PURPOSE: To determine the effects of combined CLA and n-3 administration independently or with RT on body composition, strength, and sensorimotor function in middle aged male mice during 20 wks of a high fat diet**.**

METHODS: Nine-month old C57BL/6 male mice were randomly assigned to five groups (n=10/group): 1) normal diet (CON), 2) High fat diet (HFD), 3) HFD+RT (HFD/RT), 4) HFD+CLA/n-3 (HFD/CN), and 5) HFD+RT+CLA/n-3 (HFD/RT/CN). Progressive RT (4 sets of 3 repetitions with 1-min inter-set rest) was conducted using a ladder climbing device 3x/wk for 20 wks. The combined supplement was comprised of 1% CLA (0.5% of c9, t11 and 0.5% of t10, c12) and 1% n-3. Lean (LM) and fat mass (FM) were determined using dual energy x-ray absorptiometry while grip strength and sensorimotor function were evaluated with a strain gauge and the incline-plane test, respectively. All measures were obtained pre- and post-intervention. Data were analyzed with a group x time repeated measures ANOVA, and significance was set at $p<0.05$**.**

RESULTS: There were significant group x time interactions for LM, FM, grip strength, and sensorimotor function. FM increased in HFD (+30%) and HFD/RT (+34%), while LM decreased in HFD (-32%) and HFD/RT (-55%). No significant changes in LM or FM were observed for CON, HFD/CN, and HFD/RT/CN. Strength significantly declined in HFD (-15%) and HFD/CN (-17%) but was maintained in both CON and HFD/RT. Sensorimotor function declined significantly in HFD (-11%) with no change in CON, HFD/CN and HFD/RT. Interestingly, CLA/n-3 administration appeared to facilitate greater RT-mediated improvements in strength (+22%) and sensorimotor coordination (+17%)**.**

CONCLUSION: Body composition and functionality were negatively altered following 20 wks of high fat diet. Daily CLA/n-3 intake appears to attenuate these negative alterations while even facilitating RT-induced improvements in body composition and functionality.

Study partly supported by Sekwang Inc., Vital Pharmaceuticals, and Ocean Nutrition

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Muscle Regeneration: Impact of Mast Cells on Inflammatory Cell Recruitment and Muscle Cell Proliferation

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(No relationships reported)

Inflammatory cells are traditionally associated with pain, heat, redness and swelling. However, accumulating studies have shown that some of these cells can also contribute to tissue repair. Indeed, neutrophils and macrophages can contribute to the resolution of inflammation and to skeletal muscle regeneration via the release of cytokines and growth factors. We recently showed that tryptase, the most abundant mediator in mast cell granules, could potentially support muscle regeneration by increasing skeletal muscle cell proliferation**.**

PURPOSE: To evaluate if mast cells can stimulate skeletal muscle cell proliferation**.**

METHODS: In vitro: mast cells were isolated from peritoneal cavity of female Wistar rats. L6 muscle cells were cultured with either mast cells activated with compound 48/80 or mast cell-derived conditioned media. L6 cell number was determined with CellTiter assay 24h post-seeding. In vivo: muscle injury was induced through a bupivacain injection into the right EDL muscle. Rats received a daily intra-peritoneal injection of 5 bromo-2'deoxyuridine (BrdU) and were treated or not with the mast cell stabilizer cromolyn from 24h before injury. Rats were sacrificed 48 h post injury and immunohistochemistry analyzes were performed**.**

RESULTS: In vitro proliferation of L6 cells cultured with either activated mast cells or mast cell-conditioned media was significantly increased above control (1.30±0.08 fold and 1.24±0.04 fold), respectively. The proliferative effect of conditioned media was lost when APC-366, a tryptase inhibitor, was added. In vivo results shown that, compared to control, mast cell stabilization increased the density of proliferating cells (109,033±8,186 vs 79,678±10,833 cells/mm3), neutrophils (34,116±6,167 vs 15,636±4,201 cells/mm3), macrophages ED1 (35,426±7,517 vs 13,075±4,108 cells/mm3) and macrophages ED2 (21,671±1,676 vs 16,922±715 cells/mm3), respectively. $P<0.05$**.**

CONCLUSION: Activated mast cells can stimulate skeletal muscle cell proliferation via tryptase release in vitro. However, in vivo this effect was masked by the influence of mast cells on the recruitment of other mitogenic cells such as neutrophils and macrophages.

Supported by grants from NSERC.

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Signaling Responses In Unloaded Rat Soleus Muscle To Combination Of Heat Stress And Intermittent Reloading

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(No relationships reported)

Heat stress and intermittent reloading are known as the effective countermeasure for skeletal muscle atrophy. However, it remains unclear whether the combination of heat stress and intermittent reloading are effective to attenuate muscle atrophy.

PURPOSE: To examine the effect of the combination of heat stress and intermittent reloading on signaling responses in unloaded rat soleus muscle induced by hindlimb unloading.

METHODS: Forty male Wistar rats (10wk of age, 261.7±1.17 g) were randomly divided into four groups: control (CON, n=10), hindlimb unloading (HU, n=10), hindlimb unloading with intermittent reloading (IR, n=10), hindlimb unloading with intermittent reloading and heat stress (IR+H, n=10). The HU, IR and IR+H group were unloaded for seven days. IR and IR+H group were released from unweighting for 1h every second days. During this time, IR+H group was exposed to environmental heat stress (41.5-42°C for 30 min) in a heat chamber without anesthesia. After seven days unloading, the soleus muscle were removed and analyzed by western blotting.

RESULTS: Seven-days unloading resulted in a 31% reduction in the soleus muscle mass, but only IR+H significantly prevented the reduction (CON; 168.2±6.7, HU; 116.3±3.7, IR; 121.0±3.7, IR+H; 131.1±2.4 mg). In soluble fraction, although 80-kDa form of calpain 1 was significantly increased in IR+H group compared to CON and HU group (HU; 102, IR; 132 and IR+H; 147% of CON), the autolyzed form of IR+H group was lower than CON group. Moreover, autolyzed form of calpain 2 (HU; 267, IR; 236 and IR+H; 105% of CON) and ubiquitinated protein (HU; 164, IR; 140 and IR+H; 112% of CON) in particulate fraction was significantly increased in HU group, but IR+H group prevented the increase. There were no significant changes in the phosphorylation of Akt, mTOR, S6K1 and eIF-4E.

CONCLUSION: The combination of heat stress and intermittent reloading attenuates soleus muscle atrophy through the prevention of calpain autolysis and protein ubiquitination, but independent of Akt/mTOR pathway.

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Developmental Change in Domain Size of Endplate Nucleus in the Rat Diaphragm

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(No relationships reported)

PURPOSE: Nuclei around the neuromuscular junction play an important role for the maintenance of motor endplate morphology and function in skeletal muscle. In this study, we examined the number of endplate nuclei, 3-dimensional morphological properties of the motor endplate, and the expression level of muscle-specific kinase (MuSK) in rat muscles during the postnatal development.

METHODS: Segments of the mid-costal diaphragm (DIA) and sternocleidomastoideus (STM) muscles were removed at 1, 2, 3, 4, 11 and 25 weeks after birth (n=7 in each age group). Motor endplates were labeled with a-bungarotoxin conjugated tetramethylrhodamine. Then, several single muscle fibers with labeled endplate were isolated from the segments under fluorescent microscopy, and myonuclei, including endplate nuclei, were stained with DAPI (4', 6-diamino-2-phenylindole) to examine the space distribution of these nuclei. Thirty to fifty single fibers from each muscle segment were imaged using a laser-scanning confocal system, and the domain sizes of endplate nuclei were calculated from endplate volume and number of endplate nuclei. Furthermore, total RNA was extracted from the remains of muscle segments and the level of Pax7 mRNA expression was determined using real time RT-PCR analysis system.

RESULTS: In DIA collected during the developmental period (1 to 25 weeks after birth), the mean values of muscle fiber diameter and endplate volume were significantly increased from 24±6μm to 60±15μm and from 451 ± 304μm^3 to 1045 ± 781μm^3, respectively. The mean values of the number of endplate nuclei were increased from 3.9±1.2 at 1 week to 10.2±7.8 at 25 weeks; therefore, domain sizes of endplate nuclei were identical during the developmental period (115$\pm$77μm^3 at 1 week and 102.5$\pm$75μm^3 at 25 weeks). The level of MuSK mRNA expression was dramatically decreased by 60% during this period. There were no obvious differences in the above data between DIA and STM muscles.

CONCLUSIONS: The domain sizes of endplate nuclei were speculated to be constant during postnatal development and between muscles, indicating that a regulation mechanism of the domain size exists in the neuromuscular junction. It was also speculated that the domain size was not directly regulated by the expression of MuSK.