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Rapid Ryanodine Receptor-1 Phosphorylation In Response To High Intense Resistance Exercise In Human Skeletal Muscle

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(No relationships reported)

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 (Ca^{2+}) which is vital for electromechanical coupling of myofilaments. Rapid Ca^{2+} modulation is mediated by ryanodine receptor-1 (RyR1), which, when phosporylated at serine^{2844} (pRyR1Ser^{2844}) 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 pRyR1Ser^{2844} phosphorylation in human skeletal muscle**.**

PURPOSE: It was aimed to investigate the effect of acute resistance exercise (EX) on pRyR1Ser^{2844} 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 pRyRSer^{2844} and pAMPK^{Thr172} phosph. levels at the respective time points.

RESULTS: pRyR1Ser^{2844} 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 phosph. levels of RyR1 up to 60 min post EX ($p<0.05$) than type II myofibers. pAMPK^{Thr172} 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.

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Ryr-1 Phosphorylation Responds Differently Between Concentric And Eccentric Workload In Rat Skeletal Muscle

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(No relationships reported)

BACKGROUND: Calcium (Ca^{2+}) handling in skeletal muscle regulates to diverse pathologies and performance capacities as Ca^{2+} is involved in muscle contraction machinery. Ca^{2+} 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, time patterns of RyR1 hyperphosphorylation (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 hyperphosporylated 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 RyR1 hyperphosphorylation, at least in lateralis. These findings give new insights into RyR1 regulation by exercise.

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Igf-1 Downstream Signaling Response To Protein Rich Supplementation During Hindlimb Suspension

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(No relationships reported)

It was known that anabolic signals were suppressed and catabolic signals were activated during prolonged muscle disuse, but the exact underlying molecular mechanisms are unclear. High protein supplementation has been recognized to increase the rate of muscle protein synthesis and activate anabolic signaling pathways (ex. IGF-1) and was employed in this study.

PURPOSE: To examine whether high protein supplementation would elevate phosphorylation of Akt and downstream pathways and maintain muscle function during hindlimb suspension (HS).

METHODS: Lower limbs of female rats were subjected to be unloaded by tail suspension for 28 days. During this period, one group (HS-PRO) was provided high protein supplementation (5ml/kg body weight), but others (HS) was received water via gavage twice per day, and ambulatory rats were served as control group (CON). After 28 days, contractile function of lateral gastrocnemius (LGAS) was evaluated, and both LGAS were excised and stored at -80°C. Total and phosphorylated levels of Akt, mTOR, p70S6K, and FOXO3a were measured from the sampled muscle by Western blot analysis.

RESULTS: LGAS weight was significantly decreased in HS and HS-PRO compared to that of CON at 28 days after HS, -19 and -22.5% respectively ($P<0.01$). However, in-situ peak tetanic force (Po) of HS-PRO was significantly higher than HS by 12% ($P<0.05$) but lower than CON by 25% ($P<0.01$). Phosphorylation of Akt in HS-PRO was significantly increased to CON, and pFOXO3a was also elevated in HS-PRO compared to CON and HS ($P<0.05$ and $P<0.01$ respectively). In addition, the supplementation prevented the reduction of mTOR phosphorylation during HS, -31 and -34% compared to CON and HS-PRO respectively ($P<0.05$), but there was no difference in p70S6K phosphorylation between groups. Total protein contents of all detected signals were not changed.

CONCLUSION: Although protein rich supplementation was not able to prevent the loss of muscle mass during prolonged HS, it reduced the decrease of contractile function, and elevated pAkt and pFOXO3a while maintained pmTOR .

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Influence Of Age On Leptin Induced Skeletal Muscle Signalling

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(No relationships reported)

Aging is a multifactorial process that is characterized by decreased physical activity, low fat-free mass and reduced ability to mobilize fat. Leptin is an adipocyte-derived hormone where systemic levels increases in proportion with adiposity. Chronic hyperleptinemia leads to leptin resistance, as indicated by a lower abundance of leptin receptors (OBRb), and reduced phosphorylation of STAT3 and AMPK in human skeletal muscle. The potential contribution of leptin resistance to the increase of fat mass with aging has not yet been elucidated.

PURPOSE: To determine if there is indication of increased skeletal muscle leptin resistance with aging, the basal amount of leptin receptors and the phosphorylation levels of STAT3 and AMPK, as well as an the protein amount of SOCS3 and PTP1B (the last two leptin signalling inhibitors) was assessed in healthy young and aged non-obese adults.

METHODS. Muscle biopsies were obtained in fourteen young (YG), seventeen middle-aged (MG), and eight aged (AG) healthy lean men (age: 25 ± 2, 46 ± 1, 62 ± 2 yrs, and BMI: 24.3 ± 0.5, 25.5 ± 0.3, 25.0 ± 0.7 kg/m2, respectively). Protein expression of OBRb, STAT3, AMPK, PTP1B and SOCS3 was measured by Western Blot. Plasma leptin, glucose, insulin and FFA were measured by conventional assays. Insulin sensitivity was estimated by HOMA. Body composition was measured by DXA.

RESULTS. Fat mass, FFA and leptin plasma concentrations were higher in MG and AG group than YG ($P < 0.05$), whereas HOMA values did not differ between the groups. OBRb protein abundance was similar among groups; however, Thy705STAT3 phosphorylation was lower in AG and MG compared to YG (0.29 ± 0.05; 0.32 ± 0.11 and 0.63 ± 0.20 a.u., respectively, $P < 0.05$). Surprisingly, Thr172AMPKα phosphorylation was 2-fold higher in AG than in MA and YG groups (2.94 ± 0.05; 1.32 ± 0.29 and 1.74 ± 0.17 a.u., respectively, $P < 0.05$). SOCS3 expression remained unchanged, whereas PTP1B expression was higher in the AG compared with MG and YG ($P < 0.05$).

CONCLUSION. Skeletal muscle 170 KDa OBRb protein amount is not affected by aging. However, a lower basal phosphorylation of Thy705STAT3 and a higher abundance of PTP1B suggest that leptin resistance is increased with aging in healthy lean males. It is possible that increased phosphorylation of Thr172AMPKα is a compensatory mechanism to attenuate this.<br

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

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(No relationships reported)

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: Nebulin, titin and α-actin RNA levels changed pre to post by -11%, +26% and +7% with ULLS, and by +25%*, +56%* and +16% with ULLS+T, respectively (*$p<0.05$ pre to post). STARS, atrogin1 and MCIP1 mRNA levels changed pre to post by -75%*, +77%* and -40%* with ULLS, and by -63%*, +30%* and -26%* with ULLS+T, respectively (*$p<0.05$ pre to post).

CONCLUSIONS: While changes in transcription of the Z-disc associated structural proteins nebulin and titin following10d 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 mechano-sensitive 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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Sumo-1 Rapidly Translocates In Human Skeletal Muscle Myonuclei In Response To High Intense Resistance Exercise

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(No relationships reported)

INTRODUCTION: 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 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 years; 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 7µm 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 significant ($p<0.01$) 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**.**

CONCLUSION: 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 recurring homeostasis.

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Novel Transcriptional Changes Associated with Age-related Muscle Insulin Resistance

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(No relationships reported)

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*, *COL8A2*), 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**.**