45. Abrupt increase in MUMS during voluntary contraction associated with a change in myostiffness

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[Aim] Amplitude of motor unit mechanical signal (MUMS) abruptly increased during voluntary prolonged contraction (PICC) below 20% MVC. We could not propose the mechanism of abrupt increase from the MU activities. We investigated the myostiffness during PICC or intermittent contraction (IICC) on the relationship with MUMS amplitude. [Methods] MUAP and MUMS were recorded from m. vastus lateralis or medialis in five volunteers using surface electrode and condenser type microphone. Target tension was set at the recruitment threshold of the object MU. Conduction time of the onset of initial negative going phase of distal MUMS from the onset of MUAP at the end-plate is defined as the myostiffness (Ctvib). [Results and discussion] Ctvib initially remains constant and then decreased during PICC, though Ctvib was constant through IICC. Myostiffness increased during PICC, there could not be seen meaningful or significant relationship with MUMS amplitude. It was the suggestive result that Ctvib was significantly longer under voluntary twitching condition compared with the train discharged condition. There would be two phases in Ctvib shortening, stiffness increase during PICC, 1st phase in the difference between recruitment and subsequent train discharge at initial phase of the tension increment, and next phase was decrement of Ctvib during PICC following the repetitive discharges for long period.

Keywords: motor unit, motor unit mechanical signal, myostiffness

46. Vastus intermedius fascicle behavior during concentric and eccentric contractions

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[Aims] Vastus intermedius (VI) contributes largely to knee extension force; however, its fascicle behaviors during concentric and eccentric contractions are not well understood. We aimed to compare changes in VI and vastus lateralis (VL) fascicle length during concentric and eccentric contractions. [Methods] Thirteen healthy men (Age 27 ± 3 yr) performed maximal concentric and eccentric knee extensors contractions between 35° and 105° of knee joint angles at an angular velocity of 30/°s whilst longitudinal images of VI and VL were recorded using ultrasonography. VI and VL fascicle lengths at knee joint angles of 40° and 100° were measured from ultrasound images, and then change in fascicle length between 40° and 100° was calculated. [Results] Fascicle lengths of VI and VL at 100° were 108 ± 12 mm and 104 ± 12 mm, respectively, and shortened 36 ± 12 mm for VI and 28 ± 13 mm for VL from 100° to 40° in concentric contraction, without a difference between muscles (P=0.05). Fascicle lengths of VI and VL at 40° were 72 ± 7 mm and 75 ± 8 mm, respectively, and lengthened 35 ± 9 mm for VI and 24 ± 5 mm for VL from 40° to 100° in eccentric contraction, with a significant difference between VI and VL (P<0.05). [Conclusions] VI fascicles were lengthened more than VL fascicles during eccentric contraction. It suggests that VI received greater mechanical stress compared with VL.

Keywords: Fascicle length, Vastus intermedius, Dynamic contraction

47. Influence of AGEs intake on skeletal muscle growth in mice

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Aims: Advanced glycation end products (AGEs) are the products of proteins and lipids that result from complex reactions involving a combination of hyperglycemia, hyperlipidemia and oxidative stress. Recent studies have suggested that accumulation of AGEs are related with induction of skeletal muscle atrophy. However, it remains unclear whether dietary AGEs affect muscle mass regulation. In this study, we aimed to examine the effect of AGEs intake on skeletal muscle growth in mice. [Methods] Male ICR mice (5-week old) were divided into two groups: low-AGEs fed group (L-AGEs, n=10) and high-AGEs fed group (H-AGEs, n=10), and treated with standard-diet and a heat (160°C for 1 h) treated diet for 16 weeks, respectively. [Results] There were no changes of initial body weight, final body weight, and food intake (kcal/day) between L-AGEs and H-AGEs groups (p>0.05). Normalized muscle weight (soleus, extensor digitorum longus, plantaris) by body weight were tended to be lower in H-AGEs group compared with L-AGEs group (p<0.10). Furthermore, grip strength of four limbs (L-AGEs: 0.050 ± 0.002, H-AGEs: 0.044 ± 0.002 N/g body weight, p=0.05) and wire hanging time (L-AGEs: 4245.2 ± 574.9, H-AGEs: 2990.1 ± 579.4 second/g body weight, p=0.06) were lower in H-AGEs group than L-AGEs group. [Conclusions] The findings suggest that dietary AGEs intake suppresses skeletal muscle growth in young mice.

Keywords: glycation, skeletal muscle, growth

48. Involvement of Ca²⁺ leakage out of sarcoplasmic reticulum in impaired Ca²⁺ release with prolonged low-frequency force depression

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[Aims] The purpose of this study was to investigate whether Ca²⁺ leak from sarcoplasmic reticulum (SR) contributes to impaired Ca²⁺ release with prolonged low-frequency force depression (PLFFD). [Methods] Gastrocnemius muscles from Wistar male rats were electrically stimulated at 70 Hz until force was reduced 50% of the initial value. Thirty minutes after fatiguing stimulation, the muscles were excised and single fibers were isolated from these muscles. After mechanical removal of sarcolemma, the fibers were used to measure action potential stimulation-induced force, depolarization-induced (depol-) force, endogenous Ca²⁺ content in the SR lumen (esR Ca²⁺) and SR Ca²⁺ leak properties. [Results] In fibers (fatigued fibers) from stimulated muscles, significant decreases in the ratio of force at 1 Hz to that at 50 Hz and in depol-force were observed. Additionally, in fatigued fibers, the esR Ca²⁺ was depressed and Ca²⁺ leakage from SR was increased. Increased Ca²⁺ leakage in fatigued fibers was unchanged by blockage of SR Ca²⁺-ATPase with 2-tetrabuty-1,4-benzoquinone. On the other hand, increases in the free Mg²⁺ concentration from 1 to 3 mM decreased SR Ca²⁺ leakage in fatigued fibers to control levels. [Conclusions] Impaired Ca²⁺ release of the SR with PLFFD is ascribable, at least in part, to reduced SR Ca²⁺ content caused by the Ca²⁺ efflux through the ryanodine receptor.

Keywords: skinned fiber, muscle fatigue, ryanodine receptor
49. **Stimulation of lactate receptor induces hypertrophy of cultured skeletal muscle cells**

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[Aims] Lactate receptor, G protein-coupled receptor 81 (GPR81), is expressed in not only adipocytes but also skeletal muscle cells. However, the physiological role of GPR81 in skeletal muscle cells remains unclear. Therefore, we investigated a possible role of GPR81 on skeletal muscle hypertrophy. [Methods] Mouse myoblast C2C12 cells were cultured with growth medium to stimulate proliferation and then differentiation medium for 5 days. After myotube formation, the effects of 3,5-dihydroxybenzoic acid (3,5-DHBA), a specific agonist for GPR81, or lactate on the myotube diameter and protein content of C2C12 cells were evaluated. [Results] Administration of 3,5-DHBA increased the myotube diameter and muscular protein content in a dose-dependent manner. Lactate also induced myotube hypertrophy. [Conclusions] Our results suggested that GPR81-associated signal(s) that is stimulated by 3,5-DHBA and lactate may mediate skeletal muscle hypertrophy. This study was supported, in part, by Grants-in-Aid for Exploring Research (16K12942, Y.Ohno; 16K13022, K.G.), and Grants-in-Aid for Scientific Research (C, 26350818, T.Y.) from the Japan Society for the Promotion of Science, the Naito Foundation (K.G.), and Graduate School of Health Sciences, Toyohashi SOZO University (K.G.).

**Keywords** : skeletal muscle, hypertrophy, lactate receptor

50. **Background muscle activity and prediction influence mechanical response elicited by maximum voluntary contraction**

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[Aims] This study was designed to examine whether background muscle activity and prediction of timing to exert force influence the mechanical response elicited by the maximum voluntary contraction (MVC) during passive shortening (SHO), lengthening (LEN), and isometric muscle contractions (ISO). [Methods] Fifteen men performed 100 knee extension-flexion movements passively (90°/s), 50 of which were performed at maximum knee extension. A light cue was switched on to start MVC at a 60° knee joint angle. MVC was repeated during SHO or LEN in every second trial (C1) or at random intervals (C2), and during ISO with a 60° knee angle every 4 s (C1) or at random times per 20 s (C2). Subjects were informed of the cue timing beforehand in C1 only. After the experiment, they graded the MVC force and timing and the degree of fatigue on a scale of 1 to 5. [Results] In SHO and LEN, reaction times (RT) and increases in MVC torque (dMVC) were shorter and greater in C1 than in C2. RT decreased with MVC repetitions in C1. dMVC was greatest in LEN and smallest in SHO. Differences in dMVC between C1 and C2 were largest in LEN and smallest in ISO. dMVC and the degree of fatigue increased with MVC repetitions. Self-evaluation of the MVC force and timing remained unchanged; however, it was different between C1 and C2 in SHO and LEN. [Conclusion] Background muscle activities influenced MVC torque. Informing subjects beforehand of the MVC timing had a strong effect on dMVC during LEN and little effect during ISO.

**Keywords** : reaction time, torque, self-evaluation

51. **The effects of taper on exercise performance and on glycolytic metabolism of skeletal muscles in mice**

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[Aims] The aim of this study is i) to establish a taper (reduced exercise load at the end of training period) model in mice, and ii) to elucidate the effects of taper on exercise performance and glycolytic metabolism in skeletal muscles. [Methods] Seven-week-old male ICR mice were trained for seven weeks, and randomly separated in three groups: Rest (RE, n=20), Training (TR, n=18), Taper (TA, n=18). For the following 10 days, RE stops exercise, TR continues the same training, TA reduced training duration to 30-50% of TR (training intensity and frequency are the same as TR). Exercise performance tests (PT: intermittent incremental load test) were carried out after 24 hours of the training period and sampling was carried out with or without PT. The muscle was excised, followed by analyses of enzyme activities related to the glycolytic pathway (PFK: Phosphofructokinase, M-LDH: Muscle type-Lactate dehydrogenase). The muscle glycogen content was also measured. [Results] TA shows the most excellent exercise performance compared to RE and TR. M-LDH activity was kept high in the same way as TR. When PT was not carried out, TA shows the most excellent M-LDH activity compared to RE and TR. There was no significant difference in PFK activity and muscle glycogen contents for all groups. [Conclusions] TA showed the most excellent exercise performance and M-LDH activity that was established a taper model in mice.

**Keywords** : taper, performance, glycolysis

52. **Role of the intracellular buffering action in the pH homeostasis in myocytes**

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[Aims] Homeostasis of intracellular pH (pH_i) was maintained by intracellular buffer substances and membrane transporters. We reported that two phase exist in pH_i recovery after low pH condition. However, it is unknown whether intracellular buffer substances are associated with these recovery dynamics. Using an in vivo bio-imaging model, we pharmacologically inhibited buffer substance (carbonic anhydrase, CA) and evaluated the pH_i recovery profiles following imposition of a discrete H^- challenge loaded into single muscle fibers by microinjection. [Methods] The intact spinotrapezius muscle of adult male Wistar rats (n=21) was exteriorized and muscle fibers were then loaded with low pH solution (PIPES buffer, pH 6.5) by microinjection over 3 s. The rats were divided into groups for the following treatments: 1) no inhibitor (CONT), 2) CA inhibition (ACZ, 0.5 mM). The fluorescence ratio (F500 nm/F445 nm) was measured from images captured during 1 min (1 image/sec), 5, 10, 15, and 20 min after injection. [Results] The pH_i at 1-2 s after injection significantly decreased from resting pH_i in both groups (CONT: -0.73 ± 0.03, CA: -0.86 ± 0.05). Subsequently, both groups were significantly recovered by 20 min. However, mean response time for pH_i recovery dynamics at 5 min after injection was significantly delayed by carbonic anhydrase inhibition (CONT: 62.7 ± 8.1s, CA: 139.1 ± 28.2s). [Conclusions] In conclusion, these results suggest that the buffering action of HCO_3^- plays a critical role in initial rapid pH_i recovery after injection.

**Keywords** : pH regulation, Fluorescence indicator, Membrane transporter
53. **Effect of local sex steroidogenesis on exercise training in aged rats**

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**<Aims>** During aging, there is a gradual decline in serum sex steroid hormones such as testosterone and estradiol levels. Low testosterone levels have been associated with decreased muscle mass in humans, and testosterone administration has been successful in improving general muscle function in elderly men. Previously, we demonstrated that skeletal muscle can synthesize testosterone and 5α-dihydrotestosterone (DHT) from dehydroepiandrosterone (DHEA) via steroidogenic enzymes and activate local steroidogenesis by acute exercise. However, it remains unclear whether there are sex differences in exercise training-induced alterations of sex steroid hormones in skeletal muscle among older individuals.  

**<Methods>** We divided into the control group and the training group using male and female Wistar SD rats of age for 18 months. Eight weeks exercise training performed treadmill running (running at 25 m/min for 1 hour, 5 days/week). **<Results>** Muscular DHT levels significantly increased after the training in both male and female training groups. Expression of 5α-reductase in skeletal muscle significantly increased after the training in male training group.  

**<Conclusions>** Exercise training locally activates steroidogenesis in skeletal muscle among older rats. Especially, the bioactive androgen DHT may be participating exercise-induced muscular adaptation in older individuals.  

**Keywords** : Skeletal muscle, sex hormone, aging

54. **Repeated bouts of resistance training activate ribosome biogenesis**

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**<Aims>** Recently, it has been shown that ribosome biogenesis may play an important role in the muscle hypertrophy. We reported that the magnitude of compensatory hypertrophy after synergist ablation was closely correlated with the increase of ribosome content in rats. However, it remains unclear how ribosome biogenesis is involved in the muscle hypertrophy caused by resistance training (RT). Therefore, the present study aimed to investigate the change of ribosomal RNA (rRNA) content with the change of exercise bout using rat RT model.  

**<Methods>** Male Sprague-Dawley rats were randomly assigned into four groups: Control (Cont), resistance-trained with 1 bout (1b), 3 bouts (3b) and 12 bouts (12b). The gastrocnemius muscle was subjected to resistance training protocol consisting of 50 reps of maximal isometric contractions evoked by direct electric stimulation. The muscle samples were taken 48h after the session of training, and analyzed for rRNA content. **<Results>** The content of rRNA per tissue weight gradually increased with the increase in the exercise bout. **<Conclusions>** The present results showed that strenuous muscle contractions effectively cause the increase in ribosome content in an exercise volume (contraction number) dependent fashion. The exact role played by ribosome biosynthesis in resistance training induced muscle hypertrophy needs further elucidation.  

**Keywords** : resistance training, hypertrophy, ribosome biogenesis

55. **Effects of forced eccentric contractions on histomorphometric characteristics in rat skeletal muscle**

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**<Aims>** We examined the short-term effects of forced eccentric contractions on muscle histology and muscle membrane permeability in rat skeletal muscle using Evans blue dye (EBD); a membrane impermeable dye that is used to identify muscle fibers. **<Methods>** The left tibialis anterior (TA) muscle of male F344 rats was subjected to 80 forced eccentric contractions by direct muscle electrical stimulation. The TA muscle was excised two days after the eccentric contractions. We performed immunohistochemical staining against dystrophin and laminin on frozen transverse sections of the TA, as well as GBHA staining for histochemical localization of calcium. **<Results>** The cross-sectional area (CSA) of TA muscle labeled with EBD+ was two times greater (P<0.05) than that without EBD (EBD-). Complete absence of dystrophin staining was apparent in all EBD+ fibers of the 600 fibers analyzed. EBD+ fibers were positive for laminin immunostaining and GBHA staining. The roundness of EBD+ and EBD- fibers were 1.19 and 1.41, respectively. **<Conclusions>** These findings suggest that EBD+ fibers induced by forced eccentric contractions were characterized by increased membrane permeability, higher density of calcium, apparent swelling, and a rounded.  

**Keywords** : eccentric contractions, evans blue dye, histomorphometry

56. **Effects of eccentric contractions on histomorphometric characteristics of intrafusal muscle fibers**

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**<Aims>** Evans blue dye (EBD) is used to identify muscle damage and to study cellular membrane permeability. We examined the effects of forced eccentric contractions (ECC) on the histomorphometric characteristics of intrafusal muscle fibers using EBD. **<Methods>** The left tibialis anterior (TA) muscle of male F344 rats was subjected to 80 ECCs by direct muscle electrical stimulation. The rats were injected with EBD at 24 hours after ECC, and their TA muscles were excised at 48 hours after the ECC protocol. We performed hematoxylin-eosin staining for frozen transverse sections of the TA, then analyzed EBD-positive fibers and cross-sectional area (CSA) for intrafusal and extrafusal muscle. **<Results>** Of the EBD-positive fibers, 20% and 0% were opaque in extrafusal and intrafusal muscle after ECC, respectively. EBD was infiltrated into the muscle spindle. In extrafusal muscle, the CSA of EBD-positive fibers was significantly greater than that of EBD-negative fibers. The CSA of intrafusal muscle fibers did not differ between TA muscles with ECC and without ECC. **<Conclusions>** These findings suggest that the extent of ECC-induced muscle fiber injury may differ between extrafusal and intrafusal muscle fibers.  

**Keywords** : Eccentric contractions, Intrafusal muscle fibers, Evans blue dye
57. Evaluation by X-ray diffraction of minute structural change due to the eccentric contraction

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[Aims] We evaluated minute structural changes in the sarcomere due to eccentric contraction (ECC) by X-ray diffraction. [Methods] Through tibial nerve of anesthetized 8-week-old F344 male rats, we stimulated plantar muscle with maintained blood supply to induce 10 consecutive contractions (100Hz isometric, ISO; 50Hz ECC; 75Hz ECC; 100Hz ECC) once per 3 seconds. One hour after contraction, we dissected the plantar muscle. [Results] Without contraction, we observed myosin layer-lines that most sensitively reflect degradation of periodic structure in sarcomere. However, after 75Hz and 100Hz ECC, we scarcely observed myosin layer-lines. After 50Hz ECC and ISO, we observed weakened myosin layer lines. [Conclusions] Difference in myosin layer-line intensity between 50Hz ECC and ISO is not clear at present. We continue to search for the difference in sarcomere periodic structure that senses mechanical load to strengthen skeletal muscle.

Keywords: Skeletal Muscle, Eccentric Contraction, X-ray diffraction

58. Nitric oxide regulates antioxidant enzyme in skeletal muscle

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[Objective] Nitric oxide (NO) protects against oxidative stress-induced skeletal muscle atrophy by induction of antioxidant enzyme. In vitro study shows that 24 hours NO donor treatment activates antioxidant response element (ARE). However, the mechanism for the activation of ARE by NO remains elusive. [Methods] C57BL/6 mice received daily injection of GSNO (2 mg/kg) twice a day for 3 days. At 12 hours after the last injection of GSNO, soleus, plantaris and white vastus muscles were harvested and analyzed keap1, Nrf2 and antioxidant proteins. Nuclear and cytosolic fractions were isolated from gastrocnemius muscle to measure Nrf2 translocation into nucleus by GSNO. To determine the effect of muscle contraction, plantaris muscle was harvested from trained or sedentary mice to measure Nrf2 protein. C2C12 myotube were also harvested at 1, 6 and 12 hours after stretch to measure Nrf2 mRNA. [Results] Oxidative soleus muscle expressed more antioxidant enzymes and Nrf2 than glycolytic white vastus muscle. GSNO injection significantly increased EcSOD, Nrd2 and Nrf2 translocation into nucleus. Exercise training significantly increased Nrf2 protein in plantaris muscle. Nrf2 mRNA in C2C12 myotube was significantly increased by mechanical stretch in a time-dependent manner. [Conclusions] GSNO increased Nrf2 protein and translocation into nucleus, which may contribute to NO-dependent protection of skeletal muscle atrophy.

Keywords: antioxidant enzyme, NO, Nrf2

59. Factors influencing individual and sex differences in ankle joint range of motion

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[Aims] Maximum range of motion (ROM) about a joint has been considered to be important for sport performance and injury risk. Nonetheless, factors influencing the individual and sex differences in maximum ROM remain unclear. Therefore, the purpose of the present study was to elucidate these from the viewpoints of passive stiffness and stretch tolerance, by using ultrasound shear wave elastography. [Methods] Shear modulus of each of the triceps surae was measured during passive dorsiflexion to individuals’ maximum dorsiflexion ROM in 12 males and 13 females. Shear modulus of each muscle was quantified at joint angles common to all participants (i.e., angle-specific) and at individuals’ maximum ROM. [Results] Males displayed significantly smaller ROM and greater angle-specific shear modulus than females. Maximum ROM was negatively correlated to angle-specific shear modulus and positively to shear modulus at maximum ROM for males. For females, maximum ROM was only positively correlated to shear modulus at maximum ROM. [Conclusions] These findings indicate that in males passive muscle stiffness and stretch tolerance are responsible for the individual difference in maximum ROM whereas in females the individual difference in maximum ROM is mainly due to stretch tolerance. Furthermore, the sex difference in maximum ROM is attributable to that in the angle-specific stiffness of the triceps surae.

Keywords: ultrasound shear wave elastography, flexibility, muscle stiffness

60. Effect of high frequency heat stimulation on recovery from muscle fatigue

Kazuya Hiratsuka¹, Naoya Tsunoda¹ (¹Kokushikan Univ, Tokyo, Japan)

[Aim] The aim of this study was to clarify the effect of high frequency heat stimulation on recovery from muscle fatigue in biceps brachii. [Methods] Twenty two healthy male university students were participated in this study. The all subjects were performed high frequency heat stimulation (HS) and non-stimulation as a control condition (Con). Muscle fatigue was produced by 50 times isometric elbow flexion. High frequency heat stimulation was used a Tensosys-Red Coral. Skin temperature was used a thermography. Muscle mechanical properties of biceps brachii (BB) was assessed by Tensiomyography (TMG method). Mechanical property parameters of contraction time (Tc), normalized velocity (Vrn) and maximal displacement of the muscle belly (Dm) were analyzed. Skin temperature, MVC and muscle mechanical properties were measured before (Pre) and after exercise (Post), and also 30 min recovery phase (Rec). [Results] Skin temperature of HS in Rec was significantly increased from post0 to Rec. Also, in Rec, MVC of HS was showed significantly higher value than that of Con. In Tc, Rec of HS was significantly lower value than that of Con. Vrn of Con has been increased from Post0 through Rec. On the other hand, HS was decreased. Significant difference of Dm was observed between HS and Con in Rec. [Conclusions] In this study, recovery from muscle fatigue for MVC and muscle mechanical properties due to HS were significantly promoted than that of Con. From these results, it was suggested that high frequency heat stimulation may promote to recovery from muscle fatigue.

Keywords: high frequency heat stimulation, muscle fatigue, mechanical property
61. Can dietary nitrate supplementation alleviate ECC-induced loss of contractile force?

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[Aims] We examined whether dietary nitrate supplementation can alleviate eccentric contraction (ECC)-induced loss of force in fast-twitch skeletal muscle. [Methods] Thirty male Wistar rats were randomly classified into three groups: the first group (control) which was given no NO₃; the second group (S3) which was given NO₃ (1 mmol/kg/day) for 3 days and; the third group (S6) which was given NO₃ (1 mmol/kg/day) for 6 days. After the end of NO₃ supplementation, the left anterior crural muscles were exposed to 200-repeated ECC. The contralateral muscles were used as controls. Immediately after ECC, the extensor digitorum longus and tibialis anterior muscle were removed and used for physiological and biochemical analyses, respectively. [Results] In control and S3 rats, ECC led to significant decreases (P<0.01) in the maximal tetanic force. On the other hand, in S6 rats, there were no changes between ECC-treated and contralateral muscles. ECC induced a marked depression (P<0.05) in sarcoplasmic reticulum (SR) Ca²⁺-release rate in control rats, but not in S3 and S6 rats. [Conclusions] The present results indicate that nitrate supplementation is capable to alleviating ECC-related decreases in force and suggest that the effect of nitrate supplementation may be mediated through changes in SR Ca²⁺-handling function.

Keywords: nitrate supplementation, eccentric contraction, sarcoplasmic reticulum function

62. Estimating sarcopenia by ultrasound brightness

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[Aims] Recent years, sarcopenia of an elderly person seen in the decrease in skeletal muscle mass and hypotonia becomes the social problem. A purpose of this study is to search for an occurrence factor of sarcopenia from the internal structure of the skeletal muscle and a change of the internal structure at the time of the muscular relaxation and contraction. [Methods] I assumed university student man and woman a subject. And then, I photographed a supersonic wave image at the time of the muscular strength display of the tibialis anterior muscle. In addition, I checked a brightness change and the change of the elastography level. [Results] Correlation with the muscular strength was higher in an elastography level than the brightness mean, and possibility to estimate the muscular strength display and maximum muscular strength was suggested. In addition, the possibility of the future sarcopenia estimate by the brightness rate of change was shown because a woman was higher in the brightness mean than a man, and a brightness change with the muscular contraction was small.

Keywords: sarcopenia, ultrasound, muscle

63. Myonuclear domain reduction does not affects muscle protein synthesis after resistance exercise

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[Aims] Single skeletal muscle fiber is multi nuclear cell. It is suggested that Myonuclear domain (i.e. cytosolic volume per nucleus, MND) might contribute to muscle protein synthetic capacity. In fact, Type I fiber (small MND) shows higher protein synthesis rate than Type II (large MND) fiber. However, it is unknown that whether MND size directly affects muscle protein synthetic capacity. Purpose of present study was to investigate the effect of muscle atrophy induced-MND reduction on muscle protein synthesis after resistance exercise. [Methods] Male Sprague-Dawley rats were randomly subjected to 14days of hind limb suspension (HS) group or resistance exercise. [Aims] Muscle atrophy-induced MND reduction does not influence rise in muscle protein synthesis after resistance exercise in rat skeletal muscle.

Keywords: myonuclei, muscle atrophy, muscle protein synthesis

64. Hyperbaric exposure with high oxygen concentration enhances oxidative capacity of skeletal muscle in type 1 diabetic rats

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[AIM] The effects of hyperbaric exposure on the oxidative capacity and antioxidant activity of fast skeletal muscle under hyperglycemic condition were investigated in type 1 diabetic rats. [METHODS] Male Wistar rats were divided into control, type 1 diabetic (DM) and DM with hyperbaric oxygen exposure with high oxygen concentration (HBO) groups. Diabetes was induced by injecting streptozotocin in the tail vein. The rats of HBO group were exposed to hyperbaric oxygenation at 1.25 atmospheric pressure and 36% oxygen concentration for three hours every day for 8 weeks. [RESULTS] Succinate dehydrogenase (SDH) and citrate synthase (CS) activities were decreased in the DM group compared to the control while these activities were increased in the HBO group compared to the DM group. Furthermore, the expression levels of reactive oxygen species (ROS) in the skeletal muscle of the DM group were elevated compared to control. In addition, the level of ROS in the HBO group attenuated compared to that in the DM group. [DISCUSSION] These results indicated that the exposure to hyperbaric oxygenation could prevent the dysfunction of the oxidative capacity in diabetic skeletal muscle.

Keywords: skeletal muscles, oxidative capacity, oxidative stress
65. Effects of order in concurrent training on hypertrophy and metabolic properties of rat skeletal muscle

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(Background) Induced adaptations in skeletal muscles vary between resistance training and endurance training; however, the effect on skeletal muscle when these trainings are performed continuously is not well studied. Additionally, the order of the training might affect muscle adaptation. (Purpose) In this study, we examined the effects of order in concurrent training on hypertrophy and metabolic properties of rat skeletal muscle. (Methods) Male SD rats (n= 35) were divided into five groups: 1) sedentary control (SC); 2) resistant training only (RC); 3) endurance training only (EC); 4) concurrent training A; resistance training first, then endurance training (RE); and 5) concurrent training B; endurance training first, then resistance training (ER). The training period was for 6 weeks (11 weeks old to 17 weeks old). After this, a histochemical analysis (M-ATPase, SDH and GPDH staining) in the FHL muscle of the rat was conducted to examine changes in cross sectional area (CSA) and metabolic properties. (Results and Conclusions) A significant increase in the CSA of type I fiber (P <0.05, vs. SC) and a tendency toward an increased CSA of type IIa and IIx fibers (P< 0.1, vs. SC) were observed in the RE treatment. However, in ER, the increased tendency was observed in only the CSA of IIx fiber (P< 0.1, vs. SC). Not all trainings in this study affected muscle metabolic properties. In conclusion, the order of concurrent training may affect muscle fiber hypertrophy.

Keywords: concurrent training, FHL muscle, rat

66. Effect of eccentric contraction on shear modulus of hamstrings

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[Aims] The aim of this study was to investigate the differences in muscle stress applied to the individual muscles composing the hamstrings (semitendinosus: ST, semimembranosus: SM, and biceps femoris: BF) after eccentric contractions (ECC). In this study, the shear modulus measured by ultrasound shear wave elastography was used as an index of the indirect muscle stress caused by ECC. [Methods] Fourteen healthy men performed 30 maximum ECC of hamstrings. The shear moduli of ST and SM, BF muscle bellies in the dominant leg were measured before and after ECC. Measurements of shear modulus were taken with hip at the 90 degree and knee at 45 degree of flexion. Significant differences between before and after ECC were determined for each muscle using the paired t-test. In addition, differences in change in shear modulus between before and after ECC among each muscle were determined using the Mann-Whitney U test with Holm correction. [Results] There were significant increases in shear moduli of ST and BF after ECC, but there was no significant change in shear modulus of SM after ECC. In addition, the changes in shear moduli of ST and BF between before and after ECC were significantly higher than ST (P < 0.05 and <0.052, respectively). [Conclusions] The results suggest that muscle stress applied to ST and BF caused by ECC were higher than SM.

Keywords: Hamstring muscle strain, Shear modulus, Eccentric contraction

67. Knockdown of MBNL1-associated expression of myosin heavy chain in C2C12 cells

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[Aims] Muscleblind-like (MBNL) 1 is an alternative splicing factor, and its dysfunction causes Myotonic dystrophy type 1 (DM1) that is characterized by muscular weakness, atrophy and myotonia. Furthermore, these symptoms are similar to those in age-associated skeletal muscle atrophy, so-called sarcopenia. Therefore, MBNL1 may play a key factor for the developing of skeletal muscle function in sarcopenia. In the present study, we investigated the effects of knockdown of MBNL1 on the expression of myosin heavy chain (MyHC) in C2C12 cells. (Methods) On the 3rd day of myogenic differentiation, MBNL1-targeting siRNA was transfected to C2C12 myotubes. Two days after the siRNA treatment, myotubes were collected, and MyHC expression was evaluated by real-time RT-PCR and Western blot. [Results] mRNA and protein expression levels of MBNL1 in C2C12 myotubes were decreased by ~70% and ~80% using siRNA treatment (p<0.05). Muscle protein content in myotubes was decreased by the knockdown of MBNL1 (p<0.05). On the other hand, MBNL1 knockdown-associated increase of type Ila (myh4), type Ila (myh2), embryonic (myh3), and neonatal (myh8) MyHC was observed at mRNA level (p<0.05). [Conclusion] MBNL1 might play a regulatory factor for the expression of MyHCs during myogenic differentiation.

Keywords: MBNL1, myotube, myosin heavy chain

68. Effects of herbal medicine Radix Astragali on immobilization-induced atrophy

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[Aims] To investigate whether the administration of Radix Astragali (RA) prevents both slow-twitch and fast-twitch muscle atrophy following immobilization. [Methods] In total, 27 10-week-old male F344 rats were divided into three experimental groups: control (CON, n = 9), immobilized (IM, n = 9), and immobilized and RA administered (IM + A, n = 9). In the IM and IM + A groups, both lower extremities were immobilized for 14 days. An oral decoction of RA was forcibly administered to the IM + A group for 21 days from 7 days before immobilization. At the end of the experimental period, the slow-twitch soleus and fast-twitch plantaris muscles were excised. The muscle wet mass was examined to assess changes in muscle mass following the immobilization and administration of RA. [Results] As expected, compared with the CON group, the IM group showed a significant decrease of 26% and 30% in the soleus muscle and plantaris muscle-to-body mass, respectively. However, compared with the IM group, RA administration significantly reversed the mass reduction of soleus muscle (~36%). On the other hand, the mass reduction of plantaris muscle was not reversed in the IM + A group. The IM group showed an increase in MAFbX atrogin-1 and MuRF1 mRNA, which play a pivotal role in various muscle atrophies. RA administration significantly reversed the MuRF1 increase in the soleus muscle only. [Conclusions] Our results suggest that RA administration has beneficial effects only on slow-muscle fiber-dominant soleus muscle atrophy, at least in part, via the inhibition of the ubiquitin-proteasome pathway.

Keywords: immobilization, muscle fiber type, herbal medicine
69. Pathological feature of eccentric contraction-induced nerve damage -occurrence and process-

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Aims: The purpose of this study is to monitor the process of injury after fast angular velocity eccentric contraction (EC) exercise. Methods: Twenty-four male Wistar rats were applied 180 deg/s angular EC on their medial gastrocnemius muscle (MG). EC is composed of 20 contractions (5 contractions x 4 sets). After EC, rats were dissected following different time point: immediately post (Post, n = 4), 1 day (1 d, n = 4), 3 days (3 d, n = 4), 7 days (7 d, n = 4) and 10 days (10 d, n = 8). 4 rats were assigned as the non-treated control group (CNT). To assess the muscle damage, maximal isometric tetanic torque was measured in the 10 d group. Before dissection, rats were intravenously injected with Evans blue dye (EBD, 1ml / 100g). After 24 hours, sciatic nerve and MG were harvested and tissues were cut into 5mm segments from proximal to distal positions. Intramuscular nerves in the MG were stained by fluorescent lectin that specifically stains nerve tissues. Results: Torque was significantly decreased (28%) on 1 day after EC compared with pre-exercise (p < 0.05). EBD was detected in distal portion of sciatic nerve in post, 1 d, 3 d and 7 d groups. Especially, EBD staining was observed in proximal segments close to the spinal cord on 3 d, 7 d, and 10 d. Intramuscular nerve was stained by lectin. Especially, EBD infiltrations were observed on lectin-stained intramuscular nerves only 1 d and 3 d. Conclusions: EC induced nerve damage possibly occurred near connection of neuron and muscle, and enlarged and/or transitioned from the damaged site.

Keywords: Sciatic nerve, Evans blue, Rat

70. Differences in myonuclear domain size of skeletal muscle between human and other mammals

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Aims: Myonuclear domain (MND) is the region of cytoplasm governing by single myonucleus, and MND size is an important factor for muscle fiber plasticity. To understand a basic mechanism of muscle plasticity in human, we compared the MND size among several mammals including human. Methods: Postmortem samples were taken from several muscles from 6 kinds of mammals; mouse (0.03kg, n=3): rat (0.3kg, n=3), capipara (30kg, n=1), pig (50kg, n=1), horse (500kg, n=3) and elephant (4000kg, n=1), and needle biopsy samples were taken from Vastus Lateralis (VL) muscle in 17 human subjects. Fiber type population and 2D-MND area was determined on serial cross sections of each muscle sample, which was stained for monoclonal antibodies to myosin heavy chain isoform. Thirty single fibers were isolated from 5 animals under a stereomicroscopy, and then fiber volume and myonuclear number for a given length were analyzed under confocal microscopy to measure 3D-MND volume of each single fiber. Results and Discussions: Larger animal tended to have larger 3D-MND volume, indicating that MND size relate to turnover rate of muscle protein in each animal. Although most of animals had a rank order of 2D-MND size as typeI = typeIIa < typeIIx/b, a similar MND size among three fiber types was demonstrated in human VL muscle. We speculated that muscle fiber type of human VL muscle do not have differential work roles, as compared to another mammals. Understanding the variability of MND size more deeply would provide fundamental insights into the mechanism of skeletal muscle plasticity.

Keywords: mammal, skeletal muscle, myonuclear domain

71. Effects of cold and heat treatments on recovery from injury in fast twitch muscle of rats

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Aim: In general, icing and heat treatments are applied after muscle injury. Recent studies reported that muscle regeneration was retarded by cold (icing) and promoted by heat treatments. We have examined effects of icing and heat treatments on soleus (slow-twitch) muscle, and showed that icing had a possibility to retard recovery of normal distribution of myosin heavy chain (MHC) isoforms. In this study, we examined effects of icing and heat treatments on recovery process from injury in plantaris (fast-twitch) muscle. Method: Male wistar rats (8-week-old) were randomly assigned to four groups; control (CTL), bupivacaine-injected (BPVC), bupivacaine-injected + icing (ICE) and bupivacaine-injected + heat (Heat). Icing (0°C, 20 min) was applied immediately after induction of muscle injuries, and until 3 days later. Heat treatment (42°C, 30 min) was performed every other day from 2 to 14 days after injury. The plantaris muscles were removed at 3, 7, 15 and 28 days after injury, then immunohistochemical and real time RT-PCR analysis were performed. Result: The differential effects between icing and heat treatments on recovery from the muscle injury were not detected in muscle fiber composition, cross sectional area and number of central nuclei. While, the expressions of IL-6, Paa7 and MyoD mRNA were reduced by icing treatment, suggesting a possibility to suppress inflammation and satellite cell activation in muscle fiber by cold treatment. However, the expressions of Myogenin, HGF, MHC-e, MHC-n were identical among three groups. These results suggest that icing treatment may be effective to suppress muscle inflammation at early stage of recovery from injury, but not effective for muscle regeneration at later stage.

Keywords: muscle regeneration, icing, heat treatment

72. USPs in unloaded and reloaded mouse soleus muscle

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[Aim] Ubiquitination of proteins in skeletal muscle atrophy has been extensively studied. Even though the deubiquitination is considered to be mediated by several ubiquitin-specific peptidases (USPs), a physiological role of USPs in the regulation of muscle protein content is still unclear. The purpose of this study was to investigate the responses of USPs in unloading-associated skeletal muscle atrophy with or without reloading. A possible role of USPs in skeletal muscle was also discussed. [Methods] Male ICR mice was divided into two groups, control and suspended groups. Mice in the suspended group were subjected to continuous hindlimb suspension (HS) for 2 weeks. Immediately after the 2-week HS, ambulation recovery was allowed for some mice in the 2-week suspended group. Soleus muscle was dissected from mice at baseline (untreated control; PRE), and at 1-week HS, 0, 2 weeks after 2-week HS. Protein expression level of USPs was analyzed Western blotting. [Results] Soleus muscle wet weight was decreased by HS, and was subsequently regrown by reloading. A transient increase in USP14 and USP19 expression was observed at 1-week HS. Protein expression level of USPs and Rpn11 was also increase by HS. [Conclusions] USPs including Rpn11 might play an important role in reloading-associated regrowth of unloading-induced atrophied skeletal muscle. USP14 and USP19 might participate in proteolysis in unloading-associated muscle atrophy.

Keywords: skeletal muscle atrophy, regrowth, deubiquitination
73. Effects of high concentration adiponectin concentration on the differentiation of skeletal muscle cells
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[Aims] Adiponectin, an anti-diabetic adipokine, is considered as a key mediator of obesity-associated insulin resistance as well as metabolic syndrome. Numerous studies have shown low plasma adiponectin levels are associated with obesity-related diseases such as type 2 diabetes. On the other hand, recent epidemiological studies demonstrated there is a negative relationship between skeletal muscle function and blood adiponectin level in elderly people. However, the effects of high level of adiponectin on skeletal muscle cells remain unclear. In the present study, we investigated that the effects of high level of adiponectin on myogenic differentiation by using AdipoRon, a synthetic agonist for adiponectin receptor (AdipoR).

[Methods] C2C12 cells were differentiated under a concentration of AdipoRon with or without a knockdown of AdipoR-1 or -2 by RNA interference method (siRNA). [Results] Myotube formation of C2C12 cells was inhibited by AdipoRon with a dose-dependent manner. AdipoRon-associated inhibition of myogenic differentiation was partially rescued by a knockdown of AdipoR1 or AdipoR2.

[Conclusions] Evidences suggests that higher levels of adiponectin might suppressed skeletal muscle mass as well as function.

Keywords: skeletal muscle, adiponectin agonist, myogenic differentiation

74. Effects of the number of repetitions of forced eccentric contractions on muscle damage in rat
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[Aims] Evans blue dye (EBD) is water soluble and membrane impermeable, and can be used to identify damaged muscle fibers. The aim of this study was to determine the effects of different numbers of repetitions of forced eccentric contractions (ECC) on muscle damage in rat skeletal muscle using EBD. [Methods] Twenty male rats were divided into groups (ECC-0, -20, -40, -60 repetitions), and the left tibialis anterior (TA) muscle was subjected to forced ECC with different numbers of repetitions by direct muscle electrical stimulation. EBD was injected intraperitoneally one day after ECC treatment, and the TA muscle was excised two days after ECC. [Results] The number of EBD+ fibers was significantly greater in ECC-60 than in the ECC-0, -20 and -40 groups. The cross-sectional area (CSA) of EBD-positive (EBD+) fibers of TA was significantly greater (P<0.05) than that of normal EBD-negative (EBD-) fibers. No significant difference in CSAs of EBD+ fibers was observed between the different repetition groups. [Conclusions] These findings suggest that the number of EBD+ fibers increased with repetition of ECC, and was characterized by increased membrane permeability. The CSA of EBD+ fibers was not influenced by the number of repetitions of ECC.

Keywords: eccentric contraction, muscle damage, Evans blue dye

75. Mechanical stretch increases SDF-1alpha/ CXCL12 in cultured skeletal muscle cells
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[Objective] Exercise training induces an increase in capillarity density in skeletal muscle. Chemokines, a family of 8- to 10-kDa chemotactic cytokines, provide signals that regulate cell distribution, differentiated and migration through transmembrane receptors coupled to heterotrimeric GTP-binding proteins. Among various chemokines, stromal cell-derived factor-1 (SDF-1alpha/CXCL12) is a key chemokine to control endothelial cell migration. The aim of this study was to examine whether mechanical stretch increases CXCL12 expression in cultured skeletal muscle cells. [Methods] C2C12 myoblasts and myotubes were cultured for 1, 6 and 24 hours with or without stretch (110% of original length, 1Hz) at 37 degree in a 5% CO2 humidified incubator. Total RNA was isolated at 1 and 6 hours after stretch to measure CXCL12 mRNA by RT-PCR. Protein was isolated at 24 hours after stretch to measure CXCL12 protein by ELISA. Medium was also collected at 24 hours after stretch to measure CXCL12 protein. [Results] CXCL12 mRNA and CXCL12 protein in cultured medium from C2C12 myotubes were significantly increased by mechanical stretch. There were no significantly differences in CXCL12 myoblasts. [Conclusions] Mechanical stretch increased CXCL12 mRNA expression and protein production in C2C12 myotubes, which may contribute to exercise-induced angiogenesis in skeletal muscle.

Keywords: angiogenesis, skeletal muscle, chemokine

76. Effect of electrical stimulation on molecular signals of hypertrophy in skeletal muscle
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[Aims] In order to elucidate the effects of electrical stimulation on muscle hypertrophy, we measured mTOR signaling, which is the major pathway for hypertrophy in skeletal muscle. [Methods] 7 weeks of age male ICR mice were divided into 2 groups: control (con) and Electrical Stimulation (ES). The gastrocnemius muscle was isometrically exercised with direct electrode (10 repetitions of 3-s stimulation, with a 7-s interval between contractions, for 5 sets with 3-min inter-set intervals). The voltage (30 V) and stimulation frequency (100 Hz) were adjusted to produce maximal isometric tension. Exercise with ES performed once in a week. After 3-week training, mice were sacrificed and gastrocnemius muscles were dissected quickly from each mouse for subsequent analyses. [Results] There were no significant difference in body weight and gastrocnemius wet weight between control group and ES group. We confirmed that mTOR signaling is activated in ES group. Using Western blot, we analyzed the change in signal molecules. Phosphorylation level of P70S6K, S6 and 4E-BP1 were significantly increased in ES group compared with that of control group. [Conclusions] Electrical stimulation activated molecular signaling which might be related in muscle strength and hypertrophy in skeletal muscle.

Keywords: Electrical Stimulation, mTOR signaling, hypertrophy
77. L-arginine administration attenuates eccentric contraction-induced force decrease in rat skeletal muscle

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[Aims] In this study, we examined the effect of L-arginine (ARG) supplementation on force output and protein expression in rat skeletal muscle exposed to eccentric contractions (EC). [Methods] Male Wistar rats were randomly divided into two groups: ARG and control (CON). L-ARG (500 mg/kg BW/day) were administered with drinking water continuously for 6 days. Three days after start of treatment, the left anterior crural muscles were exposed to 200-repeated EC. The contralateral muscles were used as controls. Three days following EC, torque-frequency relationship of the anterior crural muscles was measured in vivo, then tibialis anterior muscles were removed and used for biochemical analyses. [Results] In CON group, EC reduced isometric torque at all stimulation frequencies. In ARG group, decreases in the torque were observed at low frequencies (1-20 Hz), although torque values of ARG group were significantly larger compared to those of CON group. Western blot analysis showed in EC muscle homogenates that the content of dihydropyridine receptor and ryanodine receptor was decreased in CON group, but not in ARG group. Junctophilin-1 content was reduced in both group, but the level of ARG group was significantly higher compared to that of CON group. [Conclusions] These results suggest that L-ARG administration may facilitate force recovery following EC by preventing proteolysis of Ca2+-regulating proteins. **Keywords**: Eccentric contraction, Calpain, L-arginine

78. Effects of combination of neuromuscular electrical stimulation and administration of glutamine on cancer-induced muscle atrophy

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[Introduction] Neuromuscular electrical stimulation (ES) has been extensively used as a technique to improve muscle mass and function in patients with several diseases. On the other hand, it was shown that muscle protein breakdown is associated with glutamine (GLN) release from skeletal muscle in cancer cachexia. [Aims] We investigated the effects of either or both of ES and GLN administration on muscle wasting in colon 26 (C-26) tumor bearing mice. [Methods] CD2F1 mice were divided into 8 groups; control (CNT), CNT+ES, CNT+GLN, CNT+ES+GLN, C-26, C-26+ES, C-26+GLN, C-26+ES+GLN. Cancer cachexia was induced by a subcutaneous injection of C-26 cells. ES (60% of maximum torque, 50 Hz, 0.5 ms, 2 s on/4 s off) was performed to the left triceps surae muscles every other day. GLN (1 g/kg) was daily intraperitoneally administered starting one day after C-26 injection. After four weeks, medial gastrocnemius (MG) muscle was excised from each animal. [Results] Tumor-free body mass and MG muscle weight were lower in C-26 group than in CNT group (-18% and -19%, respectively). These changes were accompanied by reduced locomotor activity (-31%). The C-26+ES and C-26+ES+GLN groups showed a tendency of increased MG weight compared to the C-26 group, but it did not reach statistical significance. Moreover, GLN administration did not affect the MG weight in the C-26 mice. [Conclusions] These data show that neither ES nor GLN administration, alone or in combination, prevent the loss of muscle mass in cancer cachexia. **Keywords**: cancer cachexia, neuromuscular electrical stimulation, administration of glutamine

79. Effect of stair descending exercise on DOMS of the lower limbs and indirect measure of muscle damage after exercise

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[Aims] This study aimed to clarify the effect of different cadences of a stair descending exercise on the Delayed Onset Muscle Soreness (DOMS) in the lower limbs, and also to examine the relationship between the peak DOMS and muscle damage indices at different cadence. [Methods] Eight healthy male adults performed a stair descending exercise comprising 207 steps and 10 round trips. The subjects had 15% body weight load. Cadence was set at 90, 110, and 130 steps/min. The experimental condition was done by random order. Measurements included maximal isometric strength, thigh circumference, and muscle soreness. Muscle soreness was determined based on the visual analog scale. [Results] Muscle soreness immediately after and 12 h after exercise was significantly higher in the 90- and 130-conditions in comparison with the 110-condition (p<0.05). There was a significant negative correlation between the peak DOMS score and isometric maximum strength changes immediately after exercise and 4 days later in the 110-condition (p<0.05). There was also a significant positive correlation between the peak DOMS score and thigh circumference changes immediately after exercise and 4 days later in the 110-condition (p<0.05). There were no significant correlations between the peak DOMS and muscle damage indices in the 90- and 130-conditions. [Conclusions] This study revealed that the stair descending exercise burden on the lower limbs is reduced when the cadence is 110 steps/min. In addition, the DOMS would not be necessarily related to muscle damage indices. **Keywords**: Delayed Onset Muscle Soreness, muscle damage indices, stair descending exercise

80. The increment of calcium ion in myocyte through TRPV1 by the heat stress is inhibited by muscle contraction

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[Aims] Heat stress activates transient receptor potential vanilloid 1 (TRPV1) which expressed on the cell membrane and promotes calcium ion (Ca2+) influx from extracellular through TRPV1 channels. The effect of the muscle contraction on the TRPV1-mediated Ca2+ influx remains unexplained. We tested the hypothesis that the TRPV1 activity at heat stress is inhibited by muscle contractions. As a result, the Ca2+ influx on the heat stress is attenuated after muscle contraction. [Methods] Spionotrapezius muscles of adult Wistar rats were exteriorized in vivo and loaded with the fluorescent probe Fura-2 AM. Heat stress (muscle surface temperature 40 °C) or Capsaicin (500μM, muscle surface temperature 30 °C : CAP) were used as TRPV1 activator. Isometric contraction (100Hz, 5-10V, 30 seconds, ISO) was added 5-10 minutes after HS and CAP condition. Intracellular Ca2+ concentration ([Ca2+]i) was determined for 30 min using fluorescence microscopy. [Results] The Ca2+ influx by HS and CAP was almost inhibited by addition of the TRPV1 inhibitor. [Methods] Spinotrapezius muscles of adult Wistar rats were exteriorized in vivo and loaded with the fluorescent probe Fura-2 AM. Heat stress (muscle surface temperature 40 °C : HS) or Capsaicin (500μM, muscle surface temperature 30 °C : CAP) were used as TRPV1 activator. Isometric contraction (100Hz, 5-10V, 30 seconds, ISO) was added 5-10 minutes after HS and CAP condition. Intracellular Ca2+ concentration ([Ca2+]i) was determined for 30 min using fluorescence microscopy. [Results] The Ca2+ influx by HS and CAP was almost inhibited by addition of the TRPV1 inhibitor. [Conclusions] These results suggest that muscle contraction itself contribute to keep [Ca2+], homeostatic under hyperthermic conditions. **Keywords**: TRPV1, heat stress, muscle contraction
81. Muscle oxygenation kinetics of incremental sustained isometric knee extension exercise

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\[\text{Aims}\] The purpose of this study was to examine the relationship between muscle oxygenation kinetics of sustained incremental isometric knee extension exercise (IKEE), duration time (DT), and 20-m shuttle run test (SR). \[Methods\] Seven healthy females who specialized in ball game sports took part in the experiment. The protocol was a sustained IKEE. The load was started from 40\% MVC, was increased by 10\% every ten seconds, and the time to all-out was measured. Simultaneously, TOI at right rectus femoris (RF) was measured by a NIRS device (NIRO200NX). The decline rate (Dec-r) of TOI was calculated at 0-10 (40\%), 10-20 (50\%), 20-30 sec (60\% MVC). Arterial occlusion was used at rest and at 10 sec after exercise, and relative \(\text{mVO}_2\) was calculated from each Dec-r of TOI. In addition, SR was performed on another day, total number of completed shuttles (laps) was recorded. Pearson’s correlation was measured between each value. \[Results\] A significant negative correlation was found between the Dec-r of analysis was used for the relationships between each value. \[Conclusions\] These results suggest that a specified higher HSP content in muscles from the H24 rats compared to those from others. \[Keywords\] NIRS, incremental, isometric

82. Mutual morphological changes of the skeletal muscle and neuromuscular junction after atrophy

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\[\text{Aims}\] The purpose of this study is to examine morphological changes in muscle and neuromuscular junction (NMJ) of recovery phase from muscle atrophy due to cast immobilization. The skeletal muscles cause regression and atrophy by influence of inactivity. However, these changes are plasticity, the regaining muscle activity after the immobilization causes morphological recovery in skeletal muscles. At the time of these changes in the skeletal muscle, the NMJs also are likely to cause a reciprocal change with the muscle. \[Methods\] We used the extensor digitum longs muscle of SD male rats (9 weeks old) in this experiment. Following nine days of immobilization (IM), rats were divided into the sedentary reared (IMR) group and Clenbuterol administration (1mg / kg / day) group for promote recovery (IMC) for seven days. The morphological observation of NMJ's by optical microscope used was used for the observation cholinesterase and silver staining. Measurements items of the NMJ's were as followings: fiber diameter (FD), endplate length (LEL), endplate area (EpA), nerve terminals area (NTA). \[Results\] In this study, cast immobilization induced FD and EpA reduction. However, nerve terminal were not signs of degeneration. The ELE and EpA have shown a faster recovery than the muscle fiber during remobilization. \[Conclusion\] It is considered that the muscle hypertrophy have a control mechanism associated with the morphological changes of the nerve terminals and the endplate during recovery from immobilization-induced muscle atrophy. \[Keywords\] Neuromuscular junction, atrophy, Clenbuterol

83. Effects of heat stress on fatigue resistance in rat fast-twitch muscle

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\[\text{Aims}\] Heat shock protein (HSP) acts as a heat stress-inducible molecular chaperone. The aim of this study was to investigate the effect of heat stress on muscle fatigue resistance. \[Methods\] Wistar rats were assigned to a heat stress (H) or a control (C) group. Both hindlimbs of the H rats were immersed in a hot water (42°C) for 20 min (heat stress). The H animals were subdivided into one of four different (H0, H6, H12 and, H24) groups; 0 h, 6 h, 12 h and 24 h after heat stress, gastrocnemius (GAS) muscles of the left leg from the rats of H0, H6, H12 and H24 groups were electrically stimulated at 70 Hz for 2 min (fatiguing stimulation), respectively. Immediately after fatiguing stimulation, the GAS muscle was excised and the superficial regions of the GAS muscle were used for biochemical analyses. The right GAS muscles were muscles as non-stimulated muscles. \[Results\] Fatigue resistance was higher in the GAS muscles from the H0 and H24 rats than in those from the C rats. In the C, H0, H6 and H12 rats but not in the H24 rats, Ca\textsuperscript{2+} uptake rate of sarcoplasmic reticulum (SR) was decreased in stimulated muscles compared to non-stimulated muscles. There was a trend toward the higher HSP content in muscles from the H24 rats compared to those from others. \[Conclusions\] These results suggest that a specified time after acute heat stress, fatigue resistance is improved, which may relate to preservation of the SR Ca\textsuperscript{2+} uptake ability. \[Keywords\] heat shock protein, sarcoplasmic reticulum, gastrocnemius muscles

84. In vivo intracellular Ca\textsuperscript{2+} dynamics after few days of eccentric contractions in rat skeletal muscle

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\[\text{Aims}\] Intracellular calcium ions ([Ca\textsuperscript{2+}]) have an important role as a intracellular signal for proteins synthesis and degradation. The eccentric contractions (ECC) facilitates Ca\textsuperscript{2+} influx from the cellular outside via stretch-activated channels and causes high-level [Ca\textsuperscript{2+}], accumulation. Accumulated [Ca\textsuperscript{2+}], activates proteolysis-related enzyme and induces muscle damage. A few days after ECC, the damaged muscle region shifts from proteolysis to regeneration phase. The [Ca\textsuperscript{2+}], dynamics (i.e. time and space in the form of oscillations and waves) of the damage - recovery phase after ECC remains is unknown. We verified hypothesis that there are [Ca\textsuperscript{2+}] dynamics in the damaged muscle fiber in damage - recovery phase. \[Method\] In anesthetized Wistar rats, the tibialis anterior muscles (TA) were subjected to ECC (5 sets of 40 contractions). After 1 day, 3 days, and 7 days of ECC, TA were exteriorized (In vivo) then [Ca\textsuperscript{2+}] was observed by loading fura 2-AM using fluorescence imaging. As a control group, we also observed [Ca\textsuperscript{2+}], of unexercised TA.\[Results\] The mean [Ca\textsuperscript{2+}], was significantly increased in ECC at 1 day (+14.1\%) and 7 days (+13.2\%) compared with unexercised control group. Heterogeneous [Ca\textsuperscript{2+}], accumulation patterns and dynamics among fibers were observed under ECC groups. In particular, during the 30min observation period, the remarkable degree of Ca\textsuperscript{2+} dynamics were observed in 1day after ECC. \[Conclusion\] We observed In vivo Ca\textsuperscript{2+} dynamics in muscle fiber in damage-regenerate phase. These dynamics may produce and maintain long ranging signaling on proteins synthesis and degradation process after ECC. \[Keywords\] muscle damage, calcium imaging, intracellular signal
85. **Pulsed ultrasound prevents LPS induced muscle atrophy and p38 MAPK phosphorylation in C2C12**

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[Aims] Under sepsis, inflammatory cytokines increase systemically induced by lipopolysaccharide (LPS), and p38 MAPK phosphorylation leads to muscle protein degradation. Pulsed ultrasound radiation can provide the mechanical stimulation to the target tissue, and has been reported to show anti-inflammatory effects. Therefore, the preventive effect of pulsed ultrasound radiation on muscle protein degradation induced by LPS using C2C12 myotubes was investigated in the present study. [Methods] Pulsed ultrasound radiation was performed before LPS treatment. The pulsed ultrasound signal consisted of a series of 3 MHz, 20% burst rate, and was delivered at an intensity of 0.5 W/cm², for 30 sec. [Results] LPS treatment resulted in the increase of p38 MAPK phosphorylation and myofibril protein degradation. LPS treatment and pulsed ultrasound radiation had no effects on the expression levels of MyD88. Meanwhile, pulsed ultrasound radiation prevented the decrease of myotube diameter and myofibril protein degradation induced by LPS in C2C12 myotubes, and inhibited the phosphorylation of p38 MAPK. [Conclusions] Pulsed ultrasound radiation attenuated LPS-induced muscle atrophy through inhibiting the phosphorylation of p38 MAPK. These results indicate that mechanical stimuli with pulsed ultrasound could be a preventive intervention against cachectic muscle atrophy.

**Keywords**: sepsis, muscle atrophy, pulsed ultrasound

86. **Observation of stromal cells network following acute muscle trauma with FIB/SEM**


[Aims] It has been reported that the mononuclear cells which locate in the interstitial space of damaged muscle tissue might take part in muscle fibers repair. However, the morphology and the strain of these cells are not clear. [Methods] In this study, trauma loaded rat skeletal muscles (gastrocnemius) were observed with focused ion beam scanning electron microscope (FIB/SEM), which were reconstructed into three dimensional images by the program to develop the localization and formation of stromal cells. [Results] As a result, we observed that many cells invaded in muscle fibers, and three kinds of stromal cells were identified in the interstitial space of the gastrocnemius muscles at the 1st and the 2nd day after muscle damage. The first appeared cells had spindle shapped form. The second cells had many rough endoplasmic reticula (r-ER). The third cells were similar to granulocyte. These 3 types of cells were contacted one another and formed network. At the 5th and the 7th days after, stromal cells were surrounded the regenerate muscle fiber. [Conclusions] It was suggested that these stromal cells may exchange some information which plays some important roles in regeneration process after muscle damage. These “cell to cell contact” might have an important biomedical meaning in “the transformation of cells”, “the cell proliferation” and “the invasion to muscle fibers”. (COI:NO)

**Keywords**: FIB/SEM, muscle stromal cells, muscle regeneration

87. **Compression Garment with Medium Pressure Reduced Decrement of Jump Performance and Inflammation During Prolonged Running**

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[Aims] To examine the effect of wearing a lower body compression garment (CG) with different pressure levels on exercise performance, exercise-induced muscle damage and the inflammatory response to prolonged running. [Methods] Eight male subjects completed three exercise trials in a random order. The exercise consisted of 120 min of uphill running at 60% of VO₂max. The exercise trials included 1) wearing a lower-body CG applied 40 hPa [HIGH]; 2) wearing a lower-body CG applied 20 hPa [MED]; and 3) wearing a lower-body garment applied < 10 hPa [CON]. Time-course changes in jump height, HR, and RPE were monitored. Blood samples were collected before exercise, 60 min of the 120 min exercise period, immediately after exercise, and 1 h after exercise. [Results] Jump height was significantly higher immediately after the exercise in the MED trial compared with that in the HIGH trial (P = 0.04). Mean HR during the 120 min exercise was significantly lower in the MED trial (162 ± 4 bpm) than that in the CON trial (170 ± 4 bpm, P = 0.01). Area under the curve of plasma IL-6 was significantly lower in the MED trial (397 ± 58 pg/ml, 120 min) compared with that in the CON trial (670 ± 86 pg/ml, 120 min, P = 0.04). [Conclusions] Wearing a lower body CG exerted medium pressure (approximately 20 hPa) significantly attenuated decrease in jump performance, and increases in HR and the inflammation during prolonged running.

**Keywords**: Exercise-induced muscle damage, Inflammatory response, Compression garment

88. **Effects of glucagon-like peptide-1 analogue Exendin-4 on skeletal muscle differentiation**

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[Aims] Glucagon-like peptide-1 (GLP-1) analogues are new drugs for the treatment of type 2 diabetes. It is generally considered that GLP-1 binds to GLP-1 receptor in pancreas, enhances the secretion of insulin, and consequently lowers blood glucose level. On the other hand, skeletal muscle atrophy was observed in patients with Type 2 diabetes. Therefore, GLP-1 may have a direct effect on skeletal muscle cells. However, the effects of GLP-1 on skeletal muscle cells remains unclear. The purpose of this study was to investigate the effects of GLP-1 analogue on skeletal muscle differentiation. [Methods] Mouse myoblasts-derived C2C12 cells were incubated with Exendin-4 (Ex4), a GLP-1 receptor agonist, and the myotube formation of C2C12 cells was evaluated. The mRNA expression level of GLP-1 receptor of C2C12 myoblasts and myotubes was also investigated using real-time RT-PCR. [Results] GLP-1 receptor was observed in C2C12 myotubes at mRNA level, but not myoblasts. Ex4 stimulated C2C12 myotube differentiation. [Conclusions] Activation level of GLP-1 receptor in skeletal muscle cells might have an impact on skeletal muscle mass.

**Keywords**: incretin, glucagon-like peptide-1, C2C12
91. Time course changes of GH receptor and IGF-1 receptor gene expressions in compensatory hypertrophied skeletal muscles

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[Aims] Growth hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) strongly affects on anabolic processes of skeletal muscles, however, the regulations of each receptor expressions remain unknown during ongoing muscular hypertrophic process. We previously reported that the GH receptor (GHR) mRNA expression is down regulated at the early phase (4d) of skeletal muscle hypertrophy using compensatory hypertrophy model in rats. In the present study, we investigated the expression levels of GHR and IGF-1R mRNA in longer period (14d) using same model. [Methods] Ten-week-old Sprague Dawlay (SD) rats were used. Under anesthesia, bilateral distal tendons of Gastrocnemius muscles were dissected, and compensatory hypertrophy was induced in the Plantaris, and Soleus muscles. These muscles were taken after 4, or 14 days after surgery. Total cellular RNA was extracted, and each mRNA expression was measured by quantitative RT-PCR method. Age-matched SD rats were used as control. Statistical analysis was applied to estimate the significant effect of period and hypertrophy, using two-way ANOVA and student Newman Keuls test. [Results] The GHR mRNA expression was significantly lower in hypertrophied muscles after 4 days from surgery, but no significant difference was shown after 14 days compared to Control rats. No significant effect was observed in IGF-1 receptor levels of hypertrophied muscles. [Conclusions] It was suggested that the GHR mRNA expression is down regulated at the onset of muscular hypertrophy, then returns to normal level.

Keywords: skeletal muscle, GH, IGF-1

92. Effect of different timings of eccentric-exercise bouts on mTOR signaling in skeletal muscle

Shuo wen Chang1, Toshinori Yoshihara1, Yuri Takamine1, Hisashi Naito1 (*Hontendo University)

We previously indicated that mTOR/p70S6K-signaling transducers exhibited a circadian variation in rat skeletal muscle. However, whether different timings of exercise affect the phosphorylation of mTOR-signaling transducers is still unknown. Aim: To investigate the effect of different timings of a bout of eccentric exercise on mTOR signaling in the rat soleus muscle. Methods: Nine-week-old male Wistar rats (n = 42) were used in this study. The rats performed a 15-min bout of downhill running (eccentric exercise; inclination, -16°; speed, 16 m/min) on an animal treadmill at two different times of the day [Zeitgeber Time 6 (ZT6; light phase, n = 21) and ZT12 (dark phase, n = 21)]. The soleus muscle was removed before (control), immediately after, and 1 h after the exercise bout (n = 7 per group). The phosphorylation ratio of mTOR-signaling transducers was assessed by western blot analysis. Results: A two-way ANOVA revealed significant effects of eccentric exercise and light-dark cycle on mTOR phosphorylation ratio (p<0.05). We also found a significant interaction (p<0.05) and main effect (p<0.05) of eccentric exercise in the downstream substrate of the p70S6K phosphorylation ratio. Moreover, the p70S6K phosphorylation ratio was significantly higher at ZT6 (+42%) than at ZT12 immediately after eccentric exercise. Conclusion: The different timings of an eccentric-exercise bouts may affect the phosphorylation of mTOR signaling in the rat soleus muscle.

Keywords: circadian rhythm, intracellular signaling, downhill running
93. Effects of the combinations of different temperature stimuli on the kinetics of fibrosis and satellite cell during muscle regeneration

Tsubasa Shibaguchi¹, Kazumi Ikezaki¹, Soma Morihiro², Toshinori Yoshihara³, Hisashi Naito¹, Katsumasa Goto¹, Toshitada Yoshioka³, Takao Sugiuira¹ (Kanazawa Univ, Yamaguchi Univ, Juntendo Univ, Toyohashi SOZO Univ, Hiroaki Gakuen Univ)

[Aims] Icing and hyperthermia has been widely accepted as therapeutic treatments for skeletal muscle injury in the acute- and later-phase, respectively. However, information about these combined effects on skeletal muscle regeneration is limited. Therefore, we examined the impact of the combinations of icing and heat stress on the kinetics of fibrosis and satellite cell in skeletal muscle during the recovery from injury in male Wistar rats. [Methods] To induce muscle injury, bupivacaine (BPVC) was injected into plantaris muscles bilaterally. Icing (0°C for 20 min/day) was applied immediately after and 1, 2, and 3 days after the injury. Repeated heat stress (42°C for 30 min on alternating days) was performed during 2- or 4-14 days after BPVC injection. [Results] The progression of fibrosis in response to plantaris muscle injury tended to be prevented when only repeated heat stress was applied. Similarly, animals exposed to heat stress alone exhibited a larger increase in the number of quiescent, activated and proliferating, and differentiating satellite cells at 3 days after BPVC injection. [Conclusion] These results suggest that, compared to the combinations of icing and hyperthermia treatments, hyperthermia alone applied from acute-phase after skeletal muscle injury may be more appropriate treatment for successful muscle regeneration.

Keywords: muscle regeneration, icing, heat stress

94. Changes in proteolytic pathways during regeneration of injured rat plantaris muscle

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[Aims] The overlapping sequential stages, degeneration, inflammation, regeneration, and remodeling-repair, exist during skeletal muscle regeneration. The proteolytic systems in skeletal muscle have the lysosomal proteases system, the calpain system, the ubiquitin-proteasome system, and the myonuclear apoptosis system. Although there are a limited number of reports about these proteolytic systems during muscle regeneration, the relative contribution of each system remains unknown. Therefore, we examined the changes in the aforementioned proteolytic pathways during regeneration of injured muscles. [Methods] Eight-week-old male Wistar rats were randomly assigned to two groups: normal control and bupivacaine (BPVC)-injected. Muscle injury was induced by intramuscular injection of BPVC into the plantaris muscles. The plantaris muscles were removed at 3, 7, 15, and 28 days after BPVC injection. [Results] The peak levels of autolytic calpain I, Bax, and ubiquitinated protein were observed at 3 day after BPVC injection, while the levels of cathepsin L, calpain II, and caspase-3 expression were highest at 7 days after injury. [Conclusions] These results suggest that lysosomal proteases, calpain, and myonuclear apoptosis systems are involved in not only degeneration and inflammation but also regeneration and/or remodeling-repair after muscle injury.

Keywords: Muscle injury, Proteolysis, Muscle Regeneration

95. Influence in the combination of different temperature stimuli on the muscle growth factors during regeneration of injured rat fast-twitch muscle

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[Aims] The purpose of this study was examined that the combinations of different temperature stimuli on the muscle growth factor and myogenin expression in the experimental groups without Cont. Icing (0°C for 10 min/day) was applied immediately after injury, and 1, 2, and 3 days after the injury. Repeated heat stress (42°C for 30 min on alternating days) was performed during 2- or 4-14 days after BPVC injection. [Results] The expression of MyoD and myogenin were detected after 1, 2, and 3 days after injury. Repeated heat stress (42°C for 30 min on alternating days) was performed during 2- or 4-14 days after BPVC injection. [Conclusion] These results suggest that, compared to the combinations of icing and hyperthermia treatments, hyperthermia alone applied from acute-phase after skeletal muscle injury may be more appropriate treatment for successful muscle regeneration.

Keywords: muscle regeneration, icing, heat stress

96. SOD1 knockout induced muscle damage and satellite cell response in different muscle fiber types in the mouse

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Aim: Our previous study demonstrated that activation of satellite cell (SC) was induced in whole gastrocnemius (GAS) muscle in the superoxide dismutase 1 (SOD1) knockout mice. The activation of SC may relate to dysregulation associated with age-dependent change as reported in recent studies. In this study, we investigated the differential effects of SOD1 knockout on fast- and slow-twitch muscle fiber types.

Method: C57BL/6N mice (4-6 months old) were used as wild type control (CTL; n=6) and SOD1 knockout (SOD; n=7) mice. To determine degree of muscle damage and SC activation in type identified muscle fibers, soleus (SOL), superficial and deep portions of GAS muscles were analyzed by immunohistochemical staining and real-time RT-PCR.

Result and Discussion: In SOL mice as compared to CTL mice, fiber type shift to faster twitch fiber was detected in SOL, but not in both portions of GAS muscles. Furthermore, in the real-time RT-PCR analysis, significant increases in mRNA expression of MyoD, myosin heavy chain embryonic, interleukin-6 and endothelial NO synthase (eNOS) were showed in superficial portion of GAS, but not in SOL muscles. We speculated that the difference in fiber type shift is potentially explained by nitric oxide (NO) effect, and that higher SC activation and muscle regeneration in fast-twitch glycolytic fibers may be involved in decreases of function and number of SC in aging mice muscle.

Keywords: SOD1 knockout, muscle fiber type, satellite cell
97. Screening of compounds activating PGC-1α for the muscle endurance

Yusuke Mochizuki¹, Ayaka Takeuchi¹, Yuta Nagaïke¹, Akihito Morita¹, Naohisa Ogó¹, Akira Asai¹, Yasutomi Kamei², Shini Miura¹ (¹Univ. of Shizuoka, ²Kyoto Pref. Univ.)

[Introduction] In order to achieve the healthy protracted-life society, the elderly person has to enhance their muscular strength and/or endurance. Exercise enhances the muscular strength and/or endurance, however, elderly persons are not able to carry out exercise, furthermore, there are no participate improving the muscular endurance. PGC-1α is a transcriptional coactivator that is activated by exercise stimulation in skeletal muscle, and improves the muscle endurance. If we are able to activate PGC-1α using several compounds without exercise, it will make the elderly person high muscular endurance state. Therefore, we evaluate the compounds activating PGC-1α. [Method] We used the modified procedure of the yeast two hybrid system. HEK293 cells were transfected with GAL4DBD-PGC-1α and 9xUAS-firefly luciferase plasmids. After 24 hours of culture, cells were treated with various compounds for further 24 hours. At the end of culture, we measured the chemiluminescence using the dual-Glo luciferase assay system (Promega). [Result] The compounds activating PGC-1α, such as AICAR or resveratrol, increased the chemiluminescence, proving the validity of our assay system. Screening of total 270 compounds, 22 compounds increased the chemiluminescence more than 200 % of control. [Conclusion] We were able to establish the screening system for evaluation of compounds that activate PGC-1α. It will be useful for identifying the compound which helped improve the muscular endurance via activating PGC-1α.

Keywords: PGC-1α, muscle endurance, screening

98. Akt1 deficiency does not affect overload-induced activation of mTOR-dependent signaling in skeletal muscle

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[Aims] Skeletal muscle mass is determined by the dynamic balance between protein synthesis and degradation. It has been well documented that mTOR (mechanistic target of rapamycin)-dependent pathway plays a central role for promoting protein synthesis in skeletal muscle, however, the precise molecular regulation of mTOR activity is largely unknown. [Methods] Male Akt1 knockout mice (B6.129P2-Akt1tm1Mbb/J, the Jackson Laboratory) and littermate wild type control, 12–14 weeks of age, were used in this study. Mechanical overload of the plantaris muscle was induced by the synergist ablation. Plantaris muscle samples were collected at 12hrs following the synergist ablation surgery. [Results] Functional overload-induced activation of mTOR signaling (as determined by the phosphorylation of 4E-BP1 at Thr389 and Thr421/Ser424 and the pS6 at Ser235/236 and Ser240/244) was observed both in wild type control and Akt1 KO mice. Akt1 deficiency did not affect the activation state of mTOR signaling in response to functional overload. [Conclusions] These observations demonstrate that mTOR activation in response to the acute bout of mechanical overload occurs independently of Akt-dependent regulation in skeletal muscle.

Keywords: Akt1, mechanistic target of rapamycin, overload

99. The effect of tea catechins on age-related muscle atrophy

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[Aims] Sarcopenia is an age-related systemic syndrome with progressive deterioration in skeletal functions and loss in mass. It is highly related to hospital admission, disability, and mortality in older people. Tea catechins have been reported to possess various biological and pharmacological effects, such as antioxidative and anti-aging activity. In the present study, we examined the effect of tea catechins on age-related muscle atrophy using SAMP8 mice. [Methods] SAMP8 mice were fed with high fat diet (HF) or high fat diet with 0.5% tea catechins (HF-C) for 16-17 weeks. We measured muscle mass, fat mass, and muscle cross-sectional area. [Results] HF feeding increased in visceral fat mass and decreased muscle mass, fat mass, and muscle cross-sectional area. In contrast, these changes were not observed in the mice fed HF-C. The fast-twitch muscle cross-sectional area in HF-C fed mice was increased as compared to HF fed mice. Furthermore, we found that phosphorylation of 4E-BP in HF-C fed muscle was significantly higher than in HF fed muscle. [Conclusions] Our results indicate that tea catechins could promote protein synthesis in the muscle. Recent study suggested that tea catechins improve insulin sensitivity according to anti-aging effect. Therefore it might be involved in the increase in insulin dependent protein synthesis in the muscle.

Keywords: sarcopenia, tea catechins, obesity

100. Screening of functional ingredient for preventing muscle atrophy

Takumi Nakagawa¹, Ayaka Takeuchi¹, Yuta Nagaïke¹, Akihito Morita¹, Naohisa Ogó¹, Akira Asai¹, Yasutomi Kamei², Shini Miura¹ (¹Univ. of Shizuoka, ²Kyoto Pref. Univ.)

[Aims] In step with the graying of the population, the muscle atrophy, due to aging, inactivity or bed rest, become an object of public concern. In order to achieve the health and longevity society, the elderly person have to prevent the muscle atrophy. Although exercise enhances the muscular mass, elderly person is not able to carry out exercise, furthermore, there are no participate improving the muscular weight. FOXO1 is a transcriptional factor whose expression in a skeletal muscle induces muscular atrophy. If we are able to inactivate FOXO1 using several compounds, it will prevent the muscle atrophy in the elderly person. In this study, we evaluate the compounds which inactivate FOXO1. [Method] We used the modified procedure of the yeast two hybrid system using GAL4 DNA binding domain (GAL4-DBD) and its binding nucleotide sequence (UAS sequence). In brief, HEK293 cells were transfected with GAL4DBD-FOXO1 and 9xUAS-firefly luciferase plasmids. At the end of culture, we measured the chemiluminescence using the dual-Glo luciferase assay system (Promega). FOXO1 activity was expressed as % of control treated with vehicle. [Results] In our assay, insulin, known as a hormone in activating FOXO1 and preventing the muscle atrophy, decreased the chemiluminescence less than 40 % of control. [Conclusion] We were able to establish the screening system. The two compounds inhibiting FOXO1 activity will be useful for preventing the muscle atrophy.

Keywords: muscle atrophy, FOXO1 activity, screening of inhibitors
101. Blue tomato derived tomatine suppressed muscle atrophy
Yuma Yoshida, Shuhei Umebayashi, Haruo Nukayama, Akihito Morita, Keiji Wakabayashi, Shinnji Miura

[Background, Purpose] Muscle aging is characterized by a decline in functional performance due to progressive decrease in muscle mass and causes the locomotive syndrome. Recently, it was reported that tomatidine included in the blue tomato attenuated muscle atrophy (J Biol Chem, 2014). However, tomatidine exists in a blue tomato as tomatine which is the glucoside of tomatidine. Therefore, in this study, we examined whether tomatine or extracts form blue tomato were suppress muscle atrophy. [Methods] C57BL/6J mice (7weeks old, male) were divided into four groups. Each groups of mice were orally administrated tomatine at 0, 30, 100, 300 mg/body weight, respectively. Tomatine was administrated at the beginning and 12 h of the fasting. These mice were sacrificed after 24h of fasting. The rate of protein synthesis was evaluated by SynSET method. In addition, the concentration of 3-methylhistidine in plasma, muscle weight, and the muscular fiber diameter were also measured. [Results, Discussion] Under fasting condition, the administration of tomatine or the extract from blue tomato increased the rate of muscle protein synthesis, decreased the concentration of plasma 3-methylhistidine, and increase muscular fiber diameter, suggesting that tomatine may prevented fasting induced muscle atrophy. Hydrolyzed extract showed similar effect on the fasted muscle. However, the toxicity of tomatine should be paid attention for using the extract from blue tomato as an anti-atrophic food.

Keywords: muscle atrophy, tomatidine, tomatine

102. Effect of increased muscle glycogen on the expression of Ca2+-ATPase in the sarcoplasmic reticulum of rat skeletal muscle
Jun Shiromoto, Daiki Watanabe, Hajime Ohmori, Masanobu Wada

[Aim] A correlation between muscle glycogen and the Ca2+ handling function of sarcoplasmic reticulum (SR) during the post-exercise period was reported. This study aimed to clarify the effects of increased muscle glycogen on the expression of SR Ca2+-ATPase (SERCA). [Methods] Eight-week-old male Wistar rats were fasted for 24 h and randomly assigned to an exercise (E) group or sedentary (S) group. The E rats were made to run on a treadmill until exhaustion. After the exercise, the E rats were subdivided into an E-glycogen (EG) group and an E-fasting (EF) group. The EG rats were given 5% sucrose in water and a normal diet, whereas the EF rats were given water for 24 h post-exercise. The S rats were subdivided into an S-glycogen (SG) group and an S-fasting (SF) group. All rats were sacrificed, and the superficial and deep regions of m. gastrocnemius were excised at the end of the experiment. [Results] Glycogen concentrations was significantly higher in the muscles of the SG and EG groups than in those of the SF and EF groups. There was no significant difference in fatigue resistance, SERCA activity, or SERCA amounts. However, SERCA mRNA expression was significantly lower in the SG than in the EF group. [Conclusion] The increase in muscle glycogen did not affect the fatigue resistance in muscles or the SERCA expression. SERCA mRNA expression was reduced by the increase in muscle glycogen. The results suggest improved translational efficiency of mRNA or declined proteolytic rate.

Keywords: muscle glycogen, SERCA, skeletal muscle

103. Effects of cast immobilization on immune cell infiltration and inflammatory response in skeletal muscle
Noriaki Kawanishi, Shuichi Machida

[Background] Cast immobilization is known to induce muscle atrophy. Although autophagy and ubiquitin proteasomes play a major role in muscle atrophy, activation of these pathways is regulated by inflammatory cytokines such as TNF-alpha and IL-1beta. Inflammatory cytokines are mainly produced by immune cells (e.g., macrophages). Therefore, macrophage infiltration into skeletal muscles may play an important role in mediating the inflammatory response that leads to the development of muscle atrophy after cast immobilization. [Purpose] We examined the change in immune cell infiltration and the inflammatory response with time after cast immobilization. [Methods] The hindlimbs of C57BL/6J mice were immobilized. After immobilization for 1, 3, 7, or 14 days, muscle was collected from the anesthetized mice. Mononuclear cells from the gastrocnemius were isolated and the number of immune cells in the muscle was determined by flow cytometry. Furthermore, mRNA expression of the inflammatory cytokines was evaluated by real-time PCR. [Results] Macrophage and neutrophil infiltration into the skeletal muscle was enhanced 7 and 14 days after immobilization. Additionally, mRNA levels of TNF-alpha and IL-1beta in the muscle was elevated 7 and 14 days after hindlimb immobilization. In days 1 and 3 post-immobilization, increase in macrophage and neutrophil infiltration and upregulation of the cytokine mRNA were not observed. Our findings indicate that immune cell infiltration and inflammatory response were enhanced in skeletal muscles after hindlimb immobilization for 7 days.

Keywords: Muscle atrophy, Macrophage, Inflammation

104. Physical inactivity accelerates disuse muscle atrophy in the rat soleus muscle

[Aims] This study aimed to examine the effect of long-term physical inactivity on hindlimb unloading-induced muscle atrophy in the rat soleus muscle. [Methods] Three-week-old male Wistar rats (n = 36) were randomly assigned into control (CON, n = 18) and physical inactivity (IN, n = 18) groups. Rats in the IN group were housed in a small cage with half of the usual floor space to limit their range of movement. After 8 weeks, the rats were exposed to hindlimb unloading. The soleus muscles were removed before (PRE, n = 6/group) and after 3 days (3d, n = 6/group) and 7 days (7d, n = 6/group) after unloading. [Results] The soleus muscle weights at 3 and 7 days were decreased significantly in the IN group compared with the CON group. Although nuclear accumulation of HDAC4 and HDAC4 mRNA were significantly increased at 3d in both CON and IN groups, its downstream Myogenin and MyRF1 mRNA levels were upregulated by physical inactivity. Moreover, upregulation of Gadd45α mRNA expression levels tended to be induced by physical inactivity. There were no significant differences in the MyoD and Myogenin-1 mRNA expression levels among the groups. [Conclusion] Physical inactivity accelerates hindlimb unloading-induced disuse muscle atrophy in the rat soleus muscle mediated by the upregulation of muscle atrophy-related Myogenin and MyRF1 gene expression.

Keywords: activity restriction, histone deacetylase, histone modification
105. The time-course of intracellular signaling in response to exercise preconditioning during unloading-induced rat soleus muscle atrophy
Ikumi Yoshihara1, Toshinori Yoshihara1, Hisashi Naito1, Shuichi Machida1 (Juntendo Univ., Inzai, Japan)

[Aims] We assessed the effect of a bout of preconditioning endurance exercise on the time-course of the Signal Transducers and Activator of Transcription 3 (STAT3) signaling in the rat soleus muscle. [Methods] Forty male Wistar rats (11 wks.) were divided into two groups: sedentary control (CON, n = 20) and preconditioning exercise (EX, n = 20) groups. The rats in both groups were subjected to hindlimb unloading (HU) for 7 days. The EX group rats performed a bout of treadmill running for 30 min (25 M/m at 0° incline) immediately prior to HU. The soleus muscles were removed before (PRE) and 1, 3 and 7 days (n = 5/group) after HU. The phosphorylation ratio of STAT3 signaling was analyzed by Western blotting. [Results] Two-way analysis of variance revealed significant effects of HU and preconditioning exercise on upstream ERK1/2 phosphorylation at Tyr202/Tyr204; however, no significant effect of EX was observed during disuse. Although no significant change in the phosphorylation of STAT3 (Tyr705) in either the cytosolic or nuclear fraction was observed during HU, a significant interaction and main effect of HU and HU was found to significantly affect the STAT3 phosphorylation at Tyr727; however, no significant effect of EX was observed during disuse. Although no significant change in the phosphorylation of STAT3 (Tyr705) in either the cytosolic or nuclear fraction was observed during HU, a significant interaction and main effect of HU and preconditioning exercise on upstream ERK1/2 phosphorylation was observed. We also found a significant increase in ERK1/2 and p70S6K (Thr421/Ser424) phosphorylation in the EX group at PRE compared with the CON group. [Conclusions] Preconditioning endurance exercise attenuates disuse muscular atrophy, but the protective effect is not mediated by STAT3 signaling activation.

Keywords: disuse muscular atrophy, signal transducer, STAT3

106. The effects of polyamin to skeletal muscle
Tetsuo Ohno1, Toshiko Yamazawa1, Maki Yamaguchi1, Makiko Okhido2 (Dept Physiol., The Jikei Univ. School of Med., Tokyo, Japan, 2Dept Mol.Biol, The Jikei Univ. School of Med., Tokyo, Japan)

[Background and Purpose] Now a days, aspiration and falls are increasing because of sarcopenia accompanying with aging. Polyamins are biosynthesized in all cell and taken by food. They are thought to be physiologically active substances, but their functions are not cleared yet. Some studies show that some polyamin-related enzymes have inhibitory effects against to the muscle atrophy. While the chemical substances that lead to myopachynsis have serious side effects, it was thought that biosynthesized polyamins have little side effects. Then, to clear the physiological function of polyamins, we observed the effects to the differentiation of myocytes (C2C12) into myotubes, and increasing intake of polyamins leads to myopachynsis in adult mouse. [Methods] The myocytes (C2C12) differentiate into the myotubes in the medium with or without polyamin. After three days from the beginning of differentiation, the volume and the number of differentiated myotubes were measured with fluorescence microscope. By the electrophoretic analysis, the ratio of myosin subtypes in collected myotubes was calculated. The mice were raised with poliamine (+/-) water. After 4 weeks, we observed the cross-sectional area of triceps surae muscle with microscope, and the ratio of myosin subtypes of these muscles. [Results and Discussion] Polyamins promote the differentiation of myocytes (C2C12) into myotube, and do not affect the ratio of myosin subtypes. Polyamins do not lead to myopachynsis nor varied the ratio of myosin subtypes.

Keywords: skeletal muscle, atrophy, myosin subtype

107. IL-15 is a muscle contraction-induced myokine
Yasuro Furuchi1, Yasuko Manabe1, Kaede Miyata2, Honami Satoh1, Mayumi Takagi1, Miho Aoki1, Nobuharu Fujii1 (Tokyo Metropolitan Univ., The University of Tokyo)

[Aims] Skeletal muscles produce bioactive proteins, termed myokines. Although myokine secretion is believed to be regulated in response to muscle contraction, no experimental evidence exists to support this. Here, we provide evidence of acute contraction-induced myokine secretion, using a cell culture system. [Methods] C2C12 myotubes were placed in an electrical stimulation apparatus and stimulated for 1 hour. After contraction, secreted proteins in the conditioned media, such as IL-6, which is the best-known regulatory myokine, were analyzed by western blotting. [Results] We found that abundant proteins are released from myotubes when the culture medium is changed, and these proteins mask the contraction-induced myokine secretion. As long as the abundant proteins are eliminated, the acute regulation of IL-6 secretion can be detected. We observed that secretion of IL-15 was increased by 1-hour contraction, induced by electrical stimulation without cell damage. Contraction-induced IL-15 secretion was completely abolished by Bts (N-benzyl-p-toluene sulfonamide), which is a specific inhibitor of myosin ATPase. [Conclusions] We established an experimental condition for cultured muscle cell contraction to study the myokine secreteme and demonstrated that IL-15 is an acute muscle-contraction-induced myokine.

Keywords: Skeletal muscle, Muscle contraction, Myokine

108. Different responses of signal phosphorylation, metabolome and transcriptome to low and high frequency electrical stimulation in C2C12 myotubes
Daisuke Hoshino1, Katsuyuki Kunida1, Takumi Wada1, Atsushi Hatano1, Katsuyuki Yagi1, Tomoyoshi Soga2, Yutaka Suzuki1, Shinya Kuroda1 (The University of Tokyo, Keio University)

[Background and Purpose] Now a days, aspiration and falls are increasing because of sarcopenia accompanying with aging. Polyamins are biosynthesized in all cell and taken by food. They are thought to be physiologically active substances, but their functions are not cleared yet. Some studies show that some polyamin-related enzymes have inhibitory effects against to the muscle atrophy. While the chemical substances that lead to myopachynsis have serious side effects, it was thought that biosynthesized polyamins have little side effects. Then, to clear the physiological function of polyamins, we observed the effects to the differentiation of myocytes (C2C12) into myotubes, and increasing intake of polyamins leads to myopachynsis in adult mouse. [Methods] The myocytes (C2C12) differentiate into the myotubes in the medium with or without polyamin. After three days from the beginning of differentiation, the volume and the number of differentiated myotubes were measured with fluorescence microscope. By the electrophoretic analysis, the ratio of myosin subtypes in collected myotubes was calculated. The mice were raised with poliamine (+/-) water. After 4 weeks, we observed the cross-sectional area of triceps surae muscle with microscope, and the ratio of myosin subtypes of these muscles. [Results and Discussion] Polyamins promote the differentiation of myocytes (C2C12) into myotube, and do not affect the ratio of myosin subtypes. Polyamins do not lead to myopachynsis nor varied the ratio of myosin subtypes.

Keywords: skeletal muscle, atrophy, myosin subtype

Keywords: electical stimulation, C2C12, omics
109. **Chronic exercise with diet restriction improves metabolic functions of skeletal muscle and prevents diabetes in WBN/Kob-Fatty rats**

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**Aim:** Leptin-receptor-deficient WBN/Kob-Fatty (WKF) rat was developed for a model of chronic pancreatitis and diabetes with obesity. We recently reported that hyperlipidemia, insulin resistance, and pancreatic dysfunction could be more improved by chronic exercise (CE) with diet restriction (DR) than the DR alone. The purpose of this study was to investigate whether metabolic improvement in skeletal muscle contributes retard of development of diabetes. **Methods:** Male WKF rats (age, 6 weeks) were divided into a fatty-obese, a fatty-DR, and a fatty-exercise groups. Lean WBN/Kob rats were used as control. Food intake of fatty-DR and fatty-exercise groups was restricted to 69% and 70% of the fatty-obese group, respectively. The exercise was voluntarily wheel running. **Results:** After 6 weeks of intervention, the CE increased protein expressions which associated with glucose uptake (glucose transporter 4) and phosphorylation (hexokinase II) in skeletal muscle. And the CE increased protein expressions of mitochondria biomarkers (cytochrome c oxidase IV and heat shock protein 60) and autophagy-related protein (LC3-II). Moreover, the CE inhibited FoxO3 signal via an enhancement of PGC-1α protein expression. The effects of the DR alone were generally lesser than those of the CE with DR. **Conclusion:** We concluded that the CE with DR has beneficial effects than the DR alone, and that metabolic properties of skeletal muscle may contribute to development and prevention of diabetes. **Keywords:** WBN/Kob-fatty rat, skeletal muscle, metabolic function

110. **The effects of high fat diet-induced obesity and exercise training on nNOS expression and DNA methylation levels in skeletal muscle**

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[Aims] Skeletal muscle (SM) neuronal nitric oxide synthase (nNOS) is involved in glucose homeostasis. While nNOS in type 2 diabetic SM are undetectable, those in obese were comparable to control SM. Thus, it suggested that the importance of early intervention for glucose homeostasis. However, the epigenetic regulatory mechanisms of nNOS expression when exercise intervention during the obesity is unclear. The aim of this study was to determine the effects of high fat diet (HFD) -induced obesity and exercise training on nNOS expression and DNA methylation levels, which is major epigenetic modification, in SM. **Methods** We used twenty C57BL/6J male mice (6 weeks old). Mice were divided into three groups; 12 weeks of control diet (C), or HFD (H), and 12 weeks of HFD combined with wheel running for last 6 weeks (HE) groups. We measured nNOS protein and gene expression, and DNA methylation levels from extensor digitorum longus (EDL) and Soleus (Sol) muscles. **Results** nNOS were decreased in HE group compared with H group in Sol, (p < 0.05), but unchanged in EDL. nNOS gene expression levels corresponded with those of protein expression levels in EDL, but not in Sol. There were no significant differences of DNA methylation levels in EDL and Sol between three groups. **Conclusions** These results suggested that nNOS expression levels in SM in HFD-induced obese and exercise training were not regulated by DNA methylation. **Keywords:** nNOS, high fat diet, epigenetics

111. **Effect of aerobic exercise on compensatory skeletal muscle hypertrophy**

Guleng Siri\(^1\), Teruhiko Koike\(^1,2\), Yukie Natsume\(^2\), Shintaro Iwama\(^2\), Yoshiharu Oshida\(^1,2\) (\(^1\)Univ. Nagoya, Nagoya, Japan, \(^2\)Univ. Nagoya, Nagoya, Japan)

[Aims] To examine the effect of aerobic exercise on overload-induced skeletal muscle hypertrophy and its protein anabolic signaling pathway. **Methods** Male C57BL mice were randomly divided into three groups: rest group (REST), low-intensity aerobic exercise group (LOW) and high-intensity aerobic exercise group (HIGH). Mice in the exercise groups were assigned to run 30 mins/day and five days/week for four weeks at a speed of 10m/min (Low) or 25m/min (HIGH). Then, a gastrocnemius muscle of a right hind leg was surgically removed to overload plantaris and soleus muscles, and the left hind leg was sham-operated. **Results** Both plantaris and soleus muscles grew larger in the overloaded limbs compared to the sham-operated limbs. Both at one week and two weeks during overload, the muscle growth in plantaris muscles in the mice of the LOW group increased compared to the muscle growth in REST or HIGH group. The enhancement effect observed in plantaris muscles was not found in soleus muscles. Consistently, we observed the changes in the anabolic intracellular signaling containing Akt, mechanistic target of rapamycin (mTOR), and p70S6 kinase in plantaris muscles but not in soleus muscles. The protein expression of AMP-activated protein kinase (AMPK), forkhead box protein O1 (FoxO1), and muscle RING-finger protein-1 (MuRF1) decreased in the LOW group in plantaris muscles. **Conclusions** Chronic low-intensity aerobic exercise can enhance overload-induced muscle growth. **Keywords:** aerobic exercise, skeletal muscle hypertrophy, protein synthesis

112. **Atrophy-induced change in skeletal muscle phospholipid profile and involvement of FOXO1**

Nanami Senoo\(^1\), Eri Kobayashi\(^1\), Noriyuki Miyoshi\(^1\), Akihiro Morita\(^1\), Yasutomu Kamei\(^2\), Shinji Miura\(^1\) (University of Shizuoka, Kyoto Prefectural University)

[Aims] Phospholipid fatty acid composition is associated with the skeletal muscle physiological phenotype. However, the molecular mechanism or physiological role of the changes induced by atrophy remains unclear. Forkhead box O1 (FOXO1) is up-regulated under several pathophysiological atrophic conditions, which consequently induce muscle degradation. This study reports an association between atrophy and phospholipid profile, and the role of FOXO1. **Methods** Comprehensive phospholipid analyses of glycolytic and oxidative muscles were performed using three types of atrophy mouse models, denervation, fasting, and mdx mouse models, and genetically modified mice overexpressing FOXO1. [Results] Atrophy induced a decrease in 22:6-containing phospholipids and an increase in 18:2 and 20:4-containing phospholipids in the skeletal muscle. FOXO1 overexpression also decreased 22:6-containing phospholipids. Denervation, fasting, and FOXO1 overexpression suppressed the expression of long-chain acyl-CoA synthetase (ACSL6), which preferentially incorporates 22:6 into phospholipids. **Conclusions** We revealed that 22:6-containing phospholipids decreased during atrophy, and FOXO1 and ACSL6 may partially explain these changes. **Keywords:** lipidomics, phospholipids, transcriptional factors
113. Chronological change in muscle damage and inflammation in early onset muscle soreness during prolonged exercise

Katsuyuki Tokinoya, Keisuke Ishikura, Son-Gyu Ra, Yasuko Yoshida, Jun Shimoto, Kai Aoki, Shohiei Morita, Atsushi Aoyagi, Yoshitaru Nabekura, Kazuhiro Takekoshi, Hajime Ohmori (Univ of Tsukuba, Tsukuba, Japan, Sojo Univ, Kumamoto, Japan, Fukui Univ, Fukui, Japan)

[Introduction] We named the onset of muscle soreness during or immediately after prolonged exercise, such as a full marathon “early onset muscle soreness (EOMS)”. It is unclear when EOMS is generated and the relationship between muscle damage, inflammation, and EOMS. [Aim] We investigated the relationship between muscle damage, inflammation, and EOMS during a 30-km run. [Methods] Ten male subjects who regularly exercised were recruited. A numeric rating scale (NRS) that estimated muscle soreness in lower limbs and trunk, serum creatine kinase, lactate dehydrogenase (LDH) activity, white blood cells (WBC), muscle damage, and inflammation markers were measured at 0, 10, 20, and 30 km during a 30-km run. [Results] Each part of NRS was significantly increased at 10 or 20 km. LDH was significantly increased at 20 and 30 km. An increase in LDH isozymes (LDH3, LDH4, and LDH5) at 20 and 30 km suggested that these isozymes were mainly derived from skeletal muscle. WBC count started to significantly increase at 20 km. [Conclusion] The results suggest that EOMS and muscle damage markers start to increase at 20 km during a 30-km run. Moreover, inflammation might occur during prolonged exercise.

Keywords: 30-km run, LDH isozymes, white blood cell

114. Effect of endurance training and high-fat diets on lipid molecular species in rat skeletal muscle

Kana Takagi, Noriaki Kawanishi, Daiki Nakano, Hyeon-Cheol Lee, Toshiaki Okuno, Takehiro Yokomizo, Shuichi Machida (Juntendo Univ, Japan)

[Aims] In diabetic patients, intramuscular lipid accumulation is associated with insulin resistance. Excess intramuscular lipid accumulation in endurance-trained athletes is also observed. The difference in intramuscular lipid molecular species between obese patients and athletes is unknown. We hypothesized that the difference in skeletal muscle insulin resistance might be associated with the lipid accumulation and its fatty acid composition. [Methods] Sprague-Dawley rats were randomly assigned to three groups that received a sedentary control, high-fat diet (HFD), or endurance exercise training (TR). Rats in the TR group ran on a treadmill for 120 min/day; 5 days/week for 8 weeks. Lipidomic analysis of the soleus muscle was conducted using liquid chromatography-tandem mass spectrometry. [Results] Total diacylglycerol (DAG) concentration in the soleus was increased by exercise training. Importantly, exercise training increased the concentration of DAG molecular species containing palmitoleic acid. Similarly, total DAG concentration in the muscle was increased by HFD; however, in contrast with the pattern observed in exercise-trained muscle, DAG molecular species containing palmitoleic acid was decreased by HFD. [Conclusions] Our results indicate that even though DAG is accumulated in both exercise-trained and obese rat muscles, they exhibit distinct DAG fatty acid compositions.

Keywords: Intramyocellular lipid, Endurance exercise training, Lipid molecular species

115. Acute heat stress stimulates protein and glycogen metabolism in rat skeletal muscle

Ayumi Goto, Tatsuro Egawa, Keiichi Sekine, Rieko Oshima, Ichika Sakon, Satoshi Tsuda, Tatsuya Hayashi (Kyoto University, Kyoto, Japan)

[Aims] Heat stress (HS) has been implicated in the regulation of whole-body glucose homeostasis. However, there have been no reports about the effect of acute HS (<30 min) on glycogen and protein metabolism in skeletal muscle. The purpose of this study was to investigate the effect of acute HS on glycogen and protein metabolism using rat skeletal muscle. [Methods] Rat epitroneal muscle was isolated and incubated in the absence or presence of HS (42°C, 10 or 30 min) in Minimum Essential Medium α-based buffer. [Results] HS for 10 min activated both AMPK α 1 and AMPK α 2. HS increased 3-O-methyl-D-glucose (3MG) transport, and the stimulatory effect of 3MG transport was inhibited by dorsomorphin (AMPK inhibitor). HS decreased glycogen content and activated glycogen synthesis without affecting the phosphorylation of glycogen synthase kinase 3β or glycogen synthase. HS tended to decrease protein synthesis, and correspondingly, HS decreased the phosphorylation of p70 ribosomal protein S6 kinase and 4E-binding protein 1. On the other hand, HS did not affect the RNA expression of muscle-specific ubiquitin ligases: muscle atrophy F-box/atrogen-1 and muscle ring finger 1. [Conclusion] Acute HS might be a physiologically relevant stimulus that promotes the glucose transport/glycogen synthesis axis and inhibit protein synthesis, at least in part by activating AMPK in skeletal muscle.

Keywords: skeletal muscle, heat stress, glycogen metabolism

116. Bone mineral content, bone mineral density and flow-mediated dilation in regularly trained middle aged women

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Background and Aim: The association between the FMD and BMC & BMD was not entirely understood yet. Methods: Thirty-five postmenopausal middle-aged (66±6.1 years) regular swimmers and Nordic walkers were volunteered as subjects. All FMD measurements were performed by the same operator using the UNEXEF38G. BMC and BMD at the distal radius and muscle cross-sectional area at the mid-radius were measured by DXA and MRI, respectively. Body composition was estimated by InBody 430. Results: From the results of FMD measurement, subjects were divided into two groups; namely FMD normal group (n=17: %FMD>6.0%) and FMD deteriorated group (n=18: %FMD<6.0%). The FMD deteriorated group was significantly 4.6 years older than the FMD normal group. So that ANCOVA with the age as covariate was applied when appropriate. There was no significant body weight difference between the two groups, however, muscle CSA at the mid-femur was significantly greater in the FMD normal group than the FMD deteriorated group (p=0.029). Although there was no significant difference in the two groups, LBW was greater in the FMD normal group than in the FMD deteriorated group. The same tendency was observed in BMC & BMD at distal radius for both right and left hands (p=0.07~0.201). Conclusions: It would be said that less muscle CSA and BMC at distal radius may relate to the deterioration of FMD in postmenopausal women. Further studies, including DXA measurement of common place such as lumber spine and proximal femur, association of eating habit and exercise effects, are expecting.

Keywords: BMC, FMD, postmenopausal women
117. Relationship between ball speed and movement velocity of the swing leg in soccer players

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[Aim] In soccer, ball speed is one of the most important factors affecting the success rates of shooting and passing. The purpose of this study was to reveal the relationship between ball speed and movement velocity. [Methods] Sixty-one amateur male soccer players participated in this study. We measured maximal ball speed, movement velocity, muscle strength, and flexibility. Movement velocity was determined by measuring the maximal angular velocity without external resistance during hip flexion and knee extension as single joints, and in the whole lower limb (multiple joints). Muscle strength was measured by determining the maximal isometric strength during hip flexion and knee extension. [Results] Ball speed was found to be significantly correlated with the movement velocity of multiple joints (r = 0.46, p<0.01). However, no significant correlations were observed between ball speed and the other factors (the movement velocity of single joints, muscle strength, and flexibility). [Conclusions] These results suggest that the movement velocity of multiple joints is important for ball speed, but the movement velocity of single joints, muscle strength, and flexibility are not related to ball speed. We consider that the coordination between multiple joints is important for ball speed.

Keywords: soccer, ball speed, movement velocity

118. Study on effects of clenbuterol enantiomer on leg bone of male rats

Takashi Kitaura1 (*Inst. Liberal Arts and Science, Univ. Kanazawa, Ishikawa, Japan)

[Aims] Clenbuterol (Cb) is one of the β2 adrenergic receptor agonists with powerful anabolic effects and is prohibited to use as doping drug for athletes. Previously we have reported that Cb inhibited the longitudinal growth of bones in young male rats. But the mechanism of the inhibitory effect was unclear. Generally Cb is manufactured as a 1:1 racemic mixture of (+)-R-Cb and (+)-S-Cb isomers and it is believed that only (-)-R-Cb is pharmacological activity. The (+)-S-Cb is described as responsible for the mimetic effect on β2-receptors while the (-)-R-Cb reveals a blocking effect on the β2-receptors. This study was to make clear the effects of these enantiomers on the bone. [Methods] Eighteen male Sprague-Dawley rats (8-wk-old) were randomly assigned to either a control (Con, n = 6) or two Cb groups ((+)-S-Cb: n=6, (-)-R-Cb: n=6). Both Cb enantiomers of 2 mg/kg body wt/d were administered subcutaneously for 2 wk. After treatment, the femurs (FE) and tibiae (TI) bones were analyzed. The bone lengths were measured with the Vernier calipers and the BMD of FE and TI were measured by DXA. [Results] The length of bones showed no significant difference except TI of (+)-S-Cb (-1.2%). The BMD of FE decreased in both (+)-S-Cb (-5.8%) and (-)-R-Cb (-8.2%). [Conclusions] These results suggest that the two Cb enantiomers showed different effects on bone of young male rats. It might be indirectly affected by hormonal control of hypothalamus. It is necessary to carry out more experiments to explain the specific adaptation mechanism and to use the drug effectively.

Keywords: clenbuterol enantiomer, femur, tibia

119. Effects of electrical stimulation of denervated rat skeletal muscle on trabecular bone and osteoid formation

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[Aims] The goal was to examine the effects of electrical stimulation-induced muscle contractions (ESMC) on the histological profiles in trabecular bone architecture and osteoid formation after sciatic denervation. [Methods] Direct ESMC was performed on the tibialis anterior muscle after denervation in seven-week-old male rats divided into groups as follows: control (CON); denervation (DN); and denervation with direct ESMC (DN+ES). ESMC was performed at an intensity of 16 mA and a frequency of 10 Hz for 30 min/day, six days/week, for one week. The metaphyseal trabecular regions of the tibiae were analyzed using 3D micro Computed Tomography (CT) and histomorphometry. [Results] Marked trabecular bone loss in tibiae was evident in the DN rats. Trabecular bone volume fraction thickness (Tb.Th); connectivity density and osteoid thickness (O.Th); number of osteoid-osteocytes (Os); and the area of DMP1 immunoreactivity were greater in DN+ES compared with DN rats one week after denervation. Tb.Th showed significant linear correlations with O.Th and the number of Os (r=0.001). [Conclusions] These findings suggest that the beneficial effects of ESMC on osteoid formation related to trabecular bone architecture may be associated with mechanical stress applied to bone in the denervated rat hind limb.

Keywords: electric stimulation, muscle contraction, osteoid-osteocyte

120. The relationship between toe flexor strength and explosive power performance in American football players

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[Aims] The purpose of this study was to investigate the relationship between toe flexor strength (TFS) and explosive power tests in American football players. [Methods] Twenty-four American football players were participated in this study. Maximal voluntary strength of TFS and squat were measured as a parameter of muscle strength. 40-yds sprint, three-points-set 10-yds sprint, pro agility and standing forward jump were measured as an explosive power parameter. [Results] There were no significant correlations between TFS and explosive power tests. However, relative TFS (adjusted by body mass: rTFS) was significantly correlated with all explosive power tests (40-yds sprint, r=0.625, p<0.01; three-points-set 10-yds sprint, r=-0.584, p<0.01; pro agility, r=-0.692, p<0.001; standing forward jump, r=0.582, p<0.01). In addition, relative squat (adjusted by body mass) was significantly correlated with all explosive power tests too. After partial correlation analyses adjusting for relative squat, it was showed that rTFS was significant correlating factor of 40-yds sprint (r=-0.417, p<0.05) and pro agility (r=-0.426, p<0.05). On the other hand, relative squat was not related any explosive power tests after partial correlation analyses adjusting for rTFS. [Conclusions] These results suggest that rTFS was associated with explosive power performance in American football players.

Keywords: toe flexor strength, American football, explosive power
121. Study on structural changes of tibial articular cartilage by mechanical loading in rats

Shota Kusaka¹, Masato Takahashi¹, Tsuyoshi Kan’o¹, Shingo Nakai¹, Masafumi Ohsako² (Welfare Social Design. Toyo, Japan., Life design. Toyo, Japan.)

[Aims] The purpose of this study was to investigate an effects of various mechanical loadings on articular cartilage in rats. [Methods] Seven weeks old rats (wistar strain, male) were used as materials and were divided into jump group (EX) and control (CO). Furthermore, EX was divided into three subgroups (1)30% of the maximum jump height (E30), (2)45% of the maximum jump height (E45), (3)90% of the maximum jump height (E90). And, rats were jumped 100 times/day, five days/week for 7, 14, and 21 days. CO were fed normally in same periods as EX. Tibiae in each group were excised from them under euthanization and those specimens were observed histologically. [Results] Thickness of an articular cartilage was thin at CO-7, but it increased with growth. Cell density was high in CO-7 and it decreased with growth. Decrease in the cell density and increase in the thickness of articular cartilage in EX-45 and EX-90 were recognized after 7 days. In addition, the cell density of the EX-90 was lower than the CO and the cells were lined neatly in a vertical direction. As for results of Masson’s trichrome staining, the EX-90 were lower than the CO and the cells were lined neatly in a vertical direction. [Conclusions] It was thought that thickness of articular cartilage and the matrix fibers in deep layer increased accompanied with increase of mechanical loading, and these gave an enhancing of resistance to a mechanical loading.

Keywords: articular cartilage, structure, jump training

122. Study on structural changes of femur and characteristics of fracture line in growing rats

Shingo Nakai¹, Shota Kusaka¹, Tetsuro Suzuki², Masafumi Ohsako² (Grad sch. Welfare Society Design, Toyo univ., Faculty Human Life Design, Toyo univ.)

[Aims] A purpose of this study was to investigate a relationship between a fracture line derived from three-point-bending test and a bone structure in growing rats. [Methods] Sixty male rats (wistar strain, 3,5,7,9 and 13-week-old) were used as materials, and their femurs were excised after euthanization. Their right femurs were immersed in fixation fluid. Their left femurs were immersed in the same fluid immediately after broken by an apparatus for three-point-bending test. The samples in each group were observed histologically and structural characteristics of the breaking portions were compared. [Results] When classifying the bones from the point of view of the densities and the arrangements of the bone matrix fibers, the bones that showed different maturation degrees existed in a same cortical bone at the younger stage. Loose bone matrix fibers arranged irregularly in the immature bone. However, the arrangements of dense matrix fibers of the cortical bone were regular in the case of a matured bone and formed the lamellar structure. Many lamellar bones were formed gradually according to growth. Several cracks appeared around the fracture line and those cracks spread along each lamellar bone, by the bone breakage with the three point bending test. [Conclusion] It was understood that the cracks in the cortical bone spread from the fracture lines by the bone breakage were related to the bone structure and their maturation.

Keywords: growth phase, bone matrix, bone fracture

123. Study on site-differences of fiber structures of articular capsule and effects of immobilization on them in rat’s knee joint

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[Aim] Purpose of this study was to investigate the effects of immobilization of hind limbs on fiber structures of whole articular capsules, using knee joints of rats. [Methods] Twenty-four rats (6-week-old) were used as materials and they were divided into immobilization group (IM) and control(CO). Knee joints of IM were maintained at the angle of 90 degree for three weeks, using an apparatus for immobilization. Rats of CO were fed ordinarily, for same period of IM. Rats of both groups were euthanized, their knee joints were excised. Those articular capsules were observed macroscopically and morphologically. [Results]Adipose tissues occupied widely in the synovial fold in CO but they decrease and the folds also decreased. Most of fiber bundles arranged in oblique direction and surround the knee joint. A density of those fiber bundles in IM was higher than CO. A structural changes of infrapatellar fat body were observed at anterior face of the articular capsules in IM. [Conclusion] Increase in density of matrix fiber bundle, decrease in the synovial folds and structural changes of corpor adiposum infrapatellare were recognized and it was suggested, from these facts, that the immobilization or inactive daily life could also cause obstacles of motion other than knee extension and flexion.

Keywords: Articular capsule, Immobilization, Histological structures

124. Effect of tail suspension on osteogenic process of patellae in rats

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[Aims] The purpose of this study was to investigate effects of the inactive condition that was caused by the tail suspension on osteogenesis of the patella. [Methods] Seven-week-old rats (wistar strain, male, n=36) were divided into the tail suspension group (TS) and control group (CO) randomly. Tail was suspended in TS and rats of CO were normally for two weeks. Rats of both groups were euthanized and their patellae were excised at the end of the experimental period. Those specimens were analyzed and observed histologically. [Results] An articular cartilage existed at the posterior face (femur side) of the patella. Periosteum didn’t exist at the anterior face (surface side) of the patella and this portion was covered with a tendon and an aponeurosis of femoral quadriceps. Tendon fibers of the femoral quadriceps were embedded, as Sharpy’s fibers, at the proximal portion of the patella, and the fibers of the patella ligament were embedded in the distal portion of that. The fiber bundles of the tendon and the ligament of TS were thinner than that of CO. A fibrocartilage existed near the attaching portion of those fiber bundles and they were thicker in TS compared to CO. Trabecular bones of the cancellous bone in TS was thinner and lower dense than CO. [Conclusions] It was understood that the patella wasn’t the bone that resisted to mechanical stress like a limb bone but it showed an embrittlement by decrease in tractive forces from the muscle and ligament.

Keywords: patella, tail suspension, histological structure
125. Study on the healing process after bone injury of tibias and patellae in rats

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[Aims] We aimed to investigate effects of the existence or non-existence of the periosteum on healing processes after bone damages morphologically, using tibias and patellae of rats. [Methods] Twenty four male rats (wistar strain, seven-week-old) were used as materials, and they were divided into three groups (bone injury group: BG, periosteum or aponeurosis damage group: PAG and control group: CO). Their right legs were used for the patella observations, and their left legs were used for observation of the tibial diaphyses. The skin and the aponeurosis or the periosteum were incised in BG and PAG, and small holes were formed at the surface of the patella and tibia by drilling in BG. The skin was sutured immediately after that surgeries in both groups. One week after the beginning of the experiment, the patellae and the tibias were excised from each group, and were observed morphologically. [Results] As for the tibia, thick bone trabeculas were formed in the small holes in BG, and they reached till the edge of the small holes. The cartilage-like tissue was formed just under the periosteum, and they fused with the bone trabeculas in the hole. As to the patella, thin bone trabeculas were formed in BG, and they were slightly fused with the existing cortical bone. The cartilage-like tissue was not formed at the entrance of the damaged portion. [Conclusions] It was suggested that the existence of the periosteum and cartilage-like formation at the periosteum was important to form the callus after the bone injury.

Keywords: patella, bone injury, periosteum

126. Effects of a high fat diet and treadmill running on bone tissue in young mice

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[Aims] Bone development is significant during the growth period; thus, lifestyle or exercise habits have profound effects on bone tissue in children. However, whether a high fat diet and exercise during childhood affects bone tissue is still unknown. Therefore, we investigated the effect of a high fat diet and treadmill running on bone tissue in young mice. [Methods] Four-week-old male C57BL/6J mice (n=24) were randomly assigned to four groups: standard diet control (STD, n=6), standard diet + exercise (STD+EX, n=6), high fat diet (HFD, n=6), or high fat diet + exercise (HFD+EX, n=6). Mice were fed either a high fat diet (32% fat) or standard diet (4.6% fat) over 16 weeks. Mice in the exercise groups performed treadmill running training (6-18 m/min for 30 min) 5 days/week for 16 weeks. After the experimental period, the femurs and tibiae were removed, and bone strength (N) was measured using the three-point bending test. Bone mineral density (mg/cm3) was measured using microscopic computed tomography (micro-CT). [Results] Body weight was highest in the HFD group at the end of the experiment (p < 0.05). However, the strength of the femur or tibia was not significantly changed. Similarly, there were no significant differences in bone mineral density of the femur or tibia after 16 weeks of a high fat diet and exercise training. [Conclusion] Our data suggest that a high fat diet and treadmill running for 16 weeks may not affect bone strength and bone mineral density in young mice.

Keywords: High Fat Diet, Treadmill Running, Bone

127. The sex difference in respiratory muscle work during maximal exercise with He-O2 breathing

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[Aims] Because women have smaller chest walls and narrower airways than men, pulmonary ventilation (Vₐ) during intensive exercise is mechanically limited, and it partly limits the maximal oxygen uptake (VO₂max) in women. It is well known that helium oxygen mixture gas (HeO₂) breathing which reduces the airway resistance can increase the exercise Vₐ. Thus, we tested the hypothesis that the effect of He-O₂ breathing during maximal exercise on respiratory response and respiratory muscle work would be greater in women compared to men. [Methods] Fourteen healthy men (n = 7) and women (n = 7) performed the incremental exercise test until exhaustion under ambient air (Air) and HeO₂ (20.9% O₂) conditions. During the exercise test, we measured VO₂max and Vₐmax. To mechanically assess the respiratory work, we calculated work of breathing (WOB) as the integrated area of the transpulmonary pressure-volume loop. [Results] Vₐmax was significantly higher in HeO₂ than in Air in both genders (p<0.05). The magnitude of increase in Vₐmax with HeO₂ breathing was greater in women than in men (p<0.05). WOB in HeO₂ was significantly lower than in Air only among men (p<0.05). Both in women and men, VO₂max was not different between Air and HeO₂ conditions. [Conclusion] Our findings demonstrated that the effect of HeO₂ breathing on maximal exercise Vₐ was greater in women than men. However, the respiratory muscles work decreased by HeO₂ breathing only in men.

Keywords: respiratory muscles, women, respiratory response

128. Effects of vocalization during intermittent exercise on ventilation and CBF

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[Aims] Kendo kaku-keiko with vocalization (about 80%VO₂max) significantly increased the value of FetCO₂ (=PaCO₂) compared with that without vocalization. This exercise may increase cerebral blood flow (CBF), because PaCO₂ is one of the vasodilatation factors. In this study, we investigated whether intermittent exercise with vocalization, not kendo, increased the value of FetCO₂ and CBF. [Methods] 8 male subjects participated in this study. They performed the intermittent exercise (20-sec, 8 times) at 80% and 60%VO₂peak using a cycle ergometer with (Voc) or without vocalization (non-Voc). We measured ventilatory (FetCO₂, etc) and CBF (cerebral blood flow) variables at before and just after exercise. [Results] In Voc, the value of FetCO₂ was significantly higher than that in non-Voc at 80%VO₂peak. The value of %change of CBF in Voc tended to be higher at both intensities, but more increased at 60%VO₂peak. [Conclusion] Intermittent exercise with vocalization at 80%VO₂peak, not kendo, increased the value of FetCO₂. But, the increase of PaCO₂ caused by vocalization during exercise did not necessarily affect CBF.

Keywords: vocalization, intermittent exercise, FetCO₂