BACKGROUND: Resistance exercise is a common mode to increase muscle strength. However, severe resistance exercise leads to rapid and enduring muscle fatiguing which functional mechanisms have not been fully resolved, yet. One candidate involved in the regulation of myocellular fatigue is calcium (Ca²⁺) which is vital for electromechanical coupling of myofilaments. Rapid Ca²⁺ modulation is mediated by ryanodine receptor-1 (RyR1), which, when phosphorylated at serine²⁰⁴⁴ (pRyR1Ser²⁰⁴⁴) results in leaky ryanodine calcium channels, impaired calcium homeostasis and thus decreased abilities to sustain the molecular basis of electromechanical coupling. However, little is known about the time course and magnitude of acute exercise on pRyR1Ser²⁰⁴⁴ phosphorylation in human skeletal muscle.

PURPOSE: It was aimed to investigate the effect of acute resistance exercise (EX) on pRyR1Ser²⁰⁴⁴ phosphorylation (phosph.) in human type I and II myofibers.

METHODS: Six healthy male subjects (age: 23 ± 2 years, height: 185 ± 7 cm, and weight: 82 ± 5 kg) performed 3 sets with 8 repetitions of maximum eccentric knee extensions. Muscle biopsies were taken PRE exercise, 15 min, 30 min, and 60 min post EX. Immunohistochemistry, western blots and confocal microscopy were used to determine pRyR1Ser²⁰⁴⁴ and pAMPKThr¹⁷² phosph. levels at the respective time points.

RESULTS: pRyR1Ser²⁰⁴⁴ phosph. increased rapidly at 15 min in both type I and II myofibers (p<0.01) and further showing a sustained phosph. pattern up to 30 min (p<0.01) post EX. Compared to baseline levels, type I fibers showed higher increases in levels of phosph. in RyR1 up to 60 min post EX (p<0.05) than type II myofibers. pAMPKThr¹⁷² phosph. showed significant increases 15 to 30 min post EX (p<0.01) in type I and II myofibers with a higher increase in phosph. levels in Type I myofibers.

CONCLUSION: Severe resistance exercise contributes to temporarily increased phosph. of RyR1 and AMPK due to active recruitment of myofibers. This result supports the hypothesis that RyR1 can be rapidly phosphorylated by resistance exercise and very likely contributes to muscle fatiguing by a decline in calcium handling properties. Enhanced phosph. of RyR1 is sustained up to 60 min post EX in both myofibers what may contribute up to this time point to impaired skeletal muscle contraction abilities.

1495 Board #276 MAY 30 11:00 AM - 12:30 PM
Ryr-1 Phosphorylation Responds Differently Between Concentric And Eccentric Workload In Rat Skeletal Muscle
Kristina Braun, Sebastian Gehlert, Wilhelm Bloch, Lena Willkorn, Frank Suhr. German Sport University Cologne, Cologne, Germany. (No relationships reported)

BACKGROUND: Calcium (Ca²⁺) handling in skeletal muscle regulates to diverse pathologies and performance capacities as Ca²⁺ is involved in muscle contraction machinery. Ca²⁺ homeostasis is mainly regulated by transmembrane channel complexes of sarcoplasmic reticule, called ryanodine receptor-1 (RyR1). RyR1 associates to many regulatory proteins, of which calstabin-1 plays a key role. It was described that long-lasting exercise models hyperphosphorylate RyR1 at Ser²⁸⁴³ and thus induce a leaky channel leading to impaired muscle function by dissociating calstabin-1 from RyR1. However, it was described that phosphorylation of RyR1 (pRyR1) is unknown wherefore short-term exercise stimuli and their effects on pRyR1 were investigated.

PURPOSE: It was aimed to investigate whether concentric and eccentric exercise offers a different impact on ryanodine receptor phosphorylation in loaded rat skeletal muscle.

METHODS: 32 Sprague-Dawley rats were assigned to one of following groups: age-matched control (AC, sedentary), concentric (Conc, 0° decline) or eccentric (Ecc, -20° decline) exercise (each exercise lasted 15 min). pRyR1 was investigated by immunohistochemistry in both medial gastrocnemius and vastus lateralis.

RESULTS: In lateralis muscle 15 min of either concentric or eccentric led to markedly increased levels of pRyR1 compared to AC. Furthermore, concentric led to significantly higher amounts of pRyR1 compared to eccentric exercise. In gastrocnemius muscle a similar pattern was observed. However, there was no difference between concentric and eccentric stimuli. Discussion: The present results demonstrate that RyR1 is hyperphosphorylated very fast, which is an additional finding compared to data from the literature. Importantly, different muscle types react in a comparable manner. Interestingly, concentric exercise seems to exert a more severe effect on pRyR1 hyperphosphorylation, at least in lateralis. These findings give new insights into RyR1 regulation by exercise.
**Methods**

Muscle biopsies were obtained in fourteen young (19–28 yrs) and older (65–76 yrs) men (7 young, 4 older) and women (8 young, 4 older). Gene expression profiling was performed using the Affymetrix Human Genome U133 Plus 2 chip. Differential gene expression between older and young muscle was analyzed, separately for men and women, using an intensity-based Bayesian moderated t-test (IBMT). A logistic regression-based method (LRpath) was used to test for significant (FDR<0.01) enrichment of biological functions based on Gene Ontology (GO) terms and KEGG pathways. LRpath analysis in combination with IBMT revealed different patterns of transcriptional changes with aging in men and women. A much greater number of GO and KEGG were associated with age-related muscle insulin resistance (e.g., AMPK, SOCS3, AKT2, PPARC) and converge at the Z-disc. Since evidence suggests that loading forces in the muscle may be transmitted by structural support proteins that integrate mechanical stress at the Z-disc, we also examined several signaling proteins that reside in this region (STARS, atrogin-1, and calcineurin via its modulator MCIP1) and can in-turn regulate the expression of sarcomeric genes.

**Results**

Two groups of healthy inactive human subjects participated in unilateral lower limb suspension (ULLS; male N=5; female N=4) for 10 days alone or with a combination of aerobic and resistance exercise training (ULLS+T; male N=5; female N=5). Soleus biopsies were obtained before and after ULLS; ULLS+T biopsies were obtained ~24 hrs after the last training session. RT-PCR was used to quantify pre-mRNA and mRNA levels of select genes (arbitrary units/mg).

**Conclusions**

While changes in transcription of the Z-disc associated structural proteins nebulin and titin following 10d unloading was not statistically significant, there was significant upregulation with unloading + training stimuli. The unfavorable unloading-induced signaling response was ameliorated when combined with training as suggested by differential mRNA levels of mechanosensitive Z-disc factors that can promote atrophy (atrogin) and fiber-type shifts (calcineurin/MCIP1). These data suggest that exercise countermeasures to short-term unloading of the loading-sensitive slow soleus muscle can promote favorable transcriptional responses of proteins associated with the stress-sensitive Z-disc. (Supported by NSBRI-NASA NCC 9-58)

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**1498 Board #279 MAY 30 11:00 AM - 12:30 PM**

**Short-Term Unloading and Exercise Effects on Mechanical Stress-Sensitive Structural and Sensor Proteins in Human Soleus**


**Methods**

Loading forces are critical to maintaining homeostasis in the muscle cell. Perturbations, such as unloading/disuse, upset the normal gene expression of structural and signaling proteins. Exercise countermeasures can offset these alterations.

**Purpose**

To examine gene expression of several large sarcomeric proteins that provide structural support to the myofilament (titin, nebulin and α-actin) and converge at the Z-disc. Since evidence suggests that loading forces in the muscle may be transmitted by structural support proteins that integrate mechanical stress at the Z-disc, we also examined several signaling proteins that reside in this region (STARS, atrogin-1, and calcineurin via its modulator MCIP1) and can in-turn regulate the expression of sarcomeric genes.

**Methods**

Two groups of healthy inactive human subjects participated in unilateral lower limb suspension (ULLS; male N=5; female N=4) for 10 days alone or with a combination of aerobic and resistance exercise training (ULLS+T; male N=5; female N=5). Soleus biopsies were obtained before and after ULLS; ULLS+T biopsies were obtained ~24 hrs after the last training session. RT-PCR was used to quantify pre-mRNA and mRNA levels of select genes (arbitrary units/mg).

**Results**

Two groups of healthy inactive human subjects participated in unilateral lower limb suspension (ULLS; male N=5; female N=4) for 10 days alone or with a combination of aerobic and resistance exercise training (ULLS+T; male N=5; female N=5). Soleus biopsies were obtained before and after ULLS; ULLS+T biopsies were obtained ~24 hrs after the last training session. RT-PCR was used to quantify pre-mRNA and mRNA levels of select genes (arbitrary units/mg).

**Conclusions**

While changes in transcription of the Z-disc associated structural proteins nebulin and titin following 10d unloading was not statistically significant, there was significant upregulation with unloading + training stimuli. The unfavorable unloading-induced signaling response was ameliorated when combined with training as suggested by differential mRNA levels of mechanosensitive Z-disc factors that can promote atrophy (atrogin) and fiber-type shifts (calcineurin/MCIP1). These data suggest that exercise countermeasures to short-term unloading of the loading-sensitive slow soleus muscle can promote favorable transcriptional responses of proteins associated with the stress-sensitive Z-disc. (Supported by NSBRI-NASA NCC 9-58)

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**1499 Board #280 MAY 30 11:00 AM - 12:30 PM**

**Sumo-1 Rapidly Translocates In Human Skeletal Muscle Myonuclei In Response To High Intense Resistance Exercise**

Sebastian Gehler1, Suhr Frank1, Lena Willkommi1, Franz-Josef Klunz1, Wilhelm Bloch1, 1German Sport University Cologne, Cologne, Germany, 2University of Cologne, Cologne, Germany.

**Methods**

The small ubiquitin-like modifier “SUMO” regulates target protein functions via post-translational modification and regulates cellular integrity. Sumoylation is triggered in vivo by stressors like oxidative stress but also acute hypoxia. Exercise induced myocellular adaptation requires a complex network of nuclear protein shuttling as well as post-translational modification of proteins and thus may include also the SUMO system as an acute modulator of loaded skeletal muscle.

**Purpose**

It was aimed to investigate whether SUMO-1 is involved in the early response towards exercise in sarcoplasmatic and myonuclear compartments of loaded skeletal muscle myofibers.

**Methods**

6 healthy male subjects (age: 23±4 yrs; height: 180±8 cm; weight: 79±10 kg) performed 1 single set of 20 maximum eccentric and concentric isokinetic knee extensions. PRE, 15, 30, 60, 240 min and 24h after exercise (EX) muscle biopsies were taken from vastus lateralis muscle. DAB and Fluorescence staining was performed on 7 μm cross-sections of skeletal muscle. The density and sub-cellular localization of SUMO-1 in sarcoplasmatic and nuclear compartments of type I and II myofibers were determined via optical densitometry and confocal laser microscopy as well as in whole skeletal muscle lysates by western blotting.

**Results**

Sarcoplasmatic SUMO-1 density was higher in type I than in type II myofibers (p<0.05) PRE exercise, increased 15 to 30 min POST EX significantly (p<0.01) in both fiber types but returning to PRE levels within 60 min. SUMO-1 positive nuclear areas increased significantly (p<0.01) from PRE values within 15 min up to 60 min POST EX but returning below PRE levels within 24 hours. Confocal microscopy offered a predominant sub cellular localization of SUMO-1 towards the nuclear envelope at baseline and 24h post EX. Western blotting offered substantial changes in the pattern of SUMO-1 positive bands within the first hour POST EX.

**Conclusions**

The present investigation reveals time dependent changes in SUMO-1 density in nuclear and sarcoplasmatic compartments of human skeletal muscle fibers as early response towards high intense resistance exercise. This modulation offers a potential role of the SUMO system in myocellular adaptation towards acute exercise induced stress but also in re-occurring homeostasis.

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**1500 Board #281 MAY 30 11:00 AM - 12:30 PM**

**Novel Transcriptional Changes Associated with Age-related Muscle Insulin Resistance**

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**Methods**

Skeletal muscle insulin resistance is a characteristic feature of the normal aging process and plays a key role in the pathogenesis of age-related type II diabetes mellitus and Alzheimer’s disease. However the mechanisms associated with age-related muscle insulin resistance are not completely understood.

**Purpose**

Through a global view of transcriptional changes in young and older skeletal muscle, we sought to identify the molecular mechanisms underlying age-associated insulin resistance.

**Methods**

Muscle biopsies were obtained basally from young (19–28yrs) and older (65–76 yrs) men (7 young, 4 older) and women (8 young, 4 older). Gene expression profiling was performed using the Affymetrix Human Genome U133 Plus 2 chip. Differential gene expression between older and young muscle was analyzed, separately for men and women, using an intensity-based Bayesian moderated t-test (IBMT). A logistic regression-based method (LRpath) was used to test for significant (FDR<0.01) enrichment of biological functions based on Gene Ontology (GO) terms and KEGG pathways.

**Results**

LRpath analysis in combination with IBMT revealed different patterns of transcriptional changes with aging in men and women. A much greater number of GO and KEGG were found to be significantly enriched with differentially expressed genes in older women than in older men (259 vs. 57). Transcriptional changes in older women presented a coordinated up-regulation of immune activation (e.g., FABP4, LOX, C1R), extracellular matrix (ECM) remodeling (e.g., COL6A2, COL1A2), lipids storage (e.g., CD36, SCD); and down-regulation of mitochondrial biogenesis and function (e.g., PPARGC1, CS ), muscle regeneration (e.g., MYF6, AKT2). In contrast, no consistent biological themes can be inferred based on the limited number of significant GO/KEGG in older men.

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