercise conditions in children and adolescents. Fifty-seven participants (56% male) aged 7 to 17 years (13.0 ± 2.6) who were scheduled for routine exercise stress tests were recruited from the Royal Children's Hospital. Simultaneous bilateral blood pressures were measured in triplicate before exercise (PRE), immediately after a Bruce protocol stress test (MAX), and in triplicate during recovery (REC) using a WatchBP Office Central (Microlife), an automated oscillometric device equipped with two cuffs that inflate simultaneously to assess IAD. The median of triplicate measures was used for analysis. Resting IAD was 3.7 mmHg (IQR; 2.5-6.7) (PRE), which increased to 7.0 mmHg (IQR; 3.0-14.0) (MAX, $p=0.01$), and returned to 4.0 mmHg (IQR; 1.5-6.0) (REC, $p=0.04$ (Compared with MAX)). When assessing exercise SBP percentiles, the median absolute difference between arms was 9% (IQR; 2-24%). 10.5% (95% CI; 5%-21%) of participants had an exercise SBP ≥ 90 th percentile (defined as 'elevated' SBP response) in one arm but < 90 th percentile (normal exercise SBP) in the other. 7% (95% CI; 3%-17%) of participants had an exercise SBP ≥ 95 th percentile ('hypertensive' response) in one arm but not the other. IAD+ was observed in 12% ($n=7$) at PRE, 37% at MAX ($n=21$) ($p=0.004$, compared with PRE) and 11% ($n=6$) ($p=0.002$) (compared with MAX) during REC. In conclusion, IAD was augmented with exercise and returned to that of PRE during REC. The frequency of IAD+ was similar to that seen in healthy adults and IAD followed a similar pattern of

increase with exercise. IAD may substantially influence the clinical assessment of exercise blood pressure in children. 1. Clark CE et al. J Vasc Surg. 2012;56(3):875. doi:10.1016/j.jvs.2012.07.015 2. Holmstrup ME et al. Blood Press Monit. 2017;22(2):68-71. 3. Walsh MA et al. ACSM-TJ. 2020:1-6.

APSIPE.109

Bilateral differences in quadriceps muscle strength associated with dominance and non-dominance characteristics, are not explained by muscle size or motor unit firing rate

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Muscle strength, a function of muscle size and neural mechanisms, is important in tasks of daily living and can be enhanced e.g. by resistance exercise training (RET) [1]. While unilateral RET studies are commonly adopted to study the regulation of muscle strength and mass, little consideration is given to dominant and non-dominant limb characteristics, despite the potential for limb asymmetry in muscle strength and neuromuscular function [1]. Although no strength asymmetry for the knee extensors (KE) was evident in the "preferred" leg of soccer players [2], KE of the "dominant" limb are typically stronger than the "non-dominant" limb [3] with neural activation patterns of lower limb muscles exhibiting bilateral differences during static standing and dynamic exercise [4]. We thus investigated if bilateral differences are evident for KE muscles with respect to muscle strength and individual motor unit (MU) structure/function. Twenty young individuals (12F/8M, 24 ± 5 y, 24 ± 4 kg/m²) volunteered for the study, with all anatomical, kinesiology and electromyography assessments assessed bilaterally. The vastus lateralis (VL) muscle cross-sectional area (CSA) was assessed by ultrasound. KE strength was determined through monitoring maximal voluntary contractions (MVC). Force steadiness (FS) was assessed at 10, 25 and 40% MVC during a series of voluntary contractions. Intramuscular electromyography (iEMG) was utilised to sample individual MUs from the VL during sustained contractions, at each contraction intensity [5], of which MU firing rates (FR; consecutive firings of MU potentials) were calculated. FR variability was expressed as the mean absolute consecutive difference (MACD) of MU discharge times. Muscle CSA, strength and FS data were analysed using paired Student's t-tests. Multi-level mixed effects linear regression models were used to analyse individual MU data. Statistical significance was accepted where $p < 0.05$. Muscle CSA ($n=10$; $p=0.73$) was indistinguishable, bilaterally. However, KE strength was greater in the dominant right leg (482.00 ± 159.50 N) compared to the non-dominant left (455.50 ± 152.3 N) leg ($p=0.005$). No bilateral differences were observed at any contraction intensity for motor unit potential (MUP) area (10%MVC, $p=0.42$; 25%MVC, $p=0.47$; 40%MVC, $p=0.53$), MUP turns (10%MVC, $p=0.11$; 25%MVC, $p=0.30$; 40%MVC, $p=0.23$), MU FR

(10%MVC, $p=0.50$; 25%MVC, $p=0.72$; 40%MVC, $p=0.68$), MU FR variability (10%MVC, $p=0.95$; 25%MVC, $p=0.79$; 40%MVC, $p=0.28$) (all $n=17$) and FS (10%MVC, $p=0.75$; 25%MVC, $p=0.10$; 40%MVC, $p=0.25$). Bilateral differences in strength, presumably reflecting dominance, are independent of muscle size, MU FR or MU FR variability. Strength asymmetry may instead be explained at the single muscle fibre level i.e. greater specific force or efficiency of excitation-contraction coupling. Consideration should be afforded to the potential for introducing bias in unilateral RET studies by random selection. References: [1] Carroll T. J. et al. 2001. *Sports Med*, 31, 829-840. DOI: 10.2165/00007256-200131120-00001 [2] Rahnama N. et al. 2005. *Ergonomics*, 48, 1568-1575. DOI: 10.1080/00140130500101585 [3] Lanshammar K. & Ribom E. L. 2011. *Physical Therapy in Sport*, 12, 76-79. DOI: 10.1016/j.ptsp.2010.10.004 [4] Liang S. et al. 2016. *PeerJ*, 1-17. DOI: 10.7717/peerj.2315 [5] Piasecki M. et al. 2016. *J Physiol*, 594, 4525-4536. DOI: 10.1113/JP271087 Research supported by MRC Versus Arthritis Centre for Musculoskeletal Ageing Research and NIHR Nottingham BRC.

APSIPE.111

Chronic oxidative dysfunction following volumetric muscle loss injury

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The first objective was to systematically review prior work on chronic comorbidities of skeletal muscle following volumetric muscle loss (VML) injury; the second objective was to evaluate how VML injury impairs the capillarity and oxidative skeletal muscle capacity chronically. VML injury presents a chronic deficit in contractile function that cannot be accounted for simply by the volume of muscle lost. We posit that there is a disruption in the underlying oxidative processes following VML injury which may account for a component of the functional impairment. First, using PRISMA guidelines, we evaluated prior work on models of VML that evaluated markers of oxidative physiology; defined as any evaluation of biochemical or histological marker that constitutes the ability of a muscle cell to generate ATP through oxidative processes. Included studies had measures of capillarity, oxidative histology, mitochondrial abundance, mitochondrial function, or myosin heavy chain isoform (MyHC) expression. In total, 140 studies were evaluated and 7 met inclusion criteria for review. The literature suggested that following VML injury the remaining myofibers tend to have a slower more oxidative phenotype, but might not have an increase in the supporting oxidative architecture (i.e., capillaries) to support this change. Furthermore, there is increased mitochondria around two weeks post-VML, but mitochondrial size and quality is reduced. Therefore, a second primary study was designed to understand oxidative capacity. Adult male C57BL/6 mice ($n=24$)

underwent a sham procedure or a full-thickness multi-muscle VML injury to the gastrocnemius, soleus, and plantaris muscles (21.1 ± 0.7 mg). One- and two-months post-injury terminal histology (capillarity, mitochondrial content, and MyHC distribution), biochemical (citrate synthase), and maximal contractile function (isometric torque and force-frequency of the posterior muscle compartment) were evaluated. Myofiber capillary content, evaluated as capillary number per myofiber or area, were not significantly different between VML and sham muscles. There was a shift in the distribution of capillaries per myofiber across groups, such that the VML injury at both one- and two-months post-VML had a rightward shift in distribution. Following VML there was also a rightward shift in the cross-sectional area of myofibers, with an increasing proportion of MyHC type I expressing fibers. Surprisingly, there was not a difference in mitochondria content between sham and VML injured muscles. Maximum isometric torque was significantly less in VML (334 ± 28 mN·m) compared to sham (557 ± 66 mN·m) muscles. VML injury resulted in slower torque production and an elevated twitch:tetani ratio, perhaps supporting the slower muscle fiber phenotype in the tissue remaining. Taken together with the systematic review, results herein suggest that there is a slower phenotype after VML injury chronically with no additional increase in capillarity. This work suggests a miss-match in oxidative properties of skeletal muscle following VML as a limiting factor to muscle function. Collectively, this work suggests that the metabolic health of the skeletal muscle should be an important consideration when developing a treatment approaches for VML injury and should be a physiologic target for intervention. Supported by W81XWH-18-1-0710 & T32AR050938

APSIPE.112

Effects of One Hour Race Pace Running in Middle Age Elite Runners on Cardiac Functions

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Introduction Marathon, half-marathon and long-distance running require high-level aerobic capacity for athletic achievements. Due to high exercise intensity, running at race pace may cause serious strain on the cardiovascular system. However, cardiovascular complications such as sudden cardiac death rarely observed during or after exercise, especially in individuals who perform long-distance running exercises over a certain age. The aim of the study is to examine some haematological parameters and cardiac damage markers of individuals (over 35 years old) at individual race pace on the treadmill, before, after, and within the following 24 hours. Methodology Nineteen healthy male amateur runners (running for 11.8 ± 2.0 years, 70.5 ± 6.4 km/week training, mean age 47.4 ± 1.7) were

enrolled in this study. Measurements were performed following the approval of the local ethics committee and carried out in accordance with the Declaration of Helsinki. At first visit day, the athletes' maximal aerobic capacities were determined (47.1 ± 1.2 ml.kg⁻¹.min⁻¹) by a maximal cardiopulmonary test (Maxtest) protocol on a treadmill. Transthoracic echocardiography was performed among all patients. At second visit day, athletes performed one-hour running exercise test (with running average heart rate 161.9 ± 3.0 bpm) in which personal pace determined from Maxtest data. Venous blood samples were obtained just before the one-hour running exercise, just after the exercise, four and twenty-four hours after the exercise. ECG signals recorded together with blood sampling. Creatine Kinase (CK), Creatine Kinase Muscle Brain Fraction (CK-MB), Troponin-I, N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) were analysed as cardiac damage biomarkers. Hemogram data analysed to evaluate dehydration level. Normally distributed data were evaluated using paired t-test and repeated measures of ANOVA with Bonferroni for post hoc analyses. Non-normally distributed data were evaluated using the Friedman and Wilcoxon test. Significance was set at $p < 0.05$. Results We observed a significant difference at all cardiac biomarker levels after the one-hour running exercise trial which were within the normal range before the test (CK 161.21 ± 22.51 U/L, 222.05 ± 31.94 U/L; CK-MB 4.27 ± 0.71 ng/mL, 5.81 ± 0.95 ng/mL; Troponin-I 5.04 ± 1.12 ng/mL, 11.48 ± 2.43 ng/mL; NT-proBNP 31.24 ± 5.29 pg/mL, 64.40 ± 8.45 pg/mL; before and after test respectively, $p < 0.05$). Average CK and CK-MB values did not return to normal reference values at the twenty-fourth-hour running trial (411.90 ± 139.70 U/L and 10.14 ± 3.03 ng/mL, respectively). We did not observe any ECG signal abnormalities. Transthoracic echocardiographic evaluations were in accordance with long-distance runners. One-hour running exercise caused $6.19 \pm 1.22\%$ plasma volume loss. Discussion Race pace one-hour running caused cardiovascular strain and increased some of the cardiac damage markers significantly. Changes of these biochemical markers had shown inter-individual variability and data indicate cardiac damage in four volunteers. However, ECG signal data did not indicate any abnormalities. With this in mind, individual evaluation of middle-aged elite long-distance runners is required for prevention of sudden cardiac complications.

APSIPE.113

The Genetic and Epigenetic Regulation of Skeletal Muscle Ribosome Biogenesis in Response to Acute Exercise

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Ribosomes are the engines of protein synthesis. The production of new ribosomes (ribosome biogenesis) to augment protein translational capacity is thought to be a key component for successful skeletal muscle adaptation to exercise. The factors mediating ribosome biogenesis in

response to an exercise bout are multi-factorial, but may be related to ribosomal DNA (rDNA) dosage and/or epigenetic modifications to the rDNA repeat. To investigate these possibilities, skeletal muscle biopsies were obtained from human subjects ($n=30$; 18 men and 12 women; 31 ± 8 yrs, 25 ± 4 kg/m²) at rest and 30 minutes, 3 h, 8 h, and 24h after acute endurance (45 min biking, 70% VO₂max) or resistance exercise (4 x 7 x 2 exercises). rDNA transcription and copy number were assessed using qRT-PCR and whole genome sequencing (WGS). Targeted methylation of the rDNA promoter (-189 to +47) in biopsy samples was investigated with the Agena massARRAY EpiTYPER, and global methylation along the rDNA repeat was assessed using reduced representation bisulfite sequencing (RRBS). The induction of ribosome biogenesis coincided with resistance exercise, but not endurance exercise. In response to resistance exercise, ribosome biogenesis was associated with rDNA dosage (copy number), but not rDNA promoter methylation. We then utilized a genetic mouse model of in vivo myonuclear labeling to validate the effects of an acute hypertrophic stimulus on rDNA methylation specifically in myonuclei, and corroborated the human RRBS data. We also report that myonuclear methylation throughout the rDNA repeat is robustly modified by an acute hypertrophic stimulus. These data collectively provide novel information on genetic and epigenetic factors affecting ribosome biogenesis in response to a bout of resistance exercise that may ultimately contribute to variability between individuals in training-induced muscle fiber hypertrophy.

APSIPE.114

Effects of Acute Exercise and Obesity on GLO1 Abundance and Acetylation in Human Skeletal Muscle

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Glyoxalase I (GLO1) is the primary enzyme for detoxifying the reactive dicarbonyl methylglyoxal. Pre-clinical models demonstrate that loss of GLO1 promotes the development of diabetes in obesogenic conditions which may be related to the altered redox state and subsequent accumulation of acetylation on GLO1. However, there is limited clinical data examining the relationship between obesity and GLO1 expression especially in tissues of high relevance to glucose regulation such as skeletal muscle. Our objectives were to determine if GLO1 is attenuated in the muscle of individuals with obesity, and if acute aerobic exercise (AE) could regulate GLO1 acetylation, protein abundance or gene expression. We compared GLO1 expression in skeletal muscle biopsies from 5 obese (OB) and 15 age-matched lean, healthy individuals (LH) at baseline and the time-course following AE at 80% of VO₂max. All human research was reviewed and approved by our governing IRB and was carried out in accordance with the guidelines set forth by the Declaration of Helsinki. GLO1 protein was ~50% lower ($p < 0.05$), whereas GLO1 mRNA was ~2-fold higher in OB muscle ($p < 0.001$) at baseline but there was no effect of

the acute AE on GLO1 protein or mRNA immediately, or 3 hours post exercise. The NRF2/Keap1 axis, which regulates GLO1 transcription, was unaffected by obesity. However, acute AE increased NRF2 mRNA ($p=0.02$) and mRNA of its transcriptional target HMOX1 ($p=0.003$) in the LH group only. Given the NAD⁺-dependent deacetylase SIRT1 is capable of deacetylating GLO1 which may prevent GLO1's degradation, we measured the expression of SIRT1 as well as NAMPT and HIF1 α which regulate NAD⁺ bioavailability. HIF1 α mRNA was 3-fold ($p=0.002$) higher and NAMPT protein was ~50% lower ($p=0.2$) in OB muscle suggesting a pseudo-hypoxic environment which may attenuate NAD⁺ bioavailability. SIRT1 was ~50% lower in OB than LHC ($p=0.09$), which was rescued by AE and correlated to GLO1 protein ($Rho=0.639$, $p=0.007$). In addition, there was a trend for exercise to reduce GLO1 acetylation ($p=0.17$) in OB muscle. Contrarily, GLO1 acetylation was increased by ~50% and GLO1 protein was decreased ~40% in human primary myotubes depleted of NAD⁺ via treatment with the NAMPT inhibitor FK866 for 48 hours. This phenotype was rescued by replenishing NAD⁺ levels by treating the cells for 4 hours with the NAD⁺ precursor nicotinamide riboside. Silencing of NAMPT or SIRT1 for 48 hours also reduced GLO1 protein by 50%. These data suggest that OB muscle may be in a pseudo-hypoxic state that limits NAD⁺ bioavailability and SIRT1 activity promoting GLO1 acetylation and degradation. Future work should explore the consequences of loss of GLO1 in OB skeletal muscle and establish if interventions such as caloric restriction or exercise training are able to reverse this phenotype.

APSIPE.116

The Effect of Intensity and Duration on the Development of Exercise-induced Diaphragm and Expiratory Abdominal Muscle Fatigue

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INTRODUCTION: Exhaustive 'long-duration' severe intensity exercise (~90% of VO_{2peak} ; ~10 min) elicits respiratory muscle fatigue, likely due to a high power of breathing (PoB) combined with a competition for available cardiac output. The PoB during submaximal heavy intensity exercise ($\leq 80\%$ VO_{2peak}) does not engender a substantial locomotor vs. respiratory muscle competition for available blood flow; however, whether such exercise results in respiratory muscle fatigue is unclear. Moreover, whether the cumulative respiratory force output during exhaustive 'short-duration' severe intensity exercise ($>95\%$ VO_{2peak} ; ~5 min) is sufficient to elicit respiratory muscle fatigue remains unknown. **PURPOSE:** To investigate the effect of exercise intensity and duration on the development of diaphragm and expiratory muscle fatigue. **METHODS:** In 10 healthy adults (25 $\pm$ 5 yr, 2 females), critical power (CP), peak ramp power (Ppeak) and VO_{2peak} (57 $\pm$ 9 ml·kg⁻¹·min⁻¹) were determined via a 'ramp sprint' test. Subjects then performed three exhaustive constant-power cycling tests: 1) 5% <CP (191 $\pm$ 52 W, heavy intensity); 2) ~25% of the

difference (δ) between CP and Ppeak (625; 234 $\pm$ 53 W, severe intensity 'long'); and 3) ~50% Δ ($\Delta 50$; 263 $\pm$ 53 W, severe intensity 'short'). Diaphragm and expiratory muscle fatigue were quantified as the pre- to post-exercise reduction in the transdiaphragmatic (Pditw) and gastric (Pgaw) twitch pressure response to magnetic stimulation of the cervical and thoracic nerves, respectively. **RESULTS:** Exercise time was 35 $\pm$ 6 min, 10 $\pm$ 3 min, and 5 $\pm$ 1 min for <CP, $\Delta 25$, and $\Delta 50$, respectively. End-exercise VO_2 was lower during <CP vs. $\Delta 25$ and $\Delta 50$ (87 $\pm$ 5% vs. 98 $\pm$ 4% and 99 $\pm$ 5% of VO_{2peak} , respectively; both $P < 0.001$ vs. <CP). Immediately after <CP, $\delta 25$, and $\delta 50$, group mean Pditw and Pgaw were reduced below pre-exercise baseline values. The exercise-induced decrease in Pditw was greater after $\delta 25$ (-22 $\pm$ 12%) vs. <CP (-13 $\pm$ 8%; $P = 0.50$) and $\Delta 50$ (-14 $\pm$ 12%; $P = 0.045$). Conversely, the pre- to post-exercise decrease in Pgaw was not different between trials (<CP: -23 $\pm$ 15% vs. $\Delta 25$: -29 $\pm$ 15% vs. $\Delta 50$: -25 $\pm$ 16%) (all $P > 0.05$). Cumulative diaphragm pressure-time product (PTP) was higher during <CP (17337 $\pm$ 6794 cmH₂O·s) vs. $\delta 25$ (6697 $\pm$ 1581 cmH₂O·s) and $\delta 50$ (2707 $\pm$ 992 cmH₂O·s) (all $P \leq 0.01$). Similarly, cumulative gastric PTP was higher during <CP (8914 $\pm$ 3063 cmH₂O·s) vs. $\Delta 25$ (3331 $\pm$ 1415 cmH₂O·s) and $\delta 50$ (1321 $\pm$ 418 cmH₂O·s) (all $P \leq 0.01$). **CONCLUSION:** Exhaustive heavy intensity as well as short- and longer-duration severe intensity exercise elicits diaphragm and expiratory muscle fatigue. While the magnitude of exercise-induced diaphragm fatigue appears to be a function of both cumulative force output and exercise intensity (i.e. competition for blood flow), the severity of exercise-induced expiratory muscle fatigue was unaffected by exercise intensity or duration. This may be due to a less fatigue-resistant phenotype and/or the additional non-ventilatory roles of the expiratory muscles during exercise.

APSIPE.117

Insulin receptor blockade in the nucleus tractus solitarius augments exercise pressor reflex function in normal rats

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[Background] In type II diabetes mellitus (T2DM), insulin resistance is associated with abnormal cardiovascular responses to exercise. As evidence, we recently demonstrated in T2DM rats that activation of the exercise pressor reflex (EPR) evokes potentiated cardiovascular responses compared to healthy controls. Whether alterations in brainstem cardiovascular control center pathways mediate this augmented responsiveness remains unknown. The first site critical for processing EPR sensory information in the brainstem is the nucleus tractus solitarius (NTS) within the medulla oblongata. Brain insulin is known to modify neural activity in the NTS. Central hypoinsulinemia results from impairment of insulin transport within the brain in T2DM. Taken together, it is

hypothesized that decreasing the activity of the insulin signaling pathway within brainstem cardiovascular control centers contributes to exaggerated EPR function in this disease. [Purpose] The aim of the present study was to evaluate whether acute pharmacological central insulin receptor blockade within the NTS alters EPR function in normal healthy rats. The intent was to establish a role for the insulin signaling pathway in the expression of the EPR. [Methods] We measured blood pressure responses evoked by i) passive stretch of the hindlimb (mechanoreflex activation) and ii) intra-arterial capsaicin injection (0.3ug) into the hindlimb circulation (metaboreflex activation) in paralyzed, decerebrated rats. Both the muscle mechanoreflex and metaboreflex are functional components of the EPR. After control responses to stretch and capsaicin were recorded, an insulin receptor antagonist (GSK1838705, 10mM; GSK) or vehicle (DMSO) was injected into the NTS region (6 injections, 50nL each). The blood pressure response to each stimulus was again recorded 30- and 60-min following injections. The pressor response (peak changes in mean arterial pressure from baseline) was evaluated by 2-way ANOVA, comparing pre-, 30min, and 60min after GSK or DMSO injections. [Results] The pressor response to passive stretch (GSK vs DMSO, $P=0.04$; time, $P<0.05$; interaction, $P<0.05$; GSK $n=12$, DMSO $n=11$) was potentiated following injection of GSK compared to vehicle. Likewise, the blood pressure response evoked by capsaicin was also augmented by insulin receptor blockade (GSK vs DMSO, $P<0.05$; time, $P=0.72$; interaction, $P=0.11$; GSK $n=11$, DMSO $n=7$). [Conclusion] The results suggest that the insulin signaling pathway within the NTS modulates the blood pressure response to activation of the mechanically and metabolically sensitive components of the EPR. Moreover, the data is consistent with the possibility that decreased activation of this pathway with the advent of central hypoinsulinemia in T2DM may contribute to the abnormal exaggerated cardiovascular response to exercise characteristic of the disease. [Funding] Lawson & Rogers Lacy Research Fund in Cardiovascular Disease and National Heart, Lung, and Blood Institute (R01HL-151632).

APSIPE.118

Novel Insight on Creatine-Dependent and Creatine-Independent Mitochondrial Bioenergetics in the C26 Model of Cancer Cachexia

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Cancer cachexia is the ongoing loss of skeletal muscle mass that affects 25-80% of cancer patients and reduces survival. Currently, there is no cure for this disease. It was recently identified that cancer can induce mitochondrial dysfunction in skeletal muscle. Identifying the precise mechanism by which this dysfunction occurs could guide future therapy development as this may be linked to muscle protein homeostasis. As mitochondria can produce

ATP and ROS through both creatine-dependent and -independent pathways, we determined whether these systems were impaired in cancer cachexia. 8-week-old CD2F1 male mice ($N=12$) were injected subcutaneously with 5×10^5 colorectal 26 adenocarcinoma (C26) cancer cells or phosphate-buffered saline (PBS control, $N=10$) per flank. Tumours developed for 26-29 days. Tumour volume and body weight were measured daily and weekly respectively. Mitochondrial bioenergetics were assessed in both quadriceps and diaphragm muscles using permeabilized muscle fibers. After 26-29 days of tumour bearing, there were significant reductions in body weight (-19.6%) and muscle mass (-15.2-28.1%) in extensor digitorum longus, plantaris, tibialis anterior, gastrocnemius and quadriceps compared to PBS. Surprisingly, C26 cancer significantly increased complex I-supported ADP-stimulated mitochondrial respiration by 21-51% in the quadriceps and diaphragm. C26 cancer increased creatine-independent respiration in quadriceps and diaphragm, whereas creatine-dependent respiration was increased solely in the diaphragm. C26 cancer significantly decreased mitochondrial reactive oxygen species (mH_2O_2) emission in the quadriceps (30-115%) and diaphragm in the presence of ADP (31-61%) but this was not consistent in all ROS-generating pathways that were assessed in either creatine condition. In summary, quadriceps and diaphragm show surprising increases in mitochondrial respiration and decreases in mitochondrial H_2O_2 emission in association with cachexia at 4 weeks of tumour development. Furthermore, these dysfunctions were due to changes in the ability of mitochondria to respond to increased energy demand (ADP) although the role of creatine metabolism in facilitating these altered functions was heterogeneous across muscles. These results might represent a compensation to the metabolic stress created by cancer. Future studies should compare different durations of cancer to determine the relationship between metabolic dysfunction and hormesis during cachexia with consideration of muscle-specific responses.

APSIPE.119

Role of Morning versus Evening Chronotype on Insulin Sensitivity and Central Hemodynamics in Adults with Metabolic Syndrome

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PURPOSE: Chronotype represents an individual's preferred time of day to perform daily activities. Late chronotypes (i.e. "evening people") tend to have higher risk for obesity, hypertension and type 2 diabetes than morning chronotypes. However, whether insulin sensitivity or central hemodynamics relate to chronic disease risk per chronotype remains unclear in people with metabolic syndrome (MetS). To evaluate the relationships between insulin sensitivity and central hemodynamics with chronotype in patients with MetS. **METHODS:** According to self-reported Morning-Eveningness Questionnaire (MEQ)

scores, 19 morning (MEQ = 63.5 ± 1.2 , $n=16$ F, 54.6 ± 1.2 yrs, 35.26 ± 0.9 kg/m², 3.5 ± 0.1 ATP criteria) and 20 evening (MEQ = 45.47 ± 1.3 , $n=16$ F, 53.1 ± 1.6 yrs, 37.42 ± 1.1 kg/m², 3.5 ± 0.2 ATP criteria) chronotypes were compared. Daytime sleepiness was assessed using the Epworth questionnaire. A 120 min euglycemic clamp (40 mU/m²/min, 90 mg/dl) was performed to test metabolic insulin sensitivity (GIR; glucose infusion rate). Central hemodynamics was characterized before and after the clamp by augmentation index corrected to 75 bpm heart rate (Alx75), augmentation (AP), pulse pressure (PP) as well as forward (Pf) and backward (Pb) pulse wave. VO₂peak (ml/kg/min; indirect calorimetry) and body composition (DEXA; fat mass and lean body mass) were also measured. RESULTS: There were no statistical differences in age, BMI, ATP III criteria or daytime sleepiness between groups. However, morning chronotype tended to have less total fat mass ($P=0.07$) and higher VO₂peak ($P=0.09$) and GIR ($P=0.065$) than evening chronotype. Insulin-stimulation also tended to reduce Alx75 ($P=0.09$), AP ($P=0.054$), and Pf ($P=0.06$) in morning versus evening chronotype. Higher MEQ score was linked to lower fasting glucose ($r=-0.32$, $P=0.04$), HDL ($r=-0.36$, $P=0.03$) and Pf 120 min ($r=-0.32$, $P=0.058$). BMI was also associated with increased AP ($r=0.32$, $P=0.054$), PP ($r=0.44$, $P<0.01$), Pb ($r=0.45$, $P<0.01$) and Pf ($r=0.39$, $P=0.02$) 120 min after insulin infusion. Fat mass was associated with increased AP ($r=0.30$, $P=0.09$) and Pf ($r=0.31$, $P=0.08$), whereas lean body mass was related to elevated Pb ($r=0.41$, $P=0.02$) during insulin-stimulation. CONCLUSIONS: Despite classification of MetS, morning chronotype may confer more favorable cardiometabolic health than self-identified "evening people". Additional work is needed to discern how chronotype physiology modifies insulin action for glucose regulation and vascular health. Funding: NIH RO1-HL130296.

APSIPE.120

Iodine-enhanced micro-CT of atherosclerotic plaques complements routine histology

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The study of coronary artery disease requires visualization of arterial lesions, including plaque formation. Conventional histology methods for imaging and characterizing atherosclerotic lesions are robust but are destructive to tissue and may prevent further analyses. We tested the hypothesis that micro-computed tomography (micro-CT) paired with an iodine-based radiopaque stain would effectively image and and characterize atherosclerotic plaques in a manner comparable to routine histology while maintaining sample integrity and providing whole-volume data. We examined atherosclerotic, porcine coronary arteries using micro-CT in the absence and presence of iohexol (240 mg/ml). Following iohexol washout, histological analyses of the samples were performed with hematoxylin and eosin and Masson's trichrome. Iohexol staining generated soft tissue delineation and aided

subsequent atherosclerotic plaque assessment via augmented radiopacity of adjacent tissue, permitting three-dimensional (3D) reconstruction of these lesions while maintaining in situ architecture of plaques. Calcium phosphate deposition was readily located and visualized using micro-CT, independent of iohexol staining. Although adjacent tissue layers were discernible using micro-CT and iohexol, the cellular make-up of the tissues, including atherosclerotic plaques, was not achieved using micro-CT but completed with subsequent histology sectioning and staining. The results of this study demonstrate that micro-CT, paired with a diffusible radiopaque stain, is a powerful imaging modality for visualizing in situ architecture of the vessel wall and atherosclerotic plaque morphology, independent of and complementary to routine histological analysis.

APSIPE.121

Kinetics Analysis of Mean Arterial Pressure Following the Onset of Exercise: A Prospective Tool for Discerning Cardiovascular Health

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The regulation of mean arterial pressure (MAP) during exercise has important physiological and clinical implications. The use of kinetics analysis on numerous physiological variables following the onset of exercise, such as oxygen uptake and blood flow, has revealed important information regarding their control systems. Surprisingly, the dynamic response of MAP following the onset of exercise remains to be quantified. Therefore, ten healthy participants (Young, 24 ± 3 years) completed four transitions each from unloaded to moderate- (80% of gas exchange threshold (GET)) and heavy-intensity (30% of the difference between GET and peak oxygen uptake) dynamic single-leg knee-extensor exercise to investigate the on-kinetics of MAP. Further, six patients with essential hypertension (HTN, 50 ± 4 years) and five age-matched normotensive controls (NTN, 53 ± 4 years) completed four heavy-intensity transitions. These experiments were performed in accordance with the Declaration of Helsinki. For Young, MAP increased in an exponential manner with the speed (τ) not differing between intensities (moderate: 59 ± 30 ; heavy: 66 ± 19 s, $P > 0.05$), but the MAP amplitude was doubled during heavy-intensity exercise (moderate: 8 ± 12 mmHg; heavy: 24 ± 7 mmHg, $p < 0.001$). Further examination revealed that the Young moderate-intensity MAP response was not a true exponential (potentially not error signal driven), demonstrated by the correlation between the amplitude and τ ($p < 0.001$); while heavy-intensity lacked correlation for these parameters (potentially error signal driven, $P > 0.05$). The heavy-intensity work rate was not different between the groups: Young (36 ± 8), HTN (42 ± 10), NTN (45 ± 5 Watts); all, $P > 0.05$. Both HTN ($6 \pm 39 \pm 12$ mmHg) and NTN ($6 \pm 36 \pm 8$ mmHg)

had larger MAP amplitudes compared to Young (both, $P < 0.05$) but they were not different from each other ($P > 0.05$). The MAP tau was longer for HTN (117 ± 33 s) than both NTN (75 ± 28 s) and Young ($P < 0.05$). Taken together, this investigation establishes that kinetics modeling of MAP is practical for heavy-intensity knee-extensor exercise and has the potential to differentiate cardiovascular health status from the effect of aging, per se. Whereby, age-matched and hypertensive individuals display an exaggerated MAP response to exercise compared to young individuals, but only the patients with hypertension demonstrated a longer period of adjustment (i.e. tau). Funding sources: NIH 2T32HL007576, 1T32HL139451, R01HL14203; VA I01CX001999.

APSIPE.122

Secondary denervation and poor neuromuscular junction remodeling is a chronic pathophysiologic consequence of volumetric muscle loss

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The objective of this study was to systematically examine neuromuscular junctions (NMJs) in the remaining tissue following a volumetric muscle loss (VML) injury, which occurs when a significant portion of muscle tissue is abruptly removed following military combat, motor vehicle and industrial accidents, or even surgery. The defining characteristics of VML injury are a loss of regenerative capacity and muscle function. The most notable consequence of VML injury is the abrupt removal of muscle fibers, however the destructive nature of VML also results in the damage or removal of the extracellular matrix, vasculature and peripheral nerves. An appreciation of how other components of the neuromuscular system are affected following VML injury remains relatively unknown. We hypothesized that VML injury would result in a reduction to NMJ size, increase the appearance of irregular morphological characteristics such as axon sprouting and poly-innervation, and result in secondary denervation. A standard full thickness VML injury was used to remove ~20% of the tibialis anterior (TA) muscle in adult male inbred Lewis rats ($n=43$), the non-injured contralateral leg served as an intra-animal control. The TA muscles were harvested at 0, 3, 7, 14, 21, and 48 days post-VML surgery and the pre- and post-synaptic terminals were identified immunohistochemically. Quantitative confocal microscopy was used to examine ~50 individual en face NMJs per muscle. When compared to controls, quantitative analysis showed significant denervation in the VML injured muscle at 21 and 48 days post-VML ($p \leq 0.018$). Qualitative analysis indicated that denervation was ~16% through the first two weeks post VML, followed by an increased prevalence of denervation to ~32% by 48 days post-VML ($p < 0.001$). Terminal axon sprouting and poly-innervation facilitate NMJ remodeling during myofiber reinnervation and development, respectively, both were confirmed by 7 days post-VML ($p \leq 0.008$). However, the combination of axon sprouting and poly-innervation was only seen at 48 days

post-VML ($p=0.001$), suggesting a possible dysfunction in NMJ remodeling. Contrary to the original hypothesis VML had no consequence on pre- or post-synaptic NMJ area or volume ($p \geq 0.089$). Stabilization of NMJ area and volume may be beneficial for permitting reinnervation, especially when the use of a regenerative interventions or physical therapy is used. Overall, this study provides solid evidence that VML injury has a negative impact on the remaining NMJs. Furthermore, NMJ denervation combined with a poor NMJ remodeling response likely contributes to the long-term loss of muscle function and regenerative capacity following VML. Supported by W81XWH-19-1-0075

APSIPE.123

Impact of basal energy expenditure on VWR-mediated systemic energy metabolism in male and female mice.

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Background: Voluntary wheel running (VWR) is used to increase physical activity in mice and hypothetically to increase energy expenditure (EE). However, total EE is ~40% lower (resting EE ~ 60% less) when mice are housed at thermoneutral housing temperatures (~28-30°C). Whether the reduced basal EE of thermoneutral housing temperatures impacts the energetic response to VWR is unknown. As such, we performed indirect calorimetry experiments in mice housed at 20°C & 30°C to investigate the impact of divergent baseline EE on the capacity of VWR to change EE. Methods: Six-week old male and female C57Bl/6J mice with or without (SED) access to running wheels were housed at 20°C & 30°C for 3 weeks. At 9-weeks of age, mice were acclimated to the indirect calorimetry system for 5 days and experiments were performed to analyze the components of EE during 7-days of low-fat diet (LFD). All animals were weighed at the start and end of the 7-day experiment. Results: SED males were heavier than SED females (20°C ~18% & 30°C ~30%), and VWR males were ~18% heavier than VWR females at both temperatures. No difference in initial body weight was observed due to the 3-weeks of VWR access by sex or housing temperature. Weight gain was similar between groups during the 7-day LFD. As expected, mice housed at 30°C have ~40% lower total EE and energy intake regardless of sex or physical activity. SED male mice had ~5% higher total EE than SED females at both temperatures. Total EE was greater in VWR mice (20°C females ~15% & 30°C male ~20% & female ~30%), except 20°C males. Also as expected, mice house at 30°C had ~60% lower resting EE compared to 20°C groups. Importantly, VWR had no impact on resting EE by sex or temperature. Activity EE was ~40% greater in 30°C mice for both males and females regardless of activity level compared to 20°C mice. Interestingly, activity EE was ~55% higher in the VWR groups compared to SED in both sexes and at both temperatures. However, as a percent of total EE, 30°C male and female activity EE accounted for 46% of total EE, compared to ~20% of 20°C females and males. Conclusions: While VWR did increase EE at both

temperatures, 30°C had a greater VWR-mediated difference in total EE compared to SED, absolute activity EE compared to 20°C, and activity EE as a percent of total EE. Our data suggests that thermoneutral housing is more appropriate for studying the impact of VWR, and increases in EE, on disease phenotypes.

APSIPE.124

Biogenesis of Mitochondrial Membranes

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One of Dr. Holloszy's many accomplishments is pioneering our understanding of skeletal muscle mitochondria. In his seminal paper published in 1967, he was the first to show that exercise training increases the activities of mitochondrial respiratory enzymes in skeletal muscle. His legacy is well represented in this and other subsequent publications that cemented the role of this organelle in skeletal muscle bioenergetics, substrate metabolism, and metabolic adaptation. Not only was he rigorous in experiments but he was also incredibly gifted in his foresight. For example, in a review published with Dr. Ed Coyle in 1984, they accurately predicted the possibility that an increase in AMP might trigger metabolic adaptations induced by exercise. This occurred at least a decade before we knew about the AMP-activated protein kinase (AMPK). It is incredible to note that Dr. Holloszy's team made these landmark discoveries when much of the modern molecular biology techniques we now use were unavailable. Led by Dr. Holloszy and other contributors, we know much more about how enzymes of mitochondrial oxidative phosphorylation are regulated, including substantial advances in their transcriptional control. My laboratory attempts to add to these important discoveries by studying how mitochondrial bioenergetics is regulated by the lipid composition of the inner mitochondrial membrane.

APSIPE.125

Mitochondrial quality control regulation by ATF4 in skeletal muscle

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The complexity of mitochondrial quality control involves the synchronous activation of multiple pathways that converge on the organelle. In skeletal muscle, the coordinated regulation of mitochondria is essential to the overall health and function of this highly metabolic tissue, as well as systemic health more broadly. ATF4 is a transcription factor and the primary regulator of the mitochondrial unfolded protein response (UPR^{mt}), which serves to preserve and promote mitochondrial function by enhancing the expression of important mitochondrial chaperones and proteases when subjected to a stress stimulus such as exercise. In this way, ATF4 is central to both mitochondrial biogenesis by augmenting the capacity for expansion of

the mitochondrial reticulum, while also facilitating in turnover of dysfunctional organelles through the mitophagy-lysosome system. Furthermore, mitochondria rely on the coordinated transcription of nDNA- and mtDNA-derived mitochondrial genes and this synergistic expression is necessary to preserve proteostasis, as imbalances in nDNA:mtDNA expression can disrupt mitochondrial viability and elicit UPR^{mt} activation. As ATF4 responds to acute cellular stresses, it has been implicated in regulating skeletal muscle health by mediating aging-related, and disuse-induced muscle atrophy and decline. However, while it is understood that ATF4 is involved in mediating the mitochondrial stress response, it remains to be determined whether ATF4 is necessary for mitochondrial biogenesis in skeletal muscle. We measured ATF4 expression in C2C12 cells prior to, and following 4-days of differentiation, and found ATF4 protein levels to be elevated more than 3-fold in differentiated myotubes. This induction of ATF4 coincided with 3-5-fold increases in protein expression of mitochondrial content markers COX I and IV, despite modest decreases in UPR^{mt} factors, mtHSP70, HSP60, and CPN10. Following acute contractile activity, ATF4 mRNA and protein content were upregulated 1.5-1.8-fold in both rat tissue and cultured cells, respectively. Additionally, these increases preceded the observed changes in mitochondrial content. Conversely, in a mouse skeletal muscle disuse model of 7-days hindlimb denervation, ATF4 protein levels were induced 90%, which coincided with a 20% reduction in mitochondrial volume. Together, our data indicate that ATF4 plays an important role in regulating stress-specific signaling across a variety of conditions such as contractile activity and chronic inactivity, and is thus a potential regulator of mitochondrial adaptations in muscle. We continue to explore the necessity of ATF4 in mitochondrial remodelling via ATF4 knockdown and overexpression experiments, thus solidifying whether ATF4 is a suitable target for mitochondrial-based therapies in order to promote overall metabolic health. Work supported by NSERC, Canada.

APSIPE.126

Mitophagy undergoes time-dependent changes in response to hindlimb denervation

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During chronic muscle disuse, fibre atrophy occurs concomitantly with reductions in mitochondrial content and function. This is due to depressed mitochondrial biogenesis and elevations in degradation. The processes that underlie the degradation of mitochondria have yet to be fully elucidated. The breakdown of the organelle is due to a selective form of autophagy, termed mitophagy. When a mitochondrion becomes dysfunctional it dissociates from the mitochondrial network and is subsequently degraded by lysosomes. Impaired degradation has been associated with an accumulation of dysfunctional organelles, which augments the atrophy process. Cumulatively, the objective of this work is to better understand the process of mitophagy in the context of muscle disuse. We employed a

hindlimb denervation protocol in which we unilaterally sectioned the peroneal nerve of one hindlimb, using the contralateral limb as a control in Sprague-Dawley rats for 1, 3 or 7 days. We observed significant 10% and 30% reductions in tibialis anterior (TA) mass by 3- and 7-days post-denervation respectively, along with significant 10-25% reductions in mitochondrial protein levels. To assess organelle function, we isolated subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria and measured their respiratory capacity and reactive oxygen species (ROS) production. We observed significant functional deficits at 3-days post-denervation, and further at 7 days post-denervation. Additionally, in permeabilized fibres we measured significant increases in ROS-emission at 1-day post-denervation. Cumulatively, these data suggest that mitochondrial quality is altered prior to the decay in content. We treated a subset of rats with the microtubule destabilizer colchicine (4mg/kg/day) for 2 days prior to sacrifice to inhibit mitophagy at the stage of transport to the lysosomes. We show that in SS mitochondria there are 10 and 25% elevations in degradation at 1 and 3 days respectively, whereas no changes were apparent in the IMF subfraction. However, in both subfractions, this was reduced in denervated muscle at 7 days. These measurements provide insight into mitochondrial breakdown to the point of autophagosomal formation, but fail to capture the terminal degradation step within the lysosome. In order to assess mitochondrial compartmentalization in response to denervation, we utilized a transgenic mt-keima mouse model and unilaterally denervated them for the same time course as above. Keima, a pH sensitive fluorophore is green in the pH of the cytosol and red in the acidic pH of the lysosomes. Using confocal microscopy of freshly excised TA muscles, we observe enhanced red:green fluorescence in 7-day denervated muscle, suggestive of elevations in mitochondria housed in lysosomes. This may be due to either enhanced transport of mitochondria to the lysosome or reduced degradative activity within the lysosome at 7 days of denervation. Lipofuscin accumulation by day 7 is suggestive of lysosomal dysfunction, which could lead to insufficient mitochondrial degradation and an accumulation of dysfunctional organelles.

APSIPE.127

Human skeletal muscle responses to microgravity

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Purpose: Microgravity induces significant skeletal muscle atrophy, equivalent to 50 years of human aging on Earth, in only two to three months. As international space agencies and private contractors plan for human's first journey to Mars (1/3 g) and colonization of Earth's moon (1/6 g), it is important to understand the current state of knowledge in this critical area of crew member health. Exercise has been shown to be the most promising countermeasure to microgravity-induced atrophy. Thus, it is important to also understand the state of knowledge for mitigating muscle atrophy and identify any remaining gaps to focus future investigations. **Methods:** We performed an exhaustive

review of the literature on ground-based analogue (bed rest & unilateral lower limb suspension) and spaceflight publications. This revealed ground-based studies on 699 subjects at 25 different timepoints (942 total observations from 93 publications), with exercise countermeasures examined in 264 of the subjects at 18 timepoints (384 total observations from 56 publications). Spaceflight has been reported on 48 crew members, all of whom completed varying levels of exercise countermeasures inflight, over 9 publications. **Results:** Magnitude of muscle atrophy throughout the body directly relates to the magnitude of typical daily 1 g muscle activity removed upon entry into the microgravity environment. A reduction plateau occurs by three months without countermeasures: lower leg (-30%) > upper leg (-20%) > torso (-10%) > upper limb (-6%) muscles. Exercise countermeasures have provided varying levels of atrophy protection. The most successful programs, using a minimalistic approach to free crew time, have effectively used high intensity low volume resistance exercise coupled with continuous aerobic and interval training (cycle and treadmill) exercise. Specifically, ground-based exercise programs for the thigh have been completely effective and could be adapted for the torso and upper limb musculature. The best programs have been only partially successful (~75%) for the lower leg. Inflight implementation of a completely effective exercise program for muscle atrophy has yet to be achieved. **Conclusions:** Exercise programs and associated hardware have been adapted and improved over the last 50 years of scientific investigation. Challenges remain for the hard to target but very important lower limb muscles. The development of exercise hardware that can appropriately implement the successful exercise programs within the constraints of long duration space capsules and reduced gravity outposts also remains a challenge.

APSIPE.128

Low Dose Aspirin and PGE2/COX Pathway Inhibition in Human Skeletal Muscle: Influence of Aerobic Exercise

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Purpose: Skeletal muscle health has been shown to benefit from regular consumption of cyclooxygenase (COX) inhibiting drugs. Aspirin, especially at low doses, is one of the most commonly consumed COX inhibitors, yet investigations of low dose aspirin effects on skeletal muscle are nonexistent. The goal of this study was to examine the efficacy of low dose aspirin on skeletal muscle COX production of the inflammatory regulator prostaglandin (PG) E2 at rest and following exercise. **Methods:** Skeletal muscle biopsies (vastus lateralis) were taken from eight individuals (4M, 4W; 25±1y; 81.4±3.4kg; VO2max: 3.33±0.21L/min) before and 3.5 hours after 40 minutes of cycling at 70% of VO2max for the measurement of ex vivo PGE2 production. Muscle strips were incubated in Krebs-Henseleit buffer (control) or supplemented with one of two aspirin concentrations that reflected blood

levels following a low (10 μ M; typical oral dose: 75-325mg) or standard (100 μ M; typical oral dose: 975-1000mg) dose. Results: Low (-22 $\pm$ 5%) and standard (-28 $\pm$ 5%) dose aspirin concentrations both reduced skeletal muscle PGE2 production, independent of exercise ($P < 0.05$). There was no difference in PGE2 suppression between the two doses ($P > 0.05$). Conclusion: Low dose aspirin levels are sufficient to inhibit the COX enzyme in skeletal muscle and significantly reduce production of PGE2, a known regulator of skeletal muscle health. Aerobic exercise does not appear to alter the inhibitory efficacy of aspirin. These findings may have implications for the tens of millions of individuals that chronically consume low dose aspirin.

APSIPE.129

Overview of Preclinical Animal Study Protocol and Results of Training Studies

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Exercise and regular physical activity are widely recognized to be important for maintaining overall health and wellness. Furthermore, exercise has been proven to be an effective therapy for the prevention and treatment of a variety of diseases including diabetes, obesity, cardiovascular disease, and cancer. The physiological response of selected tissues, such as skeletal muscle, heart, liver, adipose, and brain to exercise has been studied to varying degrees in healthy and unhealthy individuals; however, our understanding of the cellular and molecular mechanisms underlying the acute responses and training adaptations to exercise is incomplete. The Molecular Transducers of Physical Activity Consortium (MoTrPAC) is an NIH Common Fund program established to elucidate how exercise improves health and ameliorates diseases by building a map of the molecular responses to acute and chronic exercise. Within the consortium, animal studies are being performed at the Preclinical Animal Study Sites (PASS), which include the University of Iowa, University of Florida and the Joslin Diabetes Center. A primary goal of the animal studies is to enable an analysis of the systemic effects of exercise on multiple organs and tissues in response to both acute and chronic endurance exercise. Acute and chronic treadmill exercise was performed in 6 and 18 months old male and female Fischer344 (F344) rats, obtained from the National Institute on Aging Rodent Colony. For acute exercise, rats performed a single 30-minute bout of treadmill exercise at ~80% VO2max. Rats were euthanized and organs and tissues harvested immediately, 0.5, 1, 4, 7, 24 and 48 hours post-exercise. For chronic exercise, rats underwent 1, 2, 4, or 8 weeks of treadmill training 5 days/week at ~70% VO2max, with incline, duration, and speed of exercise progressively increased on a daily-to-weekly basis during the initial 6 weeks; tissues and organs were harvested 48 hrs after completing the last training bout. For both exercise studies, a total of 19 different tissues and organs were collected from exercised and sedentary, control animals. All biospecimens were shipped to the biorepository at the University of Vermont for storage and distribution to the Chemical Analysis Sites. Preliminary data will be presented

on the response of the rats to progressive endurance exercise training. Maximum oxygen consumption (VO2max) was measured in 6 and 18 months old prior to exercise training using a protocol adopted from Wisloff et al. (AJP Heart 280:H1301, 2001). The data show that females have a significantly higher VO2max and maximum running speed than males at both 6 and 18 months. Both VO2max and maximum running speed decreased significantly in females and males with age. The 8-week training protocol resulted in significant improvements in VO2max and maximum running speed in females and males at both 6 and 18 months of age. Body composition was measured using NMR and revealed significant changes in body composition in both males and females. Interestingly, gender specific alterations were apparent in response to the 8 weeks aerobic training. All animal procedures were approved by the Institutional Animal Care and Use Committees at the University of Iowa, University of Florida and the Joslin Diabetes Center.

APSIPE.130

Low Dose Aspirin and PGE2/COX Pathway Inhibition in Human Skeletal Muscle: Influence of Resistance Exercise and Sex

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Purpose: Prostaglandins (PGs) have been shown to regulate inflammation, resistance exercise adaptations, and skeletal muscle health. This study examined low dose aspirin inhibition of skeletal muscle cyclooxygenase (COX) production of the inflammatory mediator PGE2 at rest and following resistance exercise. We also examined sex-specific effects of aspirin by comparing these findings with previous investigations. Methods: Skeletal muscle biopsies (vastus lateralis) were taken from six individuals (4M, 2W; 25 $\pm$ 1y; 81.5 $\pm$ 3.4kg; VO2max: 3.45 $\pm$ 0.29L/min) before and 3.5 hours after a whole-body resistance exercise bout (8 exercises, 3x10RM/exercise) for the measurement of ex vivo PGE2 production. Muscle strips were incubated in Krebs-Henseleit buffer (control) or supplemented with one of two aspirin concentrations that reflected blood levels following a low (10 μ M; typical oral dose: 75-325mg) or standard (100 μ M; typical oral dose: 975-1000mg) dose. Results: Low and standard dose aspirin similarly ($P > 0.05$) reduced muscle PGE2 production (-18 $\pm$ 5%; $P < 0.05$), independent of resistance exercise. Skeletal muscle of men was 60% more sensitive to aspirin inhibition than women ($P < 0.05$). Conclusion: Low dose aspirin inhibitory effects on the PGE2/COX pathway do not appear to be altered with resistance exercise and may chronically influence skeletal muscle of women and men differently. More sex-specific investigations regarding low dose aspirin, skeletal muscle health, and exercise are needed.

APSIPE.131

Absence of TFEB or TFE3 results in divergent mitochondrial phenotypes that are rescued by exercise

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Endurance exercise is known to promote an oxidative phenotype in muscle, which is largely characterized by improvements in mitochondrial content and function. This was long thought to be due to the exercise-induced drive in mitochondrial biogenesis, that primarily focuses on PGC-1 α . Recently, mitochondrial recycling, or mitophagy, has been implicated in mediating mitochondrial adaptations to exercise as old and damaged mitochondria can be removed from the reticulum to promote the optimization of the organelle pool. TFEB and TFE3, two related transcription factors, regulate the expression of lysosomal and autophagy-related genes, and their activation following exercise is thought to support the drive in mitophagy. Thus, our purpose was to understand the role of these two transcription factors in mediating exercise-induced mitochondrial adaptations. We hypothesized that their loss would impair mitophagy and thus attenuate the benefits of chronic contractile activity (CCA) in improving mitochondrial function. Using siRNA, the expression of TFEB and TFE3 was reduced in C2C12 murine muscle cells, and then cells were subjected to electrical stimulation (5Hz, 10V, 3hr/day, 3 days) to induce CCA. The absence of TFEB resulted in a significant reduction in PGC-1 α protein content basally and abolished the CCA-induced increase, however this was not seen in TFE3-negative conditions. Despite this reduction in biogenesis signaling, a surprising 2-fold increase in mitochondrial-encoded COX I was seen basally and following CCA in the absence of TFEB, although no change was observed for nuclear-encoded subunit COX IV. Interestingly, the absence of TFE3 resulted in a 21% decrease in COX I protein, illustrating divergent roles for TFEB and TFE3 in mitochondrial regulation. We hypothesized that the increase in COX I in the absence of TFEB was a result of dysfunctional mitochondria accumulating as PGC-1 α was downregulated, thus we evaluated mitochondrial respiration using Seahorse technology and found a reduction in basal and maximal respiration in the absence of TFE3, but not with TFEB. Although, CCA elicited significant improvements in the respiratory profile, and was able to rescue the TFE3-induced deficit. These data suggest that the absence of TFE3 may result in poor basal mitochondrial maintenance, however exercise is capable of ameliorating this phenotype. It is possible that as these two transcription factors play similar roles, and the absence of one may be compensated by the other family member. Thus, future work will evaluate the mitochondrial phenotype in the absence of both TFEB and TFE3, as well as the potential ameliorative effect of contractile activity. This work is supported by the Natural Sciences and Engineering Research Council.

APSIPE.132

Acute Cardiovascular, Hormonal, and Metabolic Responses to Aerobic Exercise

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Purpose: The purpose of this study was to investigate the cardiovascular, hormonal, and metabolic responses to aerobic exercise (AE) during and after a 40-minute cycling bout at 70% of maximal aerobic capacity (VO₂max). **Methods:** Healthy young individuals were recruited and assigned to the AE group (n=8, 25 $\pm$ 1y) or the non-exercising control group (CON) (n=6, 24 $\pm$ 1y). Heart rate (HR) was recorded throughout the AE bout. Analyses of norepinephrine (NE), epinephrine (EPI), cortisol, insulin, glucose, free fatty acids (FFA), and lactate were completed from blood samples collected pre, 20 min during, 40 min during, 10 min post, 2 h post, and 3.5 h post AE or rest. **Results:** The average HR during the AE bout was 160 $\pm$ 7 bpm which corresponded to 83 $\pm$ 3% of HRmax. AE observed increased (P<0.05) concentrations of NE [+764% (AE = 2717 $\pm$ 251 vs. CON = 315 $\pm$ 106 pg/mL)], EPI [+458% (AE = 254 $\pm$ 48 vs. CON = 46 $\pm$ 7 pg/mL)], glucose [+29% (AE = 103 $\pm$ 1 vs. CON = 80 $\pm$ 2 mg/dL)], and lactate [+328% (AE = 3.9 $\pm$ 0.1 vs. CON = 0.9 $\pm$ 0.1 mM)] compared to CON at the 40 min AE/rest timepoint. At the 10 min post timepoint, glucose [+40% (AE = 109 $\pm$ 2 vs. CON = 78 $\pm$ 1 mg/dL)] and lactate [+180% (AE = 2.7 $\pm$ 0.1 vs. CON = 1.0 $\pm$ 0.1 mM)] concentrations remained elevated (P<0.05) in AE compared to CON; AE also observed increased (P<0.05) concentrations of cortisol [+102% (AE = 21.1 $\pm$ 1.1 vs. CON = 10.6 $\pm$ 0.9 μ g/dL)], insulin [+161% (AE = 12.5 $\pm$ 1.1 vs. CON = 4.8 $\pm$ 0.5 mIU/L)], and FFA [+83% (AE = 1.15 $\pm$ 0.05 vs. CON = 0.63 $\pm$ 0.03 mEq/L)] compared to CON. Additionally, there were main time effects (P<0.05) for cortisol (-47%) and FFA (+85%) concentrations. **Conclusions:** The present data support large and transient cardiovascular, hormonal, and metabolic responses to an acute bout of AE at 70% VO₂max on a cycle ergometer. The observed changes in cortisol highlight the circadian nature of this hormone and the potential of AE to alter its normal rhythmicity; such response may imply an influence of the time of the day on the responses and adaptations to AE. Overall, these findings emphasize the demands in energy homeostasis during and following AE and expand our knowledge on the characterized physiological responses to aerobic stimuli.

APSIPE.133

Maintaining bone and muscle mass in space: The astronaut perspective

Michael Fossum¹

¹self, self

Astronauts on a space mission are subjected to physical risk during dynamic flight (launch and landing), as well as additional risks during spacewalks, radiation exposure, and the ever-present threat of an onboard fire or catastrophic

failure. The solutions to mitigating these risks lies in the technical realm of engineering design and the careful adherence to procedures. A more insidious risk with long-term consequences to an astronaut's health is the accelerated loss of muscle mass, bone density, and cardiovascular fitness can only be overcome with an aggressive exercise program. After a decade of flying long-duration crew on the International Space Station, NASA had developed exercise equipment and protocols which had proven effective at maintaining astronaut health, but it did require a significant amount of valuable time on orbit. The SPRINT protocols using high-intensity exercise have the potential to maintain an astronaut's health with less overall time required, but the physiological effects needed to be proven as well as the astronaut's ability to tolerate the new protocol. As the first on-orbit test subject, I will be discussing my personal experience.

APSIPE.134

The effect of exercise preconditioning on VO₂peak and lean mass in breast cancer patients treated with anthracyclines – A preliminary study

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Estimates indicate that 1 in 8 US women will develop breast cancer in her lifetime. Improvements in early detection and treatments have improved survival, however, relative to patients not treated with chemotherapy, breast cancer patients receiving chemotherapy are at increased risk for cardiovascular disease, functional impairments and the loss of cardiorespiratory fitness that have implications for early morbidity and mortality. The purpose of this ongoing trial is to test the hypothesis that high-intensity exercise preconditioning (exercise commenced prior to initiating chemotherapy and continued throughout treatment cycles) preserves health in breast cancer patients treated with anthracycline-containing chemotherapy. Here, we present preliminary data from the ongoing trial that is focused on VO₂peak and skeletal muscle outcomes from the first 10 participants that have enrolled and 8 (N=2 dropout) that have completed the protocol. Breast cancer patients (N=8 completed; 50±11y; 168±4cm; 92±37kg; 32.3±12.3 BMI) scheduled to receive anthracycline-containing chemotherapy were randomly assigned to one of two interventions: 1) exercise preconditioning, (3d/wk of supervised exercise throughout treatment) or 2) standard of care (attention-control). Pre-testing occurred 1-2wk prior to chemotherapy. The interventions were initiated 1wk prior to chemotherapy and continued throughout anthracycline treatment (4 doses administered every other week). Post-testing occurred within 3-7d following the last anthracycline

treatment. VO₂peak (L/min) was reduced by 16% from pre (1.50±0.48 L/min) to post (1.27±0.46 L/min) in the standard of care group (P<0.05), whereas VO₂peak was preserved in the exercise preconditioning group from pre (1.37±0.38 L/min) to post (1.43±0.41 L/min) (P>0.05). Importantly, this difference in VO₂peak response corresponded to a Cohen's d effect size (ES) of 3.25. Medium to large ES for the change from pre to post also indicated greater preservation and/or improvement in the exercise preconditioning group for lean body mass (ES=0.81), ultrasound-derived measures of vastus lateralis muscle thickness (ES=0.73), and peak heart rate (ES = 0.48). These preliminary data highlight that exercise preconditioning may serve as a strategy to preserve cardiorespiratory fitness and perhaps lean mass during anthracycline treatment of breast cancer. There remains a need for larger, definitive clinical trials to identify strategies to prevent the array of chemotherapy-induced toxicities that are observed in cancer patients treated with anthracyclines. Funding: Virginia Piper Foundation, Mayo Clinic Arizona, and UACC.

APSIPE.135

Dietary Quality of Muscle Builders Who Do or Do Not Consume a Protein Supplement

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Athletes who are actively building muscle often use a protein supplement. Karimian and Esfahani (J Res Med Sci 2011;16;1346) reported 49% of bodybuilders used a supplement while 51% did not. Little is known about the impact of protein supplements on an athlete's diet, so this study examined the dietary quality of muscle builders who used a protein supplement versus those who got their protein from food alone. The Institutional Review Boards at LSUHSC and APUS approved this study. All subjects met these inclusion criteria: 1) age > 25 years; 2) actively building their muscle, 2) free of chronic disease, and 3) not taking any prescribed medications except for oral contraceptives. Thirty-nine people met the study criteria and completed a 24-hour recall using the validated ASA24 Dietary Assessment Tool and the International Physical Activity Questionnaire. Twenty-two subjects (P) consumed a protein supplement, and seventeen subjects (NP) did not. The average age of the subjects was 32 years, and the groups were similar in height, weight, and BMI (27±5). Eighty-nine percent of the subjects had a high physical activity level (>3,000 MET-min/week). The group consuming the protein supplement spent significantly more time in total physical activity (12081 + 8567 (P) vs. 7040 + 4795 (NP) MET-minutes/week), P=0.03) because they spent significantly more time in vigorous exercise (6331 + 5881 (P) vs. 2535 + 6331 (NP) MET-minutes/week), P=0.02). Calorie intake did not differ significantly between the two groups. Those consuming a protein supplement consumed significantly more protein (169 + 82 g (P) vs. 118 + 49 g (NP), P=0.02), and both groups were at the extremes of the protein recommendation (2.2 + 0.9 g/kg (P) vs. 1.5 + 0.6 g/kg (NP), P=0.009). The protein supplemented group consumed significantly more iron, magnesium, and fiber (27

+ 14 g (P) vs. 19 + 9 g (NP), $P=0.03$) reflective of a significantly higher intake of beans and peas as a vegetable ($0.24 + 0.45$ oz eq (equivalent) (P) vs. $0.03 + 0.12$ oz eq (NP), $P=0.045$), and intact fruit of citrus, melons, and berries ($0.44 + 0.69$ cup eq (P) vs. $0.07 + 0.15$ cup eq (NP), $P=0.02$). The percent of fat calories was significantly higher in the non-supplemented group's diet ($33.9 + 9.0$ (P) vs. $40.8 + 11.0$ (NP), $P=0.046$). Although not statistically different, the protein supplement group tended to have a healthier diet as assessed by the Healthy Eating Index ($61.8 + 15.1$ vs. $54.0 + 13.3$ HEI, $P=0.088$). In this study, a clear dietary difference existed between supplement users and non-protein supplement users. The diet of the athletes who consumed a protein supplement was of better quality, demonstrated by the higher fiber, bean, and fruit intake and lower fat intake. (Funding source: American Public University System)

APSIPE.136

Single Muscle Fiber Contractile Properties in Competitive Lifelong Aerobic Exercising Men

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Purpose: The purpose of this study was to examine single muscle fiber performance in competitive lifelong aerobic exercising men (LLE, 74 ± 1 y, $n=8$), aged-matched, non-exercising healthy controls (OH, 75 ± 1 y, $n=9$), and young exercisers (YE, 25 ± 1 y, $n=8$). On average, LLE exercised ~ 5 d/wk for ~ 7 h/wk over the past 52 ± 2 y. **Methods:** Each subject had a vastus lateralis muscle biopsy to examine myosin heavy chain (MHC) I and IIa single muscle fiber size and function (strength, speed, power). Briefly, single fiber diameter was measured digitally and cross-sectional area (CSA) was calculated; fiber force (P_o) and unloaded contractile velocity (V_o) were measured using the slack test technique; and maximum shortening velocity (V_{max}) and power were assessed using submaximal isotonic force-velocity measurements fit to the Hill equation. Fiber type (MHC) was determined using SDS-PAGE. **Results:** LLE MHC I size ($7698 \pm 670 \mu m^2$) was 25-40% larger ($P < 0.05$) than YE ($6083 \pm 440 \mu m^2$) and OH ($5440 \pm 612 \mu m^2$). LLE MHC I fibers were $\sim 20\%$ stronger ($P < 0.05$), $\sim 6\%$ faster ($P < 0.05$), and $\sim 30\%$ more powerful ($P < 0.05$) than YE and OH. In contrast, LLE MHC IIa size ($6073 \pm 350 \mu m^2$) was similar to OH ($6337 \pm 715 \mu m^2$), with both groups $\sim 30\%$ smaller ($P < 0.05$) than YE ($8151 \pm 529 \mu m^2$). MHC IIa contractile function was variable across groups, with a hierarchical pattern (OH > LLE > YE, $P < 0.05$) in normalized power (muscle quality) among OH (16.4 ± 1.7 W·L⁻¹), LLE (15.2 ± 1.2 W·L⁻¹), and YE (12.6 ± 0.9 W·L⁻¹). **Conclusion:** These data suggest that competitive aerobic training throughout the lifespan benefited slow muscle fiber size and function. The similar fast muscle fiber size between LLE and OH along with varied functional profiles suggests that lifelong aerobic exercise was less impactful for these fibers. Collectively,

these data suggest that other exercise modes (e.g., interval or resistance training) or myotherapeutics may be necessary to more adequately preserve fast fiber performance with advancing age.

APSIPE.137

Human Adipose and Skeletal Muscle Tissue DNA, RNA and Protein Content

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Purpose: This investigation examined DNA, RNA, and protein content in adipose tissue in order to gain additional insight into the amount of tissue that may be required for advanced analyses. Skeletal muscle was also studied to provide a direct comparison into potential differences between these two important and highly metabolic tissues. **Methods:** Basal adipose and skeletal muscle tissue samples were obtained from 10 (7M, 3W) recreationally active participants (25 ± 1 y; VO_{2max} : 3.5 ± 0.2 L/min). DNA, RNA, and protein were extracted and subsequently analyzed for quantity and quality. **Results:** Average DNA content of adipose and skeletal muscle tissue was 52 ± 4 ng and 189 ± 14 DNA·mg tissue⁻¹, respectively ($p < 0.05$). Average RNA content of adipose and skeletal muscle tissue was 46 ± 5 and 537 ± 23 ng RNA·mg tissue⁻¹, respectively ($p < 0.05$). Average protein content of adipose and skeletal muscle tissue was 4.0 ± 0.3 and $177 \pm 3 \mu g$ protein·mg⁻¹, respectively ($p < 0.05$). **Conclusion:** The primary finding was that human adipose tissue had 28% of the DNA, 9% of the RNA, and 2% of the protein amounts found in skeletal muscle per mg tissue. This information should be useful across a wide range of human clinical investigation study designs and various laboratory analyses, including large scale omics applications.

APSIPE.138

An In Vitro Model of Exercise to Study Skeletal Muscle Signaling Events During Contraction in Humans

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Background. Skeletal muscle is a central organ in whole-body metabolism, but its role in signaling metabolic adaptations at rest or during exercise is often overlooked. Indeed, muscle cells secrete signaling molecules including RNAs, metabolites, extracellular vesicles and small peptides called myokines. Myokines can act in an autocrine/paracrine or endocrine fashion to modify the metabolism and/or function of muscles, as well as other organs. The secretion of some myokines can be increased by muscle contraction. A major challenge for the study of myokine secretion in response to exercise is that

circulating levels do not represent their release from muscle as other organs can secrete these peptides. In this regard, the use of biopsy-derived human primary muscle cells prevents interference from other tissues when measuring myokine secretion. To mimic muscle contraction in vitro, electrical pulse stimulation (EPS) can be used, as it induces metabolic adaptations similar to those observed in the muscle of exercising humans. Most studies published to date on the effect of EPS on the secretion of myokines by human primary muscle cells have employed stimulation conditions mimicking chronic exercise (i.e. 24-48h in duration at a low voltage and low frequency). We set out to establish conditions to reproduce acute exercise in human primary muscle cells cultured in vitro, so as to better understand the impact of muscle contraction on the secretion of certain myokines. Objective. Li et al. (2018) demonstrated that EPS for 1h could mimic contraction-related adaptations in C2C12 mouse muscle cells (1). Their stimulation conditions activated the AMP-activated protein kinase (AMPK) pathway and the translocation of GLUT4, the main glucose transporter in muscle cells. Our objective was to validate their model of acute EPS treatment and adapt it for human primary muscle cells. Methods. We reproduced the EPS conditions of Li et al. (20V, 24ms impulses per second, at 1Hz for 1h) on differentiated human primary muscle cells derived from biopsies of clinical participants. The phosphorylation of AMPK, Acetyl CoA Carboxylase (ACC), AS160 and TBC1D1, all mediators of AMPK pathway activation leading to GLUT4 translocation during muscle contraction, was then measured by Western blot in whole-cell lysates. Total phospho-GLUT1 and GLUT4 was also quantified in isolated membrane, cytosol and total fractions of the cell lysates by Western blot. Secretion of a myokine extensively studied and known to be induced by muscle contraction, interleukin (IL)-6, was measured in the cell culture supernatant by ELISA. Significance of the findings. The optimization of our model of exercise in vitro could help better understand the signaling events occurring in skeletal muscle that lead to improvements in energy metabolism. This model would serve for the study of metabolic diseases such as obesity and type 2 diabetes, for which skeletal muscle is a key organ in the development and progression of the disease. Funding. This research project is funded by the Société Francophone du Diabète. 1. Li Z, Yue YY, Hu F, Zhang C, Ma XF, Li N, Qiu LH, Fu ML, Chen LM, Yao Z, Bilan PJ, Klip A, and Niu WY. Electrical pulse stimulation induces GLUT4 translocation in C2C12 myotubes that depends on Rab8A, Rab13, and Rab14. *American Journal of Physiology-Endocrinology and Metabolism* 314: E478-E493, 2018.

APSIPE.139

Acute Resistance Exercise-Induced Hormonal Response Increases Mitochondrial Fusion and Mitophagy Markers Only in Untrained Young Men

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Introduction: Mitophagy is imperative for maintaining quality mitochondria for efficient energy production and overall cellular health. In muscle cells, cellular mitochondria health maintained by mitophagy is critical for muscle regeneration. Prior published work by our group has demonstrated resistance exercise (RE)-induced hormonal changes (i.e., testosterone, growth hormone, cortisol) could result in a sex-specific response to satellite cell myogenic state after muscle damage. Testosterone and cortisol have been shown to regulate autophagic initiation, though the effect of these hormones on mitophagy remains uninvestigated. Given cellular mitochondria health is critical for muscle regeneration, it suggests the importance of determining whether there is a sex-specific hormonal response associated with mitophagy after muscle damage. **Purpose:** To examine the effect of the acute RE-induced hormone response on the mitochondrial fusion (OPA1 and MNF1), fission markers (DRP1), and mitophagy markers (PINK1 and PARKIN) in untrained young men and women. **Methods:** Sixteen untrained men (n= 8, 22 ± 3y) and women (n= 8, 20 ± 1y) performed 80 (8 sets of 10 repetitions; 3-min rest between sets) unilateral maximal eccentric knee extension exercise followed either by an upper-body RE (EXP) or seated rest for 20 min (CON). Muscle samples were analyzed for intramuscular gene expression for PINK1, PARKIN, DRP1, OPA1, and MNF1 at baseline (BL), 12h, and 24h post-exercise using real-time PCR. Relative fold change in transcript abundance was determined using the $\Delta\Delta Ct$ method. **Results:** We found a significant condition x time x gender interaction effect ($p \leq 0.05$) for PINK1. In men, intramuscular PINK1 gene expression was greater at 12h for EXP (2.76 ± 0.53 fold) than CON (0.88 ± 0.28 fold) and also greater at 24h for EXP (1.71 ± 0.29 fold) than CON (0.50 ± 0.18 fold). Further, in men, PINK1 was increased at 12h (2.76 ± 0.53 fold) from BL for EXP; whereas, PINK1 was decreased at 24h (0.50 ± 0.18 fold) from BL for CON. For EXP, PINK1 was greater in men (2.76 ± 0.53 fold) than in women (1.05 ± 0.53 fold) at 12h. For CON, there was no difference for PINK1 between genders. We also identified a significant condition x gender interaction effect for OPA1. For CON, OPA1 was greater in women (1.75 ± 0.28 fold) than men (0.87 ± 0.28 fold). Further, in men, OPA1 was greater in EXP (3.47 ± 0.77 fold) than CON (0.87 ± 0.28 fold), whereas there was no difference in women. We found a significant time effect for PARKIN, with it decreased at 24h (0.06 ± 0.01 fold) from BL and 12h (1.28 ± 0.22 fold). Furthermore, PINK1 and OPA1 were significantly correlated to growth hormone (PINK1: $r^2 = 0.25$; OPA1: $r^2 = 0.60$) and cortisol (PINK1: $r^2 = 0.36$; OPA1: $r^2 = 0.25$) in men regardless of condition. **CONCLUSION:** In response to muscle damage, our results suggest that acute RE-induced hormonal changes appear to increase mitochondrial fusion

(OPA1) and mitophagic response (PINK1) in untrained young men but not in untrained young women.

APSIPE.140

Systems-genetics interrogation of exercise-induced drivers of mitochondrial DNA content and function

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The physiological demands of exercise elicit beneficial metabolic effects systemically. Although the health benefits of repeated bouts of physical activity are well documented, the molecular mechanisms by which exercise combats metabolic dysfunction remain inadequately understood. A molecular hallmark of the exercise training response in skeletal muscle is an increase in the number and function of mitochondria. In addition to ATP-linked oxidative metabolism, mitochondria are critical signaling hubs responsible for calcium and iron homeostasis, cholesterol and fatty acid synthesis, and steroidogenesis. We explored whether similar to muscle, exercise promotes an increase mtDNA copy number (CN) in liver and adipose tissue, and determined whether this molecular adaptation is associated with specific health benefits of exercise. We utilized the UCLA Exercise Hybrid Mouse Diversity Panel (ExchMDP), a genetic tool comprised of ~100 distinct inbred and recombinant inbred mouse strains, to probe complex physiological and molecular traits that arise in response to exercise training. Female mice from the ExchMDP remained sedentary (SED) or performed volitional exercise training (TRN) by in cage running for 30 days. We quantified mtDNA copy number (CN) and performed RNA sequencing on skeletal muscle, liver, and gonadal white adipose tissue from 4 SED and 4 TRN mice following a 6h fast, 30h after the last bout of exercise. TRN mice showed elevated mtDNA in all three tissues (muscle 8.9% , P=0.0001; liver 7.0% , P=0.0008; adipose 18.5% , P=0.007). Because of the strain variability in daily running (average daily running distance across all strains = 5.9 ±3.3 km), we determined whether changes in mtDNACN are associated with exercise volume. Surprisingly, mtDNACN did not correlate with average daily running distance, running duration, or running speed for the three tissues studied (P>0.05). We observed a significant gene by exercise interaction that was confirmed by genome wide association studies of mtDNA copy number in each tissue. We conducted candidate gene analysis studies to identify genes causal for the increase in mtDNA with exercise TRN in liver and adipose tissue. Findings indicate unique regulatory control of mtDNACN under sedentary and exercise trained conditions. The marked increase in the mtDNACN in adipose tissue was inversely correlated with gWAT weight (bicolor -0.144, P=0.00018), while liver mtDNACN was inversely associated with circulating triglycerides (bicolor -0.191, P=0.00428) and HOMA-IR (insulin resistance index bicolor -0.148, P=0.028) following TRN.

Studies to identify the tissue-selective causal driver genes controlling the increase in mtDNACN and analyses to determine whether increases in mtDNACN underlie specific metabolic health benefits associated with exercise training are underway.

APSIPE.141

Tolerance to maximum exercise and quality of life in survivors of the severe form of COVID-19 in a city in Brazil

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Introduction: The new coronavirus (SARS-CoV-2) is responsible for causing acute respiratory syndromes that can vary from mild symptoms to severe conditions, with hospitalization and a significant mortality rate. Patients who require mechanical ventilation in the most acute phase of the disease may experience serious side effects, such as muscle dysfunction, fatigue, pain and dyspnoea, which can impact the quality of life. There may also be physical sequelae resulting from prolonged immobility, including cardiorespiratory deconditioning. Objective: To draw a cardiopulmonary and quality of life profile in patients affected by COVID-19 in the state of Pernambuco-Brazil. Methods: This is a cross-sectional study, in which patients were asked about their quality of life before and after Covid-19 using the Medical Outcomes Study Short - Form 36 (SF-36) questionnaire and underwent TECP. Results: 10 post-SARS-CoV-2 patients (4 men and 6 women, 51.1 ±10.89 years) were evaluated. The average hospital stay was 47.5 days, 60% of the patients were obese and 70% were hypertensive. The mean total SF-36 value before COVID-19 (590.5 ±159.9) and after (424 ±178.1) (p = 0.038) the other domains did not show differences. The mean value of the TECP variables were: VO2 peak 18.32 ±6.62, oxygen consumption at the first ventilatory threshold (VO2L1) 13.70 ±4.21, VE/CO2slope 35.68 ±5.38. Final considerations: Patients who were hospitalized by Covid-19 have a reduction in maximum functional capacity, which may have been influenced by the length of stay and obesity present in most of the sample. There was also a reduction in the total quality of life scores after Covid-19.

APSIPE.142

Severing the bond! The impact of DEPTOR-less mTOR on cellular anabolism

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The mechanistic target of rapamycin (mTOR), a protein kinase, is a central component of cellular anabolism, and of great interest in the study of cellular growth. Anabolism via the mTOR pathway is regulated by several molecules that are responsive to changes of energy status of the cell, which can lead to the activation or suppression of the

kinase. One protein known for the suppression of the mTOR kinase is known as DEPTOR. The binding of DEPTOR to mTOR results in the inactivation of mTORC1 and mTORC2 leading to the reduction of cell survival and autophagy. Conversely, anabolic stimulation of the cell and activation of mTOR results in the phosphorylation and subsequent degradation of DEPTOR. It is generally thought that an increase in protein synthesis in response to anabolic stimuli requires activation of the mTOR pathway, and full activation can only be achieved by the degradation of the DEPTOR-mTOR complex, allowing for the downstream phosphorylation of p70-S6 kinase (p70-S6K) and eIF4E binding protein 1 (4EBP1) by mTOR. A novel pharmacologic treatment, NSC126405, has been used to prevent the binding of DEPTOR to mTOR in multiple myeloma cells¹. To our knowledge this has not been applied to skeletal muscle. As the main site of metabolic regulation, skeletal muscle provides a lucrative target for the study of anabolism. Therefore, the aim of this study was to determine whether preventing the binding of DEPTOR to mTOR for 24 hours using with varying concentrations of NSC126405 drug would alter myotube anabolism. Results: Fractional synthesis rates were not affected by the 24-hour NSC126405 treatment at any dose ($p>0.05$). At low doses, 0.5 μM , total DEPTOR and the ratio of phosphorylated to total 4EBP1 are significantly elevated compared to control ($p=0.051$ and $p=0.03$). DEPTOR expression and the p4EBP1/4EBP1 ratio were not different than control at the 2 and 4 μM concentrations ($p>0.05$). However, at the higher concentrations of NSC126405 (2.0 and 4.0 μM , but not 0.5 μM), the ratio of phosphorylated to total p70-S6K were significantly greater than control ($p=0.04$ and $p=0.01$, respectively). Methods: L6 myoblasts were cultured on 10 cm plates in standard culture medium supplemented with 1% penicillin/streptomycin, and 10% fetal bovine serum until 70% confluence. Differentiation was induced by switching to medium supplemented with 1% penicillin/streptomycin, 2% horse serum, and 0.5% insulin-transferrin-selenium. On the fifth day myotubes were treated with media containing 4 concentrations of NSC126405 (0.5, 1.0, 2.0, 4.0 μM); all treatment media contained 4% deuterium oxide. Media and myotubes were harvested 24-hours after treatment. Protein expression of common anabolic markers was measured using western blot techniques. Fractional synthesis rates were assessed using deuterium oxide and mass spectrometry. Conclusion: These studies indicate that the pharmacologic loss of the DEPTOR/mTOR bond is sufficient to elevate mTORC1 activity, as indicated by the activation/phosphorylation of mTORC1 substrates. However, the prevention of DEPTOR/mTOR complexation did not affect the magnitude of fractional synthesis rates in these cells. While overall FSR was not affected by the intervention, the shift toward mTORC1 signaling suggests that the specific proteins being manufactured in treated cells are different than in untreated cells, which may ultimately serve to support anabolism beyond the 24 hours.

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APSIPE.143

Modulation of Muscle Specific MicroRNA and Growth Genes during Alaska Backcountry Expeditionary Hunting

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Introduction. Arduous physical and environmental conditions can negatively affect optimal human performance. We developed an Alaska Backcountry Expeditionary Hunting (ABEH) paradigm in remote, unscripted environments, which encompassed chronic physical activity, significant load carriage, limited nutrient supply and negative energy balance. Despite weight loss resulting from ABEH, skeletal muscle and lean tissue mass was largely preserved in our previous participants. Recognizing the regulatory importance of muscle microRNAs (myomiRs) as central mechanisms in the regulation of muscle anabolism, the objective of the current study was to measure the influence of a 12-day ABEH on changes in myomiRs and muscle growth gene expression in healthy human participants. Methods. Four female and male participants (48 $\pm$ 12 years; 26 $\pm$ 1 kg/m²) were recruited for this investigation. All data and samples were collected during pre- and post-ABEH visits in Fairbanks, AK. Body composition was measured using dual energy x-ray absorptiometry scans. The cross-sectional area of upper leg muscles (XT) was measured using magnetic resonance imaging. Muscle biopsies were obtained from the vastus lateralis using a 6-mm Bergström needle using sterile procedures under local anesthesia by a physician. Results. Real-time PCR was utilized to obtain expression values for five myomiRs (mi-1-3p, -206, -208b, -23a, 499a) and three muscle growth related genes (FSTL1, MEF2C, MyoD1). Data are reported as mean $\pm$ SD and considered significant at $P<0.05$. ABEH promoted significant reductions in body weight (6-1.7 $\pm$ 0.8 kg), fat mass (6-1.8 $\pm$ 0.3 kg) and body fat (6-1.9 $\pm$ 0.3%), but lean tissue mass (6+0.2 $\pm$ 0.8 kg) and XT (6-2.6 $\pm$ 3.9 cm²) were preserved. There was a >4fold upregulation in myomiR-206 and ~3fold increased expression of muscle FSTL1. Conclusion. Based on these preliminary findings in a small number of participants, we cautiously suggest that alterations in myomiR-206 and muscle FSTL1 may be important in the maintenance of skeletal muscle, even under conditions of negative energy balance elicited by vigorous levels of physical activity.

APSIPE.144

role of the central nucleus of the amygdala in blood pressure regulation during appetitive and aversive classical conditioning task in rats

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Predicting outcomes and preparing actions for future events based on current contexts is important, especially in

sports competitions owing to the dynamically changing environment. Motor control is essential for appropriate and rapid action, and autonomic cardiovascular tuning is likewise critical to supply energy to active skeletal muscles. However, the neuronal mechanisms underlying the dynamic adjustments of autonomic cardiovascular responses are still unclear. Previously, we showed that electrical and chemical stimulation of the central nucleus of the amygdala (CeA) in anesthetized rats induced bidirectional (facilitatory or inhibitory) cardiovascular responses in a region-specific manner, indicating that the CeA displays the neuronal circuitry underlying the modulation of autonomic responses (Yamanaka et al., 2018). Conditioned cardiovascular responses are classically recorded during anticipation of either appetitive or aversive outcomes (Harris and Brady, 1974). However, when facing an environment where the emotional context can dynamically change, cardiovascular responses are also dynamically regulated, and amygdala involvement has not been demonstrated. Thus, we hypothesize that the CeA plays a role in autonomic cardiovascular tuning for adaptive behavioral control. To test this hypothesis, we recorded blood pressure and heart rate of head-restrained male Wistar–Kyoto rats ($n = 7$) using telemetry during appetitive and aversive classical conditioning tasks. Rats learned varying associations between conditioned stimuli (CS) and unconditioned stimuli (US) in three contexts: reward (RW), neutral (NA), and aversive (AV) blocks. One tone (CS+, 10 kHz) cue was associated with reward delivery (reward CS+ predicts 5% sucrose, 0.08 mL) in the RW context, no outcome (neutral CS+) in the NA context, and punishment (aversive CS+ predicts an air puff, 30–40 psi, 1 s) in the AV context. The other tone (CS-, 4 kHz) cue was associated with no outcome in all contexts (reward, neutral, and aversive CS-). Blood pressure and heart rate in the RW block gradually increased after reward CS+, followed by a vigorously increased response to the actual reward. The predictive response was significantly higher than the responses in the NA and AV blocks. Blood pressure and heart rate responses to the air puff-predicting cue in the AV block were significantly lower than the responses in the NA block, although unanticipated context switching from NA to RW blocks produced blood pressure responses to reward CS+ similar to responses in the previous NA context block. In addition, responses to reward CS+ were dramatically increased after receiving reward US+. Finally, to test whether the CeA plays a causal role in the context-dependent regulation of blood pressure, we examined the effects of bilateral CeA inactivation by microinjection of the GABA receptor agonist muscimol. Inactivation of the bilateral CeA caused significant decrease in the blood pressure before and after switching from the RW to NA context ($p = 0.006$). Notably, a decrease in the blood pressure was observed during the second half of the RW context block ($p < 0.001$), but not immediately after NA→RW switching ($p > 0.05$). Our findings demonstrate that blood pressure is rapidly regulated in a context-dependent manner and that the CeA likely assists in maintaining pressor response tuning in dynamically changing emotional contexts. This work was supported by Grant-in-Aid for Scientific Research for Young Scientists (B) 16K16485 (to K.Y.), MEXT-Supported Program for the Strategic Research

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APSIPE.145

Early neuromuscular and contractile maladaptations to short-term bed rest

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Background: Inactivity and unloading induce rapid skeletal muscle atrophy and functional decline, detectable after 2-3 days (1). Importantly, muscle force is lost to a much greater extent than muscle mass (2, 3). We aimed to investigate the early determinants of the disproportionate loss of muscle force with respect to the decrease in muscle size in response to unloading caused by short-term bed rest. Methods: 10 male volunteers (22.9 ± 4.7 years; 181.2 ± 3.9 cm; 77.5 ± 10.0 kg) underwent 10 days of bed rest (BR). At baseline (BR0) and at the end (BR10), quadriceps femoris (QF) volume and knee-extensors force (MVT) were obtained by MRI and dynamometry, respectively. At BR0, BR5 (mid-BR) and BR10 blood samples and a biopsy from the vastus lateralis (VL) were collected. Damage to the neuromuscular junction (NMJ) was assessed from serum levels of C-terminal Agrin Fragment (CAF) and presence of denervated fibers was determined by positivity for neural cell-adhesion molecule (NCAM) staining. Mechanical properties of single fibers were also assessed. The study was approved by the local Ethics committee. Results: from BR0 to BR10, QF volume and MVT respectively decreased by 5.2% ($p < .01$) and 14.3% ($p < .01$). A significant increase of NCAM positive fibers was observed with respect to baseline: 5.5 (n.s.) fold at BR5 and 26.1 fold at BR10 ($p < .01$), accompanied by a 10.6% increase in serum CAF levels at BR5 (n.s) and 19.2% at BR10 ($p < .05$), reflecting increased instability of the NMJ. In addition, fast fiber CSA decreased by 15% ($P = .055$) at BR10, while single fibers maximal tension (force/CSA) was unmodified. However, the handling of Ca^{2+} by the sarcoplasmic reticulum was altered as calcium release induced by caffeine exposure decreased at BR10 by 35.1% ($P < .01$) and 30.2% ($P < .001$) in fast and slow fibers, respectively. Myofiber functionality expressed as pCa_{50} (Ca^{2+} concentration at which 50% of the maximum force is produced) did not change. Discussion: These findings show that early onset of NMJ damage, denervation and impairment in calcium dynamics are major

determinants of the greater loss of muscle force than of muscle size accompanying disuse. The identification of such mechanisms warrant the implementation of early countermeasures against the maladaptive changes of the neuromuscular system to unloading. Funding by ASI, MARS-PRE Project, n. DC-VUM-2017-006, is acknowledged. References 1. R. Demangel, et al., Early structural and functional signature of 3-day human skeletal muscle disuse using the dry immersion model. *J. Physiol.* 595, 4301–4315 (2017). 2. B. T. Wall, et al., Substantial skeletal muscle loss occurs during only 5 days of disuse. *Acta Physiol.* 210, 600–611 (2014). 3. M. D. de Boer, C. N. Maganaris, O. R. Seynnes, M. J. Rennie, M. V. Narici, Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. *J. Physiol.* 583, 1079–1091 (2007).

APSIPE.146

Passive stretch improves insulin-stimulated glucose transport together with downregulation of TXNIP in rat immobilized soleus muscle

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PURPOSE An acute bout of exercise is known to increase susceptibility of glucose transport to insulin and improves insulin resistance in skeletal muscle. This is possibly mediated by decrease in cellular energy status (decrease in phosphocreatine and ATP) and following activation of AMP-activated protein kinase (AMPK). Although passive stretch stimulates glucose transport independently of insulin action, it is unclear whether stretch increases susceptibility of muscle glucose transport to insulin and improves insulin resistance. Therefore, we examined the effect of stretch on insulin-stimulated glucose transport in insulin resistant soleus muscles of immobilized rats. In addition, we examined the possibility that stretch decreases protein expression of TXNIP which is known to be a key negative regulator of insulin signaling. **METHODS** Rats were divided into non-immobilized control and immobilized groups. Non-immobilized control rats were allowed to move freely in their cages. Immobilized rats were anesthetized and their both hindlimbs were immobilized for 6 h. Unilateral soleus muscles of immobilized rats were shortened by plantarflexing the ankle joint throughout 6 h immobilization. Contralateral muscles were stretched for 1 h by dorsiflexing the ankle joint following 5 h shortening by plantarflexing. We measured basal and insulin (50 μ U/ml) stimulated 2-deoxyglucose (2DG) uptake rate in isolated soleus muscles. Moreover, phosphocreatine and ATP concentration were measured in these muscles. In addition, AMPK phosphorylation and TXNIP protein expression were evaluated. **RESULTS** Although insulin (50 μ U/ml) increased glucose transport by 1.9-fold in soleus muscles of non-immobilized control rats ($p < 0.05$), insulin did not

significantly increase glucose transport in shortened muscles of immobilized rats. This result shows that insulin resistance is induced in these shortened muscles. On the other hand, 1 h passive stretch restored insulin resistance of glucose transport in muscles of immobilized rats (insulin-stimulated 2DG uptake in control, 3.62 ± 0.21 ; shortened, 1.95 ± 0.12 ; stretched, $3.66 \pm 0.21 \mu\text{mol/g muscle}/20\text{min}$). However, 1h passive stretch did not decrease phosphocreatine and ATP concentration, additionally it did not change AMPK phosphorylation. In addition, TXNIP protein was increased in shortened muscles of immobilized rats as compared with muscles of control rats ($p < 0.05$). Moreover, elevated TXNIP expression in these muscles was returned to control level after 1 h passive stretch (TXNIP in control, 100 ± 9 ; shortened, 166 ± 8 ; stretched, 107 ± 9 arbitrary units). **CONCLUSION** Passive stretch improves insulin-stimulated glucose transport in insulin-resistant soleus muscles of immobilized rats, although the cellular energy status and AMPK activation were not changed. Thus, the mechanism by which stretch improves insulin action is unlikely to be similar to that of exercise. The effect of stretch may be due to downregulation of TXNIP protein expression.

APSIPE.147

The Acute Effect of High-intensity Interval Exercise with Blood Flow Restriction on Circulating Progenitor Cell Number in Chronic Heart Failure

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Peripheral vascular dysfunction is both a precursor to and a consequence of heart failure with reduced ejection fraction (HFrEF) and is involved in propagating disease severity. In HFrEF, in addition to endothelial damage, peripheral vascular dysfunction may be driven via a reduced endogenous repair capacity (reduced number of circulating hematopoietic and endothelial progenitor cells [PCs]). In healthy adults, there are both short- and long-term benefits of exercise on endogenous repair capacity. A single session of aerobic exercise induces a transient increase in circulating PC number, and exercise training leads to a sustained increase in baseline PC number. However, in HFrEF, although exercise training can increase baseline PC counts, the transient response to a single session of aerobic exercise is severely attenuated, negating the short-term benefits of exercise seen in healthy adults. However, combining high-intensity interval exercise (HIIT), a common exercise strategy in HFrEF, with blood flow restriction (BFR) may provide an additional stimulus to evoke a short-term increase in exercise-induced PC mobilisation via alterations in peripheral vascular shear stress, decreases in muscle oxygenation and increases in the release of angiogenic factors. **Purpose:** To investigate the effect of a single session of HIIT with BFR on PC number in HFrEF. **Methods:** 12 participants (71 ± 11 y; 1 female) with HFrEF (New York

Heart Association I-III; LVEF: $34 \pm 9\%$) were recruited. Participants completed HIIT (5 x 2 min cycling bouts at 80% ramp-incremental peak power, separated by 2 min recovery), either with BFR (HIIT+BFR; $n = 6$; thigh cuff pressure 100 mmHg) or without BFR (HIIT; $n = 6$; thigh cuff pressure 10 mmHg). BFR cuffs were inflated around the proximal portion of the thighs for the first 90 s of recovery between each exercise bout. The number of PCs (CD34+ and CD34+/KDR+ cells) in venous blood was measured before and 10 min after exercise using flow cytometry. Results: Pre-exercise, there was no difference in CD34+ and CD34+/KDR+ cell number in HIIT+BFR and HIIT groups ($P > 0.05$). There was no increase in CD34+ PC number pre- to post-exercise, and no difference between groups (pre vs. post: HIIT+BFR: 2469 ± 1537 vs. 2343 ± 1362 / 106 total events; HIIT: 2491 ± 1218 vs. 2408 ± 1137 / 106 total events; time effect: $P = 0.71$; interaction: $P = 0.94$). Likewise, the number of CD34+/KDR+ PCs remained unchanged pre- to post-exercise, with no difference between groups (pre vs. post: HIIT+BFR: 78 ± 75 vs. 49 ± 27 / 106 total events; HIIT: 66 ± 21 vs. 71 ± 41 / 106 total events; time effect: $P = 0.40$; interaction: $P = 0.23$). Conclusion: Circulating PC number is not increased by a single session of HIIT with or without BFR in HFrEF, providing further support that exercise-induced PC mobilisation is impaired, and a single session of exercise is unable to engender a meaningful transient increase in endogenous repair capacity in HFrEF. Therefore, adherence to exercise training is necessary to positively impact vascular health via an increase in endogenous repair capacity.

APSIPE.148

Mechanisms of intolerance for long- and short-duration high-intensity exercise: a possible role for the inspiratory rib cage muscles

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INTRODUCTION: At the limit of tolerance (LoT), the maximal isokinetic power-generating capacity of the locomotor muscles (PISO) greatly exceeds the task-specific power required by high-intensity cycling with a tolerable duration of >7 minutes (TlimLONG), i.e. there is a 'power reserve'. Conversely, a power reserve is typically not observed following high-intensity cycling with a tolerable duration of <6 minutes (TlimSHORT). This suggests that the mechanisms associated with intolerance are different for TlimLONG and TlimSHORT. Preliminary data from our lab suggest that the magnitude of exercise-induced inspiratory muscle fatigue is greater after TlimLONG vs. TlimSHORT. PURPOSE: To determine whether the magnitude of the power reserve at the limit of high-intensity constant-power cycle exercise is related to the severity of inspiratory muscle fatigue. METHODS: Ten healthy adults (25 ± 5 yr; 2 females) completed a ramp-sprint test to determine critical power (CP) and peak ramp power (RITpeak). Constant-power cycling was performed to the LoT at 1) 25 % (TlimLONG) and 2) 50 % (TlimSHORT) of the difference between CP and RITpeak. PISO was measured at the LoT

during a 6-s maximal effort at 80 rpm. Cervical magnetic stimulation (CMS) was used to discriminate between the relative contributions of diaphragm and rib cage muscle fatigue to global inspiratory muscle fatigue. A reduction in transdiaphragmatic (Pditw) and gastric twitch pressure (Pgw) without a decrease in esophageal twitch pressure (Pestw) to Pgw ratio (Pestw/Pgw) is suggestive of predominant diaphragm fatigue. Conversely, a reduction in Pditw and Pestw/Pgw with maintenance of Pgw is suggestive of predominant rib cage muscle fatigue. RESULTS: Tlim was 10.2 ± 2.6 min and 4.9 ± 0.7 min for TlimLONG and TlimSHORT, respectively ($P < 0.01$). At the LoT, PISO was greater than task power in TlimLONG (356 ± 110 vs. 234 ± 53 W; $P < 0.01$) but was not different to task power in TlimSHORT (315 ± 115 vs. 263 ± 53 W; $P = 0.11$). The exercise-induced reduction in CMS-derived Pditw and Pgw was greater in TlimLONG vs. TlimSHORT (Pditw: -22 ± 12 % vs. -14 ± 12 %; $P = 0.03$) (Pgw: -35 ± 13 % vs. -25 ± 20 %; $P = 0.04$). There was no difference between TlimLONG and TlimSHORT in the pre- to post-exercise change in Pestw/Pgw ratio (0.8 ± 0.8 vs. 0.8 ± 1.1 , respectively; $P = 0.88$) or the decrease in Pestw (-15 ± 15 % vs. -9 ± 13 %, respectively; $P = 0.22$). There was a significant negative relationship between the difference in power reserve (δ power reserve) and the difference in Pestw (δ Pestw) between TlimSHORT and TlimLONG ($r = -0.76$; $P = 0.01$). No such relationships between δ power reserve and δ Pditw ($r = -0.47$; $P = 0.17$) or δ Pgw ($r = 0.53$; $P = 0.11$) were found. CONCLUSION: Diaphragm fatigue was greater following TlimLONG than TlimSHORT, but no relationship between δ Pditw or δ Pgw with the δ power reserve suggests that diaphragm fatigue is unlikely to have a role in the different mechanisms of intolerance between trials. However, although speculative, the presence of a negative relationship between δ Pestw and δ power reserve may suggest that inspiratory rib cage muscle fatigue differentially contributes to the LoT in TlimLONG vs. TlimSHORT.

APSIPE.149

Reproducibility of skeletal muscle oxygen consumption and oxidative capacity measured using Near Infrared Spectroscopy (NIRS) in young and old adults

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Background: Skeletal muscle dysfunction contributes to the decline in exercise capacity with age. Developing methods to monitor skeletal muscle in older adults is important to mitigate decline. NIRS has been applied in skeletal muscle for local assessment of oxygen consumption (mVO₂) and oxidative capacity (τ), however, reproducibility of NIRS measures in older adults has not been established. The objective of this study was to assess reproducibility of NIRS-measured mVO₂ and τ in young and old adults within one study. Methods: Older adults (OA) were enrolled in a tri-ethnic, population-based cohort study, the Southall and Brent Revisited (SABRE) study, young adults (YA) were

University students or staff. Oxygenated and deoxygenated haemoglobin were measured from the gastrocnemius using NIRS (Portamon, Artinis), arterial occlusions were applied proximal to the measurement at rest to measure mVO₂ and following a resistance band exercise protocol to measure the time constant for the recovery of mVO₂ (τ). All measurements were repeated during a single NIRS evaluation. Data are mean $\pm$ SD, agreement between measurements is presented as mean difference (δ) and limits of agreement (LOA); rho refers to Lin's concordance correlation coefficient. Results: 10 young adults (male 4(40%), mean age $\pm$ SD 25 $\pm$ 4 years) & 14 older adults (male 9(64%), mean age $\pm$ SD 74 $\pm$ 9 years) completed all measurements. Resting mVO₂ was reproducible in both groups (OA: δ [LoA] was -0.49[-1.62, 0.65] $\Delta\mu$ M/s; rho=0.92, p<0.001; YA: δ [LoA] was 0.00[-0.003, 0.002] $\Delta\mu$ M/s; rho=0.98, p<0.0001). Similarly, the time constant for the recovery of mVO₂ was reproducible in both groups (OA: δ [LoA] was -7.76[-29.29, 13.77]s; rho=0.91, p<0.0001; YA: δ [LoA] was -5.47[-59.85, 48.92]s; rho=0.73, p<0.0001). Conclusion: NIRS can be applied in young and old adults to reproducibly measure local resting mVO₂ and τ . This provides a simple non-invasive tool for assessment of age-related decline in skeletal muscle function. All procedures were in accordance with the principles of the Helsinki declaration, all participants provided written informed consent. The older adult protocol was conducted as part of the SABRE study and was approved by the National Research Ethics Service (NRES) Committee London-North Fulham. The young adult protocol was approved by the UCL Research Ethics Committee. Funding source: British Heart Foundation.

APSIPE.150

Administration of loquat leaf extract enhance resistance training-induced muscle strength in aged female rats

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The progressive loss of skeletal muscle mass, strength, and physical function that occurs with aging is known as sarcopenia. Resistance training improves skeletal muscle mass and strength. Moreover, loquat (*Eriobotrya japonica* (Thunb.) Lindl.) has been shown to effectively protect against aging-induced skeletal muscle loss, but its effects on resistance training-induced muscle hypertrophy and strength require further research. The aim of this study was to investigate the effects of loquat leaf extract (LLE) administration on resistance training-induced muscle hypertrophy and strength in aged female rats. Female adult rats (16 months old) were divided into the following 4 groups: control sedentary, control climbing training, LLE administration sedentary, and LLE administration climbing training groups. The rats in the LLE administration group were given LLE (1.5 g/kg/day) once a day by oral gavage for 8 weeks. The rats in the climbing training group were trained (one session every 3 days for 8 weeks) to climb a ladder while bearing a load. At 18 months old, the flexor

hallucis longus (FHL) muscles were collected and analyzed. The total training load and maximum load-carrying capacity were increased (p < 0.05) by climbing training. LLE administration did not affect the total training load, but the maximum load-carrying capacity was higher in the LLE administration climbing training group than in the control climbing training group (p<0.05). The weight of the FHL muscles was significantly increased in the sedentary LLE administration. The weight of the FHL muscles was significantly increased in the control and LLE administration climbing training groups but was not significantly different between the groups. These results suggest that LLE administration enhanced the resistance training-induced muscle strength in the aged female rats.

APSIPE.151

In the D2.mdx mouse model of Duchenne muscular dystrophy, restoring mitochondrial creatine metabolism is associated with partial improvements in muscle quality

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Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease with limited treatments. We recently reported that skeletal muscle mitochondrial creatine metabolism is impaired in a mouse model of DMD (D2.mdx). Rescuing this pathway might improve muscle health given creatine can amplify ADP's ability to stimulate mitochondrial ATP production while attenuating mitochondrial reactive oxygen species production. The objective of this study was to identify whether the mitochondrial-targeted drug Olesoxime (TRO19622) would improve mitochondrial creatine-dependent bioenergetics and muscle health in D2.mdx mice. Male D2.mdx mice received a daily oral gavage of Olesoxime (DRUG) (30mg/kg b.w.) or corn oil (VEH, vehicle) from days 10-28 of age. Age-matched wildtype (WT) animals served as a healthy control. Quadriceps and diaphragm muscle were collected, and mitochondrial bioenergetics were assessed using high resolution respirometry and high resolution spectrofluorometry in permeabilized fibre bundles. Creatine sensitivity, an index of mitochondrial creatine kinase (mtCK) activity, was assessed by comparing conditions saturating mtCK (20mM creatine) to conditions lacking creatine. The ability of creatine to stimulate respiration in WT quadriceps (+37-62% across 100-500 μ M ADP) and diaphragm (+39-55%) was lost in VEH only for quadriceps but rescued by DRUG (+18-28%). Likewise, the ability of creatine to attenuate mitochondrial H₂O₂ emission in WT quadriceps (-30 to -38%) and diaphragm (-33 to -34%) was lost in VEH but was rescued by DRUG in quadriceps (-42 to -43%) and diaphragm (-23 to -26%). These improvements in creatine metabolism were related to lower serum creatine kinase (-54%, DRUG vs VEH; muscle damage marker), recovery from fatigue in quadriceps (+25-38% vs VEH), longer cage hang time

(+53%), and a small increase in whole body lean volume (+3.0%) and hindlimb muscle volume (+6.5%) as assessed by microCT. Decreases in grip strength, voluntary wheel running distance, maximal stimulated force in quadriceps (in situ) and diaphragm (in vitro) observed in VEH were not improved by DRUG. In summary, short-term treatment with Olesoxime rescued mitochondrial creatine metabolism which occurred in parallel to partial improvements in some indices of muscle health in D2.mdx mice.

APSIPE.152

DNA and Protein Extraction from Human Adipose and Skeletal Muscle Tissue: Methods Comparison

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Purpose: The goal of this investigation was to determine the homogenization and extraction technique pairing that led to the highest DNA and protein extraction yields from adipose and skeletal muscle tissue. **Methods:** Basal adipose and skeletal muscle tissue samples were obtained from healthy, recreationally active individuals. A variety of homogenization and extraction protocols were tested. Briefly, two homogenization techniques (plastic tube and pestle and Omni soft tissue probe) and three extraction methods (TRIzol, DNAzol, and DNeasy Blood and Tissue kit) were tested for DNA extraction. For protein extraction, six homogenization techniques (glass homogenizer, plastic tube and pestle, PowerGen, glass tube and pestle, Omni hard tissue probe, and Omni soft tissue probe) and three extraction methods (House buffer, RIPA buffer, and Minute Total Protein column for adipose tissue) were tested. **Results:** Across the different methods, the DNA content ranged from 0.9 to 55 ng DNA·mg tissue⁻¹ in adipose and from 24 to 225 ng DNA·mg tissue⁻¹ in skeletal muscle tissue. Protein content across the various methods ranged from 1.4 to 3.5 µg protein·mg tissue⁻¹ in adipose and from 48 to 246 µg protein·mg tissue⁻¹ in skeletal muscle tissue. **Conclusion:** The DNA and protein yields obtained from adipose and skeletal muscle tissue were method dependent. The combination of the DNeasy Blood and Tissue kit with manual plastic tube and pestle homogenization yielded the highest quantity of DNA for both tissues. Homogenization via the Omni Tissue homogenizer with the plastic soft tissue homogenizing probe in RIPA buffer yielded the highest adipose and the second highest skeletal muscle tissue protein contents.

APSIPE.154

Acute Hormonal and Metabolic Responses to Whole-Body Resistance Exercise

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Purpose: This investigation examined hormonal and metabolic responses in blood to a single bout of whole-body resistance exercise (RE). **Methods:** Healthy, physically active individuals (n=6, 25±1y) were recruited to the RE group and were compared to a group of non-exercising control (CON) subjects (n=6, 24±1y). The CON group was included to account for normal circadian variations in blood markers. The RE bout consisted of 3 sets of ~10 repetitions on 8 movements including: chest press, overhead press, seated row, triceps extension, biceps curl, leg press, leg curl, and knee extension on cable assisted equipment. Analyses of norepinephrine (NE), epinephrine (EPI), cortisol, insulin, glucose, free fatty acids (FFA), and lactate were completed for both groups from blood samples collected pre, 10 min post, 2 h post, and 3.5 h post RE or rest. **Results:** RE observed increased (P<0.05) concentrations of cortisol [+93%, (RE = 20.5±1.2 vs. CON = 10.6±0.9 µg/dL)], insulin [+233%, (RE = 16.0±0.9 vs. CON = 4.8±0.5 mIU/L)], glucose [+53%, (RE = 119±5 vs. CON = 78±1 mg/dL)], and lactate [+840%, (RE = 9.2±0.3 vs. CON = 1.0±0.1 mM)] compared to CON at the 10 min post timepoint. EPI was lower (P<0.05) in RE at baseline compared to CON [-66%, (RE = 17±3 vs. CON = 50±21 pg/mL)]. There were no differences in NE or FFA concentrations at any timepoint between RE and CON (P>0.05). Additionally, there were main time effects (P<0.05) for cortisol (-47%) and FFA (+81%) concentrations from pre to 3.5 h post timepoints. **Conclusion:** These data support large and transient hormonal and metabolic responses after whole-body RE in select blood markers. Differences in the cortisol response of the RE group imply that time of day may influence responses and adaptations to strength training. The present findings underscore the fluctuations in energy homeostasis after RE and expand our knowledge of basic physiological responses to this stimulus.

APSIPE.155

Development of a Human Muscle-on-Chip to Study the Effect of Microgravity and Electrically Stimulated Contraction Onboard the International Space Station

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Introduction: Microgravity-induced loss of muscle as experienced by astronauts provides a unique opportunity to study accelerated muscle degeneration. Recent studies

indicate that reduced gravity can negatively impact cell autonomous proliferation and differentiation and is an area of growing research focus. Significant progress in microphysiological systems, or organ-on-chip technologies, have the potential to advance the study of microgravity's impact on human cell biology. This work describes the development of a microphysiological system to evaluate the adaptation of primary human muscle cell-laden hydrogels in a microfluidic perfusion system that also enables electrical pulse stimulated contraction. Our long-term goal is to leverage the microgravity conditions on the International Space Station (ISS) to study changes in human skeletal muscle cell biology. **Materials and Methods:** Biopsies of the vastus lateralis were obtained from young (<40yrs) and older (>60yrs) study volunteers. Myoblasts were isolated from the biopsies and enriched for CD56+ myogenic cells and were encapsulated into 30 μ L of a biomimetic collagen-matrigel hydrogel and injected into a PDMS-based chip designed to enclose the cells while a surrounding channel facilitated diffusion of culture medium. Tissue chips were perfused with growth medium for 2 days, followed by 7 days of intermittent perfusion with differentiation-conductive media. **Results:** The tissue chip was evaluated for its ability to maintain muscle bundle viability and sterility and to induce electrically stimulated muscle contraction matching the delivered frequency. Immunofluorescent staining of myosin and actin proteins indicated a fusion index >80% and myotube alignment within 30°, while also revealing sarcomere structures, the basic contractile unit of the muscle fiber. We tested different daily electrical stimulation regimes and captured images to assess mean displacement of the muscle bundle using a MATLAB algorithm. Displacement magnitudes recorded over 60secs ranged from 0.2-4.5 mm for non-stimulated and stimulated conditions. Muscle bundles were then preserved with RNALater for gene expression studies. We found significant upregulation of MyoD, myogenin, MRF4, and MLP, all of which are regulatory proteins involved in skeletal muscle myogenesis. **Conclusion:** This work demonstrates the successful implementation of a microphysiological system for the study of muscle structure and function. We expect the first human skeletal muscle-on-chip study to be delivered to the ISS in Nov 2020. We hypothesize that muscle microtissues isolated from young athletic and older sedentary adults and exposed to microgravity may show a significant difference in expression of atrogenes, fiber size, and contraction rate compared to time-matched and phenotyped-matched ground controls. This work is supported by NCATS/NIH (UG3TR002598 awarded to SM)

APSIPE.156

Downhill running elicits positive cardiac adaptations in growth-restricted mice

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Introduction: Growth-restriction (GR) during postnatal life is linked to increased cardiovascular disease risk in

adulthood, with greater impairments seen in females. Regular aerobic exercise training can promote cardiovascular health, but the effect on GR mice remains unclear. Downhill running (DHR) is consistently used for skeletal muscle growth but it can also stimulate a moderate intensity aerobic load. Therefore, the purpose of this study was to examine the effect of DHR on cardiovascular capacity in GR female mice. We hypothesized that DHR would improve cardiovascular capacity as measured by echocardiography. **Methods:** To induce postnatal growth-restriction, FVB mouse dams were fed either a 20% protein control diet or an 8% low-protein isocaloric diet beginning 2 weeks before mating. Pups born to control-fed dams were cross-fostered to either a low-protein fed dam to induce growth-restriction (PUN) or a different control fed dam (CON). On postnatal (PN) day 21 all pups were weaned and fed a control diet. At PN45 mice were allocated to down-hill running (PUN-DHR; n=4, CON-DHR; n=3) or sedentary (PUN-SED; n= 5, CON-SED; n= 4) The DHR protocol was defined as treadmill running 3 days/week for 12 weeks at 18 m/min at a -16% decline for 28 minutes. At PN 128 echocardiography and Doppler analysis measured cardiac structure and function while the mice were under 1% isoflurane anesthesia. Statistical analysis was performed using JMP Pro, utilizing a two-way ANOVA with body weight and heart rate as covariates, with an alpha level of .05 set a priori and if significant Tukey's post hoc for multiple comparisons was conducted. **Results:** Echocardiography revealed a diastolic dysfunction pattern with impaired relaxation that was improved with DHR. The PUN-SED group exhibited reduced E-wave deceleration rate (EWDR; p=.0001) and systolic left ventricular internal diameter (LVIDs; p=.0426) and increased isovolumic relaxation time (IVRT; p=.0001) and deceleration time (DT; p<.0001). Structurally, the PUN-SED group displayed increased left ventricular mass (LVM; p=.0121), LV Mass Index (p=.0152), diastolic LV anterior wall (LVAWd; p=.0175) and systolic posterior wall (LVPWs; p=.0343) thickness but displayed no change in ejection fraction or isovolumic contraction time. DHR improved these variables in PUN-DHR, except for DT, such that there was no significant difference between CON-DHR and PUN-DHR. The DT of PUN-DHR decreased (positive adaptation) but only to levels similar of CON-SED. CON-SED and CON-DHR were not significantly different, except for DT, IVRT & EWDR. **Conclusion:** DHR reversed the cardiac impairments caused by growth-restriction but there is a possible adverse effect on the control group. This data suggests that diastolic impairments caused by postnatal growth-restriction can be mitigated with downhill exercise training, but this modality may not be appropriate for healthy populations.

APSIPE.157

Acute Cardiovascular Response to Whole-Body Resistance Exercise

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Purpose: This investigation examined cardiovascular responses to a single bout of whole-body resistance exercise (RE). **Methods:** Healthy, recreationally active individuals (n=6, 25±1y) were recruited to the RE group and a group of non-exercising control (CON) subjects (n=6, 24±1y) were included to account for normal circadian variation. The RE bout consisted of 3 sets of ~10 repetitions on 8 movements including: chest press, overhead press, seated row, triceps extension, biceps curl, leg press, leg curl, and knee extension on cable assisted equipment. Heart rate (HR) was recorded continuously throughout the RE bout for the RE group and during a 40-min rest period in the CON group. **Results:** Average HR during the RE bout was 136 ±3 bpm which corresponded to 70 ±2% of HRmax and 50 ±10% of heart rate reserve (HRR). During the bout HR peaked at 170 ±2 bpm, corresponding to 87 ±1% of HRmax and 79 ±6% HRR. The HR response was cyclic in nature and tended to peak shortly after completing each set and decreased during each 90-s rest period. Average HR in CON subjects was 67 ±1 bpm during the rest period, which was lower (P<0.05) than the average HR during the RE bout. **Conclusion:** These data support large and transient cardiovascular responses during whole-body RE. While not typically viewed as an aerobic stimulus, these data illustrate the potential for RE to facilitate cardiovascular and oxidative adaptations. Due to the short bursts of maximal effort followed by a rest period, the expected adaptations could be similar to those typically seen when undergoing high intensity interval training.

APSIPE.158

Effect of maternal aerobic exercise on infant mesenchymal stem cell metabolism: The ENHANCED By Mom Study

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Aerobic exercise in nonpregnant populations has been shown to enhance certain aspects of the metabolic phenotype including glucose and lipid handling. Recent preclinical data suggests exercise during pregnancy can improve the metabolic phenotype not only of the mother, but of the developing offspring as well. We utilized an in-utero cell culture model (mesenchymal stem cells, MSC) to characterize the effects of maternal aerobic exercise on the offspring's metabolic phenotype. Expecting mothers, were

randomized into one of two groups: aerobic exercise (AE) or non-exercise control (CON). The AE group completed 150 min of weekly moderate-intensity exercise during pregnancy (24+ weeks) while the CON attended stretching sessions. Following delivery, MSCs were isolated from the umbilical cord of the offspring. One set of MSCs from each subject were serum starved and incubated with ¹⁴C-glucose in the presence or absence of insulin to measure rate of glucose oxidation and glycogen synthesis (an index of insulin sensitivity, Si). A second set of cells were incubated with ¹⁴C-Oleate to measure rates of fatty acid oxidation (complete and incomplete), uptake, and esterification. Our preliminary data suggests MSCs from AE-trained mothers had elevations in complete fatty acid oxidation (0.021±0.006 vs. 0.023±0.005 nmol/mg/min., CTRL vs AE, n=4) and reductions in incomplete fatty acid oxidation (1.03±0.1 vs. 1.01±0.07 nmol/mg/min., CTRL vs AE, n=4). These data provide evidence of a programmed metabolic phenotype in human offspring resulting from maternal aerobic exercise.

APSIPE.159

MicroAge: Functional, contractile 3-D human muscles constructs for studies of the effects of microgravity on muscle mass and function on board the International Space Station.

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Shifting age demographics are increasingly affecting modern societies, contributing to increasing numbers of older adults with relatively poor health and quality of life. The patho-mechanistic basis for age-related muscle loss has yet to be fully resolved. However, we have previously demonstrated that skeletal muscle from aged pre-clinical species and humans shows maladaptation to exercise, thus compromising their ability to maintain muscle mass and function. Previous studies have highlighted that exercising muscle in young or adult animals and humans generates reactive oxygen species (ROS) which subsequently activate specific transcription factors leading to increased generation of cytoprotective proteins. These effects are not recapitulated in the muscles of older animals or humans in response to exercise. In an analogous, albeit accelerated manner, muscles of astronauts and animals exposed to microgravity also rapidly lose mass and are relatively unresponsive to resistance training under microgravity. Funded by the UK Space Agency (UKSA) for an upcoming national mission to the International Space Station (ISS), we will utilise in vitro studies of bio-engineered, human skeletal muscle constructs to determine whether analogous maladaptation to contractile activity occurs in muscle constructs exposed to microgravity as that which occurs with older adults on earth. For this purpose, we have developed a contractile, synthetic muscle model using immortalised human myoblasts encapsulated in fibrin hydrogels and anchored onto bespoke, 3-D printed polylactic acid (PLA) scaffolds. The resultant muscle constructs

will be integrated into fully sealed 'plug-and-play' experimental units, specifically designed to execute fluid exchanges at defined intervals, perform electrical field stimulations, simultaneously monitor muscle contractions via impedance sensing and maintain construct viability for ~9 days. On board the ISS, the experimental units will be interfaced with the 'KUBIK' incubator, allowing for automated execution of the experimental sequence with minimal crew involvement. The custom flight hardware to maintain the muscle constructs and permit electrical field stimulation has been designed in collaboration with Kayser Space Ltd., (Harwell, UK), and the experiment is anticipated to fly to the ISS in November 2021.

APSIPE.160

Does stretch activation contribute to slow-twitch muscle endurance?

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Stretch activation (SA) is a delayed increase in force following a rapid muscle length increase. SA is best known from asynchronous insect flight muscle where its high magnitude replaces calcium's typical role of modulating muscle force levels during a contraction cycle. SA also occurs in mammalian skeletal muscle, but previously was thought to be too low in magnitude, relative to calcium-activated (CA) force, to be a significant contributor to force generation during locomotion. However, during extended muscle use, i.e. fatigue conditions, calcium-activated force is impeded, in part, by high [Pi] and poor calcium cycling, thus a compensatory force modulation mechanism such as stretch-activated force generation could be beneficial. To test this supposition and investigate the mechanism behind SA, we compared SA and CA tension at different Pi concentrations (0-16 mM) in skinned mouse soleus (slow-twitch) and extensor digitorum longus (EDL, fast-twitch) muscle fibers. CA isometric force decreased 54-59% in both muscles with increasing [Pi]. In EDL, SA force decreased by 40% with [Pi], leaving the SA to CA force ratio relatively constant, 17-25%, at all [Pi]. In contrast, SA force increased 42% in soleus, causing a quadrupling of the SA to CA force ratio, 11% at 0 mM Pi to 43% at 16 mM Pi. Our results agree with previous studies that found SA was modest at low [Pi], but our novel work shows that SA is a significant force modulator in slow-twitch mammalian fiber types at higher [Pi]. For the SA force modulation we measured to be useful during locomotion, a muscle must be going through cyclical lengthening and shortening contractions. For extensor muscles such as soleus, many of which are known to be actively stretched prior to shortening, the delayed tension increase brought about by this stretch must be timed to occur with soleus shortening. This results in higher force during shortening, producing greater work and power for locomotion. To test this possibility, we overlaid soleus muscle length change patterns of running mice with SA traces from our *in vitro* soleus fiber experiments. Synchronizing the start of the

lengthening portions revealed that the SA force peak would occur when the soleus muscle powering running is shortening. This is promising for skeletal FSA to be physiologically relevant because augmenting force during soleus shortening would increase its work and power output. Based upon our previous *Drosophila* myosin studies and this work, we propose two mechanisms to explain the different SA responses of EDL and soleus. A rapid stretch of a slow-twitch fiber, in the presence of Pi, reverses myosin's power stroke and drives some myosins back to a weakly-bound pre-power stroke state. These myosins can then quickly rebind to actin and increase force production. Reversing the power stroke could be a mechanism to store and return energy that a muscle might gain from a preceding eccentric contraction. In contrast, for fast-twitch fibers stretch and Pi may cause myosin to detach from actin without reversing the power stroke. Supported by NIAMS R01 grant AR064274 to D.M.S. Correspondence: swankd@rpi.edu

APSIPE.161

Hepatokines-a novel group of exercise factors

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Regular physical activity not only improves the exercise capacity of the skeletal muscle performing the contractions, it is beneficial for the whole body. An extensive search for exercise factors mediating these beneficial effects has been going on for decades. Particular skeletal muscle tissue has been investigated as a source of circulating exercise factors, and several myokines have been identified. However, exercise also has an impact on other tissues. The liver is interposed between energy storing and energy utilising tissues and is highly active during exercise. Hepatic O₂ uptake and CO₂ delivery can increase two-fold in order to maintain energy homeostasis. Studies in exercising mice revealed pronounced regulation of key signal transduction pathways and the hepatic transcriptome after one acute bout of exercising, indicating the adaptation of the hepatic metabolism and stress defense. These adaptative processes may be supported by muscle-derived metabolites as succinate, which shows a pronounced hepatic uptake during exercise. The data also revealed the upregulation of > 50 potentially secreted proteins. Analysis of arterial-to-venous differences of proteins over the hepato-splanchnic bed in exercising humans confirmed the release of the hepatokines fibroblast growth factor-21, follistatin, angiopoietin-like protein 4, heat shock protein 72, and insulin-like growth factor binding protein from the liver and the increase in the bloodstream during or in the recovery after an exercise bout. The exercise-induced release of hepatokines is blocked by somatostatin, which implicates a hormonal regulation by glucagon/insulin or by plasma free fatty acids. Thus, a novel group of exercise factors termed hepatokines has emerged. These hepatokines may convey some of the beneficial whole-body effects of exercise that could ameliorate metabolic diseases, such as obesity or type 2 diabetes.

APSIPE.163

The response to physical activity in mouse and man: an interplay between genetics and exercise

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Increased physical activity is routinely prescribed by physicians as a defense to combat and prevent cardiometabolic diseases. Engagement in such activities has been extensively proven to be at least mildly effective regardless of age, sex, ethnicity, or cardiometabolic disease status. Despite the overwhelming evidence supporting physical activity, long-term adherence among the general population is low. In addition, the molecular mechanisms by which participation in physical activity leading to improvements in health are needed for the development of personalized medicine and next generation therapeutic approaches. To address these needs, we undertook an unprecedented massive scale genetics project where female mice from the Hybrid Mouse Diversity Panel (HMDP) performed voluntary wheel running for approximately one month. The HMDP is a powerful and unique genetic tool that has been used in the intricate dissection of complex traits, especially cardiometabolic ones, and consists of over 100 inbred and recombinant inbred strains of mice. Due to the genetics of the HMDP, high-resolution association mapping or genome wide association studies (GWAS), gene-by-environment (e.g. physical activity), and multi-omics analyses and integration (systems genetics) can and have been performed to elucidate causality of complex traits. Twelve samples were taken from each animal resulting in thirty different traits allowing for a comprehensive whole-body and tissue specific assessment of the exercise response to be conducted. Specifically, we sought to determine the consequences of sustained physical activity upon physiologically and clinically relevant traits. Furthermore, our exercise HMDP was integrated with the human Skeletal Muscle, Myokines and Glucose Metabolism (MyoGlu) study. This study consisted of twenty-six normal or overweight male Norwegians who were subjected to a 12-week exercise training program with pre- and post-acute exercise bouts. Subcutaneous white adipose tissue and skeletal muscle biopsies were taken at multiple time points within MyoGlu which nicely complement, substantiate, and expound upon our mouse data. Using a multi-tissue and multi-omics approach in mouse and man, our discoveries fit into four main categories: 1) phenotypic response to exercise for clinically and physiologically relevant traits (highlighted by reductions in liver triglycerides), 2) molecular response to exercise within skeletal muscle, white adipose tissue, brown adipose tissue, liver, and heart (distinct reprogramming of brown adipose tissue metabolism), 3) a personalized exercise response due to the interaction between exercise dose (duration, intensity, type) and natural genetic variation, and 4) insight into the biology of volitional exercise where strong central nervous system regulation was uncovered. Our results are also being made publicly available in a user friendly and simple

web app designed to allow researchers to visualize, compare, and interrogate data at all levels.

APSIPE.164

High Intensity Exercise in Space: Human Skeletal Muscle Fiber Types After 6 Months Aboard the International Space Station

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Purpose: To examine skeletal muscle fiber types from the leg muscles of six male astronauts (48±3y) before and after a long duration stay (166±7 d; range 135-193 d) on the International Space Station (ISS). While on the ISS, astronauts performed an exercise program incorporating continuous aerobic and high intensity interval training (cycle and treadmill) coupled with high intensity, low volume whole body resistance exercise six days per week. Methods: Skeletal muscle biopsies of the vastus lateralis (VL) and soleus (SOL) were obtained ~45 days before flight and within 24h of landing back on earth. A total of 6137 single muscle fibers were isolated from the VL (253±22 from each biopsy) and SOL (258±25 from each biopsy) and analyzed via SDS-PAGE for myosin heavy chain (MHC) profile. Hybrid fiber types were a composite of MHC I/IIa, IIa/IIx and I/IIa/IIx fibers. Results: Average fiber type profile for the VL was similar pre to post flight for MHC I (40±7 vs. 39±9%), MHC IIa (45±5 vs. 51±7%) and hybrid (15±4 vs. 10±3%) fibers, although there was considerable variation among two astronauts (±20% change in slow fiber population). There was a slow-to-fast fiber shift in the SOL with a reduction (P<0.05) in MHC I fibers (78±3 vs. 62±7%) that was distributed across the MHC IIa (17±3 vs. 24±3%) and hybrid (5±2 vs. 15±6%) fiber types. Conclusion: These are the first upper leg muscle (vastus lateralis) fiber type data with long duration space flight and the limited alterations suggest an effective countermeasures program, although a degree of heterogeneity was observed among the astronauts. The soleus data show a slow-to-fast fiber type transition that is comparable to our previous work when astronauts performed a moderate intensity exercise program and suggest that challenges remain to protect the lower leg muscles. This contractile protein phenotypic shift has several health implications for skeletal muscle (i.e. fuel use, reduced oxidative capacity, fatigue, insulin resistance, organ cross-talk) and may require additional exercise and/or loading paradigms to fully mitigate this multifaceted risk.

APSIPE.165

Effects of pulmonary hypertension on oxygen exchange in contracting rat skeletal muscle

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Introduction: Pulmonary hypertension (PH) is a disease characterized by pulmonary vascular dysfunction and exercise intolerance due, in part, to gas exchange impediments and impaired cardiac function. In the rat model of PH, we sought to determine whether there are peripheral (i.e. muscle) aberrations in O₂ delivery (QO₂)-to-O₂ utilization (VO₂) matching and vascular control that might contribute to poor exercise tolerance. Furthermore, we investigated the peripheral effects of nitric oxide (NO), a potent vasodilator, in attenuating these anticipated decrements. **Methods:** Adult male Sprague-Dawley rats were administered a one-time intraperitoneal injection of monocrotaline (MCT; 50 mg/kg) to induce progressive PH. Disease progression was monitored via echocardiography and experiments were conducted when moderate PH, but not right ventricular (RV) failure, became evident. Phosphorescence quenching protocols determined the partial pressure of O₂ in the interstitial space (PO_{2is}) in the spinotrapezius muscle at rest and during contractions under control (SNP (-)) and NO-donor (SNP (+)) superfusion conditions. **Results:** MCT rats displayed RV hypertrophy (RV/LV+S: 0.44 ±0.04 vs. 0.28 ±0.01), pulmonary congestion, increased right ventricular systolic pressure (48 ±6 vs. 20 ±3 mmHg), and arterial hypoxemia (PaO₂: 63.5 ±3.5 vs. 83.5 ±3 mmHg) compared to healthy rats (all $P \leq 0.05$). No differences were observed in SNP (-) PO_{2is} kinetics or steady state contracting PO_{2is} between healthy and MCT ($P > 0.05$), although PO_{2is} was significantly lower in MCT animals during seconds 10-26 of contractions. SNP (+) revealed a significantly lower PO_{2is} and total muscle oxygenation (Area under the curve: 1730 ±215 vs. 2789 ±165) throughout contractions in MCT animals versus healthy ($P \leq 0.05$). **Conclusions:** Our findings reveal that, for small muscle mass exercise in MCT rats, muscle oxygenation is impaired across the rest to exercise transition but not at rest or during contraction steady state. The capacity for exogenous NO to elevate the QO₂-to-VO₂ ratio in contracting muscle is attenuated by PH. These data support QO₂-VO₂ mismatch as a potential contributor to slowed VO₂ kinetics, and thus reduced exercise tolerance, in PH patients, and suggest smooth muscle dysfunction as a mechanism for this impaired muscle QO₂ response to exercise. This work was supported in part by the Sustained Momentum for Investigators with Laboratories Established (SMILE) Grant.

APSIPE.166

AS160 is Essential for Greater Insulin-stimulated Glucose Uptake in Isolated Rat Skeletal Muscles after AICAR Stimulation

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Background: 5' AMP-activated protein kinase (AMPK) activation has been proposed to play a role in the improved insulin sensitivity that is observed in skeletal muscle after acute exercise. It has also been suggested that enhanced phosphorylation of the insulin signaling protein Akt Substrate of 160 kDa (AS160; also known as TBC1D4) is involved in the greater insulin sensitivity observed post-exercise. Supporting this idea, it was reported that incubation of isolated muscles with 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), a pharmacological AMPK activator, induced subsequently enhanced insulin-stimulated glucose uptake (GU) in isolated skeletal muscles from wildtype (WT), but not AS160-knockout (KO) mice. Rats are frequently studied to elucidate the mechanisms that regulate post-exercise, insulin-stimulated GU in skeletal muscle. Accordingly, the objective of this study was to use our recently created AS160-KO rat model to test if AS160 is essential for AICAR-induced elevation in insulin-stimulated GU in rat skeletal muscle. **Hypothesis:** AS160 is essential for greater insulin-stimulated glucose uptake in rat skeletal muscle after AICAR-induced activation of AMPK. **Methods:** Male, AS160-KO and WT control rats (16-22 weeks-old) were studied. Both epitrochlearis muscles from each rat were isolated and longitudinally split into two muscle strips. Each muscle strip underwent a four-step incubation. During step 1 (60 minutes), muscle strips were incubated with Krebs-Henseleit Buffer (KHB) ±AICAR (2mM). During step 2 (180 minutes), all muscle strips were incubated in KHB supplemented with glucose in the absence of AICAR. During steps 3 (30 minutes) and 4 (20 minutes), muscle strips were incubated ±insulin (1.2nM). Radiolabeled 3-O-methyl-D-glucose was included during step 4 to allow the measurement of GU. Muscles were also assessed for phosphorylated levels of: AMPK α (pAMPK α), AS160 (pAS160), Akt (pAkt), acetyl-CoA carboxylase (pACC), and TBC1D1 (pTBC1D1) by immunoblotting. **Results:** Prior AICAR-treatment induced greater GU in insulin-stimulated muscles from WT, but not AS160-KO rats. Enhanced insulin-stimulated GU in WT rats was accompanied by greater pAS160 on Ser704 and Thr642. Prior AICAR-treatment induced similar increases in multiple indicators of AMPK-activation in the muscles from WT versus AS160-KO rats, including: pAMPK α -Thr172 and the phosphorylation of AMPK's downstream targets pACC-Ser79 and pTBC1D1-Ser237. Neither genotype nor AICAR effects were observed for pAkt-Thr308 or pAkt-Ser473. **CONCLUSIONS:** AS160 is essential in isolated rat skeletal muscles for the AICAR-induced improvement in insulin-stimulated glucose uptake.

APSIPE.167

Exercise-induced metabolite signatures in skeletal muscle are similar between young and old adults

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Introduction: Impairments in skeletal muscle mitochondrial and amino acid metabolism may contribute to age-related diseases such as sarcopenia. Endurance exercise is known to improve skeletal muscle metabolic health in part by enhancing metabolic flux to meet the energetic demands of contractile activity. It is unclear, however, whether aging and sarcopenia influence acute exercise induced perturbations in skeletal muscle metabolite signatures. The purpose of this investigation was to define resting and exercise-induced metabolite signatures in skeletal muscle from groups of active (OA), sedentary (OS), and sarcopenic (OSC) older adults and a group of active young adults (YA). **Methods:** A total of 51 participants (OA=13, OS=16, OSC=7, YA=15) were examined over 3 study visits which included assessments of cardiorespiratory fitness (VO₂peak), muscle mass (skeletal muscle index (SMI)) and strength (maximal strength (1-RM) of the quadriceps muscles). Percutaneous biopsies of the vastus lateralis were obtained before, immediately after, and 3 hours following a bout of endurance exercise (cycle ergometry, 45 minutes at 70% heart rate reserve). Metabolite profiles were generated from a portion of the biopsy samples by tandem mass spectrometry. All experimental procedures were conducted in accordance with guidelines set forth in the Declaration of Helsinki and approved by the institutional review board at AdventHealth Orlando. **Results:** Of the 431 metabolites identified in the analysis, 257 were differentially expressed between groups at baseline, and 176 metabolites were altered with exercise. We identified age-, activity-, and sarcopenic-specific metabolite signatures using an unbiased data-driven cluster analysis. Individual metabolites within these clusters associated with VO₂peak, SMI and strength quadriceps 1-RM. When data from all groups was combined, the abundance of short-chain acylcarnitines (butyrylcarnitine and hydroxybutyrylcarnitine) corresponded with VO₂peak, while several amino acids (leucine, phenylalanine, tryptophan) were positively associated with SMI and 1-RM. At the whole-body level, endurance exercise induced a greater reliance on fat utilization as evidenced by reduced respiratory quotient and increased whole-body fat oxidation. These responses were similar between groups. At the level of skeletal muscle, endurance exercise induced an increase in short-chain acylcarnitines (butyrylcarnitine and hydroxybutyrylcarnitine) and ketone bodies (2-hydroxybutyric acid and 3-hydroxybutyric acid) in a specific temporal pattern. This metabolite response to endurance exercise was similar amongst the groups. **Conclusion:** Despite baseline differences, the metabolite response in skeletal muscle was remarkably comparable between young and older groups both immediately after and 3

hours following an acute bout of endurance exercise. These findings are consistent with beneficial effects of endurance exercise to improve metabolic health in older adults. This study was funded by National Institute on Aging (K01AG044437 awarded to PMC).

APSIPE.168

Effect of age and exercise on in vitro endothelial cell migration

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Introduction: Endothelial cell migration is an important step in physiological angiogenesis. Physiological angiogenesis in striated muscle is an important adaptation to exercise training. The effect of age and acute exercise on physiological angiogenesis is not well understood. We hypothesized that the serum of younger adults would induce a higher level of endothelial cell migration in vitro than serum of older adults after a single bout of dynamic exercise. **Methods:** 11 healthy adults, 5 older and 6 younger, provided written, informed consent and participated in the study. They completed a single bout of dynamic exercise, which consisted of 45 minutes of cycle ergometry at 60% age-predicted HR_{max}. Serum was collected before and after the exercise bout and was used in an in vitro transwell cell migration assay. Human umbilical vein endothelial cells (HUVEC) were plated on a porous membrane at a density of 5 x10⁴ cells/well. The cells were allowed to attach overnight and were then treated with basal cell media containing 2% serum collected from subjects. After an overnight incubation, the cells were stained with DAPI so that they can be visible using fluorescent microscopy. The membranes were imaged and the number of cells migrated to the bottom of the membrane were manually counted. **Results:** The results of a 2-way ANOVA suggest that there is no significant difference in cell migration within each age group from pre-exercise to post-exercise ($p > 0.05$). There was also no difference in cell migration between age groups at either pre-exercise and post-exercise ($p > 0.05$). **Conclusion:** These results suggest that in vitro endothelial cell migration induced by human serum is not affected by a single bout of acute exercise in either older or younger adults. Additionally, age does not appear to have an effect on endothelial cell migration at rest or immediately after exercise.

APSIPE.169

Effects of aging and endurance exercise training on bone structure and blood flow

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The link between bone blood flow and bone formation/resorption is well-established. Advancing age is linked to decreases in bone blood flow and bone density. Endurance exercise training has been shown to improve both bone blood flow and bone formation rates. We hypothesized that long-term endurance exercise training restores bone blood flow and increases bone density in advancing age. Young (n=24, 4-6 months) and old (n=28, 20-22 months) male Fischer-344 rats underwent treadmill exercise training (15 m/min, 15° incline, 1hr/day, 5 days/wk for 10-12 wks) or remained sedentary in cages. At the end of the training period, quantitative analysis of distal femur metaphysis, including bone volume density (BV/TV, %), connectivity, number, thickness, and separation of trabeculae, was performed using microcomputed tomography equipped with an x-ray tube working at 70 kV and 114 μ A with a 20- μ m voxel size. Additionally, regional blood flow to the femur was measured at rest and during exercise using radiolabeled microspheres. Advancing age reduced bone volume density (30%), trabecular connectivity (66%), and number (35%), and increased spacing (66%) of trabeculae compared to young age (p<0.05). Contrary to expectations, bone structure was not changed by endurance exercise training in either young or old rats. Regional blood flow to the proximal and distal metaphysis and epiphysis of the femur, as well as diaphyseal marrow was lower in aged rats than in young rats at rest. However, exercise-trained old rats showed significantly enhanced blood flow to the proximal and distal metaphysis and epiphysis and diaphyseal marrow during exercise when compared to old-sedentary rats (p<0.05). These data suggest that impaired bone structures and reduced regional bone blood flow are closely associated with advancing age. However, endurance training intervention in old rats can restore skeletal blood flow during exercise to that similar to young-sedentary and -trained rats. Supported by NIH grant AG055029

APSIPE.170

Stay-At-Home Mandate during COVID-19 Pandemic Negatively Impacted Dietary, Activity, and Alcohol Use Patterns in People Living with HIV with At-risk Alcohol Use

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The COVID-19 pandemic, leading to mandated limits to non-essential activity, has instigated paradigm shifts in health-related behaviors that may have a more profound impact on individuals with comorbidities. People with pre-existing conditions are at higher risk for complications of COVID-19 yet vulnerable to maladaptive changes in dietary and activity patterns. People living with HIV (PLWH) often have comorbid conditions that may place them at increased risk of more severe COVID-19 illness which may be complicated by at-risk alcohol use, and at-risk alcohol use could adversely impact health-promoting behaviors. The objective of this study was to assess health-related behaviors in PLWH with at-risk alcohol use during the early phases of the COVID-19 pandemic. Eighty PLWH with fasting dysglycemia participating in the Aging in Louisiana: Immunosenescence, HIV, & Socioenvironmental Factors-Exercise (ALIVE-Ex) study consented to a brief phone survey during the Louisiana stay-at-home order. Participants were categorized based on pre-pandemic Alcohol Use Disorder Identification Test (AUDIT) score into low-risk (AUDIT<5; N=24 males & 23 females, age: 50 $\pm$ 13 yrs, BMI: 29.8 $\pm$ 7.3 kg/m²) or at-risk (AUDIT $\geq$ 5; N=27 males & 6 females, age: 53 $\pm$ 8 yrs, BMI: 26.5 $\pm$ 6.4 kg/m²) alcohol use categories. Prior week self-reported health-related behaviors were assessed using modified versions of the National Health Interview Survey (NHIS) Dietary Screener (2015), International Physical Activity Questionnaire (IPAQ), and National Institute on Alcohol Abuse and Alcoholism (NIAAA) Recommended Alcohol Questions. Measures were compared between pre-pandemic risk categories and were further correlated with alcohol use measures during the stay-at-home order. People with at-risk alcohol use reported less frequent fruit intake (p<0.01), more frequent meat intake (p=0.03), and fewer days engaged in moderate-intensity physical activity (p<0.01). People with at-risk alcohol use reported a greater maximum number of drinks in one sitting (p<0.001), more frequent binge drinking (p<0.001), and feeling drunk or tipsy more often (p=0.03). Among participants with low-risk alcohol use, 9% reported more frequent binge drinking and 7% reported more frequent episodes of becoming drunk or tipsy compared to pre-COVID-19. Among participants with at-risk alcohol use, 16% reported more frequent binge drinking and 22% reported more frequent episodes of becoming drunk or tipsy compared to pre-COVID-19. Regardless of pre-pandemic risk category, binge drinking p=0.30, p=0.008) and feeling drunk or tipsy p=0.42, p<0.001) were associated with salty snack food intake. Becoming drunk or tipsy was associated with emotional eating p=0.23,

$p < 0.05$), and binge drinking was associated with a greater frequency of vigorous physical activity ($p = 0.25$, $p = 0.03$). Overall, participants with at-risk alcohol use before or during the stay-at-home order reported less healthy dietary and activity patterns during the stay-at-home order. These data urge consideration of patients' alcohol use habits and the need for integrative interventions to encourage healthy eating and physical activity, especially when self-isolating or under stressful conditions such as during the COVID-19 pandemic. This work was supported by grants from the NIH/NIAAA: P60AA009803 & UH3AA026198 (PEM).

APSIPE.172

Cathepsin B Release is Blunted in Obese Adults and Males when Performing High Intensity Exercise

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It is generally accepted that exercise induces interorgan crosstalk by releasing exerkines into the circulation. In particular, Cathepsin B (CTSB) is a lysosomal cysteine protease that is released as a myokine in response to exercise. Importantly, CTSB is able to cross the blood brain barrier to act in a neuroprotective manner. Animal models demonstrate the ability of skeletal muscle to secrete CTSB in response to aerobic exercise, therefore, increasing spatial memory and cognitive function. Interestingly, there is growing evidence that the chronic inflammatory state of obese individuals is a risk factor for cognitive impairment. Given the positive association of exercise induced CTSB release and cognition, the goal of the current study was to investigate effect of acute aerobic exercise (AE) intensity on CTSB release. Age-matched lean healthy (LH) ($n = 15$) and 5 obese (OB) ($n = 5$) subjects volunteered to complete acute AE sessions at 40%, 65%, 80% or at VO₂ max where the submaximal intensities were energy matched. Blood samples were taken before and 30 minutes after treadmill exercise. All human research was reviewed and approved by our governing IRB and was carried out in accordance with the guidelines set forth by the Declaration of Helsinki. There was no effect of lower intensity AE (40% and 65%) on serum CTSB in either of the LH and OB cohorts. Interestingly, only the LH cohort had an increase in serum CTSB in response to acute 80% AE ($p = 0.03$). Further, AE at VO₂ max elicited an increase in LH serum CTSB ($p = 0.04$) and trended for increase in OB ($p = 0.05$). When stratified by sex, there was a trend for blunted CTSB response to maximal exercise in males ($p = 0.06$). There were no correlations between the change in serum CTSB and energy expenditure. These data suggest that exercise induced CTSB secretion is blunted in OB individuals and potentially in males. CTSB remains a promising therapeutic target to protect from loss of spatial memory and cognitive function. Understanding the mechanisms by which exercise induces CTSB secretion are imperative to preserving memory and cognitive function.

APSIPE.173

Chronic Attenuation of Muscle Metaboreflex Induced Increases in Cardiovascular Performance During Exercise Via Ablation of TRPV1 Sensitive Afferents

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Dynamic exercise poses a major challenge in the ability to provide adequate blood flow to the active skeletal muscle. When O₂ delivery is insufficient for O₂ demand, metabolite accumulation occurs which activates the muscle metaboreflex via stimulation of skeletal muscle afferents, some of which express TRPV1 cation channels. In healthy subjects this reflex improves skeletal muscle perfusion through robust increases in cardiac output that drive increases in mean arterial pressure. In heart failure, the cardiac output response is significantly attenuated, and thus, reflex characteristics shift to profound increases in systemic sympathetic activity which elicits widespread vasoconstriction including to the ischemic active muscle from which the reflex originates. This vicious positive feedback likely impairs exercise tolerance in heart failure as well as potentially triggering adverse cardiovascular events due to the high sympathetic drive. Administration of an ultra-potent analog of capsaicin known as Resiniferatoxin (RTX), can attenuate cardiac sympathetic afferents, systemic vascular responses, and peripheral sensation of nociceptive pain through epicardial, systemic, and intrathecal administration respectively. In this study we utilized healthy, chronically instrumented, conscious canines exercising at 3.2 k/hr with successive partial reductions in hindlimb blood-flow to evaluate muscle metaboreflex responses before and after ablation of TRPV1 expressing afferents via intrathecal administration of RTX at the level of L4-L6. We observed that RTX significantly attenuated muscle metaboreflex induced strength (gain) in the ability to raise cardiac output (70%), heart rate (50%), and mean arterial pressure (50%) and that these reductions in reflex gain were maintained 78 ± 10 days from initial RTX administration. We conclude that RTX significantly attenuated muscle metaboreflex gain. Furthermore, administration of RTX in heart failure may reduce excessive sympathetic activation during exercise thereby improve cardiovascular function and exercise tolerance. Supported by NIH grants: HL-55473, HL-126706, HL-120882, and in part supported by NIGMS/NIH R25-GM058905.

APSIPE.174

Preliminary findings from a pilot study investigating the effects of an aerobic exercise intervention in persons living with HIV with at-risk alcohol use

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Chronic alcohol consumption is a common and costly form of substance abuse in the U.S. and is highly prevalent in persons living with HIV (PLWH). Combination antiretroviral therapy (ART) has significantly increased longevity in PLWH with over 50% of PLWH in the U.S. being ≥ 50 years old. Metabolic dysregulation is often a consequence of aging, at-risk alcohol use and chronic ART in PLWH. The primary goal of this pilot study was to test the hypothesis that a 10-week moderate intensity (40-60% heart rate reserve) aerobic exercise intervention (30 minutes, 3x/week) would improve fasting plasma glucose (FPG) and 2-h oral glucose tolerance test (OGTT) values in PLWH with at-risk alcohol use. Secondary outcomes included the effects of the exercise intervention on body composition, body mass index (BMI), waist/hip ratio (WHR), 24-h diet recall (ASA-24) and quality of life (SF-36). A subset of adult, in-care PLWH (n=7) enrolled in the ongoing ALIVE-Ex study with a FBG of 95-124mg/dL and an "Impaired" 2-h plasma glucose value of 140-199mg/dL, completed the exercise intervention with a greater than 85% compliance rate. At-risk alcohol use was determined by the Alcohol Use Disorders Identification Test (AUDIT). An AUDIT score ≥ 5 was defined as at-risk alcohol use (n=4) and an AUDIT score of < 5 (n=3) was considered low-risk. Alcohol use, pre- and post-exercise intervention, was determined by 30-day Timeline Followback (TLFB). Prior to the exercise intervention, abdominal fat was higher in PLWH with low-risk alcohol use compared to PLWH with at-risk alcohol use ($p < .05$). Additionally, 66.6% of PLWH with low-risk alcohol use were considered obese (BMI > 30), while none of the PLWH with at-risk alcohol use had a BMI > 30 . The exercise intervention increased $\text{VO}_{2\text{max}}$ by $7.4 \pm 5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in participants with at-risk alcohol use. Based on the TLFB, alcohol intake remained relatively constant, as did daily caloric intake and macronutrient composition of the diet. However, total fat intake (kcal) was lower in PLWH with at-risk alcohol use compared to PLWH with low-risk alcohol use following the exercise intervention. PLWH with at-risk alcohol use achieved "normal" values for FPG ($92.8 \pm 12.5 \text{ mg/dL}$) and 2-h OGTT plasma glucose levels ($128.8 \pm 23.3 \text{ mg/dL}$). Self-reported physical health, energy and social functioning in participants with at-risk alcohol use were moderately improved ($p < .10$) following the exercise intervention, but did not reach statistical significance with the small sample size. Following the exercise intervention, a trend toward a smaller waist circumference and lower percent body fat was detected in participants with at-risk alcohol use compared to those with low-risk alcohol use.

BMI and WHR were not altered by the exercise intervention in either group. Overall, preliminary data from this pilot study suggest that moderate intensity aerobic exercise improves dysglycemia in PLWH with at-risk alcohol use. Based on our preliminary findings, moderate aerobic exercise may be an appropriate intervention strategy to decrease the risk of developing metabolic comorbidities in PLWH with at-risk alcohol use. Supported by UH3AA026198, 5P60AA009803, T32AA007577 to PEM

APSIPE.175

Calcium and caloric restriction: A new perspective on old ideas for treatment of diabetes.

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The work of Dr. John O. Holloszy, and his many trainees, helped to define the discipline of exercise biochemistry. I was fortunate enough to be mentored by two of Dr. Holloszy's trainees in my scientific career, and influenced by numerous others. I plan to share these influences and how Dr Holloszy's work surrounding the role of calcium in exercise-mediated glucose uptake and caloric restriction for improved insulin sensitivity, lead me towards a hypothesis of how these mechanisms may converge to resolve inflammation with exercise.

APSIPE.176

Moving the exercise biology field beyond MoTrPAC 1.0

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The Molecular Transducers of Physical Activity Consortium (MoTrPAC) aims to discover the molecular basis for some of the health benefits of exercise in healthy, disease-free participants. MoTrPAC will use the latest technology and platforms in transcriptomics, metabolomics, lipidomics, (epi)genomics to interrogate the acute and chronic (training) responses to both aerobic and resistance exercise. This large-scale investigation will be the first to systematically investigate how these molecular responses vary among individuals, and to better understand why some people respond better to exercise than others. MoTrPAC will begin to decipher important questions regarding gender differences, whether or not older adults respond similarly to middle-age or younger adults, or whether children respond differently. MoTrPAC will also determine whether or not some people or demographics respond better to resistance exercise, and molecular factors that play a role in the variation of these improvements. This is the Main Study. Let's call this MoTrPAC 1.0. MoTrPAC also supports additional studies, or ancillary studies, which can provide knowledge beyond MoTrPAC 1.0, by adding more specialized tests in subsets of participants (phenotyping MoTrPAC 1.0 is limited), perform additional analyses in blood or tissue specimens, or in some cases, utilize MoTrPAC data in order to compare to other subjects, or diseases, for example, diabetes patients. Ancillary studies could also compare the moderate intensity aerobic and resistance exercise

performed in MoTrPAC to higher intensity interval training or combined aerobic and resistance training. Ancillary studies can also access the large biobank of tissues that have been collected from the animal studies. While MoTrPAC will be the largest mechanistic study of exercise ever performed, a myriad of questions will remain. Much is still left to discover why exercise can be powerful medicine.

APSIPE.178

Loss of sirtuin 1 (SIRT1) does not impair contraction-stimulated glucose uptake in mouse skeletal muscle

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Exercise (i.e. contraction) potently stimulates an increase in skeletal muscle glucose uptake, making it a cornerstone non-pharmaceutical intervention for treating clinical hyperglycemia and insulin-resistance. Remarkably, however, the molecular signaling steps that regulate contraction-stimulated (C-Stim) glucose uptake by skeletal muscle remain incompletely defined. The mammalian ortholog of Sir2, sirtuin 1 (Sirt1), is a protein deacetylase that is thought to link perturbations in energy flux associated with exercise, particularly at the level of NAD⁺ and NAD⁺/NADH, to subsequent cellular adaptations. Nevertheless, its role in C-Stim glucose uptake has not been defined. The objective of this study was to determine the contribution of SIRT1 to C-Stim glucose uptake in mouse skeletal muscle. Using the radioactive 2-deoxyglucose uptake (2DGU) approach we measured ex vivo glucose uptake in unstimulated (NoStim; i.e. rested) and electrically-stimulated (Stim; 100 Hz contraction [2s train, 0.2ms pulse, 35V] every 15s for 10 min) soleus and extensor digitorum longus (EDL) from ~15 week old male and female mice with muscle-specific knockout of SIRT1 (SIRT1 mKO) and their wildtype (WT) littermates; force production was measured throughout the protocol. As expected, force output decreased over the 10 min protocol, although there were no differences in maximal force or the rate of fatigue between genotypes. In soleus and EDL from WT mice, 2DGU was ~2.5-fold higher in Stim vs. NoStim, regardless of sex, and this effect was not impaired in SIRT1 mKO mice. Interestingly, absolute values for C-Stim 2DGU was ~1.3 fold higher in female vs. male, regardless of muscle type or genotype. Taken together, our findings demonstrate that SIRT1 is not required for C-Stim glucose uptake in mouse skeletal muscle. In addition, future work will investigate the mechanisms underlying higher contraction-stimulated glucose uptake in the skeletal muscle of female mice. Cantó, C., Jiang, L. Q., Deshmukh, A. S., Matak, C., Coste, A., Lagouge, M., ... Auwerx, J. (2010). Interdependence of AMPK and SIRT1 for Metabolic Adaptation to Fasting and Exercise in Skeletal Muscle. *Cell Metabolism*, 11(3), 213–219. Jørgensen, S. B., Richter, E. A., & Wojtaszewski, J. F. P. (2006). Role of AMPK in skeletal muscle metabolic regulation and adaptation in relation to exercise. *Journal of Physiology*, 574(1), 17–31. Kjøbsted, R., Munk-Hansen, N., Birk, J. B., Foretz, M., Viollet, B., Bjørnholm, M., ... Wojtaszewski, J. F. P. (2017). Enhanced muscle insulin sensitivity after contraction/exercise is

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APSIPE.179

Effects of soluble guanyl cyclase activator on skeletal muscle capillary hemodynamics in heart failure rats with reduced ejection fraction

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Introduction: Heart failure (HF) impairs microcirculatory function and thus alters the structural and functional elements of convective and diffusive oxygen (O₂) transport, in part, by decreased nitric oxide (NO) bioavailability. Soluble guanyl cyclase (sGC) activators were developed to target downstream effects of NO directly, but their effects on muscle microcirculation are not known. This investigation tested the hypothesis that chronic administration of sGC activator (BAY 60-2770) would increase red blood cell (RBC) velocity, RBC flux, and capillary hematocrit (HCT). Furthermore, we investigated whether exogenous NO (sodium nitroprusside, SNP) superfusion, in addition to the sGC activator, would further improve O₂ transport. Methods: HF was induced in adult male Sprague-Dawley (3-4 mo. old) rats via surgically induced myocardial infarction (MI). Following ~3 weeks of HF progression, 0.3mg/kg BAY 60-2770 in 1 ml vehicle (10% Transcutol, 20% Cremophor, Sigma Aldrich, St. Louis, MO, and 70% water) was administered via oral gavage twice a day (HF + sGC; n = 10) for 5 days prior to intravital microscopy experiments. The control HF group (HF; n = 9) received vehicle only. High resolution intravital microscopy recorded RBC velocity, flux, and HCT under resting conditions at ~2.4 μm sarcomere length in the spinotrapezius muscle. Results: MI size was determined via planimetry (29 ± 2 vs 28 ± 3 %) and pressures via Millar catheterization (16 ± 1 vs 14 ± 1 mmHg) HF + sGC vs HF, respectively (P > 0.05). RBC flux (70 ± 9 vs 25 ± 8 RBC/s) and velocity (490 ± 43 vs 226 ± 35 μm/s), were both increased in HF + sGC vs HF (P < 0.05). Capillary HCT was greater in HF + sGC vs HF (16 ± 1 vs 10 ± 1%), respectively (P < 0.05). In addition, RBC flux was highly correlated with velocity (r = 0.958). Conclusion: sGC activator increases resting skeletal muscle capillary hemodynamics ultimately improving perfusive and diffusive O₂ conductances. This suggests a strong therapeutic potential for sGC activators in cardiovascular disease by improving blood-myocyte O₂ flux, VO₂ kinetics, and potentially exercise capacity.

APSIPE.180

AMPK signaling, not Rac1 activation contribute to the insulin sensitizing effects of exercise following moderate-intensity exercise in humans

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PURPOSE: A single session of moderate-intensity exercise is sufficient to improve insulin sensitivity in most humans; however, the determinants of such improvements are not completely understood. Ras-related C3 botulinum toxin substrate 1 (Rac1), a small membrane-associated GTPase, facilitates glucose uptake by reorganizing actin filaments when independently activated by insulin or muscle contraction. The purpose of this study was to investigate whether enhanced activation of Rac1 contributes to post-exercise improvements in insulin sensitivity. **METHODS:** This study was conducted in accordance to guidelines set forth by the Declaration of Helsinki. Sedentary lean adults (n=14 [4M/10F], BMI 22.2 ± 2.1 kg/m², VO₂max 32.2 ± 4.5 ml/kg/min) completed two metabolic study visits in a randomized crossover design. Trials were identical other than completing 1-hour of moderate-intensity cycling exercise (65% VO₂max) or remaining sedentary. Insulin sensitivity was determined via variable glucose infusion rate in response to constant insulin infusion (2.3 mU/kg FFM/min) measured during the final 30 min of a 3-hour hyperinsulinemic-euglycemic clamp (2-5 h post-exercise or rest). Vastus lateralis muscle biopsies were obtained 15 min post-exercise or rest (basal) and 1 h into a hyperinsulinemic-euglycemic clamp for each trial to determine Rac1-GTP binding by activation assay and insulin signaling by western blotting. **RESULTS:** Phosphorylation of AMP-activated protein kinase (AMPK), Rac1-GTP binding, and activation of downstream p21-activated kinase (PAK) were increased 15 min post-exercise (P≤0.05 vs. basal); whereas neither Akt phosphorylation (Thr308 and Ser473) nor phosphorylation of downstream TBC1 domain family member 4 (TBC1D4) at Ser318 were altered (P>0.10 vs. basal for all comparisons). Exercise-induced improvements in insulin sensitivity were evidenced by significantly increased glucose infusion rates (+12 ± 16.5 %, P=0.03) during the exercise trial compared with the rest trial. Despite measures of improved insulin sensitivity, exercise did not further enhance insulin-stimulated phosphorylation of Akt (P=0.40), TBC1D4 at Ser318 (P=0.36), Rac1-GTP binding (P=0.36) or phosphorylation of PAK (P=0.71) compared with rest. However, AMPK-specific phosphorylation of insulin-independent TBC1D1 at Ser231 and insulin-dependent TBC1D4 at Ser704 were increased 1 h into hyperinsulinemic-euglycemic clamps during the exercise trial compared with the rest trial (P≤0.01 for both). **CONCLUSION:** We interpret these findings to indicate that AMPK-specific signaling and not Rac1 signaling contribute to the insulin sensitizing effects of exercise in the hours post-exercise among lean individuals.

APSIPE.181

Physiological and Nutritional Ketosis Have No Effect on Endurance Exercise Performance in Female Mice

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Elevation of serum ketone body (KB) levels and promotion of ketone body metabolism have been suggested as strategies to increase exercise performance. However, studies in humans and animals have not produced consistent results. In this study, we employed two strategies to induce ketosis in female mice to evaluate the effects on exercise performance and metabolism. Physiological ketosis was induced via fasting and nutritional ketosis was induced via short-term administration of a ketogenic diet (KD). In Study 1, we compared exercise performance in fed and fasted female mice (n=5 each group). After 8 hours of fasting, serum KB significantly increased (0.82±0.05 vs. 0.36±0.07 mM) and serum glucose significantly decreased (93±7 vs. 136±6 mg/dl) in fasted compared to fed mice. Fed and fasted mice were then subjected to an endurance exercise capacity (EEC) test on a motorized treadmill. Fasted mice had similar EEC performance as fed mice (100.4±0.4 vs. 94.2±4.6 min) and similar serum glucose and KB at the end of exercise. In Study 2, female mice were fed a KD (90% total calories from fat) for 5 weeks. At the end of 5 weeks, KD mice gained significantly more weight than chow-fed mice (12.8±1.9% vs. 6.2±2.3%, n=6-9 each group) and had 65% greater adipose tissue weight (P<0.05). Serum ketone bodies were significantly higher in KD mice (0.72±0.06 vs. 0.33±0.04 mM). When subjected to the EEC test, KD and chow-fed mice achieved similar exercise times. At the end of exercise, both serum glucose and KB were significantly higher in the KD groups. Biochemical tissue analysis showed that KD led to significant increases in triglyceride concentration in the heart (~25%) and liver (~65%), and significant decreases in glycogen content in the muscle (~40%) and liver (~60%). These data show that physiological ketosis, induced by fasting, or nutritional ketosis, due to short-term KD, does not significantly increase exercise performance. The data also demonstrate that short-term KD in female mice leads to increased body weight and adiposity with lipid accumulation in the heart and liver and glycogen depletion in the liver and skeletal muscle. Therefore, the findings suggest that the short-term use of the KD is not an effective strategy to enhance exercise performance and may lead to aberrant endogenous tissue storage.

APSIPE.182

A.V. Hill, Celebrating a Century of VO₂max

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Archibald Vivian Hill (1886-1977) was a British physiologist who made vital contributions to our understanding of integrative physiology. In 1922, he published a paper with Otto Meyerhoff, a German biochemist, that revolutionized

the field of bioenergetics. They identified, for the first time, an anaerobic pathway in frog muscle. This work was immediately recognized as a breakthrough, and they were awarded the 1922 Nobel prize in Physiology or Medicine. Hill was credited with discoveries related to heat production in muscle, and Meyerhoff with discovering the fixed relationship between the consumption of oxygen and lactic acid metabolism in muscle. Hill then sought to translate these findings to the exercising human, and in doing so, he established the concept of maximal O₂ intake (VO₂max). Hill and colleagues studied the time course of changes in VO₂ during running, and in recovery. In the course of their research, they made the critical distinction between the oxygen intake at the lungs, the oxygen uptake by the muscles, and the oxygen requirement of exercise. Hill also introduced the term “oxygen debt” to accompany the term “oxygen deficit” that August Krogh introduced. The VO₂max paradigm of Hill proposed that: (a) there is an upper limit (i.e.- ceiling) on a person’s ability to take in and consume oxygen, (b) a person can exercise at intensities in excess of VO₂max, but only for brief periods of time, (c) there is inter-individual variability in VO₂max, and (d) VO₂max is a determinant of running performance. Hill also discussed, from a theoretical standpoint, the physiological factors that limit a person’s VO₂max. He correctly proposed several factors (cardiac output, lung diffusion capacity for oxygen, and oxygen carrying capacity of the blood), but he lacked knowledge of peripheral factors in skeletal muscle that limit VO₂max in certain situations. Hill’s discovery of VO₂max started a revolution in the field of exercise physiology. It explained the observable facts with sufficient clarity and brilliance to win supporters, but it left enough questions about the “limiting factors” unanswered, and this has kept physiologists busy over the past century. With minor refinements, Hill’s VO₂max paradigm has withstood the test of time.

APSIPE.183

High- and low-carbohydrate diets do not affect swimming economy in recreational swimmers

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Swimming economy is inversely related to the energetic cost of swimming, and is as a key factor influencing endurance swimming performance. It is measured as the rate of energy expenditure relative to swimming speed. The objective of this study was to determine if high-carbohydrate, low-fat (HCLF) and low-carbohydrate, high-fat (LCHF) diets affect energetic cost of submaximal swimming. Eight recreational swimmers consumed two 3-d isoenergetic diets in a crossover design. Diets were tailored to individual food preferences, and macronutrient consumption was 69-16-16% and 16-67-18% carbohydrate-fat-protein for the HCLF and LCHF diets, respectively. Following each 3-d dietary intervention, participants swam in a flume at velocities associated with approximately 50, 60, and 70% of their maximal aerobic capacities (VO₂max).

Expired breath was collected and analyzed in real time while they swam that enabled calculation of the energetic cost of swimming. A paired t-test compared macronutrient distribution between HCLF and LCHF diets, while repeated-measures ANOVA determined effects of diet and exercise intensity on physiological endpoints. Respiratory exchange ratio was significantly higher in HCLF compared to LCHF ($p = 0.003$), but there were no significant differences in the rate of oxygen consumption ($p = 0.499$) or energetic cost of swimming ($p = 0.324$) between diets. Heart rate did not differ between diets ($p = 0.712$), but oxygen pulse, a non-invasive surrogate for stroke volume, was greater following the HCLF diet ($p = 0.029$). Results from this study suggest that a 3-d high-carbohydrate diet increased carbohydrate utilization but did not affect swimming economy at 50-70% VO₂max. Although these intensities are applicable to ultramarathon swims, future studies should use higher intensities that would be more applicable to shorter duration events such as marathon (10-km) and 1,500-m swim races. There was no funding source for this study.

APSIPE.184

Skeletal Muscle Fiber Type-Specific Redox Signatures in Lean and Obese Individuals in Response to Exercise

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The balance between reduction and oxidation of molecules (redox) drives many cellular processes such as adaptation to metabolic challenges, mitochondrial biogenesis, metabolic flexibility and longevity of organisms. At the center of redox balance are the coenzymes flavin adenine dinucleotide, a main component of flavoproteins (Fp) and Nicotinamide Adenine Dinucleotide (Phosphate) [NAD(P)] for their ability to both sequester and donate electrons. Recent evidence has suggested that the beneficial effects of exercise on skeletal muscle may be exhibited, in part, by balancing the redox state. Therefore, we set out to determine 1) differences in skeletal muscle redox state between lean healthy (LH) and obese (OB) individuals, 2) the effect of acute aerobic exercise (AE) on skeletal muscle redox state, and 3) fiber type-specific effects of obesity or exercise on skeletal muscle redox state. Muscle biopsies (vastus lateralis) were obtained from 16 LH and 3 age-matched OB individuals before (pre), 30 minutes after (post), and 3h after AE at 80% VO₂max. Muscle bundles were fixed in OCT via liquid nitrogen cooled isopentane. Serial sections (6µm) were probed for auto-fluorescent properties of NAD(P)H (excitation at 340-360nm) and flavoproteins (Fp) (excitation at 440-470nm) to quantify the redox signatures of individual muscle fibers. Redox state was calculated as $[Fp]/[Fp+NAD(P)H]$. Fiber type was determined by MHC I and IIa immunofluorescence staining in serial sections. All human research was reviewed and approved by our governing IRB and was carried out in accordance with the guidelines set forth by the Declaration of Helsinki. OB muscle was in a more reduced state than LH ($p=0.049$). LH MHC I fibers were in a more oxidized state than LH MHC IIx at baseline ($p=0.0001$), post

($p=0.0007$) and post 3hr ($p=0.048$). There was no difference in redox state within the OB fiber-types. Fp intensity increased post AE in all fiber types in both groups ($p=0.043$). In both groups, MHC I displayed higher NAD(P)H intensity than MHC IIx ($p=0.003$) and higher Fp intensity than both MHC IIa ($p=0.006$) and MHC IIx ($p=0.0001$). These results suggest OB muscle has a differential and potentially dysfunctional redox response to AE. In LH muscle, oxidative fibers appear to effectively utilize reducing equivalents at rest and in response to exercise. Future studies will explore the cause of the altered redox state in OB muscle and if redox balance can be rescued by interventions such as chronic caloric restriction or exercise training.

APSIPE.185

Magnetic resonance imaging-derived measurement of muscle mass: evaluation of analytical methods

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Introduction. Magnetic resonance imaging (MRI) represents one of the most accurate techniques available to determine changes in skeletal muscle that may occur with exercise or dietary interventions. Manual segmentation of MRI-derived anatomical images demands tedious and tireless effort, reducing enthusiasm for the application of this technique despite its inherent value. The primary objective of this study was to determine potential bias in the measurement of thigh muscle cross sectional area (CSA) between the laborious, manual OsiriX program and the efficient, semi-automated 3D Slicer program. **Methods.** Twenty-seven females and males (69 ± 5 yrs/age; 32 ± 4 kg/m²) were recruited to participate in a double-blind, randomized dietary supplementation study (outcome data published previously). In order to address the objective of the current study, T2 weighted images were utilized from pre- and post-supplementation MRI scans using soft tissue contrast. OsiriX and 3D Slicer image segmentation of CSA was performed by two technicians using standard operating procedures to verify consistency and accuracy. Agreement between the manual and semi-automated methods of CSA analysis was determined using the Bland Altman method. **Results.** A comparison of the changes in CSA obtained by OsiriX and 3D Slicer programs provided limits of agreement of -2.004 cm² (95% Confidence Intervals: $[-2.806, -1.202]$) and 2.294 cm² (95% Confidence Intervals: $[1.492, 3.096]$). We conclude that these limits of agreement are acceptable for intervention-based investigations. Therefore, the 3D Slicer method may be a viable alternative for the determination of MRI-derived muscle mass in longitudinal and/or large-scale studies.

APSIPE.186

Astaxanthin Supplementation Enhanced Improvements in Exercise Efficiency with Endurance Training in Older Men but Not Women

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Background: Older sedentary adults tend to have low levels of exercise efficiency that can prevent them from accomplishing daily activities and reduce their quality of life. Endurance training can increase exercise efficiency, thus decreasing the energy required to perform a given amount of work. Astaxanthin (AX) is an antioxidant and anti-inflammatory dietary supplement that has been shown to improve endurance capacity, but its effects on enhancing endurance trained adaptations in the elderly have not been investigated. **Purpose:** We tested a formulation of AX in healthy male and female subjects (65-82 yrs) to determine if AX promotes an increase in exercise efficiency with endurance training (ET). **Method:** We conducted a randomized, double-blind, placebo-controlled study. Forty-two subjects were supplemented with a daily oral dose of AX or placebo (PL) during 3 months of ET consisting of a high intensity interval walking program. A standard Balke treadmill graded exercise test (GXT) was conducted in conjunction with indirect calorimetry before and after intervention to measure energy expenditure. **Results:** Participants were able to perform more work and consequently increase their energy expenditure on the GXT after ET. Energy expenditure decreased when performing the same amount of work achieved during their baseline visit. While exercise efficiency increased in all groups, AX supplementation increased exercise efficiency relative to PL in males only. **Conclusion:** AX supplementation can help improve exercise efficiency in the elderly undergoing endurance training, especially in men. This work was supported by Astavita, Inc.; National Institutes of Health grants (T32 AG000057, T32 HL007028-42, P01 AG001751).

APSIPE.187

Physiological profile of trained, older male swimmers: insight into the influence of aging on maximal aerobic power and efficiency while swimming.

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Cardiorespiratory fitness and skill level have been assessed extensively for trained, younger (<30 yrs) swimmers via measures of maximal oxygen uptake, swimming economy and efficiency. Little information has been reported regarding physiological characteristics of middle-aged to older, trained swimmers. The purpose of this investigation was to identify the physiological profile of middle-aged to older swimmers to gain insight into the effects of aging on cardiorespiratory fitness and swimming skill. Eighteen swim trained males volunteered for this study (52 ± 11 yr, range: 35-77 yrs; BMI 26.2 ± 3.4 kg.m⁻²; training regularly for 13 ± 8 yrs; 4.2 ± 0.8 sessions.wk⁻¹; 2590 ± 741 m.session⁻¹). After a warmup, swimmers completed four to six submaximal and one maximal swim

effort and metabolic responses (indirect calorimetry), heart rate (HR, telemetry), blood lactate (Bla, finger stick), and RPE (Borg Scale, 6-20) were assessed. Power equivalence (Peq, Watts; metabolic power) for each submaximal effort was estimated from oxygen uptake and the respiratory exchange ratio. Swimming efficiency was indirectly assessed at a Peq of 800 and 1000 Watts using the relationship between swimming velocity and Peq for each swimmer. Maximal aerobic power (MAP) while swimming ranged from 2.5-4.7 L.min⁻¹, (3.6±0.6 L.min⁻¹; >12 METS), while max HR, Bla, and RPE ranged from 144-192 bpm (166±14 bpm), 4.5-14.1 mM (9.6±2.7 mM) and 15-20 (18±1), respectively. Further, MAP, max HR and max O₂ pulse were found to be inversely related to age [R = -0.74, -0.52 and -0.61, respectively (P<0.05)]. The submaximal efforts elicited a relative physiological load of ~43 to 76% MAP with swimming velocity and Peq strongly correlated (R = 0.98±0.02, P<0.05). Estimated swimming velocity at a given Peq of 800 (~65% of MAP) and 1000 W (~76% of MAP) ranged from 0.80 – 1.11 m.s⁻¹ (0.94±0.09 m.s⁻¹) and 0.89-1.28 m.s⁻¹ (1.05±0.11 m.s⁻¹, respectively. Further, swimming velocity at a Peq of 800 and 1000 W was not correlated with age. Cardiorespiratory fitness, as noted by maximal aerobic power and heart rate, decreased as a result of advancing age within a group of middle-aged to older trained, male swimmers. However, similar swimming velocities across age for a given metabolic power suggests that swimming efficiency (skill/technique) is preserved with advancing age. This study provides a general physiological profile of middle-aged to older, trained male swimmers and suggests that decrements in swimming performance with advancing age may be more related to a loss in maximal aerobic power and not necessarily decrements in skill level.

APSIPE.188

Satellite cell incorporation in myofibers from anterior crural muscles of Pax7CreERTdTomato transgenic mice during the recovery of lengthening contractions.

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It is known that intense exercise with lengthening contractions leads to skeletal muscle damage. However, little is known regarding satellite cells incorporation after exercise-induced damage, and how it affects muscle regeneration. The goal was to investigate the time-course of satellite cell incorporation into myofibers after in vivo muscle lengthening contractions, by tracking Pax7-dependent TdTomato fluorescence in myofibers. Procedures were approved by UCSD-IACUC and followed APS guidelines for animal research. Experiments were performed in 7-8 months-old Pax7CreERTdTomato mice, separated into three groups (3d, 5d, and 7d). Mice were treated for 5 days with tamoxifen (2 mg/mouse/day, i.p.),

and on the last day, the right leg was subjected to a lengthening contraction protocol (LCP), while the contralateral leg was used as a control. LCP consisted of contracting the anterior crural muscles via electrical stimulation of the peroneal nerve for 600 ms (0.1 ms pulses, 150 Hz pulse-frequency), implementing active plantar flexion in the last 400 ms of stimulation, with 12 s intervals between contractions for a duration of 30 min (total of 150 contractions). The LCP resulted in a decrease of the initial torque by 80 ± 2 % at the 150th contraction which was consistent between the groups of mice (79 ± 5 %, 82 ± 5 %, and 80 ± 2 %, for 3d, 5d, and 7d, respectively). After 3, 5, or 7 days post-LCP (groups 3d, 5d, or 7d, respectively), tibialis anterior (TA) and extensor digitorum longus (EDL) muscles from both legs were dissected. EDLs were placed in chambers, perfused with Tyrode's (95% O₂, 5% CO₂, 22°C), and tested ex-vivo for contractility with 300 ms trains (0.5 ms pulses, 1-200 Hz). EDL muscles from the LCP legs showed an average of 40 ± 6 %, 48 ± 15 %, and 25 ± 8 % less force production than the control leg at 3, 5, and 7 days post-LCP, respectively. EDLs following the contractility test, and TA muscles, were fixed, frozen in OCT compound, sliced (10 µm sections), then labeled with anti-laminin antibody with fluorescent secondary antibody and DAPI. Satellite cell incorporation into myofibers was determined by the appearance of endogenous TdTomato fluorescence in myofibers. TA and EDL muscles from LCP legs of Pax7CreERTdTomato mice showed a higher quantity of immunofluorescent myofibers compared to the control legs. The data suggest that lengthening contractions decrease force production of muscles in vivo and ex-vivo and are associated with activation of satellite cell incorporation into myofibers. Support: Tobacco-Related Disease Research Program (T29KT0397)

APSIPE.189

Effects of Broad Spectrum Antibiotic Administration on Running Wheel Behavior in Mice.

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The gut microbiota modulate a variety of physiologic processes. Previous work from our group has shown that regular endurance exercise, independent of diet, alters the composition of the gut microbiome and metabolome. We hypothesize that microbes contribute to endurance training adaptations. One way to test this is to deplete microbes using broad spectrum antibiotics (ABX). In order to test this, we needed to determine if wheel running behavior was affected by ABX. In a 2 x 2 design (wheel/sedentary, control/ABX), male and female C57Bl mice ran for 25 days to measure baseline running behavior. This was followed by 4 week administration of ABX (Vancomycin Hydrochloride, Metronidazole, Kanamycin Sulfate, and Ampicillin) in the drinking water. All procedures were approved by UIUC Institutional Animal Care and Use Committee prior to enactment. There were no differences

in water or food intake between groups. Wheel running resulted in significant reductions in body weight, perigonadal fat pad weight, and improved time to fatigue on a treadmill test. These effects were not altered by ABX administered in the last 4 weeks of training. ABX increased cecum weight and tended to improve glucose tolerance. Importantly, ABX treatment did not significantly affect wheel running behavior when compared to controls. Therefore, we conclude that ABX may be used to test whether microbial depletion contributes endurance exercise-induced training adaptations. We are currently conducting experiments to determine whether ABX, started just prior to wheel exposure, alters training adaptations including performance and biochemical adaptations in muscle and other tissues. This work supported by UIUC RB 20044 and NIH AG-059622 (to RWJ). NT Hutchinson is supported by USDA NIFA 2019-38420-28973.

APSIPE.190

Random forest regression model fails to predict oxygen uptake kinetics during moderate and heavy intensity cycling from wearable sensor data

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Easy-to-obtain wearable sensor data have been used in combination with machine learning techniques to estimate dynamic pulmonary oxygen uptake ($\dot{V}O_{2p}$) responses during moderate intensity exercise and activities of daily living. However, it is unknown if these prediction algorithms are applicable to heavy intensity exercise, where the $\dot{V}O_{2p}$ response is more complex. It was hypothesized that a machine learning model of similar design to the previously validated moderate intensity algorithm would accurately estimate $\dot{V}O_{2p}$ during both moderate and heavy exercise, and predict slower kinetics during heavy compared to moderate exercise. Fifteen young healthy adults (7 females; peak $\dot{V}O_{2p}$: 42 ± 5 ml·min⁻¹·kg⁻¹) performed a 25W·min⁻¹ ramp-incremental exercise test to volitional exhaustion, and three different pseudorandom binary sequence (PRBS) exercise tests consisting of low to moderate, low to heavy, and ventilatory threshold to heavy transitions in work rate, respectively. Wearable sensor data (percent heart rate reserve, estimated minute ventilation, and breathing frequency) collected during the exercise tests were used as inputs for a random forest regression model to predict instantaneous $\dot{V}O_{2p}$, which was then compared to directly measured breath-by-breath values. Frequency domain analyses were used to assess $\dot{V}O_{2p}$ kinetics in the measured and predicted responses. Predicted $\dot{V}O_{2p}$ had low bias (0.022 l·min⁻¹), and was strongly associated with the measured $\dot{V}O_{2p}$ (rrm = 0.911, $p < 0.001$). Comparison of predicted and measured kinetics revealed a Condition x Data type interaction ($F(1.369, 19.167) = 5.339$, $p = 0.023$), and a bias for slower prediction kinetics. There was a moderate within-participant repeated measures correlation between predicted and measured kinetics (rrm = 0.413, $p = 0.021$), but no significant between-participant relationships were observed. These findings suggest that the random forest algorithm is able to predict overall changes in $\dot{V}O_{2p}$, but it is not able to accurately predict the kinetics of the

response when extended to heavy intensity exercise. Therefore, new machine learning models should be explored to improve the prediction of dynamic $\dot{V}O_{2p}$ responses. (Supported by NSERC).

APSIPE.191

Tumor Growth Reduced and Tumor-Infiltrating Lymphocytes Increased with Exercise Training in Mouse Model of Pancreatic Cancer

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Moderate intensity treadmill running performed five times weekly for 45 min each session has been shown to slow tumor growth in a mouse model of pancreatic cancer. The reduction is thought to be due in part to normalization of tumor vasculature. However, this volume of exercise (225 min/week) may not be possible for pancreatic cancer patients to achieve. It is also not clear if other mechanisms are at play in providing this beneficial effect. We therefore investigated the ability of a low volume of exercise training to reduce pancreatic tumor growth in a mouse model. We also compared tumor infiltrating lymphocytes between exercise trained and sedentary mice. **METHODS:** Tumors were induced in six-week old male M. musculus C57Bl/6 mice through subcutaneous injection of PDAC-4662 tumor cells. Tumors were palpable (>50 mm³) after one week and animals began their assigned intervention. Tumors were measured on alternate days for two weeks. Animals were then euthanized, tumors extracted and digested, and tumor infiltrating lymphocytes characterized by flow cytometry. This protocol was approved by MD Anderson Institutional Animal Care and Use Committee. Comparisons were made between mice performing low volume exercise (15 min treadmill running at 12 m/min, five days/week) ($n=6$) and sedentary mice ($n=6$). Group differences in tumor volume over time were compared by repeated measures ANOVA (Greenhouse-Geisser adjusted). Differences in tumor infiltrating lymphocytes were assessed by t-tests. **RESULTS:** As expected, tumor volume increased over time in both groups ($F(2.24, 17.94)=17.127$, $p<0.001$). The interaction between time and group was not significant ($F(2.24, 17.94)=2.575$, $p=0.099$). There was a significant difference in overall tumor volume between groups ($F(1, 7.2)=7.214$, $p=0.028$), where average tumor volume in exercised mice (mean=244.65mm³, 95% confidence interval [99.253, 390.047]) was less than in sedentary mice (mean=463.273mm³, 95%CI [344.556, 581.989]). This corresponds to a large effect size ($r=0.689$). Exercised mice had a greater proportion of CD8 T cells in tumors (mean=11.98, standard error=2.44) than sedentary (mean=5.18, standard error=0.53). This difference (-6.80 , 95% CI [-13.04, -0.55]) was significant ($t(5.48)=-2.727$, $p=0.038$). This difference corresponds to a large effect size ($r=0.759$). No group differences were noted in proportions of tumor infiltrating CD4 T cells ($t(6.189)=-0.817$, $p=0.444$) or NK cells ($t(8.566)=0.034$, $p=0.973$). **CONCLUSIONS:** Just 75

min/week of treadmill running significantly reduced tumor size over two weeks in pancreatic tumor-bearing mice. This was accompanied by an increase in CD8 T cells in the tumor. Whether these cells exhibited antitumor function remains to be determined. Results are limited by the small sample and should be replicated.

APSIPE.192

Effects of S-nitrosoglutathione reductase inhibition on post-fatigue force and intracellular Ca²⁺ in intact single myofibers

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Skeletal myofibers exhibit increased production of nitric oxide (NO) during contractions and exercise. Increased NO production may lead to increased production of S-nitrosoglutathione (GSNO). However, it is unknown whether GSNO is produced during exercise and what its effects are on contractile and Ca²⁺ handling in myofibers. We have shown previously [Gilmore, et al., *Experimental Biology* 2020] that acute inhibition of GSNO reductase (GSNORi; SPL-334) delays force recovery after fatiguing contractions. The goal was to investigate whether GSNORi (or vehicle control; DMSO (10 μ M)) alters post-fatigue force and Ca²⁺-handling in intact single myofibers. Single flexor digitorum brevis (FDB) myofibers (n=4 for DMSO and n=5 for GSNORi treated myofibers) from C57BL/6J mice (8-9 weeks old) were microinjected with Ca²⁺-sensitive probe FURA-2, placed in an experimental chamber to measure isometric force coupled with Ca²⁺-fluorescence during electrical stimulation. This was performed to detect fatigue-based changes in intracellular Ca²⁺ transients before and after incubation with GSNORi (10 μ M). GSNORi treated myofibers showed a trend to have a greater decrease in force 30 min after fatiguing contractions compared to DMSO-treated myofibers (P=0.06). The incubation of GSNORi did not further decrease the myofibrillar Ca²⁺ responses that occur during fatiguing contractions in the DMSO group (~23% vs 9% increase in Ca²⁺50 for DMSO vs GSNORi, respectively). Also, there were no changes between treatments in intracellular Ca²⁺ transients during contractions after fatigue or in maximum Ca²⁺ release by contracting in presence of 10 mM caffeine. This indicates that GSNO buildup does not cause changes in the release of Ca²⁺ from the sarcoplasmic reticulum. Likely, the effect detected previously in intact extensor digitorum longus muscles and in FDB myofibers was due to impairments on the contractile sites of muscle, causing decreased force production following fatiguing contractions. This result further supports the hypothesis we put forth [Gilmore, et al., *Experimental Biology* 2020] that GSNOR exhibits a protective effect on contractile function of skeletal myofibers during fatigue, and indicates that the effect does not alter intracellular Ca²⁺ handling in myofibers, and it is specifically protective against excess GSNO on the contractile sites of muscle during intense contractile activity. Support: NIH-NIAMS (R01AR06957704, to MCH).

APSIPE.193

Overtraining syndrome increases parylation and activates the UPRmt mechanism in skeletal muscle of mice.

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Long periods of high volume and/or high intensity of physical training may trigger the so-called overtraining syndrome, resulting in several abnormalities in the skeletal muscle, including low oxidative capacity and DNA damage. Poly [ADP-ribose] polymerases (PARPs) are proteins involved especially in DNA repair. On the other hand, PARPs activation generates an excessive consumption of NAD⁺, reducing the mitochondrial activity. However, the effect of overtraining on PARPs activation in the skeletal muscle is unknown. Here we combined bioinformatic and molecular assays to evaluate the effects of the overtraining on PARP and mitochondrial imbalance in skeletal muscle of mice. All the experiments were conducted in conformance with guidelines for experimental procedures as set forth in the Declaration of Helsinki and the APS "Guiding Principles in the care and Use of Animals." Male C57BL/6J mice were distributed in the follow groups: Control, Trained and Overtraining (OT). The training and overtraining protocol were described by Pereira et al. 2012. Bioinformatic analyses showed that Parp1 gene has a negative correlation with VO₂ and mitochondrial genes in the skeletal muscle of several strains of BXD mice. Besides that the PARP1 gene has a negative correlation with genes codified by the mitochondrial DNA and has a positive correlation with genes codified by the nuclear DNA. The OT protocol promoted a reduction in the exercise performance, increased skeletal muscle pain, increased skeletal muscle inflammation, reduced locomotor activity after the exercise and increased grooming. Interestingly, the overtraining increased the global protein parylation in the skeletal muscle, reduces the mitonuclear imbalance induced by the exercise training and increases the activation of the UPRmt mechanism. The results indicate that the overtraining syndrome increases PARPs activation, promotes a skeletal muscle disorder, reduces mitochondrial protein content and activates de UPRmt mechanism in the skeletal muscle. Ref: Pereira BC, Filho LAL, Alves GF, et al. A new overtraining protocol for mice based on downhill running sessions. *Clin Exp Pharmacol Physiol*. 2012;39(9):793-798. doi:10.1111/j.1440-1681.2012.05728.x

APSIPE.194

Knockout of miR-16 impairs muscle contractility and mitochondrial integrity in a sex-dependent manner

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Type 2 diabetes mellitus (T2DM), induced by excess calorie intake and physical inactivity has become the most common metabolic disease in Western society. Skeletal muscle (SkM) insulin resistance is known as a major defect in T2DM, concurrently T2DM patients often have impaired SkM contractility and mitochondrial quality. Therefore, it is imperative to further investigate molecular dysregulations in SkM to attenuate insulin resistance. We and others have demonstrated consistent downregulation of the microRNA-16 (miR-16) in SkM across clinical and pre-clinical models of T2DM. However, the causal relationship between miR-16 and muscle health are not fully understood. **PURPOSE:** To investigate how deletion of miR-16 gene in mouse affects SkM contractility and mitochondrial integrity. **METHODS:** 24 wildtype (WT) and 24 SkM miR-16 knockout (KO) male mice and 21 WT and 20 KO female mice (total of 89) were used for this study. At 6 wks of age, plasmid DNA for pMitoTimer was transfected in flexor digitorum brevis (FDB) muscle using electroporation (75 V, 20 ms & 1Hz). At 10 wks of age, half of the mice were given high-fat diet (HFD; 45% fat) for 4 weeks to induce insulin resistance, while the remainder were fed normal chow (NC; 17% fat). At 14 wks, in vivo electrical stimulation was performed in lower limb muscle to determine muscle contractility (fatigability, time to max contraction, and half-relaxation [1/2RT]). 48 h later, FDB muscle was dissected to assess mitochondrial degeneration level via computation analysis of pMitoTimer fluorescence. All animal experiments were approved by IACUC. Data were analyzed by two-way ANOVA within sex and significance was denoted at $p < 0.05$. **RESULTS:** Time to maximal contraction was ~39% higher in KO group compared to WT group in males ($p = 0.058$) while an interaction was noted in females, whereby WT-HFD animals had ~35% lower time to max contraction compared to other groups ($p < 0.001$). There was a main effect in 1/2RT in males, whereby KO group had ~20% lower 1/2RT compared to WT group ($p = 0.033$). An interaction in 1/2RT was noted in females. Specifically, 1/2RT of female KO-HFD group was ~47% and ~24% lower than WT-NC and WT-HFD, respectively ($p = 0.031$). In female, there was an interaction in fatigability, with KO-HFD mice having at least ~39% to ~55% lower peak torque levels from 50 to 110 seconds compared to other groups ($p < 0.05$), but this effect was absent in male counterparts. In males, a main effect was noted that HFD had ~747% higher in pure red puncta in MitoTimer (indicator of completely degenerated mitochondria) level compared to NC ($p = 0.012$), while females showed ~207% higher pure red puncta level in KO compared to WT ($p = 0.018$). **CONCLUSION:** Our data suggests miR-16 KO impairs muscle contractility in both males and females while accumulation of degenerated mitochondria was observed in miR-16 KO female mice, indicating miR-16 may serve as a potential target for T2DM-induced SkM and mitochondrial degeneration.

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APSIPE.198

Comparing Three Exercise Interventions on Pancreatic Tumor Growth in Mice During Chemotherapy Treatment

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Introduction- Pancreatic cancer is one of the most deadly neoplastic diseases. Exercise training yields improvements in physical function and reduces fatigue in pancreatic cancer patients. There is also evidence that moderate exercise may slow pancreatic tumor growth in mice (Schadler et al. 2016). However, it is unknown if exercise of a greater intensity and/or of lower duration would also yield benefit. In this study, we compared the effect of three-exercise interventions on pancreatic tumor growth in mice receiving concomitant chemotherapy. **Methods –** Male C57BL/6 mice (6-8-week-old, $n = 55$) were inoculated subcutaneously with pancreatic tumor cells (PDAC4662). Mice began their assigned intervention ($N = 11/\text{group}$) once tumors reached $\geq 50 \text{ mm}^3$: high intensity interval training (HIIT), continuous high volume training (Cont-HV), continuous low volume training (Cont-LV), or sedentary (SED). Mice in HIIT ran 10 one-min intervals on treadmill at 20m/min, followed by one-min walking at 8m/min for 3 days/week. Cont-HV and Cont-LV mice ran at 12m/min for 45 and 15 min for 5 days/week, respectively. SED did not run. All mice received 15mg/kg gemcitabine three times in a week; an additional group of SED mice did not receive gemcitabine as a control (No GEM, $N = 11$). Tumor volume was measured every other day until euthanasia (Tumor volume = $\sim 150 \text{ mm}^3$). Differences in tumor volume between groups were assessed via Repeated Measures ANOVA. All experiments were conducted after the MD Anderson Institutional Animal Care and Use Committee approval. **Result -** Gemcitabine significantly inhibited tumor growth. There was significant difference in overall tumor volume between SED and No GEM group ($F(1,18) = 14.24$; $p = 0.001$). The average tumor volume in SED with Chemotherapy (mean = 144.396 mm^3 , 95% confidence interval [93.23, 195.56]) was lower than in No GEM group (mean = 274.385 mm^3 , 95%CI [223.22, 325.55]). There was no significant difference in tumor volume between the exercise groups and sedentary mice receiving chemotherapy treatment ($F(3,36) = 0.842$; $p = 0.480$), with a moderate effect size of $r = 0.48$. **Conclusion –** Exercise did not offer further reduction in tumor growth beyond that provided by chemotherapy treatment in a mouse model of pancreatic cancer. No adverse effect of exercise on mice was noticed. Future studies should include muscle strength and cardio respiratory fitness markers as exercise has been shown to improve muscle strength in pancreatic cancer patients (Wiskemann et al. 2019). **Reference** Schadler, N J Thomas, PA Galie, DH Bhang, KC Roby, P Addai, JE Till, et al. 2016. “Tumor Vessel Normalization after Aerobic Exercise Enhances Chemotherapeutic Efficacy.” *Oncotarget* 7 (40):

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APSIPE.199

Immunomodulation using Macrophage Colony Stimulating Factor or Voluntary Wheel Running Rescues Aged Skeletal Muscle Function following Disuse Atrophy

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Aging impairs muscle regrowth and function following a period of disuse. Poor recovery of muscle size and strength with aging coincides with a suppressed macrophage response and increased fibrosis. Immunomodulation in the form of exercise and cytokine injections has been demonstrated to improve skeletal muscle regeneration. However, it is unclear if these macrophage-promoting approaches would improve skeletal muscle recovery following disuse in aged animals. Therefore, the purpose of this study was to examine the effectiveness of macrophage colony stimulating factors (MCSF) or voluntary wheel running (VWR) on the recovery of muscle and strength following disuse atrophy in aged mice. Aged (20–22 mo) male C57BL/6 mice were assigned to be an ambulatory control, or undergo 14 days of hindlimb unloading or 14 days of hindlimb unloading followed 14 days of reloading. Mice in the recovery group received a single injection of MCSF or PBS in both triceps surae while a second group of recovery mice underwent 14 days of VWR. We found that MCSF and VWR at the initiation of reloading enhanced soleus muscle regrowth and rescued grip strength above ambulatory control and PBS injection. Moreover, following MCSF and VWR, soleus muscle cross sectional area and the ratio of BHP/COLIV (indicative of decreased fibrosis) was also elevated above control and PBS. Overall, these results suggest that immunomodulating approaches such as MCSF and VWR during the recovery phase from disuse atrophy restore aged skeletal muscle mass, myofiber size, and grip strength while also reducing muscle fibrosis.

APSIPE.200

An evolutionary perspective on strength training for endurance athletes

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Physiological adaptations to habitual physical activity are highly specific to the stimulus and generally work to match exercise capacities to demand. Performance-enhancing benefits of strength training to endurance sport are an

exception to this rule, made more surprising given that some physiological attributes underlying performance in strength and endurance activity are antagonistic. This apparent “endurance training specificity paradox” can be understood using an evolutionary lens. While human evolution is characterized by a shift towards increased endurance capacities, our transformation into endurance athletes was tempered by an emphasis on energetic thriftiness and other competing selective pressures. Further, inherited mammalian muscle biology constrained potential mechanisms through which strength gains could be accrued via endurance activity. Thus, improvements in endurance performance gained through strength training are reconciled with the specificity of training principle. This view also dispels “paleo-lifestyle” notions of “living like our ancestors” to maximize athletic potential: early humans likely lived on the margins of energy balance and almost certainly did not engage in the high-volume repetitive physical activities of modern athletes.

APSIPE.201

When GLUT4 ‘arrived’ in muscle

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While known for many decades that skeletal muscle is the major disposal site of dietary glucose, it was not until 1987 that the molecular mechanism of this process emerged. In what begun a long-term collaboration with John Holloszy, we established that glucose transporters increased in isolated membranes of insulin-injected rats, at the expense of those in intracellular membranes of the same muscles. The transporters were first recognized through binding of the exquisite ligand, cytochalasin B, and after GLUT4 cloning, through antibodies. This led to a number of key discoveries, including that GLUT4 also responds to exercise (swimming) and hypoxia, albeit the donor fraction was distinct from that responding to insulin. Through collaborations with several postdoctoral fellows and scientific mentees of John’s, we confirmed insulin-dependent GLUT4 translocation in human muscle (with Juleen Zierath and Harriet Wallberg-Henriksson) and that the process is defective in diabetic rats and humans. Fast forwarding through the decades, my lab has endeavoured to identifying the molecular signals elicited by insulin that end up mobilizing GLUT4-containing vesicles in skeletal muscle and muscle cells in culture. After identifying Akt/PKB as a key node in this process (in 1999), we found that this signal percolates downstream of AS160/TBC1d1 to the small GTPases Rab8 and Rab13 (2008–10). We call this signal transduction the ‘software’ of insulin action. The GTPase respective effectors are MyoVa and actinin4. MyoVa physically mobilizes GLUT4 vesicles out of their dynamically retaining-compartment towards the cell periphery, and actinin4 tethers them beneath the membrane for subsequent fusion via SNARE proteins. I will present this process and how it is altered in insulin-resistant states.

APSIPE.202

Effect of daily voluntary exercise on stress-induced high blood pressure and gene expression profiles of the amygdala in rats

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Background: Chronic stress induces high blood pressure (BP), whereas physical exercise has anti-stress and anti-hypertensive effects^{1,2}). However, the mechanisms underlying these physiological changes remain unknown. Given that the amygdala (AMY) is involved in stress-induced physiological responses including BP regulation³), we hypothesized that stress- and exercise-induced cardiovascular effects are both mediated by altered gene expression profiles in the AMY. **Purpose:** The purpose of this study was to clarify whether chronic restraint stress in rats alters the resting BP levels and gene expression profiles in the AMY and whether daily exercise modulates the chronic stress-induced physiological and transcriptomic changes. **Methods:** Four-week-old Wistar rats were allocated into three groups: control (Co, n = 6), chronic restraint stress (St, n = 6), and chronic restraint stress with voluntary exercise (SE, n = 6). Chronic restraint stress was induced for 3 weeks by a daily, 1-h immobilization session (5 days per week). The arterial pressure (AP) of Co, St, and SE groups was measured using a tail-cuff method before and after intervention. The body weight (BW) was also measured during the experimental period. After 3 weeks of intervention period, transcriptomic analysis at the level of the AMY was performed in each group of animals using a DNA microarray. Target AMY genes were analyzed by gene ontology and pathway analysis and their expression was correlated to the AP levels. Immunohistochemical analysis was used to investigate the localization of proteins related to the identified genes. Moreover, siRNAs were used to silence the activity of the screened genes expressed in the AMY to clarify their role in regulating the cardiovascular phenotype. **Results and Discussion:** The mean AP in the St group after chronic restraint stress was significantly higher than in the two other groups, whereas it was not significantly different between the Co and SE groups. The rat BW after intervention was significantly lower in the St and SE groups than in the Co group. Furthermore, the expression of 451 AMY genes were significantly altered in the St group compared to the Co group; however, 357 out of those 451 genes showed no expression change in the SE group compared to the Co group, suggesting that abnormal expression in about 80% of the stress-sensitive genes was rescued by daily voluntary exercise. The gene pathway analysis suggested that STAT3 (signal transducer and activator transcription 3) and NR3C1 (nuclear receptor subfamily 3 group C member 1; a glucocorticoid receptor) are potentially associated with both stress- and exercise-induced cardiovascular responses. Regarding STAT3, we identified the presence of its phosphorylated, activated form in both neurons and astrocytes in the AMY, including in the central nucleus of the amygdala (CeA) where it has cardiovascular regulating

functions³). Moreover, injection of siRNA against STAT3 in the CeA led to a cardiovascular phenotype. **Conclusions:** These results suggest that altered gene expression in the AMY is related to the mechanisms regulating both the stress-induced high BP and the preventive effect of exercise. We also propose that STAT3 is associated with both mechanisms. The functional roles of NR3C1 need to be investigated in future studies. **References:** 1. Bruder-Nascimento, T, et al. *Arq Bras Cardiol* 99, 907-914, 2012. 2. Waki, H, et al. *Physiol Genomics* 45, 58-67, 2013. 3. Yamanaka, K, et al. *J Physiol Sci* 68, 233-242, 2018. **Acknowledgement:** This work was supported by MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S1411008), Private University Research Branding Project, and JSPS KAKENHI (19K11449 & 19KK0251)

APSIPE.203

Effects of acute nitrate supplementation on running performance and muscle damage markers

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INTRODUCTION: Nitrate is an inorganic anion present in some foods such as celery, beetroot, lettuce and spinach. Several studies have shown that nitrate supplementation has benefits in physical exercise, like running, especially because of increase on mitochondrial efficiency, vasodilation, blood flow to the muscle, physiological responses of fast twitch fibers (type II fibers) and reducing the Adenosine triphosphate (ATP) production cost. **Methods:** Eight recreational runners (age 33.63 ± 5.13 years old and 53.50 ± 3.42 average 10km running time) received nitrate supplementation in a double-blind, randomized crossover study. Were divided in two groups: Nitrate (NO3) (140 mL of concentrated beetroot juice supplement (BEET IT Sport, James White Drinks Ltd., Ipswich, UK) containing ~ 12,5mmol of beetroot juice and Control (CON) (140mL of mixed redcurrant and grape juice). All groups ingested nitrate 2 hours before in the running test. Running performance were tested through 10-km running time-trial and blood makers of muscle damage concentration (creatin kinase (CK) and lactate dehydrogenase (LDH)) were tested one day before (PRE) and 10 minutes after the 10-km running time-trial test (POS). After the normality test (Shapiro-Wilk), 2 X 2 (group X moment) repeated measures analysis of variance (RMANOVA) with the Bonferroni adjustment for multiple comparisons was used to muscle damage makers. For performance, Student's t test paired samples was performed. Statistical significance was set at p < 0.05. **RESULTS:** There was no significant differences 10-km running time-trial (NO3: 52.35 ± 4.34; CON: 54.36 ± 4.47, p = 0.28). CK showed a significant increase in the CON group (PRE: 244.62 ± 139.59; POS: 348.87 ± 170.76, p = 0.016) but not in NO3 group (PRE: 177.87 ± 65.94; POS: 201.87 ± 55.97, p = 0.540). When we compare the groups,

NO₃ showed lower CK concentration results than the CON group (NO₃: 201.87 ± 55.97; CON 348.87 ± 170.76, $p=0.036$). LDH showed a significant increase in the CON group (PRE: 397.37 ± 161.06; POS: 791.37 ± 357.78, $p=0.003$) but not in the NO₃ group (PRE: 384.77 ± 169.44; POS: 580.66 ± 358.00, $p=0.080$). When comparing between groups, there are no significant differences (NO₃: 580.66 ± 358.00; CON: 791.37 ± 357.78, $p=0.244$). CONCLUSION: The NO₃ group present better results because to the effect of nitrate supplementation, mainly mitochondrial efficiency and decreased ATP cost, thus reducing work and consequently presented lower muscle damage. In summary, we can conclude that acute ingestion of nitrate supplementation not increases the blood makers of muscle damage concentration in recreational runners, result that indicates an improvement in muscle recovery after the 10km running performance.

APSIP.204

Interaction Between Inflammation and Substrate Metabolism During Recovery from an Acute Bout of Treadmill Training

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Background: The mechanisms of substrate metabolism during exercise have been well studied, and the induction of inflammatory cytokines has been demonstrated in multiple types and intensities of acute exercise. However, less is known about the recovery period following exercise, and even less is known about the interaction between substrate metabolism and inflammation in this post-exercise window. While it is known that inflammatory cytokines including interleukin-6 (IL-6) can promote glucose uptake during exercise, their impact on oxidative glucose metabolism remains unclear. Therefore, the objective of this study is to characterize the interactions between inflammation and tissue-specific metabolism during recovery from High Intensity Interval Training (HIIT) and moderate intensity steady state (MISS) treadmill exercise. Methods: 6-8 week old C57/BL6J male mice were acclimated to an Exer3/6 Treadmill (Columbus Instruments) one day prior to a two-hour bout of HIIT: 20 minutes at 12 m/min, followed by alternating two minute periods of 19 m/min. and 12 m/min. These speeds were chosen as they are above and below the average C57/BL6J lactate threshold. Food was removed from the mouse cages two hours prior to exercise. To understand the impact of inflammatory cytokines on skeletal muscle metabolism, myeloid-specific interleukin-6 (IL-6) knockout (LysM-cre IL-6^{f/f}) mice were subjected to a two-hour bout of MISS (15 m/min). An infusion of 13C6 glucose was administered in order to determine tissue-specific metabolic substrate preference in both protocols. Before exercise (pre), and 0, 0.5, 2, 4, 24, and 72 hours after exercise, blood and tissues were taken from the mice to measure serum metabolites and hormones, glycogen content, and metabolic flux. T-tests were performed when two means were compared, and a one-way ANOVA with Tukey's multiple comparisons test was performed when more than two means were compared. The mean±S.E.M. of n=2-5 are shown. Results:

Our HIIT protocol resulted in myofibrillar disruption evidenced by loss of Desmin staining, significant depletion then supercompensation of liver glycogen (226±40 umol/g pre, 93±60 umol/g at 0, and 290±36 at 72 hours (ANOVA $p=0.04$), and modest depletion then a tendency toward supercompensation of gastrocnemius glycogen 9.3±1.5 umol/g pre, 6.7±1.5 umol/g at 0, and 11.6±1.7 at 72 hours. Serum IL-6 peaked 4 hours post exercise (661±647 pg/mL vs. 134±36 pg/mL pre, $p=0.19$), and serum interleukin-10 peaked 2 hours post-exercise (543±246 pg/mL vs. 0±21 pg/mL pre, $p=0.059$). The proportion of glucose that fueled the TCA cycle (glucose oxidation) in gastrocnemius increased immediately after MISS (34.2±2.7 vs. 16.0±2.7% pre-exercise, $p=0.001$) in wild-type but not myeloid-specific IL-6 knockout mice, (4.4±1.4 vs. 8.4±3.1 pre-exercise, $p=ns$). In response to HIIT, post-exercise gastrocnemius glucose oxidation was similar post-exercise (17.5±11.4%) and trended down later into recovery (9.4±3.9% at 2 hours post). Conclusions: While liver and gastrocnemius glycogen content was low, inflammatory cytokines peaked. IL-6 and IL-10 were induced following visible muscle damage, and peaked 2-4 hours post exercise. During glycogen restoration, the skeletal muscle shifts its energy substrates for oxidation from greater reliance on glucose to a greater reliance on alternative energy substrates, including fatty acids, likely to prioritize glucose partitioning for glycogen storage. These effects may be mediated by IL-6.

APSIP.205

Proteomic Investigation of Resistance Exercise-Induced Hormonal Changes on Muscle Damage in Untrained Women: A Pilot Study

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Background: Studies have demonstrated resistance exercise (RE)-induced hormones assist muscle protein synthesis and hypertrophy. However, the effect of these hormones on other cellular processes, such as muscle repair and the immune response, is lacking. Objectives: To examine the effect of RE-induced hormone changes on skeletal muscle proteome in untrained young women. Methods: Three women (age: 19 ± 1yr; height: 162 ± 13cm; weight: 54 ± 5kg; FFM: 33 ± 3kg; FM: 32 ± 10%) completed two sessions of 80 unilateral maximal eccentric knee extensions followed by either an upper body RE (EX; induced hormonal changes) or rested for 20 min (CON). Muscle samples collected at 24h on the exercising leg after each session were used for comparison between conditions (EX and CON). Global protein expression changes were measured by liquid chromatography tandem mass spectrometry. Student's t-test analyses were carried out using Perseus software and only the proteins with a p value < 0.05 were considered to be differentially expressed between EX and CON at 24h. Results: A total of 918 proteins were identified. Forty of those were significantly ($p < 0.05$) different between EX and CON in untrained young women. At 24h, proteins associated with

cellular growth [i.e., Myosin-11 (2.5-fold), Fibrillin-1 (1.6-fold), and CDC42 (1.5-fold)] and protein synthesis [i.e., 60S ribosomal protein L4 (1.0-fold), 40S ribosomal protein S18 (0.5-fold) and 60S ribosomal protein L10 (0.4-fold)] were significantly greater for EX than CON. On the contrary, proteins associated with improving immune function (antibody activity and protease inhibition) [IGHV3-23 (2.9-fold), A1AC (2.0-fold), and IGKV4-1(2.0-fold)] were significantly lower for EX than CON. In addition, proteins associated with cell structure (Fibrillin-1, Plectin, Myomesin-1, Nebulin, and Titin; 1.6 – 0.25-fold) were significantly greater for EX than CON. Further, proteins associated with protecting against oxidative stress (Superoxide dismutase, Acylamino-acid-releasing enzyme, and PRDX6; 0.68 – 0.27-fold) were significantly lower for EX than CON. Conclusion: In response to muscle damage, the addition of the RE-induced hormonal changes resulted in an increased skeletal muscle abundance of several proteins associated with cellular repair and growth and a decreased abundance of proteins associated with immune functions as well as oxidative stress protection in untrained women. This project is supported by Texas Tech University internal funding.

APSIPE.206

Effect of Developmental Exposure to Hypobaric Hypoxia in Aerobic Capacity and Ventilatory Efficiency during Maximal Exercise in Young Admixed Andean Residents

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INTRODUCTION: Bogotá (2600 m, 28% reduction in O₂ inspired pressure) is the most populated city above 2500 m, with roughly 8 million people from diverse and admixed genetic background and high immigration levels from lower altitudes. Ancestral Andean high-altitude natives (Quechua and Aymara) exhibit specific genetic adaptations at rest and during exercise when compared to lowlanders acclimatized to the same altitude: higher hemoglobin concentration, lower hypoxic ventilatory drive, lower resting and exercise ventilation and higher alveolar-capillary diffusing capacity. Some studies propose early developmental adaptations in Andean high-altitude residents lacking altitude ancestry. It is not clear for admixed Andean population, born and raised at moderate altitude, if early developmental hypoxic exposure confers beneficial effects during exercise in altitude. We hypothesized that differential chronic altitude exposure in antenatal and postnatal development affects aerobic capacity and ventilatory response in young healthy admixed Andean residents at moderate altitude.

OBJECTIVE: To determine the effect of duration and time of exposure to hypobaric hypoxia on aerobic capacity and ventilatory efficiency during maximal exercise in young Andean residents at moderate altitude. **METHODS:** We used a cross-sectional study design to enroll 405 healthy,

non-athlete, young residents in Bogotá. Subjects were assigned to four groups according to sex and altitude exposure: native groups were born and raised in altitudes between 2500 m and 2800 m and acclimatized groups were born and raised (first 14 years of age) at altitudes below 500 m but had lived at 2600 m for at least 10 weeks before enrollment. 1. Native men (NM): n = 171, age (mean ± sd) 22.4 ± 2.3 yr, body mass index (BMI) 21.7 ± 1.9 kg m⁻²; 2. Native women (NW): n = 156, age 21.6 ± 2.0 yr, BMI 21.8 ± 1.7 kg m⁻²; 3. Acclimatized men (AM): n = 40, age 20.7 ± 1.9 yr, BMI 22.1 ± 1.6 kg m⁻²; 4. Acclimatized women (AW): n = 38, age 21.1 ± 2.0 yr, BMI 21.7 ± 1.7 kg m⁻². All subjects performed an incremental (ramp) cardiopulmonary exercise testing in an electronically-braked cycle ergometer with continuous breath-by-breath gas-analysis and 12-lead electrocardiography recording, earlobe capillary lactic acid concentration was sampled every 2.5 min. Peak work rate (WR pk), peak oxygen consumption per kilogram of body weight (VO₂ PK), ventilatory equivalent for carbon dioxide at ventilatory threshold 2 (EqCO₂ VT₂), ventilatory efficiency slope (VE-VCO₂ S) and intercept (VE-VCO₂ I) and peak heart rate (HR PK) were compared between groups by Wilcoxon Rank Sum test, α error was set at 0.05 level. **RESULTS:** For men and women WR PK and VO₂ PK were consistently higher in native groups. WR PK: 272 vs 233 W, p < 0.001 (CI95% 183, 498) for NM vs AM respectively, and 165 vs 147.5 W, p < 0.01 (CI95% 4.99, 23.99) for NW vs AW respectively. VO₂ PK: 46.18 vs 38.58 mL min⁻¹ kg⁻¹, p < 0.001 (CI95% 4.20, 8.87) for NM vs AM respectively, and 34.78 vs 32.48 mL min⁻¹ kg⁻¹, p < 0.001 (CI95% 1.59, 4.86) for NW vs AW respectively. Native groups also had lower EqCO₂ VT₂ and Ve-VCO₂ S and higher Ve-VCO₂ I in both sexes. EqCO₂ VT₂: 29.72 vs 31.53, p < 0.001 (CI95% -2.56, -0.67) for NM vs AM respectively, and 31.46 vs 33.46, p < 0.001 (CI95% -2.89, -0.91) for NW vs AW respectively. VE-VCO₂ S: 32.23 vs 36.13, p < 0.001 (CI95% -5.28, -1.84) for NM vs AM respectively, and 33.16 vs 37.17, p < 0.001 (CI95% -5.27, -1.52) for NW vs AW respectively. VE-VCO₂ I: 2260.10 vs 522.65 mL min⁻¹, p < 0.01 (CI95% 965.43, 4609.26) for NM vs AM respectively, and 2224.79 vs 343.93 mL min⁻¹, p < 0.05 (CI95% 194.87, 2848.93) for NW vs AW respectively. HR PK was similar in native and acclimatized groups. **CONCLUSIONS:** For healthy young admixed Andean residents, prenatal and continuous postnatal developmental hypobaric hypoxic exposure have a favorable effect in aerobic capacity and ventilatory efficiency during maximal exercise at moderate altitude; the relative role of oxygen transport and utilization systems in this effect should be further investigated. Supported by COLCIENCIAS Grant number 120356934972, 713-2013.

APSIPE.208

Integrative transcriptomic, proteomic and protein synthesis profiling of individual proteins in mouse skeletal muscle reveal distinct signatures between adult and old animals.

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Sarcopenia is defined as the loss of muscle mass and function in the advanced stage of life. With advancing age there is a notable decrease in muscle mass, with substantial loss of protein, suggesting that the balance between protein synthesis and degradation is net negative. This study examined the effect of age on transcript levels, rates of turnover and total abundance of individual proteins in muscle. In this study, male adult (6-8 months) and old (26-28 months) mice were dosed with deuterated water (2H₂O) to a plasma enrichment of 4.5% for a number of time-points up to 60 days (Kim et al., 2012). Gastrocnemius muscles were collected, and label free proteomics was performed to observe the fractional synthesis rates of individual proteins. Parallel to this, label free proteomics was performed on the gastrocnemius muscles of a separate group of adult and old mice. These two datasets were compared with an RNAseq dataset produced from male adult and old male mice. GO and KEGG pathway term enrichment was performed alongside Ingenuity Pathway Analysis in order to identify patterns in the data, focussing specifically on pathways and systems involved in protein synthesis and degradation in muscle. Analysis of the rates of synthesis of individual proteins identified several reactive oxygen species-mediated and structural proteins as altered with age. GO and KEGG pathway analysis of label free proteomic data revealed enrichment of the endoplasmic reticulum associated degradation pathway, unfolded protein response and ubiquitin protein ligase binding. The data also revealed no correlation between the mass or amino acid length of proteins and the fractional synthesis rates of proteins in the muscles of old mice suggesting that translation elongation was unaffected with age. Funded by National Institute of Aging (grant number AG-051442) and the University of Liverpool.

APSIPE.209

Exercise is Chemotherapy: Exercise-Derived Myokines Suppress Lung Cancer Cell Growth

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Among chronic diseases in the United States, cancer is one of the most devastating, ranking second in mortality rates among Americans. Of the primary malignancies, lung cancers are some of the most common, coming in second in both genders, and most deadly, carrying a survival rate

of roughly 18% and accounting for 25% of all cancer deaths. While smoking cessation programs have drastically reduced the burden of lung cancer, continued research into mechanisms that can halt tumor progression are clearly warranted. One novel route by which cancer growth may be halted has gained prominence in recent years: physical exercise. The discovery of myokines, pharmacologically active substances released from skeletal muscle, indicates that contracting skeletal muscle may exert mechanistic control over the biology of extra-muscular tissues. Based on previous research showing benefits of physical exercise in preventing cancer growth, the objective of this study was to investigate the effect of myokines released from metabolically active skeletal muscle on the proliferation of cultured lung cancer cells. Briefly, the hindlimb vasculature of a female Wistar rat was perfused with physiological solution (Krebs-Henseleit buffer). Electrical stimulation of the sciatic nerve was then used to elicit muscular contraction of the hindlimb musculature, with perfusate collected both before and during muscular contraction. A549 lung cancer cells were incubated in DMEM containing either 10% Krebs buffer, perfusate collected prior to contraction, or perfusate collected during contraction. Following 48 hours of incubation, proliferation of cells treated with exercise-conditioned perfusate was reduced ($p < 0.05$) by ~30% when compared to both cells treated with Krebs or non-exercised conditioned perfusate, with no differences ($p > 0.05$) observed between Krebs and non-exercising treatments. Based on these results, we can conclude that compounds released from skeletal muscle reduce cancer cell growth – and that muscular contraction is key to eliciting release of cancer fighting myokines. Experiments to identify the specific mechanism and mediators of this effect are underway, with the goal of broadening the knowledge of exercise-derived myokines and their role in the amelioration of cancer pathology.

APSIPE.210

Change of Plasma Long Non-Coding RNA Profile After Maximal Aerobic Exercise in Elite Endurance Runners

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The athlete performance can be influenced by genetic mechanisms of control and the long non-coding RNAs (lncRNAs) are a part of these mechanisms. In the last years, some studies investigate association between lncRNAs and exercise, however, this relationship is unclear. Thus, the aim of this study was to investigate possible change of plasma lncRNAs profile after maximum aerobic exercise in elite running athletes. Thirteen athletes participated of study, seven male athletes (age: 27.7 ± 6.6 years; weight: 59.1 ± 3.5 kg; height: 170.3 ± 6.0 cm) and six female athletes (age: 28.2 ± 5.0 years old; weight: 46.5 ± 2.8 kg; height: 158.8 ± 5.2 cm). A cross-sectional study was performed, with a maximum effort test and two blood

collections (before and after test). The test was carried out on a treadmill and measured oxygen consumption using indirect calorimetry (Vista Mini-CPX™, Vacumed™, Ventura, CA, EUA). The blood collected was centrifugate at 1000g for 10 min, obtaining plasma. After that, the Human LncProfiler™ qPCR Array Kit was used for plasma analysis by qRT-PCR at thermocycler StepOne Plus™ Real Time System. All procedures was approved by local ethical committee. There was a significant difference in anthropometric and physiological measurements, such as fat mass (female athletes: $12,9 \pm 2,5$ %; male athletes: $4,3 \pm 1,3$ % – $p < 0,01$) and maximum oxygen consumption (female athletes: $65,3 \pm 4,2$ ml·kg⁻¹·min⁻¹; male athletes: $76,2 \pm 2,5$ ml·kg⁻¹·min⁻¹ – $p < 0,01$). Additionally, in male athletes, 24 lncRNAs were detected during the rest and 22 lncRNAs after exercise and in female athletes, 13 lncRNAs were detected during the rest and 13 lncRNAs after exercise. Through multidimensional analysis, a trend to separation between groups and conditions was observed after maximal aerobic exercise. Therefore, our study is first to identified change of plasma lncRNA profile following maximal exercise in male and female elite endurance runners. Therefore, we speculate that exercise can modulate lncRNAs detection, modifying the lncRNA plasma profile in male and female athletes. Future studies are necessary to identify lncRNAs functions in aerobic exercise.

APSIFE.211

Investigation of skeletal muscle mitochondrial function following an ultramarathon: a case study in monozygotic twins

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The primary objective of this case study was to investigate the changes in skeletal muscle mitochondrial function that occur in response to a 100-km ultramarathon in monozygotic twins. Secondly, we sought to determine the effect of periodically consuming a low-carbohydrate, high-fat diet (LCHFD) during training on mitochondrial respiration. To accomplish these objectives, one pair of male monozygotic twins ran 100 km side-by-side on treadmills. During the 8 weeks of training leading up to this event, twin 1 (T1) and twin 2 (T2) consumed a high-carbohydrate diet where caloric and macronutrient intake were matched, with the exception of 2 consecutive days per week for the final 4 weeks, where T2 consumed a calorie and protein-matched LCHFD. The reason for this periodized approach was to deplete glycogen levels in T2 during two days of training per week. Training load was also matched between the subjects, most of which they completed together. Furthermore, the subjects were extremely well-matched in VO₂ max (61.2 and 60.3 ml/kg/min), BMI (20.4 and 20.6 kg/m²), and body mass (62 and 61.9 kg). During the 100-km run, food and fluid intake were matched, as well as the pace of the run. The run took 16 hours and 3 minutes to complete. Muscle biopsies were collected 4 weeks prior to the run, as well as 4- and 52-

hours post-run in order to measure mitochondrial oxidative capacity and reactive oxygen species (ROS) production using the Oroboros O2k-FluoRespirometer. Respiratory exchange ratio (RER) was measured at km 3, 32, 64 and 97 during the run using a gas-calibrated indirect calorimetry system. During the last 4 weeks of training, T2 lost an average of ~1 kg of body weight over the two days of LCHFD each week ($p = 0.02$), which was regained two days after re-introduction of carbohydrate, consistent with fluctuating glycogen levels. Though T2 trained with periodically low glycogen levels, RER was no different from T1 throughout the 100-km run. Four hours after the run, state 3, uncoupled, and fatty acid-supported mitochondrial respiration were reduced in T1 by 22.3%, 34.1% and 25.7%, respectively, and by 28.4%, 49.4% and 39.9% in T2. Possibly the most novel finding from this study was that 52 hours after the run, respiratory capacity in each of these states had almost fully recovered, or surpassed baseline values, indicating that the reduction in respiratory capacity was not likely due to protein degradation. It is currently unclear by what mechanism mitochondrial respiration was impaired 4 hours post-run. Interestingly, mitochondrial ROS production (when measured at concentrations of ADP ranging from 12.5 to 100 μ M) was increased by approximately 50% in both subjects following the run and remained elevated 2 days later. It does not appear that either diet granted the subjects an advantage in the way of greater fat oxidation during the run, nor did either appear to better-preserve mitochondrial energy production post-run. This study is valuable because it provides evidence of an acute reduction in mitochondrial respiratory capacity following ultra-endurance activity, which recovered in just 2 days. Future measurements will include changes in blood markers of muscle and liver damage, as well as immunohistochemical staining for structural damage to skeletal muscle. This study was approved by the Brigham Young University Institutional Review Board and conformed to the principles of human experimentation set by the Declaration of Helsinki.

APSIFE.214

Combined Effects of Lunar Gravity and Heavy Ion Exposure on Rates of Skeletal Muscle Protein Synthesis in Soleus and Plantaris Muscles

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Unloading-induced atrophy and exposure to space radiation are considered major physiological limitations to future space exploration. It is commonly purported that skeletal muscle is radio-resistant due to its post mitotic state, but recent work has indicated changes in both protein synthesis rates and muscle morphometry with exposure to high-charge high-energy particles. While unloading-induced atrophy has been widely studied in models of complete unloading, information on muscle atrophy in partial loading conditions is much less common, but there is indication that muscle anabolism is linked to

gravitational load or lack thereof. It is clear that space exploration includes both space radiation- and unloading-induced atrophy; however, the combined effects of these two environmental factors has limited investigation. The purpose of this study was to determine the impact of acute radiation exposure, with or without the addition of partial loading on skeletal muscle protein synthesis rates.

METHODS: BALB/cByJ mice were assigned either to full weight bearing or partial weight bearing equal to 1/6th bodyweight groups. These groups were then further assigned into either single 0.5 Gy of 300 MeV whole – body ²⁸Si radiation exposure (RAD) or sham. Radiation exposure occurred on Day 0 at Brookhaven National Laboratory and was followed by 21d of Earth (CC) or Lunar (G/6) loading. Soleus and plantaris muscles of the hind limb were examined for muscle weight and mixed muscle fractional synthesis rates. Protein synthesis rates (FSR) were measured over 24 hours using deuterium. **RESULTS:** In the postural soleus muscle, partial loading resulted in lower muscle mass, regardless of radiation exposure (CC = CC+RAD > G/6+SHAM = G/6+RAD). Interestingly, FSR was only lower in soleus ($p < 0.05$) after exposure to radiation in partially loaded animals (CC > G/6+RAD), and G/6+SHAM or CC+RAD groups were not different from each other or the other groups. It is noteworthy that soleus mass was greatest in the CC+RAD group ($p < 0.05$) when compared to other groups, even though FSR was similar to CC and G/6+SHAM ($p > 0.05$). In plantaris, muscle mass was only lower with G6+SHAM ($p < 0.05$), while mass of all other groups were similar (CC = CC+RAD = G/6+RAD > G6+SHAM). The apparent rescue of plantaris muscle mass in G/6+RAD occurred in spite of the observation that FSR was not different among groups ($p > 0.05$). **CONCLUSION:** This study suggests that heavy ion exposure affects skeletal muscle and can lead to discrete and combined effects in selected muscles including but not limited to a slight rescue of partial loading's reduction in muscle mass. However, these changes may not be evident through endpoint assessments of fractional synthesis rates, likely due to fractional synthesis rates normalizing to the resultant mass by day 21. Funded by NSBRI/NASA

APSIPE.215

A systematic review of exercise training protocols for mice

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There has been an increase in the number of experiments investigating the molecular mechanisms underlying the beneficial effects of exercise using mouse models. However, the design of the exercise training protocols and the efficacy of those programs to elicit established training adaptations are variable. Therefore, a systematic review of exercise training studies in mice was conducted to characterize various training protocols and the outcomes measured to support the efficacy of the training program. A literature search was performed using PubMed and EMBASE databases for articles published between 1966 and 2020. Our initial search identified 671 studies. Out of 671 studies identified, 154 studies met the following inclusion criteria: 1) inbred mice with no treatment (e.g., diet

or drug intervention), 2) inclusion of a sedentary/control group, 3) exercise training for more than 1 week, and 4) training with a treadmill. Studies with overtraining-based protocols, wheel running, transgenic animals, rats, acute exercise training (less than 5 sessions), or no exercise outcome data reported, were excluded. Random allocation to sedentary and exercise group, blinded assessment of the outcome variable, sample size calculation, animal welfare statement, and a statement regarding conflict of interest were recorded to assess the risk of bias and study quality. Most studies reported random allocation to groups and included an animal welfare statement. Half included a conflict of interest statement. However, <20 studies reported blinded assessment of any variable and only 2 included a sample size calculation, suggesting that study bias should be further investigated. Out of 154 studies, 34 studies (22%) assessed performance (e.g., exhaustion time, maximum velocity, or work) as an outcome variable. Oxidative enzyme activity such as citrate synthase or succinate dehydrogenase activity or lactate levels were reported as markers of training efficacy in 16 out of 154 (10%) studies. Other exercise-related traits such as blood pressure, mitochondrial DNA copy number, or muscle fiber type were reported in 6 other studies. There were 98 studies (64%) with no classical markers of exercise performance or training efficacy. Most studies used C57BL/6 mice (97 of 154) while other strains used were Balb (18), Swiss (16), NMRI (4), and FVB (3). Male mice were used exclusively in nearly all studies (134 out of 154) and only a few studies (17 out of 154) included female mice. Training protocols varied markedly. The most common parameters were a treadmill speed of 11-15 m/min, an incline of 0 to 10 degrees, 60 minutes per session, 5 days/week for 8 weeks. Nearly all studies reported an increase in exercise performance in the trained group relative to sedentary controls. Similarly, enzyme activity was significantly higher in exercise-trained mice compared with controls in 11 of 16 studies. Therefore, in the studies included in this review, outcome variables were significantly different between exercise and sedentary groups regardless of training protocol. Future analysis will focus on the impact of individual exercise training parameters to identify the treadmill-based exercise training protocol for mice yielding the greatest change in exercise performance and/or adaptations to training.

APSIPE.216

Mitochondrial Quality Control in Exercise-mediated Protection against Diastolic Dysfunction in Diabetes

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Diabetes mellitus (DM)-induced heart failure is preceded by cardiac diastolic dysfunction, a loss of contractility during diastole. Emerging evidence shows that diastolic dysfunction is strongly associated with poor mitochondrial quality in the myocardium. On the contrary, exercise training is the most potent method for prevention and

treatment of diabetes with superb cardiometabolic benefits; however, the mechanism(s) is unknown. We hypothesize that mitochondrial quality control is critical in exercise-mediated protection against diabetes-induced cardiac dysfunction. Here, we subjected C57Bl/6 mice to high-fat feeding (60% HFD) for 4 weeks followed by low-dose streptozotocin injections (5 daily doses of 50 mg/kg and 3 weekly doses of 20 mg/kg, i.p.) to induce severe diabetes. The diabetic mice were then randomly divided into sedentary (DM-Sed) and voluntary wheel running (DM-Ex) for 6 weeks. Sedentary mice on normal chow diet served as control (Con). Echocardiogram revealed diastolic dysfunction with preserved systolic function in DM-Sed mice as indicated by a significantly exacerbated decline of end-diastolic diameter following dobutamine injection. Transmission electron microscopy showed impairment of mitochondrial morphology in the diabetic myocardium, including accumulation of smaller and irregularly-shaped mitochondria. No evidence of cardiac fibrosis was present in the diabetic hearts via picrosirius-red staining and western blot. Despite severe hyperglycemia, DM-Ex mice showed improved exercise capacity, restored diastolic dysfunction and correction of mitochondrial abnormalities. Importantly, we have observed signs of mitophagy with increased BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (Bnip3) in the myocardium of DM-Ex mice. We have also obtained evidence that a single bout of treadmill running activity in mice results in activation of mitochondria-associated AMP-activated kinase (mitoAMPK). These findings suggest that exercise-mediated mitochondrial quality control, presumably through activation of mitophagy via mitoAMPK, is key in treatment of diabetes-induced diastolic dysfunction.

APSIPE.217

Cardiopulmonary Exercise Testing in a Large Community-Based Sample: Feasibility and Methodology

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Background: Cardiopulmonary exercise testing (CPET) is a powerful clinical and research tool for assessment of physiologic exercise responses, but protocols for expanding the use of CPET into large community-based samples are lacking. **Methods:** A cycle ergometry CPET protocol with peak-exercise blood sampling was developed and implemented in three Framingham Heart Study cohorts (Generation 3, OMNI 2, New Offspring Spouses) at their 3rd study visit (2016-2019). Standardized quality control and safety protocols were developed and evaluated. Peak oxygen consumption (VO₂) was assessed as the highest 30-second median during the final 90 seconds of incremental exercise and the ventilatory anaerobic threshold (VAT) was calculated using the V slope method. Generalized additive models were used to evaluate the associations of effort-dependent exercise

variables and peak respiratory exchange ratio (RER) achieved. **Results:** Of 3486 individuals presenting to the research center, 3116 (89%) completed exercise, demonstrating high participation rates and feasibility. The sample was middle-aged (54±9 years), with 53% women, body mass index 28.3±5.6, 48% with hypertension, 6% smokers, and 8% with diabetes. The mean exercise duration was 12.0±2.1 minutes (range 3.7-20.5) and testing was completed within a 30-minute overall protocol. There were no major cardiovascular adverse events or deaths associated with CPET. Consistency of resting VO₂ and VO₂/work slope were observed over the course of a 4-hour testing day and across the 3-year study protocol. A total of 98%, 96%, 90%, 76%, and 57% of the sample reached RER values of ≥1.0, ≥1.05, ≥1.10, ≥1.15, and ≥1.20, respectively and the mean RER was 1.22±0.10. Relating exercise performance to peak RER, there were steep changes for percent predicted peak VO₂, the VO₂ at the VAT as a percentage of peak VO₂, percent predicted heart rate achieved, and Borg (subjective dyspnea) scores up to peak RER ≥1.10; changes were less steep at higher achieved RER values. **Conclusions:** Maximum effort CPET is safe and feasible and can be done efficiently in a large sample of middle-aged, community-dwelling individuals with heterogeneous cardiovascular risk profiles. Peak RER values below 1.10 were associated with steep graded reductions in CPET performance measures that require maximum volitional effort such as peak VO₂. By contrast, peak RER values of at least 1.10 were achievable by 90% of middle-aged adults and values beyond 1.10 were associated with relatively non-dynamic increments in peak-effort dependent CPET measures.

APSIPE.218

Pulmonary Capillary Wedge Pressure Responses to Upright Exercise Refine Hemodynamic Assessment and Predict Prognosis in HFpEF.

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Introduction: A resting supine pulmonary capillary wedge pressure (PCWPrestsupine) of ≥15 mmHg is considered a hemodynamic gold standard criterion for diagnosing left-sided heart failure with preserved ejection fraction (HFpEF). How PCWP changes upon assuming the upright position in HFpEF, and what exercise PCWP threshold optimally relates to functional capacity and outcomes in HFpEF remains largely unexplored. **Methods:** We investigated patients referred for evaluation of exertional dyspnea who met resting supine hemodynamic criteria for having HFpEF. Uniform measurement of upright resting PCWP was performed, followed by maximum upright incremental ramp cycle ergometry cardiopulmonary exercise testing (CPET). Paired PCWP and direct Fick cardiac output (CO) measurements were made minute-by-minute to permit derivation of PCWP/CO slopes, which were compared to

isolated PCWPex values in relation to exercise capacity (peak VO₂ and oxygen uptake efficiency slope [OUES]) and patient outcomes, as assessed with Cox regression models. For analysis, we stratified patients according to two recently advanced abnormal exercise PCWP thresholds: a PCWP/cardiac output (CO) slope >2mmHg/L/min and a Peak PCWP ≥25 mmHg. We hypothesized that exercise PCWP patterns would be distinct from resting measures and that PCWP/CO slope ≥2 mmHg/L/min would be more closely related to cardiorespiratory fitness and event-free survival than exercise PCWP ≥ 25mmHg. Results: In 209 patients with PCWPrestupine ≥15mmHg and normal LVEF (median [IQR] age 63 [51, 71] 52% women, BMI 32 [27.4, 36.0], LVEF 65 [60, 70]) decrements in PCWP from the supine to upright position (-11 mmHg [8.5, 13 mmHg] and respirophasic changes in PCWP (3 mmHg [2, 4 mmHg] were proportionate to BMI (both $\beta >0.24$, $p < 0.001$). During exercise, PCWP/CO slope was <2 mmHg/L/min in 80 patients (38%) and peak PCWPex was <25 mmHg in 102 patients (49%). After adjustment for age, sex, and BMI, PCWP/CO >2 mmHg/L/min was associated with an increased hazard for combined HF hospitalization or death (hazard ratio HR=2.88, 95% CI [1.38, 5.99] $p=0.005$). PCWP/CO ≥2 mmHg/L/min was also associated with lower peak VO₂ (15.6ml/kg/min, IQR [11.7, 18.3] vs. 13.0ml/kg/min, [10.8, 15.9], $p=0.002$) and lower OUES (1675, IQR [1355, 2047], vs. 1308, IQR [1011, 1665], $p < 0.001$). In contrast, peak PCWPex ≥25 mmHg was not associated with prognosis or exercise capacity. Conclusion: In patients with resting hemodynamic evidence of HFpEF, upright posture results in reduction in PCWP that is proportionate to BMI. Upright PCWPex indexed to CO, but not PCWPex >25mmHg, is associated with functional capacity and HF-event free survival. These findings highlight an important role of assessing exercise PCWP/CO relationships in patients with suspected HFpEF, even when PCWPrestupine is elevated. Support: Support was obtained from the National Institutes of Health R01HL151841 (GDL and RS), R01HL131029 (GDL), K23-HL138260 (Dr. Naylor) and the Heart Failure Research Innovation Fund (GDL).

APSIPE.219

Self-chosen pace displays a better walking economy when expressed in caloric unit cost

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The study aimed to determine at which walking pace humans are the most efficient at oxidizing fat. Forty-seven healthy participants (22 men, 25 women) ranging from 20 to 64 years old participated in the study that required the completion of four exercise intensities on a treadmill (self-chosen pace, 3 METs, 5 METs, and 7 METs). Metabolic rate was collected through indirect calorimetry and substrates contribution to energy production was calculated using stoichiometric equations. Caloric unit cost (CUC) was computed to determine the energy required to cover a

given distance (kcal·kg⁻¹·km⁻¹), that is walking economy. Results showed that the absolute FAT oxidation (g·min⁻¹) reached the peak value at 5 METs (M = 0.18, SD = 0.12) but did not differ from the self-chosen pace (M = 0.18, SD = 0.07); meanwhile the absolute CHO oxidation rates increased as a function of walking intensity. The statistical analyses further revealed that the optimum CUC occurred at the self-chosen pace. The post-hoc analyses showed that relative contribution as expressed in kcal·kg⁻¹·km⁻¹ of CHO oxidation to energy production was equal to the relative contribution of FAT oxidation at self-pace (M = 0.54, SD = 0.18, and M = 0.50, SD = 0.17 respectively; $p = .497$) and then started to significantly depart from each other at 3 METs onwards ($p = .001$) [CHO at 3 METs (M = 0.64, SD = 0.15), 5 METs (M = 0.74, SD = 0.19), and 7 METs (M = 0.90, SD = 0.19); FAT at 3 METs (M = 0.40, SD = 0.15), 5 METs (M = 0.30, SD = 0.19), and 7 METs (M = 0.14, SD = 0.18)]. Overall, the results show that the optimum substrates contribution to energy production (metabolic efficiency) occurs at self-chosen walking pace.

APSIPE.223

Overview and update of MoTrPAC clinical studies

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MoTrPAC, sponsored by the NIH Common Fund, is the single largest investment ever made into better understanding potential molecular underpinnings of exercise-induced health benefits. The adult MoTrPAC clinical program is comprised of a large-scale, randomized, controlled, exercise clinical trial in sedentary volunteers ranging 18 to 60+ years of age, complemented by comparative acute response studies in highly active and highly trained volunteers. An additional complement involves a parallel, single-site trial in children. This session will provide an overview of these MoTrPAC clinical studies including: rationale, study design (i.e. clinical protocol, phenotyping, acute response molecular transducers mapping), planned analyses, and expected outcomes. The adult program is being conducted across 10 clinical sites. Their integration with other key MoTrPAC components (e.g., NIH, coordinating center, molecular mapping sites, bioinformatics center, pediatrics site, pre-clinical animal sites) will also be discussed.

APSIPE.224

Effect of Acute and Chronic Exercise on Radiosensitivity in Tumor-Bearing Rats

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Background: Solid tumors contain hypoxic regions that have long been known to be resistant to radiation therapy. Increasing the efficacy of the primary treatment (radiotherapy) of prostate cancer is of clinical importance and has typically been pursued pharmacologically. Prior evidence from our lab has shown that exercise training can improve the oxygenation of a tumor, thereby decreasing regions of hypoxia. Using a pre-clinical orthotopic model of prostate cancer, we tested the hypothesis that exercise training would result in an enhanced radiotherapy response due to mitigation of hypoxia. Further, we tested the hypothesis that a single bout of exercise would also enhance radiosensitivity of prostate tumors. **Methods:** Dunning R-3327 MatLyLu prostate adenocarcinoma cells (2x10⁵) were injected into the ventral lobe of 5-month-old male RNU (NIH nude) rats (n=20). These animals were acclimated to treadmill exercise, and then randomized into three groups, Tumor Bearing Sedentary (TBS, n=7), acute exercise (TBAEX, n=7), or Chronic Exercise (TBEX, n=6). After ~5 days of recovery from surgery, TBEX animals began progressive exercise training on a motorized treadmill at 25 m/min with a 5% incline for 10 minutes a day progressing to 60 min/day for a ~5-week period, whereas TBAEX were exercised for one 30 minute bout at the same intensity 20 minutes before irradiation. Whole body ionizing radiation was performed on all animals at 2Gy at the end of the 5-week period of training followed by clonogenic cell survival assay to assess survival fraction. Pre-injection (Pre) and post-exercise training (Post) animals also performed Vo₂max testing for assessment of aerobic capacity changes. **Results:** There were no significant differences (all p>0.05) in tumor mass between groups (TBS 8.5±1.2; TBAEX 7.8±1.1; TBEX 6.9±1.6 g). The survival fraction was significantly different in TBS and both acute and chronic exercise (TBS 56.1±6.2; TBAEX 41.2±3.2; TBEX 48.7±6.9%, p<0.05). Following training, TBEX increased Vo₂max by 6.4±1.3 ml·min⁻¹·kg⁻¹ (p<0.05) whereas TBS had a decrease of 5.6±2. ml·min⁻¹·kg⁻¹ (p<0.05). **Conclusion:** This study suggests that both acute and chronic exercise have the potential to augment the tumor microenvironment favorably to enhance radiotherapy compared to sedentary counterparts. Further, prostate cancer, independent of treatment significantly diminishes maximal aerobic capacity, but was mitigated with moderate intensity exercise training. Given prostate cancer patients often present fatigue and loss of quality of life, moderate-intensity exercise training may be useful to improve both therapeutic response of the tumor, and quality of life of patients via maintaining or improving aerobic capacity.

APSIPE.225

Inflammation, mitochondria , and exercise responsiveness in obesity

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Systemic inflammation is a hallmark of obesity and may contribute to mitochondrial dysfunction and attenuate exercise responsiveness. **PURPOSE:** The purpose of this study was to investigate how obesity and associated systemic inflammation influences mitochondrial function and acute exercise responsiveness. **METHODS:** Lean (n=8; 6F/2M; BMI:<25 kg/m²), overweight (n=11; 4F/7M; BMI:25-30 kg/m²) and obese (n=20; 13F/7M; BMI:>30 kg/m²) adults performed 30 minutes of single-leg cycling at 70% of VO₂peak. Vastus lateralis biopsies were collected before exercise and 3 and 6 hours post exercise. Oxygen consumption (JO₂) and reactive oxygen species (ROS) production were measured in isolated mitochondria from pre-exercise biopsies. Fractional synthesis rate (FSR) of muscle proteins was measured from incorporation of infused [ring-¹³C₆]phenylalanine. **RESULTS:** Regression analysis revealed a positive association between BMI and circulating TNFα (r=0.460, p<0.05) and a negative association between BMI and JO₂ (r=-0.329, p<0.05). BMI was negatively associated with the exercise-induced increase sarcoplasmic protein FSR (r=-0.350, p<0.05), but not mixed muscle protein (MMP) FSR (r=-0.226, p=0.178). Notably, there was a negative association between circulating TNFα and the exercise-induced increment of MMP (r=-0.439, p<0.05) and sarcoplasmic FSR (r=-0.458, p<0.05). There was no significant association between mitochondrial function and muscle protein synthesis. **Conclusion:** These data suggest that chronic inflammation may diminish skeletal muscle exercise responsiveness in obesity.

APSIPE.226

Characterization of 2 weeks of aerobic blood-flow-restriction (BFR) exercise training responses on skeletal muscle mitochondrial adaptations and aerobic performance: preliminary data

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INTRODUCTION: We recently demonstrated that gravity-induced blood flow restriction (BFR) augmented AMPK activation and PGC-1α mRNA expression following a single bout of aerobic exercise [1]. Because the AMPK/PGC-1α axis regulates the adaptive response to exercise training in skeletal muscle [2] we hypothesized that chronic responses (eg. mitochondrial and whole body performance) to aerobic training would be augmented by BFR. We tested this hypothesis following 2 weeks of aerobic exercise either with or without BFR. **METHODS:** Nineteen recreationally active subjects (13 females and 6 males) completed seven, 30-minute cycling exercise sessions over a 2-week period. Exercise was performed with legs below (CTL, n= 10) or

above participants hearts (BFR, n= 9). Exercise sessions were completed at the exercise intensity that immediately preceded the onset of blood lactate accumulation during a maximal aerobic test in the leg down (CTL) position. Skeletal muscle biopsies were obtained from the vastus lateralis and indices of aerobic performance were measured before and after training. RESULTS: Differences were observed between CTL and BFR for training session ratings of perceived exertion (RPE): time $p < 0.0001$; group $p < 0.001$; interaction = 0.003 – RPE in BFR was significantly higher during all training sessions (all $p < 0.05$). Training session rating of perceived pain (RPP) were also higher during BFR than CTL (time $p < 0.0001$; group $p < 0.0001$; interaction $p = 0.1031$). There were no differences between CTL and BFR for in-training heart rate (HR) (time $p = 0.74$; group $p = 0.96$; interaction $p = 0.589$), and blood lactate levels (BLa) (time $p = 0.23$; group $p = 0.068$; interaction $p = 0.335$). BFR had no effect on protein responses of: LRP130 (time $p = 0.376$; group $p = 0.769$; interaction $p = 0.671$); for NRF2 (time $p = 0.442$; group $p = 0.254$; interaction $p = 0.688$); for CV (time $p = 0.975$; group $p = 0.389$; interaction $p = 0.441$); for CIII (time $p = 0.832$; group $p = 0.368$; interaction $p = 0.578$); for CII (time $p = 0.437$; group $p = 0.320$; interaction $p = 0.744$); for CIV (time $p = 0.762$; group $p = 0.295$; interaction $p = 0.882$); and for the average of OXPHOS (time $p = 0.889$; group $p = 0.331$; interaction $p = 0.661$). The response to training was different (time $p = 0.444$; group $p = 0.337$; interaction $p = 0.018$) between CTL and BFR for PGC1a. CONCLUSION: Our findings demonstrate that BFR had no impact on the response of important mitochondrial protein content levels to aerobic training. Interestingly, PGC-1a protein decreased following BFR and increased following CTL. The current study presents preliminary findings with further analysis currently underway. REFERENCES: 1. Preobrazenski, et al. (2019) Appl. Physiol. Nutr. Metab. 45(6): 641-649. 2. Gibala, et al. (2008) J. Appl. Physiol. 106(3): 929–934.

APSIPE.227

Insulin Resistance Induced by 10 days of Bed Rest is Accompanied by Elevations in Intramyocellular Lipid in the Soleus and Tibialis Anterior of Older Adults

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Introduction: Older adults often experience periods of bed rest (BR) during illness or hospitalization resulting in a loss of skeletal muscle mass and physical function concomitant with impairments in insulin sensitivity and lipid oxidation. These adaptations are believed to be mediated in part through dysregulation of intramyocellular lipids (IMCL) storage. Whether an accumulation of IMCL contributes to the impairments in insulin sensitivity remains controversial. In addition, the time course by which muscle mass and contractile function are restored following BR is unclear. Our goal was to determine the changes in insulin sensitivity, IMCL, body composition and skeletal muscle function experienced by older adults during 10 days of BR and following 4 weeks of reambulation. Methods: Ten older

adults (65-85 yrs) completed 10 days of strict BR at the in-patient clinical research unit at the AdventHealth Translational Research Institute in Orlando, FL. The following assessments were performed pre-BR (PBR) and following BR; body composition by dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) of the mid-thigh; contractile function by isokinetic dynamometry; IMCL by magnetic resonance spectroscopy (MRS); and insulin sensitivity was measured by hyperinsulinemic euglycemic clamp. During the subsequent 4 weeks of non-exercise reambulation, body composition, contractile function, and MRI/S assessments were performed weekly. All experimental procedures were conducted in accordance with guidelines set forth in the Declaration of Helsinki and approved by the institutional review board at AdventHealth Orlando. Results: BR did not induce significant changes in body weight and whole-body fat mass. BR induced a significant decrease in leg lean mass and in quadriceps peak torque and power. There was an ~18% decrease in glucose infusion rate during the clamp, indicative of skeletal muscle insulin resistance. This occurred concomitantly with an accumulation of IMCL in the soleus and tibialis anterior (TA) muscles. Following 1 week of re-ambulation, leg lean mass and IMCL recovered to PBR levels. However, peak torque and power did not fully return to PBR levels until week 4. Interestingly, the slow recovery of quadriceps contractile function occurred along with an increase in trunk (~0.53kg) and android fat mass (~0.13kg), and mid-thigh intermuscular (~10%) and subcutaneous (~3%) adipose tissue volume compared to PBR. Conclusion: Our data indicate that insulin resistance induced by 10 days of bed rest is accompanied by IMCL elevations in the soleus and TA of older adults, suggesting a potential role for lipotoxicity. The slower recovery of quadriceps contractile function compared to muscle mass highlights the need for older adults to exercise during re-ambulation. Finally, our data suggest that bed rest may predispose accumulation of fat mass during recovery. Future work is needed to explore whether insulin sensitivity and other metabolic processes remain impaired throughout ambulatory recovery in older adults. This study was funded by National Institute on Aging (R01AG060153 awarded to PMC).

APSIPE.228

Disentangling the cardiodynamic phase of pulmonary oxygen uptake at the onset of exercise

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We examined how different breathing patterns can modulate venous return and alveolar gas transfer during exercise transients in humans. Ten healthy men transitioned from rest to moderate cycling while breathing spontaneously (SP) or with voluntary increases in abdominal (AB) or intrathoracic (RC) pressure swings. We used double body plethysmography to determine blood displacements between the trunk and the extremities (Vbs).

From continuous signals of airflow and O₂ fraction, we calculated breath-by-breath oxygen uptake at the mouth and used optoelectronic plethysmography to correct for lung O₂ stores changes and calculate alveolar O₂ transfer (V'O_{2,A}). Oesophageal (Poes) and gastric (Pga) pressure were monitored using balloon-tipped catheters. Cardiac stroke volume was measured using impedance cardiography. During the cardiodynamic phase (F1) of V'O₂-on kinetics (20 s following exercise onset), AB and RC increased total alveolar oxygen transfer compared to SP (227±32, p=0.019 vs 235±27, p=0.001 vs 206±20 ml). Poes and Pga swings increased with AB (by 24.4±9.6 cm H₂O, p<0.001) and RC (by 14.5±5.7 cm H₂O, p<0.001), respectively. AB yielded a greater increase in intra-breath Vbs compared with RC and SP (+0.30±0.14 vs +0.16±0.11, p<0.001 vs +0.10±0.05 ml, p=0.006) and increased the sum of stroke volumes compared to SP (4.47±1.28 vs 3.89±0.96 L, p=0.053), while RC produced significant central blood translocation from the extremities compared with SP (by 493±311 ml, p<0.001). Our findings indicate that combining exercise onset with AB or RC increases venous return momentarily increasing mass oxygen transport above metabolic consumption limiting the oxygen deficit incurred.

APSIPE.229

Senescent cells accumulate in skeletal muscle of aged mice following mechanical overload

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Resistance exercise training is not as effective for promoting muscle hypertrophy in aged muscle as it is in young; however, it is unknown if cellular senescence is a contributing factor. **PURPOSE:** To determine if senescent cells are elevated in aged mice following 14 days of mechanical overload. **METHODS:** All experiments were conducted in conformance with guidelines for experimental procedures in the APS Guiding Principles in the Care and Use of Animals. Adult (5 month) and old (24 month) male C57BL/6J mice underwent sham or synergist ablation surgery to induce mechanical overload of the plantaris muscle. Following surgery, the plantaris muscle underwent immunohistochemical analysis for mean and fiber type-specific muscle fiber cross-sectional area (CSA), fiber-type distribution, and senescence-associated β -galactosidase (SA β -gal) to quantify senescent cell abundance. **RESULTS:** Relative to sham controls, normalized wet weight of the plantaris muscles was greater in young than in old mice following 14 days of mechanical overload. Similarly, muscle fiber CSA was only higher in young mice; however, type 2a fibers were significantly larger in both young and old mice, while type 2x+2b fibers were only bigger in young mice. There were more type 2a fibers following mechanical overload, although there was no difference between young and old mice. Aging did not affect the abundance of SA β -gal+ senescent cells in plantaris muscle, as they were extremely rare in both young and old sham mice. Senescent cell abundance was higher than sham following 7 days of overload regardless of age, but SA β -gal+ cells

continued to accumulate only in aged muscle so that by 14 days of overload, there were significantly more SA β -gal+ senescent cells in aged muscle compared to young. **CONCLUSION:** Higher abundance of senescent cells is associated with blunted muscle hypertrophy in aged muscle in response to overload, potentially through upregulation of the senescence-associated secretory phenotype. A future study will determine if senolytic treatment can restore hypertrophic growth of type 2x+2b fibers in old mice. Supported by NIH grant AG049806 to JJM and CAP.

APSIPE.230

AICAR-induced skeletal muscle glucose uptake requires the acetyltransferases p300 and CBP

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While exercise has long been known to robustly increase glucose uptake by contracting skeletal muscle, the molecular signals underlying this effect remain to be fully elucidated. Using acetyl CoA as a substrate, acetyltransferases add acetyl groups to the lysine residues of a protein, and in turn, this acetylation can alter protein function and activity. By extension, given the central importance of acetyl CoA to cellular metabolism and signaling, it is possible that protein acetylation contributes to contraction-stimulated glucose uptake, although to our knowledge, this has not been studied. Addressing this, the objective of this study was to determine the importance of the acetyltransferases E1A binding protein p300 (p300) and cAMP response element-binding protein binding protein (CBP) to contraction-stimulated glucose uptake using the AMP analog, AICAR (5-Aminoimidazole-4-carboxamide ribonucleotide); we focused on p300 and CBP because of recent work demonstrating their critical importance to skeletal muscle contractile function. Mice with tamoxifen-inducible, skeletal muscle-specific knockout of Ep300 (p300) and/or Crebbp (CBP) were generated using Cre-LoxP methodology; the specific mice generated were mice with knockout of both p300 and CBP (i.e. mPCKO), and mice with only one allele of p300 (i.e. mPZ-CKO) or CBP (i.e. mCZ-PKO). At 13-15 weeks of age, mice were orally gavaged with tamoxifen, including wildtype/littermate controls (WT). On day 3 (D3), 4 (D4) or 5 (D5) after initiating tamoxifen dosing we assessed basal and AICAR-stimulated (2mM for 50 min) glucose uptake in the soleus and extensor digitorum longus (EDL) using an ex vivo 2-deoxyglucose uptake (2DGU) approach. At D3, AICAR-stimulated 2DGU was reduced ~55% in mPCKO vs. WT mice in both soleus and EDL, and by D4 was it was completely blocked, such that it was not different from Basal 2DGU. Remarkably, this severe impairment in 2DGU in mPCKO mice was rescued in D3 mPZ-CKO and mCZ-PKO mice. Taken together, these data demonstrate that p300/CBP are required for AICAR-induced skeletal muscle glucose uptake. Mechanistically, it is likely that this effect is due, at least in part, to the large-scale transcriptional uncoupling and ~50% decrease in GLUT4 abundance that

has previously been demonstrated in skeletal muscle from mPCKO mice.

APSIPE.231

mitoAMPK Activation in Response to Exercise and Metformin in Skeletal Muscle

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Endurance exercise is known to induce energetic stress and mitochondrial remodeling with profound metabolic improvement against type 2 diabetes. Metformin, a reversible inhibitor of mitochondrial respiratory chain complex 1, has been used for decades as an antidiabetic drug to promote insulin sensitivity and reduce body weight gain. The underlying mechanism(s) of endurance exercise and metformin treatment remain to be fully elucidated. We hypothesize that endurance exercise and metformin both activate mitochondria-associated AMPK (mitoAMPK) as an important step in promoting metabolic adaptations. To address this hypothesis, we subjected mouse skeletal muscle to electrical stimulation via the sciatic nerve at 100 hz for 30 min or treadmill running for 90 min. Immediately after exercise, mitochondrial and cytosolic fractions were isolated from the gastrocnemius for western blotting. AMPK phosphorylation at threonine 172 (pAMPK T172) was elevated in all three fractions suggesting that AMPK, including mitoAMPK, is activated by exercise. To ascertain the effects of metformin, metformin was injected daily (i.p.) for three days, and skeletal muscles were harvested 1 hour after the last injection. mitoAMPK phosphorylation at T172 was increased ~2-fold in the gastrocnemius in metformin-treated mice with no change in cytosolic pAMPK T172, suggesting that metformin selectively activated mitoAMPK. Together, we demonstrate that mitoAMPK is activated in response to exercise or metformin treatment, suggesting the importance of this step in mediating the antidiabetic effects of exercise and metformin.

APSIPE.232

Impact of Regular Aerobic Exercise on Femoral Artery Hemodynamic Profile in Middle-aged Men

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Peripheral conduit arteries often present with a bidirectional antegrade and retrograde blood flow pattern across the cardiac cycle. Antegrade and retrograde flow are considered to have antiatherogenic and proatherogenic effects, respectively. A previous cross-

sectional study demonstrated that endurance-trained older adults exhibited a significantly lower retrograde shear rate in the common femoral artery than that in sedentary peers. However, the impact of regular aerobic exercise on femoral arterial hemodynamics in middle-aged men has remained sparse and inconclusive. To address this question comprehensively, we conducted the following two studies: In the cross-sectional study, sedentary and 35 physically-active men (18 – 64 yrs) were studied. Men engaging ≥ 30 min of moderate-intensity physical activity, more than 2 times per week, were defined as physically-active. Subjects were age-wise divided into young (< 39 yrs) and middle-aged (≥ 40 yrs) groups. In the interventional study, 15 sedentary men (52 ± 9 yrs) underwent 16 weeks of aerobic exercise training (60-75% heart rate reserve, 30-45 min/day, and 4-5 days/week). Hemodynamic profile of the common femoral artery was evaluated by the Doppler ultrasound device. The cross-sectional study demonstrated higher antegrade shear rate in young physically active (52.4 ± 17.6 s⁻¹) than in young sedentary men (37.9 ± 14.3 s⁻¹, $P < 0.01$), whereas there was no significant difference between middle-aged physically-active (42.5 ± 10.5 s⁻¹) and sedentary men (50.2 ± 14.8 s⁻¹). Significant effects of age and physical activity level were observed in retrograde shear rate. In the intervention study, antegrade shear rate was significantly decreased after the exercise intervention (from 51.2 ± 20.7 to 37.7 ± 11.4 s⁻¹, $P < 0.001$), but retrograde shear rate was not changed (from -13.5 ± 4.8 to -12.3 ± 6.3 s⁻¹, $P = 0.289$). These results suggest that regular physical activity is associated with the reduced femoral artery antegrade shear rate in middle-aged men.

APSIPE.233

Accuracy of Predictive Equations for Maximal Oxygen Consumption in Healthy Native and Acclimatized Young Adults Living at Moderate Altitude

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INTRODUCTION: Maximal oxygen consumption (VO₂ MAX) is considered the gold standard for evaluation of cardiorespiratory fitness. Predictive equations for VO₂ MAX are used as a reference for performance during cardiopulmonary exercise testing (CPET) and for exercise prescription in settings in which direct VO₂ MAX measurement is not feasible. About 8 million people live in Bogota at moderate altitude (2600 m above sea level) and are exposed to 28% lower inspired oxygen pressure that affects oxygen delivery and utilization systems. VO₂ MAX and maximal work rate (WR MAX) during CPET have been reported to be about 10% lower in moderate hypobaric hypoxia. Multiple predictive equations have been developed for low-altitude populations; the accuracy of these equations is currently not known in subjects permanently exposed to moderate altitude. OBJECTIVE: To determine the accuracy of predictive equations for VO₂ MAX in healthy young residents at moderate altitude.

METHODS: 405 healthy young adults (209 men, 196 women age 21.80 ± 2.19 yr, body mass index 21.77 ± 1.76 kg/m²) living at moderate altitude in Bogotá (2600 m) completed an incremental (ramp) maximal cardiopulmonary exercise testing in an electronically-braked cycle ergometer with continuous breath-by-breath gas-analysis, 12-lead electrocardiography recording and earlobe capillary lactic acid concentration was sampled every 2.5 min. A systematic literature search in Medline® and Embase® databases identified 13 studies reporting 42 predictive equations for VO₂ MAX. All studies were conducted at low altitudes. Subjects were grouped by sex and altitude exposure: those born and raised above 2500 m (natives) and those born and raised (first 14 years of age) below 500 m but living in altitude (acclimatized). Distribution was assessed by Shapiro-Wilk test; direct and estimated VO₂ MAX values were compared using Wilcoxon Rank Sum test. Correlation between direct and estimated VO₂ MAX were calculated by Pearson's product-moment correlations. **RESULTS:** 42 equations for VO₂ MAX were selected: 16 for men, 16 for women and 10 for both sexes. In men, out of 26 equations, 22 resulted in predicted VO₂ MAX values significantly different from direct values for both native and acclimatized males ($p < 0.05$); for the remaining four equations, two showed differences only for native and two only for acclimatized men. In women, out of 26 equations, 17 showed significant differences for both native and acclimatized women, from the remaining nine equations, four showed differences only for native and five only for acclimatized women. Pearson product-moment correlations showed only three equations with strong correlation between direct and predicted values for both men and women ($r > 0.6$, $p < 0.05$). These equations had in common the inclusion of workload as a predictive variable for VO₂ MAX. In men, all three equations significantly overestimated VO₂ MAX in both native acclimatized groups, while in women two equations overestimated and one underestimated VO₂ MAX for both groups. **CONCLUSIONS:** Available predictive equations do not reliably predict VO₂ MAX in a healthy young population residing in moderate altitude. Only equations that include WR MAX had good correlation with direct VO₂max values, although they consistently demonstrate differences with direct values. These results may be due to lower VO₂ MAX attained at altitude; the role of anthropometric and lifestyle differences between high and low altitude populations should also be considered. Supported by COLCIENCIAS Grant number 120356934972, 713-2013.

APSIPE.235

The role of the circadian clock in exercising tendons

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Collagen is the most abundant secreted protein in vertebrates and also one of the most long lived with a turnover half-life exceeding hundreds of years, especially in tendons 1-4. The permanency of collagen in tendon contrasts with continued collagen synthesis by tendon

fibroblasts throughout adult life and with transcriptional/translational homeostatic mechanisms that replace damaged proteins with new copies. Here, we show circadian clock regulation of secretory pathway-resident proteins that navigate procollagen-I from its site of synthesis in the endoplasmic reticulum to secretion at the plasma membrane 5. The result is nocturnal procollagen-I synthesis and daytime collagen fibril assembly, in mice. Rhythmic degradation maintains collagen homeostasis. This circadian cycle of collagen synthesis and degradation affects a pool of newly synthesised collagen-I whilst maintaining the permanent collagen network. Disabling the circadian clock causes collagen accumulation. Thus, the circadian clock controls a sacrificial pool of collagen to maintain normal tissue function. Furthermore, we have developed a new collagen reporter system, DyProQ 6, which uses CRISPR-Cas9 to introduce nluc (encoding NanoLuciferase, NLuc) into the Col1a2 locus. This new method is upwards of 1000x more sensitive than hydroxyproline determination, maintains endogenous regulatory mechanisms, and can be used in live cell imaging and multiwell formats. We demonstrated circadian clock regulation of collagen homeostasis, imaged procollagen-containing transport vesicles in living cells, and screened a library of 1,971 FDA-approved compounds in which we identified 10 candidates for repurposing in the treatment of tendon pathology, fibrosis, and related degenerative diseases. The research was generously funded by Wellcome in the form of a Centre award (203128/Z/16/Z), a Senior Investigator Award (110126/Z/15/Z) and a 4-year PhD studentship (210062/Z/17/Z). For correspondence, karl.kadler@manchester.ac.uk 1 Verzijl, N. et al. Effect of collagen turnover on the accumulation of advanced glycation end products. *J Biol Chem* 275, 39027-39031, doi:10.1074/jbc.M006700200 (2000). 2 Thorpe, C. T. et al. Aspartic acid racemization and collagen degradation markers reveal an accumulation of damage in tendon collagen that is enhanced with aging. *J Biol Chem* 285, 15674-15681, doi:10.1074/jbc.M109.077503 (2010). 3 Heinemeier, K. M., Schjerling, P., Heinemeier, J., Magnusson, S. P. & Kjaer, M. Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. *FASEB J* 27, 2074-2079, doi:10.1096/fj.12-225599 (2013). 4 Sivan, S. S. et al. Collagen turnover in normal and degenerate human intervertebral discs as determined by the racemization of aspartic acid. *J Biol Chem* 283, 8796-8801, doi:10.1074/jbc.M709885200 (2008). 5 Chang, J. et al. Circadian control of the secretory pathway maintains collagen homeostasis. *Nat Cell Biol* 22, 74-86, doi:10.1038/s41556-019-0441-z (2020). 6 Calverley, B. C., Kadler, K. E. & Pickard, A. Dynamic protein quantitation (DyProQ) of procollagen-I by CRISPR-Cas9 NanoLuciferase tagging. *bioRxiv*, doi:https://doi.org/10.1101/2020.05.17.099119 (2020).

APSIPE.236

Scientific Contributions of John O. Holloszy, M.D.

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This Symposium is in recognition of John O. Holloszy, M.D., (1933-2018), a pioneer in the field of exercise and the father of Exercise Biochemistry. He was one of the first to recognize that a sedentary lifestyle and lack of exercise contributed to many of the age-related metabolic changes that lead to type 2 diabetes and cardiovascular disease. Since the early 1960's his research has shaped our understanding of how exercise modulates the body's molecular, cellular, and physiological systems, and provides a vital tool to counter chronic diseases. His landmark studies on muscle contraction and glucose transport, the impact of training on glucose and insulin metabolism in healthy and diseased humans, his understanding of how caloric restriction could impact longevity, and his studies showing that older healthy and diseased populations could still elicit beneficial cardiovascular adaptations with exercise training, have formed the foundation of our discipline. Holloszy's outstanding productivity was made possible by his mentoring of ~100 postdoctoral fellows and decades of continuous NIH grant funding. This symposium brings together former colleagues, collaborators, former trainees, and next generation scientists who have benefited from his discoveries, and will serve to highlight the outstanding legacy of a man who was without doubt, one of the world's most preeminent exercise biochemists/physiologists.

APSIPE.237

Alterations in Metabolic and Cardiovascular Risk Factors during Critical Training in Wildland Firefighters

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Introduction. Wildland firefighters (WLFFs) are confronted with numerous physical and mental stressors. The pre-fire season includes an intense 2-week critical training (CT) period, a preparatory phase of multiple activities that can result in metabolic disturbances. The purpose of this study was to identify physiologic changes in resting metabolic, inflammatory, and oxidative stress biomarkers during two weeks of CT in WLFFs. **Methods.** Eighteen male and three female participants were recruited from a Type I interagency hotshot crew and monitored over their 2-week CT. Fitness was assessed via the Bureau of Land Management Fitness Challenge (push-ups, pull-ups, sit-ups, 1.5-mile run). Participants were asked to fast and abstain from caffeine prior to blood draws collected on days 1, 4, 8, and 11. Blood was analyzed for changes in resting values of a lipid and glucose panel, C-reactive protein (CRP) and oxidative stress markers 8-isoprostane (8ISO), 3-nitrotyrosine (3NT), lipid hydroperoxides (LOOH), and

protein carbonyls (PC). **Results.** The high physical demands of CT resulted in a significant reduction of total cholesterol (172.6 ± 11.4 to 153.9 ± 8.1 mg-dL⁻¹, $p=0.011$), glucose (93.1 ± 2.2 to 88.4 ± 2.2 mg-dL⁻¹, $p=0.030$), and hemoglobin A1c (5.2 ± 0.1 to 5.1 ± 0.1 %, $p=0.003$) from day 1 to 11. Significance was not present for CRP ($p=0.32$) or PC ($p=0.73$). A main effect for time was observed in 8ISO (d1: 15.5 ± 1.3 , d11: 18.0 ± 1.2 pg-mL⁻¹, $p<0.001$), 3NT (d1: 2.9 ± 0.3 , d8: 2.9 ± 0.2 mg-mL⁻¹, $p=0.033$), and LOOH (d1: 2.2 ± 0.4 , d11: 4.0 ± 0.6 mM, $p=0.001$).

Conclusions. The observed alterations to baseline values of metabolic and oxidative stress markers suggest an acute, high-intensity physical stress during WLFF CT to which the crew rapidly adapted. Funded by the US Forest Service 16-CR-11138200-005.

APSIPE.238

Muscle Soreness and Damage During Wildland Firefighter Critical Training

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Introduction. Wildland firefighters (WLFFs) undergo critical training (CT) immediately before the firefighting season. The intensive nature of preparatory CT exercise regimen could lead to muscle damage, as previously reported cases of rhabdomyolysis in WLFF have been documented. The objective of this study was to document the effects of CT on physiologic markers of muscle damage and acute overreaching. **Methods.** Male ($n=18$) and female ($n=3$) Type I Interagency Hotshot WLFFs were studied during an 11-day CT period. Upper- (US) and lower-body (LS) muscle soreness and body weight (BW) were collected daily. Blood was collected on days 1, 4, 8, and 11 to measure creatine kinase (CK), lactate dehydrogenase (LDH), cortisol, and testosterone. Skinfolds were taken on days 1 and 11 to estimate body fat (BF) and lean body weight (LBW). The Bureau of Land Management fitness challenge defined fitness. **Results.** No differences in BW were observed days 1-11. BF significantly decreased (15.3 ± 1.4 % vs. 14.1 ± 1.3 %, $p=0.002$), while LBM significantly increased (67.3 ± 2.3 kg vs. 68.8 ± 2.2 kg, $p=0.002$). US and LS showed main effects of time, peaking on day 3 (US: 3.8 ± 0.5 cm, $p<0.001$; LS: 4.3 ± 0.3 cm, $p<0.001$). CK showed a significant effect of time, peaking on day 4 (73.4 ± 14.4 U-L⁻¹ vs. 132.8 ± 15.4 U-L⁻¹, $p=0.001$). LDH showed a significant effect of time (159.4 ± 5.5 IU-L⁻¹ vs. 164.4 ± 6.9 IU-L⁻¹, $p=0.04$). There was a significant increase in cortisol on day 8 (48.0 ± 4.6 ng-mL⁻¹ vs. 61.9 ± 2.9 ng-mL⁻¹, $p=0.036$), while no changes occurred in testosterone. The testosterone/cortisol ratio (T/C) decreased on day 8 (0.31 ± 0.05 vs. 0.19 ± 0.02 , $p=0.014$). Fitness and off-season training time correlated with ameliorations in these markers. **Conclusions.** These data suggest that WLFFs undergo significant physiological stressors resulting in muscle soreness, damage, and possible overreaching during CT. Fitness and preparedness appear to be protective during CT. Careful preparation and monitoring of the training stimulus is key to

avoid clinical ramifications. Funded by the USFS 16-CR-11138200-005

APSIPE.239

Blood Oxidative Stress Responses to Exercise Following 16-sessions of Heat Acclimation

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INTRODUCTION: Environmental temperature can impact exercise-induced blood oxidative stress; however, the effects of heat acclimation on this response have not been fully elucidated. The purpose of the study was to investigate the effects of hot (32°C) and neutral (7°C) environmental temperatures on post-exercise blood oxidative stress responses following 16 temperature acclimation sessions. **METHODS:** Untrained participants (N = 15, age, VO₂peak) completed 16 temperature acclimation sessions of a cycling bout at an intensity perceived as “hard” in either a warm (32°C) or neutral (7°C) environment. Pre- and post- acclimation exercise tolerance trials were conducted, which involved cycling at 50% W_{peak} for one-hour. Blood sampling occurred before (PRE), immediately after (POST), two-hours (2-HR), and four-hours (4-HR) after the exercise tolerance trials. Blood samples were analyzed for oxidative stress markers including lipid hydroperoxides (LOOH), 8-isoprostanes (8-ISO), protein carbonyls (PC), 3-nitrotyrosine (3-NT), ferric-reducing ability of plasma (FRAP), and Trolox-equivalent antioxidant capacity (TEAC). **RESULTS:** Exercise-dependent increases were observed in PC (p = 0.043), 3-nitrotyrosine (p < 0.001), lipid hydroperoxides (p < 0.001), and TEAC (p = 0.048). Significant time x temperature interactions were observed for FRAP (p = 0.021). FRAP was significantly lower at POST (p = 0.12) and 4-HR (p = 0.035) after 16 sessions in the neutral temperature. **CONCLUSION:** Exercise-induced elevations in FRAP were lower after 16 training sessions, suggesting an exercise training effect. Other blood oxidative stress responses were unaltered by 16 sessions of acclimation in a hot environment.

APSIPE.240

Integrative multi-omic analyses in MoTrPAC

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Exercise is a powerful physiological stimulus that induces a multitude of effects across different organs, and if performed regularly has many beneficial effects on health, including improved physical capacity and reduced risk for premature mortality. The NIH Common Fund Program

Molecular Transducers of Physical Activity Consortium (MoTrPAC) aims to identify the molecular signals that drive exercise adaptation across different organs, signals that ultimately improve health and prevent disease. The preclinical phase of MoTrPAC consists of 1. an acute endurance exercise bout in rats with biological sampling of 18 tissues per sex at 7 time points after exercise, and 2. endurance training for up to 8 weeks with biological sampling after 1, 2, 4 or 8 weeks of training. Chemical Analysis Sites in MoTrPAC have then generated and analyzed multiple high-throughput -omic data types from the preclinical samples. The multi-omic data covers different sequencing-based platforms (RNAseq, Reduced Representation Bisulfite Sequencing and ATAC-seq), proteomics (global-, phospho- and acetyl-proteomics), targeted and untargeted metabolomics, and lipidomics. The data is subsequently collected at the Bioinformatics Center of MoTrPAC, where it is processed and analyzed through consistent bioinformatic and analytic pipelines per our standard operating procedures, to improve reproducibility, interpretability and ease in data harmonization across data-generating sites. After processing and normalization, the data is being analyzed for differential expression, pathway enrichment analyses, correlation with clinical variables (e.g. sex, age and changes in VO₂max) and eventually multi-omic integration with the goal to build a molecular map of the response to physical activity. This is a consortium-wide effort that involves all components of MoTrPAC. All the data and results are shared with the scientific community through the MoTrPAC Data Hub (<https://motrpac-data.org/>).

APSIPE.241

Building and Expanding Your Social Media Footprint

Stacy Brooks¹

¹Communications and Social Media, American Physiological Society

APS Director of Communications and Social Media, Stacy Brooks, will lead this session on social media science communications. Topics will include setting objectives and goals for your social media feeds, the basics of social media analytics, when to use a paid social media strategy and tips for building a professional social media presence that won't come back to haunt you down the road.

APSIPE.242

Testosterone, Females and Athletic Response – Current Research and Controversy

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There is increasing evidence that androgens are beneficial for athletic performance in women. In elite female athletes, the prevalence of polycystic ovarian syndrome (PCOS) is high. There is also more common with rare conditions of severe hyperandrogenism, i.e. disorders in sex development (DSD) such as 46,XY DSD. While women with PCOS can have a slightly higher testosterone level, women

with 46,XY DSD have testosterone levels in the male range, which is 10 to 20 times higher than the normal female range. It could be considered unfair to allow female athletes with endogenous testosterone in the male range to compete against women with normal female androgen levels. This led the International Association of Athletics Federation (IAAF) and the International Olympic Committee (IOC) to establish regulations concerning hyperandrogenism in female athletes. However, it is still a controversy about who should be allowed to compete within the female category. To study if, and to what degree testosterone affects physical performance in women, we performed a randomized, double-blind controlled study. We found that a moderately increased testosterone concentration enhances physical performance in women, primarily aerobic running time as well as lean body mass. Furthermore, testosterone increases the oxidative capacity and microvasculature in lean muscle mass, supporting and effect of testosterone on the muscle level. This is the first study showing evidence for a causal performance-enhancing effect of testosterone in women.