MYONUCLEUS-RELATED PROPERTIES IN SOLEUS MUSCLE FIBERS OF *MDX* MICE

（***mdx*** マウスヒラメ筋線維における筋核関連特性）

Graduate School of Frontier Biosciences, Osaka University

Masahiro Terada

List of content

ABSTRACT

Distribution and total number of myonuclei in single soleus muscle fibers, sampled from tendon-to-tendon, were analyzed in *mdx* and wild type (WT) mice. Apoptotic myonuclei and microscopic structure around myonuclei were also analyzed. Three types of muscle fibers of *mdx* mice with myonuclear distribution at either central, peripheral, or both central and peripheral region were observed. All of the myonuclei were located at the peripheral region in WT mice. The total number of myonuclei in whole fiber was identical between *mdx* and WT mice and between fibers with different distribution of myonuclei in *mdx* mice and peripheral nucleus was noted where the central nucleus was missing. Fiber size, sarcomere number, myonuclear size, myosin heavy chain expression, satellite cell number, and neuromuscular junction were identical between each type of fiber. Apoptosis was not detected in any myonuclei located either central or peripheral region of fibers. Thus, it was suggested that apoptosis-related loss of central myonuclei and regeneration-related new accretion at the peripheral region is not the cause of the different distribution of myonuclei seen in muscle fibers in *mdx* mice. But it was speculated that cross-sectional translocation of myonuclei may be induced in response to regeneration from central to peripheral region, although microscopic evidence of any structures were not observed around myonuclei.

Key words: *mdx* mice, soleus muscle fibers, central and/or peripheral myonuclei, apoptosis

INTRODUCTION

Dystrophin-deficient (*mdx*) mouse is a well-known animal model for Duchenne muscular dystrophy (DMD). The muscles of *mdx* mice are characterized by cycles of muscle fiber necrosis followed by regeneration (5) . Further, the regenerating fibers can be easily identified, because they contain conspicuous internal (central) myonuclei (3). Williams *et al.* (24) reported that fibers with either a predominantly centrally located string of myonuclei, or a combination of central nuclei and peripheral pattern of nuclei appeared in soleus muscle fibers of *mdx* mice. However, it is unclear how the change of localization of myonuclei within a fiber is induced. It is not known whether the total number of myonuclei in a whole single muscle fiber response to necrosis/regeneration cycle, either.

It was reported that loss of myonuclei was associated with the atrophy of soleus muscle fibers (1, 2, 14). Oishi *et al.* (16) also reported that hindlimb unloading caused a significant decrease of myonuclear number in atrophied soleus muscle fibers of rats. They also observed many fibers with central nuclei at the early phase (day 3) of ambulation recovery period. However, the mechanism responsible for the relationship between the number and localization of myonuclei is still unclear.

Muscle satellite cells are myonuclear precursors lying between the sarcolemma and the basal lamina of myofiber (9), and myonuclear accretion occurs through the incorporation of satellite cell nuclei into the growing myofibers (13). Satellite cells also play one of the essential roles in the regeneration from injury (20) and growth of skeletal muscle (6). Kawano *et al.* (6) recently reported that the growth-associated increase of myonuclear number in a single fiber of rat soleus was less than the age-matched cage controls due to an inhibited elevation of satellite cell number,

suggesting a close relationship between satellite cells and myonuclei. However, role of satellite cells in the regulation of necrosis/regeneration cycle and translocation of myonuclei in muscle fibers of *mdx* mice is unclear. Therefore, the total numbers of both quiescent and mitotic active satellite cells, as well as the myonuclear number, were counted in whole fibers with various distribution of myonuclei in the present study.

Waerhaug & Lomo (22) reported that fast motoneurons form large ectopic junctions, whereas slow motoneurons form small ectopic junctions, concluding that the type of motoneuron, not muscle fiber, determines the fast or slow character of the neuromuscular junction. Changes in the distribution of acetylcholine receptors were also observed in muscle fibers of *mdx* mice (8, 11, 17, 21). However, it is not known whether the distribution or number of acetylcholine receptors is different between fibers with different localization of myonuclei. Therefore, the numbers and size of neuromuscular junctions (NMJs) were compared between fibers with different location of myonuclei in this study.

It is essential to develop a suitable method for stimulation of regeneration of damaged muscle. Therefore, the current study was performed to investigate the mechanism responsible for the cycle of necrosis and regeneration of muscle in *mdx* mice. The major purposes were to test the hypotheses that myonuclei in regenerating fibers translocate cross-sectionally from central to peripheral region, or that central myonuclei are lost due to apoptosis and new myonuclei are accreted at the peripheral region when the regeneration of fiber is advanced. The total number and location of myonuclei in single soleus muscle fibers, sampled from tendon-to-tendon, were measured. Apoptotic myonuclei were checked by using the terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) method. Further, the

existence of any evidence, which may help the translocation of myonuclei, was also investigated by analyses using electron microscopy.

MATERIALS AND METHODS

All experimental procedures were conducted in accordance with the Japanese Physiological Society *Guide for the Care and Use of Laboratory Animals* and also followed the guiding principles of the American Physiological Society. The study was also approved by the Animal Use Committee at the university.

Animal care and experimental procedures

Nine-week-old male C57BL/10-*mdx* Jic (dystrophin-deficient, *mdx*, n=10) and C57BL/6J Jcl (wild type: WT, n=10) mice were used in the present study. All mice were housed in a controlled environment with 12:12 hr of light:dark cycle and the temperature and humidity were maintained at ~23 ^{o}C and ~55%, respectively. Two to three mice were housed in a cage with 11 × 20 cm and 11 cm height. Solid food (CE-2, Nihon CLEA, Tokyo) and water were supplied *ad libitum*.

A single injection of 5-bromo-2'-deoxyuridine (BrdU, Sigma-Aldrich, 100 mg/kg body weight) in phosphate-buffered saline (PBS) was performed *i.p.* 2 days prior to the sampling. The mice were anesthetized by injection of sodium pentobarbital (5 mg/100 g body weight, *i.p.*). The soleus muscles were sampled bilaterally and cleaned of excess fat and connective tissue. For the longitudinal analyses of single fibers, the left muscles sampled from *mdx* and WT mice (n=5 each) were stored in cellbanker (Nihon Zenyaku, Tokyo) at −80 °C. The right muscles were stretched at an optimal length and pinned on a cork and frozen in isopentane cooled with liquid nitrogen. And

the mid-portion of the frozen muscle was mounted on a cork by using optimum cutting temperature (OCT) compound (Miles, IN, U.S.A) and frozen in liquid nitrogen for analyses of the expression of myosin heavy chain (MHC) and myonuclei with or without apoptosis, location of myonuclei in cross-sectional samples.

Further, soleus muscles were sampled bilaterally in 5 *mdx* and WT mice. The left muscles were frozen in liquid nitrogen for analyses of apoptotic myonuclei in longitudinal single fibers. For electron microscopic analyses, the right muscles of *mdx* mice were fixed with 2% glutaraldehyde in 0.1% cacodylate buffer at 4°C for 2 hr, rinsed with 0.1% cacodylate buffer solution, and postfixed in 2% osmium tetraoxide with 0.1% cacodylate buffer solution at 4°C for 2 hr. After rinsing with cacodylate buffer solution, the specimens were dehydrated in graded concentrations of ethanol and embedded in epoxy resin.

Longitudinal analyses

Myonuclei, satellite cells, and neuromuscular junction: The muscles stored in the cellbanker were thawed instantly at 35 °C. The muscles were placed in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 0.2% type I collagenase, 1% antibiotics, and 10% new-born calf serum (NCS) for 3 hr at 35 °C to digest the collagens. The collagenase-treated samples were fixed in 4% buffered-formaldehyde for 10 min and rinsed in PBS. Entire single muscle fibers were isolated from tendon-to-tendon using fine needles. The fibers were collected carefully using pipette to avoid any damage. The collected fibers were separated into tubes and immersed in DMEM containing 10% NCS.

For immunohistochemistry, tube No. 1 and 2 were used to label M-cadherin and

BrdU as described previously (23). The single fibers in these tubes were permeabilized with 1% triton X-100 diluted with PBS for 10 min. These fibers were, then, blocked in 10% goat serum diluted with PBS for 15 min at room temperature. The primary polyclonal antibody specific to M-cadherin (Santa Cruz Biolotechnology, Inc.) and monoclonal antibody specific to BrdU (Becton Dickinson) diluted at 1:50 with PBS containing 0.5% Tween 20 and 0.5% bovine serum albumin (BSA) was applied to tube No. 1 and 2, respectively. And the tubes were kept at 4 ^{o}C for at least 12 hr. The primary antibody was detected by secondary antibody reaction, which was performed for 2 hr at 4 ^{o}C, using goat anti-rabbit immunoglobulin G (IgG) conjugated to fluorescein (Chemicon International, Inc.) and goat anti-mouse IgG conjugated to fluorescein-isothiocyanate (Jackson Immuno Research) diluted at 1:100 with 0.5% Tween 20 and 0.5% BSA for tube No. 1 and 2, respectively. The nuclei (myonuclei + satellite cells) in the antibody-reacted fibers in the tube No. 1 and 2 were further counterstained with propidium iodide (PI, 10 μg/ml PBS) for 10 min. Fibers in tube No. 3 were incubated with α-bungarotoxin in PBS (1:100) for 10 min. The myonuclei were stained by PI for 10 min. After staining, the single fibers were rinsed with PBS and stored in tube filled with PBS at 4 °C until analyses. Immediately before the analysis, the fibers were mounted on a slide glass in 50% glycerol with coverslips with "struts" of hardened nail polish on the corners to minimize fiber compression.

Apoptotic myonuclei: The soleus muscles were gradually thawed to room temperature in a low-calcium relaxing solution with 50% glycerol after the soleus muscles were adapted to the solution at –4 °C overnight, as described previously (15). Single fiber segments (n=30 from each muscle) were mechanically isolated by using

fine tweezers under a dissection microscope, placed on gelatin-coated glass slide, and air dried. Mechanical isolation of single fibers has been shown to strip off the basal lamina of muscle fiber including satellite cells (15). Immediately, the apoptotic myonuclei were labeled using TUNEL method as described previously (1). Briefly, the fibers were fixed with 4% buffered formaldehyde for 1 hr and permeabilized in 0.1% sodium citrate containing 0.1% Triton X-100 for 5 min. Subsequently, the fibers were incubated in the reaction buffer containing terminal deoxynucleotidyl transferase (Roche Diagnostic) for 1.5 hr at 37°C. After the reaction, the fibers were rinsed with PBS and mounted in the medium containing 4', 6-diamidino-2-phenylindole (DAPI). The DAPI-labeled total myonuclei and TUNEL-positive apoptotic myonuclei in the double-stained section were observed under a confocal microscope.

Cross-sectional analyses

Myosin heavy chain expression and myonuclei: Four serial cross-sections (10 μm thickness) were cut in a cryostat maintained at –20 °C. Two sections were used for the analyses of MHC expression. The expression of MHC was analyzed by using monoclonal antibodies specific for slow (type I) or fast (type II) MHC isoform, i.e., primary antibody, NCL-MHCs and NCL-MHCf (Novocastra Laboratories, UK), as described previously (7). Briefly, the avidin-biotin immunohistochemical procedure was used for the localization of primary antibody binding according to the instructions for ABC kit (Vector Laboratories, Burlingame, CA, U.S.A.). The PBS was used as a buffer for all IgG primary antibodies. The visualization for primary antibody binding site was performed with diaminobenzidine tetrahydrochloride. The stained images were incorporated into a computer. Number of primary antibody positive fibers was

counted in the whole muscle cross-section stained with antibodies specific for slow or fast MHC. Fiber phenotypes were classified as type I, I+II, or II. The third section was stained for myonuclei using haematoxylin for 5 min at room temperature. The muscle fibers were matched with those in the first and second sections and MHC expression in fibers with or without central nuclei was determined.

Apoptotic myonuclei: The forth cross-sections were incubated in the reaction buffer added anti-laminin antibody (Sigma) diluted 1:200 with reaction buffer. Anti-laminin antibody was visualized with donky anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (Invitrogen) diluted 1:200 with PBS containing 0.5% Tween 20 and 0.5% bovine serum albumin (BSA). After the reaction, the section was rinsed with PBS and mounted in the medium containing DAPI. The apoptotic myonuclei were labeled using the same TUNEL method as mentioned above. The DAPI-labeled myonuclei and TUNEL-positive apoptotic myonuclei in the triple-stained section were observed under a confocal microscope.

Confocal microscopy

A FV-300 confocal microscope with an argon laser (488 nm of peak wavelength, Olympus) was used to analyze the fiber length, sarcomere number, fiber cross-sectional area (CSA), the number and distribution of PI- and TUNEL-positive myonuclei, and CSA of PI-positive myonuclei in the whole single fibers. The fibers with various distribution of PI-positive myonuclei were classified into three types as described previously (24); type C in which more than 80% of myonuclei were arranged as a straight chain in the central region of fiber, type P in which all myonuclei were

distributed at the peripheral region of fiber, and type C+P in which less than 80% of myonuclei were located at the central region and the residual myonuclei were located at the peripheral region (Fig. 1).

First, a maximum-intensity projection rotated orthogonally to the long axis of the fiber was produced from the stack, and the fiber CSA was measured at three non-overlapping regions randomly chosen along the fiber length. The PI-labeled nuclei were counted in the entire single fiber. Myonuclear domain size (10, 15) was also calculated by multiplying the fiber CSA and the fiber length and then dividing by the myonuclear number per fiber. The largest diameter of the myonuclei (at least 30 myonuclei in each fiber) was also measured. The fiber length and the length of 10 consecutive sarcomeres were also measured in each fiber by Normarski optic scan and the fiber CSA and the length of the fiber were normalized at a 2.5 μm sarcomere length. Sarcomere number in whole fiber was also calculated. The TUNEL-positive myonuclei in the entire single fiber and cross-section were also checked.

Electron microscopy

In the right soleus muscles of *mdx* mice, both longitudinal and cross-sectional ultra-thin sections were made with an OMU4 Ultracut microtome (Reichert, Vienna, Austria) and stained with 5% uranyl acetate and 1% lead acetate, 1% lead nitrate and 1% lead citrate. Structure around each myonucleus was examined using H-7650 transmission electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan).

Statistical analyses

Values were expressed as means ± SEM. Significant differences among groups were determined by two-way ANOVA, followed by Scheffé's post hoc test using StatView packaging software (HULINKS, Tokyo, Japan). Differences were considered significant at the 0.05 level of confidence.

RESULTS

Distribution of myonuclei, size and phenotype of fiber, sarcomere number, and NMJ. Fibers with central nuclei were observed in *mdx*, but not in WT, mice (Fig. 1). The percentages of type C, C+P, and P fibers in *mdx* mice were 20.7 and 50.1, and 29.2%, respectively (Fig. 1B). Fiber length and sarcomere number (Fig. 2) were identical between *mdx* and WT mice. And these levels were similar in any types of fibers of *mdx* mice. Fiber CSA was not different between two species and also between 3 types of fibers in *mdx* mice (Fig. 3).

The percent distribution of fibers expressing type I, I+II, and II MHC was 29.1, 4.7, and 66.2% in WT mice (Fig. 4). And that in *mdx* mice was 7.6, 38.5, and 53.9% in fibers without central nuclei and 3.7, 48.4, 47.9% in fibers with central nuclei, respectively. The percentage of type I+II fibers was less and that of type II fibers was greater than that of type I fibers in WT mice ($p<0.05$). As for *mdx* mice, the percentage was identical in fibers with or without central nuclei. The percentage of fibers expressing pure type I MHC in *mdx* mice was less than in WT mice ($p<0.05$), and that of fibers co-expressing type I and II MHC was greater than in WT mice ($p<0.05$).

All type P fibers in WT mice had a single innervation (Fig. 5A). The percent type P fiber in *mdx* mice was significantly less than WT mice. And ~20.1% of fibers had 2 NMJs and no NMJ was detected in ~6.6% fibers. These characteristics were

similar in any fibers with different distribution of myonuclei. The absolute and relative diameter, normalized with fiber CSA, of NMJs, which may have a close relationship with MHC phenotype, were not different between 2 species and was identical in fibers with or without central myonuclei in *mdx* mice (Figs. 5B and C).

Number, size, and domain of myonuclei, and satellite cells. The mean myonuclear number of type P fibers in WT and *mdx* mice was 541 and 449 ($p<0.05$, Fig. 6A). Myonuclear CSA of type P fibers was also greater in WT than *mdx* mice (16%, $p<0.05$, Fig. 6B). But the total number and size of myonuclei were identical in any types of fibers with different distribution of myonuclei in *mdx* mice. Myonuclear domain was similar in any types of fibers of *mdx* mice. However, the level of type P fibers in *mdx* mice were 23 % greater than WT mice ($p<0.05$, Fig. 6C). The numbers of both mitotic active and quiescent satellite cells in whole single fiber, as well as the percent mitotic active cells, were identical between 2 species and fibers with various distribution of myonuclei in *mdx* mice (Fig. 7A-C).

Apoptotic myonuclei. In the longitudinal fibers, the TUNEL-positive apoptotic myonuclei were not detected in type P fibers of WT mice and in all types of fibers of *mdx* mice (Fig. 8). In contrast, TUNEL-positive myonuclei were slightly detected in the cross-sectional sample of *mdx*, not WT, mice. Those nuclei were located at the peripheral region. Further, there was no relationship in the distribution of TUNEL-positive apoptotic nuclei between the myonuclei located at the central or peripheral region.

Electron microscopic images of central and peripheral myonuclei. Electron microscopic analyses were performed in both cross- and longitudinal-sections of *mdx* mice (Fig. 9). However, typical differences in the morphological characteristics, which may help the translocation of myonuclei, were not detected in both central and peripheral myonuclei.

DISCUSSION

A series of central myonuclei were observed in muscle fibers, sampled from tendon-to-tendon, in *mdx* mice (Figs. 1A and B), which is the first evidence to the author's knowledge. Most of the previous studies analyzed the location of myonuclei in muscle cross-section (12, 19) or in a portion of longitudinal fibers (24). The total number of myonuclei was identical between fibers with different distribution of myonuclei (type C, C+P, and P fibers). Further, the peripheral myonucleus was noted generally at the site where the central myonucleus was missing in type C+P fiber (Fig. 1A). Thus, these observations suggest that the cross-sectional translocation of the myonuclei from the center to periphery of the fiber may be induced. The author, therefore, speculated that the shift of the fiber properties, for example type C→C+P→P, may be induced during the regeneration process in muscle fibers of *mdx* mice.

It was reported that the fibers with mixed population of central and peripheral myonuclei were damaged (24). However, these central myonuclei were not always arranged like a chain, as was noted in the present study (type C fibers). Immature myogenic cells, such as myoblasts and myotubes, are centrally nucleated (18). Chain of central nuclei was observed in myotubes (4). The mean CSA and length of fibers were identical between *mdx* and WT mice in the present study. Furthermore, any

marked abnormalities were not noted between the fibers with different distribution of myonuclei in *mdx* mice. Many parameters, such as fiber CSA and length, sarcomere number, myonuclear number and CSA, MHC expression, and satellite cell number, were identical among the fibers with different distribution of myonuclei. Interestingly, myonuclear domain size was also similar between 3 types of fibers, even though the domain area of each myonucleus might be different between the myonuclei located at the central and peripheral region. The sarcomeres were also clearly seen throughout the fiber length even in the type C fibers, indicating that the intracellular myofibrils were formed normally. Furthermore, the type C fibers contained NMJ, suggesting the successful innervation of the motor axon even in the fibers containing central myonuclei. Therefore, the fibers with central nuclei may be not necessarily immature. It is also indicated that the cross-sectional migration of myonuclei in response to degeneration/regeneration cycle is not related to most of the characteristic of muscle fibers, including the morphologic, as well as contractile (24), properties. Meanwhile, apoptosis was not detected in any myonuclei located either central or peripheral region of fibers. Thus, it was suggested that apoptosis-related loss of central myonuclei and regeneration-related new accretion at the peripheral region is not the cause of the different distribution of myonuclei seen in muscle fibers in *mdx* mice.

In conclusion, the fibers containing the central nuclei were noted and the nuclei in these fibers were arranged like a single chain throughout the fiber length in the soleus muscle of *md*x mice. The total number of myonuclei was the same in any fibers with different distribution of myonuclei. Further, peripheral nucleus was noted generally at the site where the central nucleus was missing. These results suggest that myonuclei migrate cross-sectionally, although microscopic evidence of any structures was not

observed around myonuclei.

ACKNOWLEDGEMENTS

This study was carried out as a part of Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (19-6885, M.T.), and was also supported by the Grant-in-Aid for Scientific Research (S-19100009, Y.O. and C-20500577, N.N.) from Japan Society for the Promotion of Science and the Grant-in-Aid for the Academic Frontier Project (Waseda University: 2005-2010) of the Ministry of Education, Culture, Sports, Science and Technology (K.I.). The author acknowledges the principal support and extremely generous advice and encouragement for Professor Yoshinobu Ohira. And the author thanks Yon Bo Lan, Fuminori Kawano, Takashi Ohira and Yoko Higo at Osaka Industry University, Osaka, Japan, for their valuable comments and for their help in the data analyses.

REFERENCES

1. **Allen DL, Linderman JK, Roy RR, Bigbee AJ, Grindeland RE, Mukku V, and Edgerton VR**. Apoptosis: a mechanism contributing to remodeling of skeletal muscle in response to hindlimb unweighting. *Am J Physiol* 273: C579-587, 1997.

2. **Allen DL, Yasui W, Tanaka T, Ohira Y, Nagaoka S, Sekiguchi C, Hinds WE, Roy RR, and Edgerton VR**. Myonuclear number and myosin heavy chain expression in rat soleus single muscle fibers after spaceflight. *J Appl Physiol* 81: 145-151, 1996.

3. **Bigard XA, Janmot C, Merino D, Lienhard F, Guezennec YC, and D'Albis A**. Endurance training affects myosin heavy chain phenotype in regenerating fast-twitch muscle. *J Appl Physiol* 81: 2658-2665, 1996.

4. **Harris AJ, Duxson MJ, Fitzsimons RB, and Rieger F**. Myonuclear birthdates distinguish the origins of primary and secondary myotubes in embryonic mammalian skeletal muscles. *Development (Cambridge, England)* 107: 771-784, 1989.

5. **Haslett JN, Kang PB, Han M, Kho AT, Sanoudou D, Volinski JM, Beggs AH, Kohane IS, and Kunkel LM**. The influence of muscle type and dystrophin deficiency on murine expression profiles. *Mamm Genome* 16: 739-748, 2005.

6. **Kawano F, Takeno Y, Nakai N, Higo Y, Terada M, Ohira T, Nonaka I, and Ohira Y**. Essential role of satellite cells in the growth of rat soleus muscle fibers. *Am J Physiol Cell Physiol* 295: C458-467, 2008.

7. **Kawano F, Wang XD, Lan YB, Yoneshima H, Ishihara A, Igarashi M, and Ohira Y**. Hindlimb suspension inhibits air-righting due to altered recruitment of neck and back muscles in rats. *Jpn J Physiol* 54: 229-242, 2004.

8. **Lyons PR, and Slater CR**. Structure and function of the neuromuscular junction in young adult *mdx* mice. *J neurocytol* 20: 969-981, 1991.

9. **Mauro A**. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 9: 493-495, 1961.

10. **McCarter GC, and Steinhardt RA**. Increased activity of calcium leak channels caused by proteolysis near sarcolemmal ruptures. *J Membr Biol* 176: 169-174, 2000.

11. **Minatel E, Santo Neto H, and Marques MJ**. Acetylcholine receptors and neuronal nitric oxide synthase distribution at the neuromuscular junction of regenerated muscle fibers. *Muscle Nerve* 24: 410-416, 2001.

12. **Mokhtarian A, Lefaucheur JP, Even PC, and Sebille A**. Hindlimb

immobilization applied to 21-day-old *mdx* mice prevents the occurrence of muscle degeneration. *J Appl Physiol* 86: 924-931, 1999.

13. **Moss FP, and Leblond CP**. Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec* 170: 421-435, 1971.

14. **Mozdziak PE, Pulvermacher PM, and Schultz E**. Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading. *J Appl Physiol* 88: 158-164, 2000.

15. **Ohira Y, Tanaka T, Yoshinaga T, Kawano F, Nomura T, Nonaka I, Allen DL, Roy RR, and Edgerton VR**. Ontogenetic, gravity-dependent development of rat soleus muscle. *Am J physiol* 280: C1008-1016, 2001.

16. **Oishi Y, Ogata T, Yamamoto KI, Terada M, Ohira T, Ohira Y, Taniguchi K, and Roy RR**. Cellular adaptations in soleus muscle during recovery after hindlimb unloading. *Acta Physiol (Oxford, England)* 192: 381-395, 2008.

17. **Personius KE, and Sawyer RP**. Terminal Schwann cell structure is altered in diaphragm of *mdx* mice. *Muscle Nerve* 32: 656-663, 2005.

18. **Saito K, Fukuyama Y, Ogata T, and Oya A**. Experimental intrauterine infection of akabane virus. Pathological studies of skeletal muscles and central nervous system of newborn hamsters with relevances to the Fukuyama type congenital muscular dystrophy. *Brain Dev* 3: 65-80, 1981.

19. **Schafer R, Zweyer M, Knauf U, Mundegar RR, and Wernig A**. The ontogeny of soleus muscles in *mdx* and wild type mice. *Neuromuscul Disord* 15: 57-64, 2005.

20. **Schultz E, Jaryszak DL, and Valliere CR**. Response of satellite cells to focal skeletal muscle injury. *Muscle Nerve* 8: 217-222, 1985.

21. **Torres LF, and Duchen LW**. The mutant *mdx*: inherited myopathy in the mouse. Morphological studies of nerves, muscles and end-plates. *Brain* 110 (Pt 2): 269-299, 1987.

22. **Waerhaug O, and Lomo T**. Factors causing different properties at neuromuscular junctions in fast and slow rat skeletal muscles. *Anat Embryol (Berl)* 190: 113-125, 1994.

23. **Wang XD, Kawano F, Matsuoka Y, Fukunaga K, Terada M, Sudoh M, Ishihara A, and Ohira Y**. Mechanical load-dependent regulation of satellite cell and fiber size in rat soleus muscle. *Am J Physiol* 290: C981-989, 2006.

24. **Williams DA, Head SI, Lynch GS, and Stephenson DG**. Contractile properties of skinned muscle fibres from young and adult normal and dystrophic (*mdx*) mice. *J Physiol* 460: 51-67, 1993.

FIGURE LEGENDS

Figure 1: (A) Various distribution of myonuclei in whole soleus muscle fibers, sampled from tendon-to-tendon, of *mdx* and wild type mice. Type C: With more than 80% of myonuclei at the central region. Type P: With all myonuclei at the peripheral region. Type C+P: With less than 80% of myonuclei at the central region. (B) Percentages of fibers with different distribution of myonuclei. All of the fibers in wild type mice were peripheral-nucleated. Mean ± SEM. * and †: $p<0.05$ vs. wild type mice and type C fibers of *mdx* mice, respectively.

Figure 2: Fiber length and sarcomere number in whole fiber. Mean ± SEM. See Figure 1 for the abbreviations.

Figure3: Fiber cross-sectional area in whole fiber. Mean ± SEM. See Figure 1 for the abbreviations.

Figure 4: Percent distribution of fibers expressing slow (type I), fast (type II), or both slow and fast (type I+II) isoforms of myosin heavy chain. w/o: Fibers without central nuclei. w/: Fibers with central nuclei. Mean ± SEM. *, †, and §: $p<0.05$ vs. wild type mice and type I and I+II fibers in respective group. See Figure 1 for the abbreviations.

Figure 5: (A) Percentage of fibers with different numbers of neural innervations in whole fiber. Absolute (B) and relative diametr, normalized with fiber cross-sectional area (CSA, C), of neuromuscular junctions are also shown. Mean ± SEM. *: $p<0.05$ vs. type P fibers in wild type mice. See Figure 1 for the abbreviations.

Figure 6: Myonuclear number (A), myonuclear cross-sectional area (B), and myonuclear domain (C) analyzed in whole single fiber of wild type and *mdx* mice. Mean ± SEM. *: $p<0.05$ vs. type P fibers in wild type mice. See Figure 1 for the abbreviations.

Figure 7: Number of mitotic active (A) and quiescent satellite cells per whole fiber

(B), and the percentage of mitotic active satellite cells relative to the total satellite cells (C). Mean ± SEM. See Figure 1 for the abbreviations.

Figure 8: The distribution of myonuclei with or without {labeled using 4', 6-diamidino-2-phenylindole (DAPI)} apoptosis in muscle fibers of *mdx* and wild type (WT) mice. See Figure 1 for other abbreviations.

Figure 9: Electron micrographs of central and peripheral myonuclei in soleus muscle fibers of *mdx* mice.

Fig. 1

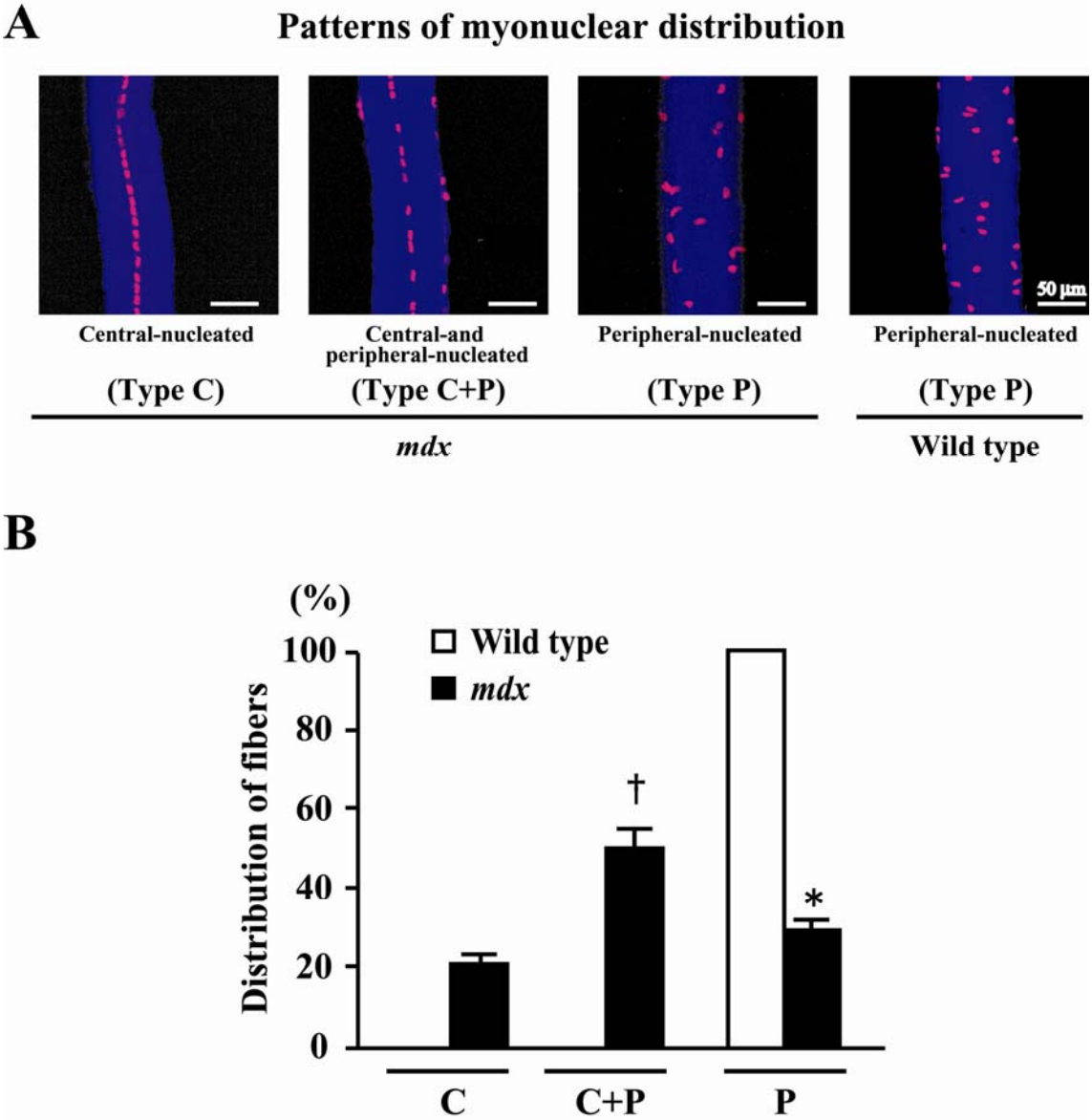

A
Patterns of myonuclear distribution
50 µm
Central-nucleated
(Type C)
Central-and peripheral-nucleated
(Type C+P)
Peripheral-nucleated
(Type P)
Peripheral-nucleated
(Type P)
mdx
Wild type
B
(%)
Distribution of fibers
100
80
60
40
20
0
Wild type
mdx
†
*
C
C+P
P

Fig. 2

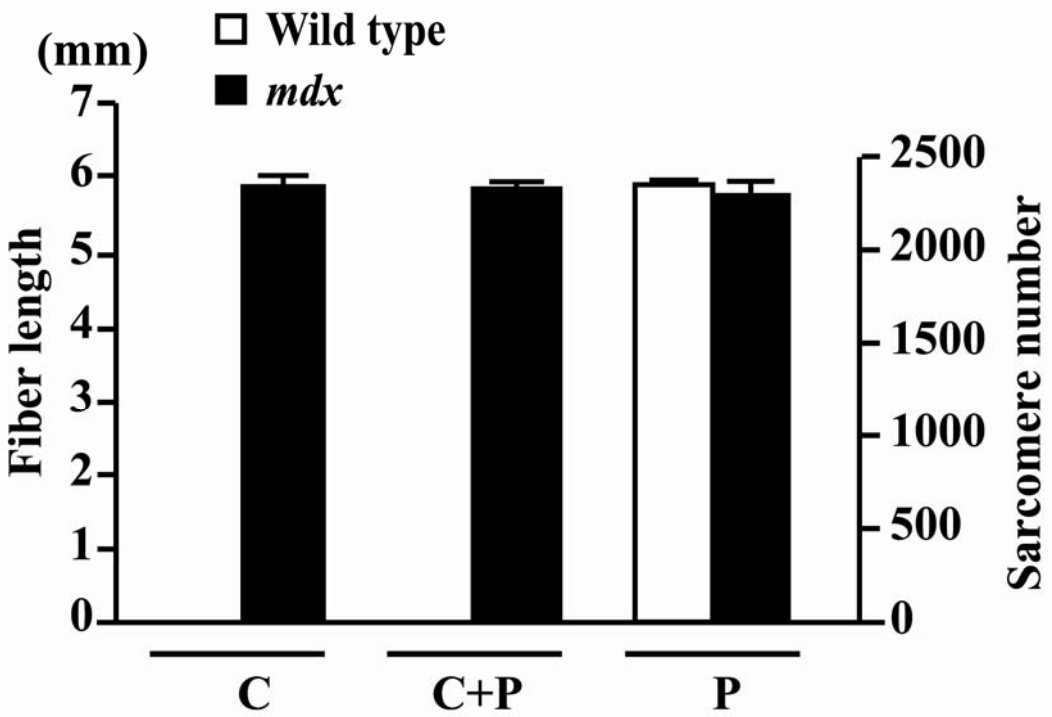

(mm)
Wild type
mdx
Fiber length
7
6
5
4
3
2
1
0
2500
2000
1500
1000
500
0
Sarcomere number
C
C+P
P

Fig. 3

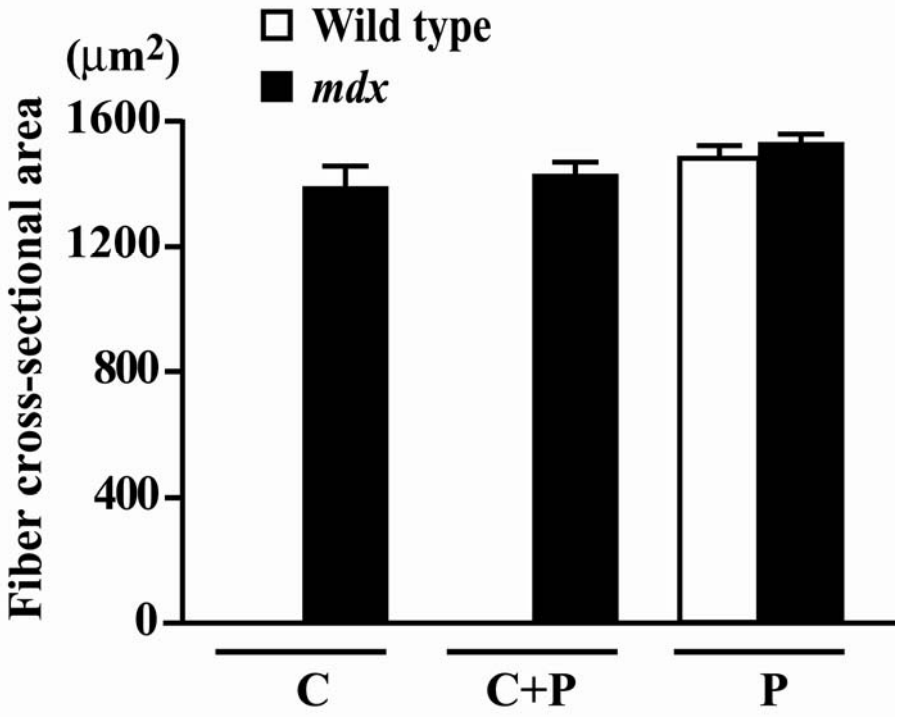

Wild type
mdx
(μm2)
Fiber cross-sectional area
1600
1200
800
400
0
C
C+P
P

Fig. 4

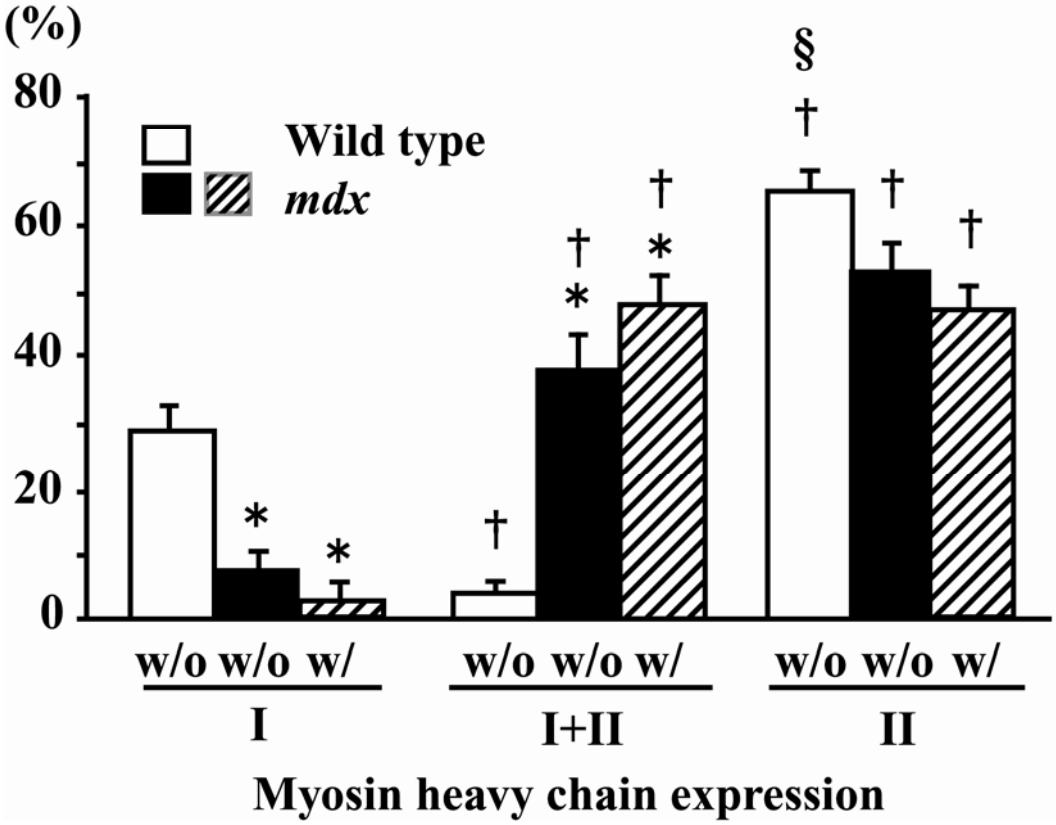

(%)
80
60
40
20
0
Wild type
mdx
*
*
†
†
*
†
*
§
†
†
†
w/o w/o w/
I
w/o w/o w/
I+II
w/o w/o w/
II
Myosin heavy chain expression

Fig. 5

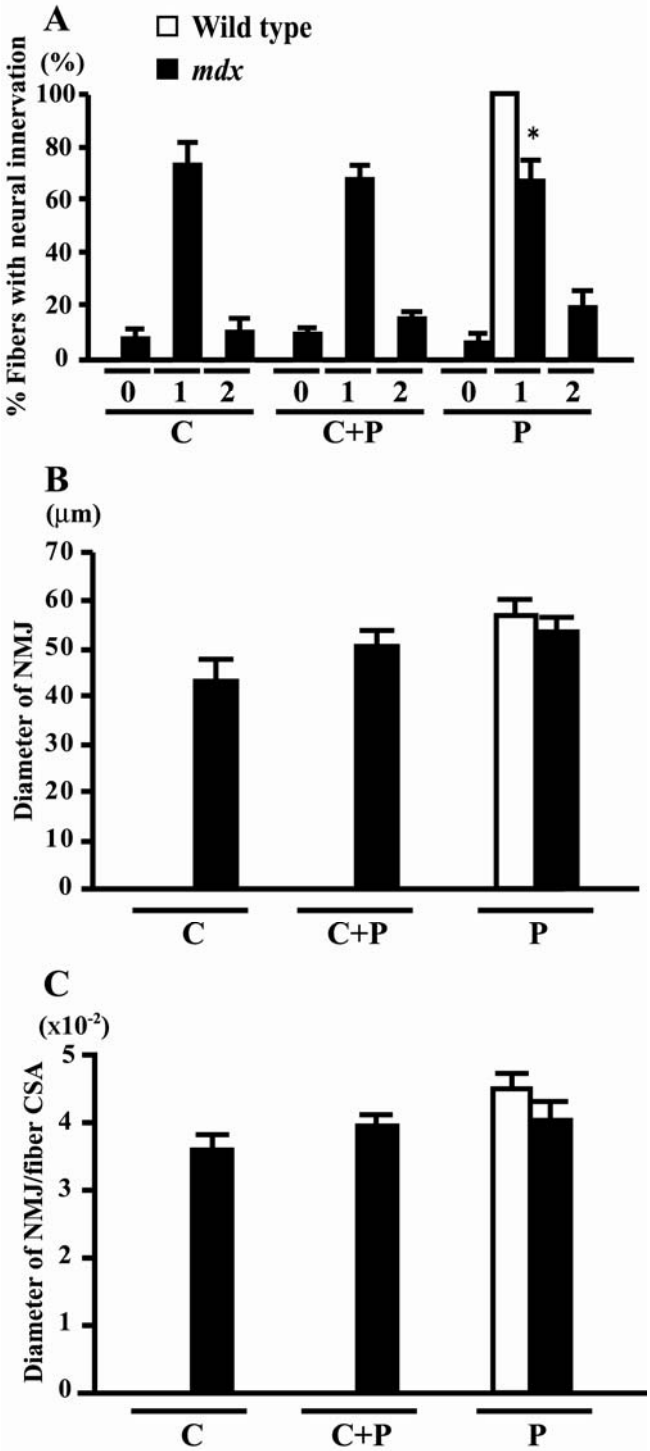

A
Wild type
mdx
(%)
% Fibers with neural innervation
100
80
60
40
20
0
*
0 1 2 0 1 2 0 1 2
C
C+P
P
B
(μm)
Diameter of NMJ
70
60
50
40
30
20
10
0
C
C+P
P
C
(x10-2)
Diameter of NMJ/fiber CSA
5
4
3
2
1
0
C
C+P
P

Fig. 6

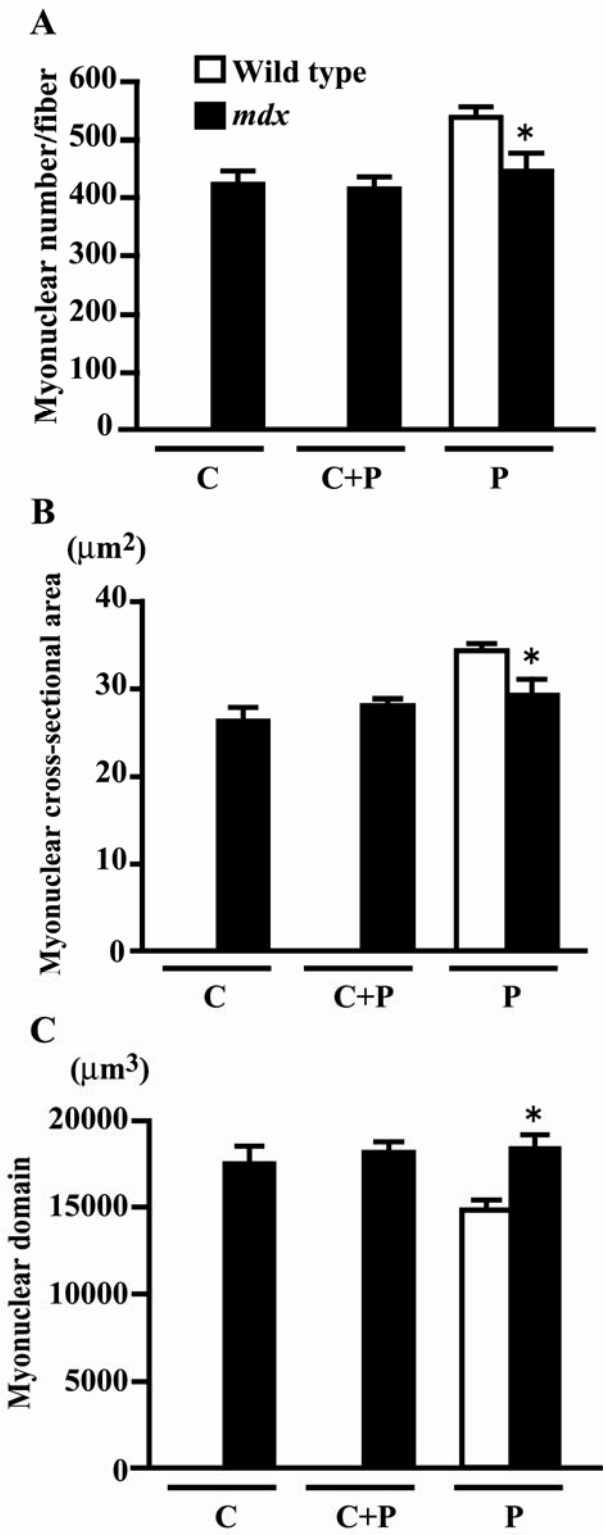

A
Wild type
mdx
Myonuclear number/fiber
600
500
400
300
200
100
0
*
C
C+P
P
B
(μm2)
Myonuclear cross-sectional area
40
30
20
10
0
*
C
C+P
P
C
(μm3)
Myonuclear domain
20000
15000
10000
5000
0
*
C
C+P
P

Fig. 7

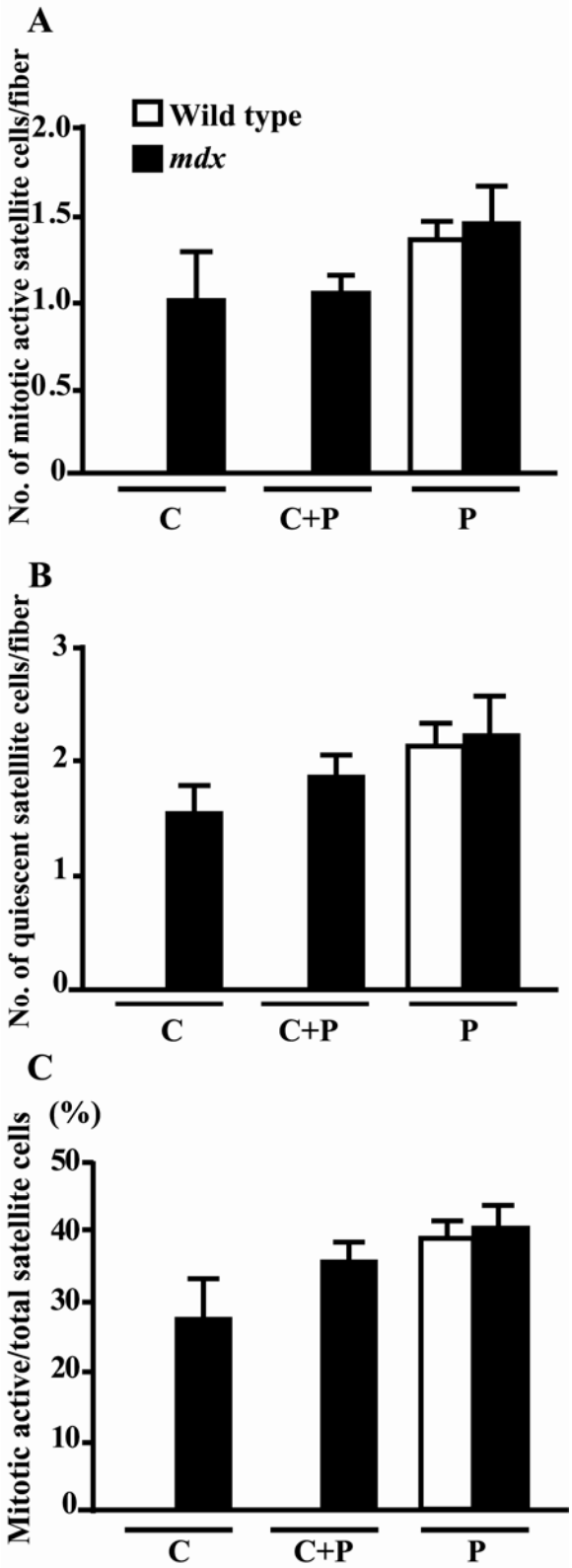

A
No. of mitotic active satellite cells/fiber
Wild type
mdx
2.0
1.5
1.0
0.5
0
C
C+P
P
B
No. of quiescent satelllite cells/fiber
3
2
1
0
C
C+P
P
C
(%)
Mitotic active/total satellite cells
50
40
30
20
10
0
C
C+P
P

Fig. 8

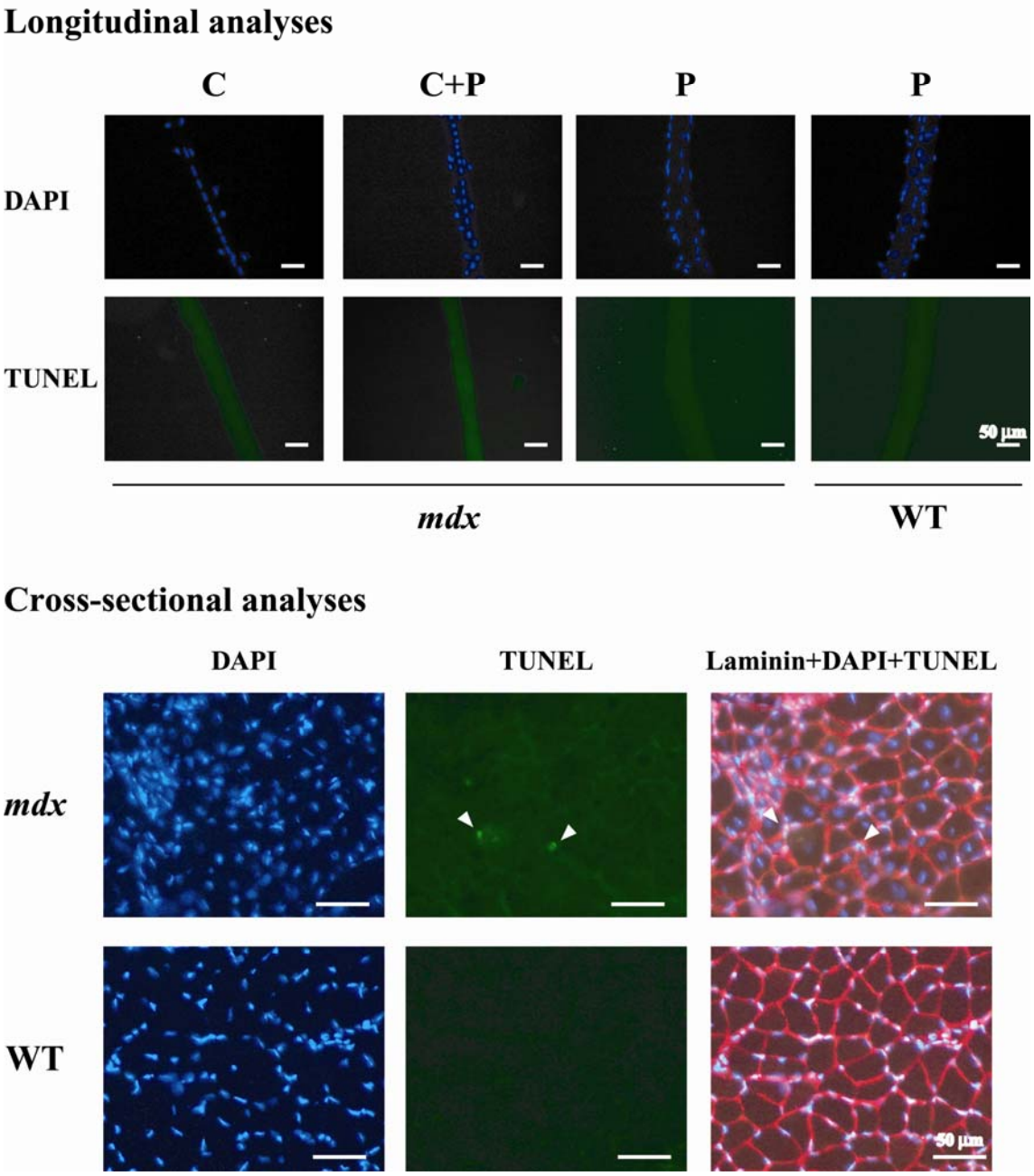
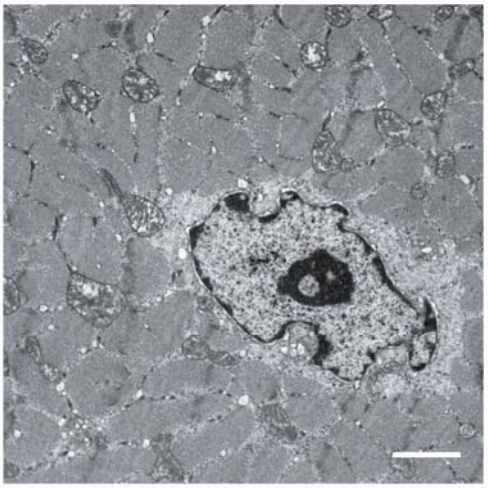
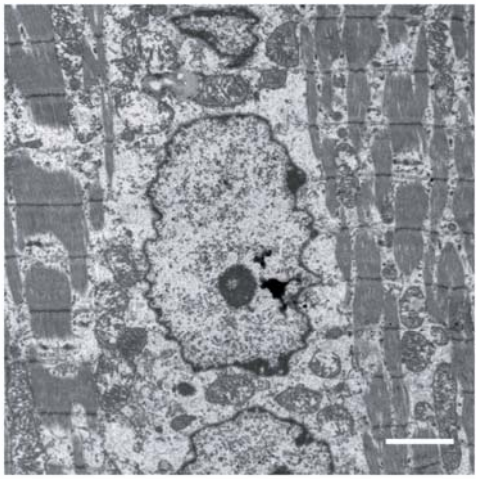

Longitudinal analyses
C
C+P
P
P
DAPI
TUNEL
50 μm
mdx
WT
Cross-sectional analyses
DAPI
TUNEL
Laminin+DAPI+TUNEL
mdx
WT
50 μm

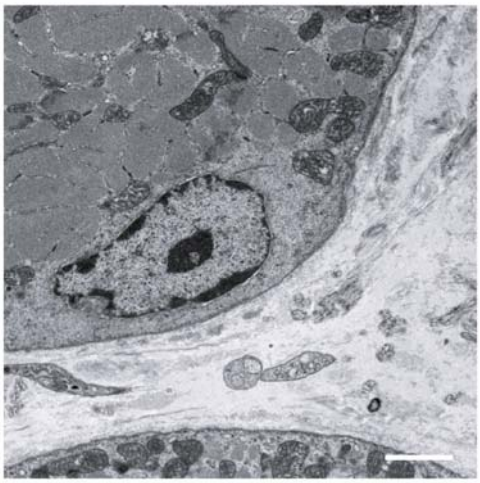
Fig. 9

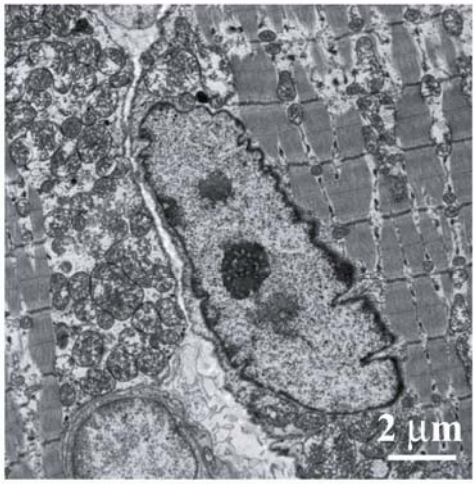
Cross sectional analyses **Longitudinal analyses**





Central nuclei





Peripheral nuclei

研究業績目録

原著論文 (英文)

1. Terada, M., Kawano, F., Ohira, T., Oke, Y., Nakai, N., Ohira, Y. Effects of mechanical overloading on the properties of soleus muscle fibers, with or without damage, in *mdx* and wild type mice. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meeting, ELGRA Symposium, P3.033. Angers, France, 22-27 June, 2008.
2. Ohira, T., Wang, X.D., Terada, M., Kawano, F., Higo, Y., Nakai, N., Ochiai, T., Gyotoku, J., Nishimoto, N., Ogura, A., Ohira, Y. Responses of electromyogram activity in adductor logus muscle of rats to the altered gravity levels. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meeting, ELGRA Symposium, P3.032. Angers, France, 22-27 June, 2008.
3. Kawano, F., Wang, X.D., Nakai, N., Higo, Y., Terada, M., Ohira, T., Nonaka, I., Ohira, Y. Gravitational loading stimulates adhesion of satellite cells and myonuclear accretion during fiber growth in rat soleus muscle. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meeting, ELGRA Symposium, P3.023. Angers, France, 22-27 June, 2008.
4. Ohira, Y., Kawano, F., Goto, K., Terada, M., Ohira, T., Nakai, N., Higo, Y., Yohioka, T. Role(s) of gravitational loading on the growth-related transformation of fiber phenotype in rat soleus. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meeting, ELGRA Symposium, p115. Angers, France, 22-27 June, 2008.
5. Nakai, N., Kawano, F., Terada, M., Oke, Y., Ohira, T., Ohira, Y. Effects of peroxisome proliferator-activated receptor alpha (PPARalpha) agonists on

leucine-induced phosphorylation of translational targets in C2C12 cells. Biochim Biophys Acta. 1780(10): 1101-5, 2008.

6. Kawano, F., Takeno, Y., Nakai, N., Higo, Y., Terada, M., Ohira, T., Nonaka, I., Ohira, Y. Essential role of satellite cells in the growth of rat soleus muscle fibers. Am. J. Physiol. Cell Physiol. 295(2): C458-67, 2008.
7. Terada, M., Lyubaeva, E., Ohira, T., Netreba, A., Kawano, F., Okabe, H., Imaizumi, K., Vinogradova, O., and Ohira, Y. Responses of vastus lateralis muscle fibers to cycling training with different loading parameter in human. Jpn. J. Aerospace Environ. 44: 59-64, 2007.
8. Oishi Y., Ogata T., Yamamoto KI., Terada M., Ohira T., Ohira Y., Taniguchi K., Roy RR. Cellular adaptations in soleus muscle during recovery after hindlimb unloading. Acta Physiol. (Oxf.). 192(3):381-95, 2007.
9. Lan, Y.B., Terada, M., Higo, Y., Kawano, F., Matsuoka, Y., Wang, X.D., Nakai, N., and Ohira, Y. Effects of mechanical and/or neural stimuli on the properties of soleus muscle fibers in *mdx* mice. Jpn. J. Aerospace Environ. Med. 44: 19-29, 2007. Lan, Y.B., and Terada, M. contributed equally to this study.
10. Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Wang, X.D., Nakai, N., Fukuda, H., Ohmi, S., and Ohira, Y. Role(s) of nucleoli, phosphorylation of ribosomal protein S6 and/or HSP27 in the regulation of muscle mass. Am. J. Physiol. Cell Physiol. 293: C35-C44, 2007.
11. Ohira, Y., Wang, X.D., Terada, M., Kawano, F., Matsuoka, Y., Higo, Y., Ohira, T., Kojima, A., Goto, K., and Yoshioka, T. Investigation of the mechanism responsible for muscle atrophy and countermeasures in microgravity environment: Role (s) of mechanical load and/or satellite cells. Proceedings of the 1st

International Space Medicine Symposium 2007, p91-93, 2007.

12. Ohira, Y., Yoshinaga, T., Ohara, M., Kawano, F., Wang, X.D., Higo, Y., Terada, M., Matsuoka, Y., Roy, R.R. and Edgerton, V.R. The role of neural and mechanical influences in maintaining normal fast and slow muscle properties. Cells Tissues Organs 182: 129-142, 2006.
13. Terada, M., Kawano, F., Wang, X.D., Matsuoka, Y., Ochiai, T., Inada, H., and Ohira, Y. Effects of acute exposure to microgravity or hyper–gravity environment on motor control of arm in human. J. Gravit. Physiol., volume 13, Number 1, p33-34, 2006.
14. Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Wang, X.D., Lan, Y.B., Nakai, N., and Ohira, Y. Mechanical stress-dependent transformation of nucleoli in myonuclei of rat soleus muscle fibers. J. Gravit. Physiol., volume 13, Number 1, p85-86, 2006.
15. Wang, X.D., Kawano, F., Matsuoka, Y., Terada, M., Yamamoto, H., Ito, T., Goto, K., Lan, Y.B., and Ohira, Y. Role of macrophage function in regeneration of soleus muscle fibers in *op/op* mice. J. Gravit. Physiol., volume 13, Number 1, p77-78, 2006.
16. Matsuoka, Y., Kawano, F., Wang, X.D., Terada, M., Higo, Y., Lan, Y.B., Ogura, A., Nishimoto, N., Adachi, Y., and Ohira Y. Blockade of interleukin-6 signaling pathway promotes muscle cell differentiation. J. Gravit. Physiol., volume 13, Number 1, p83-84, 2006.
17. Ohira, T., Morikuni, S., Wang, X, D., Terada, M., Kawano, F., Matsuoka, Y., Higo, Y., Lan, Y, B., Seki, T., Ohira, Y. Responses of adductor longus muscle to hindlimb suspension in wistar hannover rats. J. Gravit. Physiol., volume 13,

Number 1, p79-80, 2006.

18. Morikuni, S., Ohira, T., Wang, X.D., Terada, M., Kawano, F., Matsuoka, Y., Lan, Y.B., Yamamoto, H., Ito, T., Goto, K., Seki, T., and Ohira, Y. Role(s) of macrophage in the plasticity of plantaris muscle fibers in *op/op* mice. J. Gravit. Physiol., volume 13, Number 1, p81-82, 2006.
19. Higo, Y., Terada, M., Umemoto, S., Kawabe, N., Kawano, F., and Ohira, Y. Effects of Tenotomy and/or Denervation on the characteristics of plantaris muscle fibers of *mdx* mice. J. Gravit. Physiol., volume 13, Number 1, p87-88, 2006.
20. Wang, X.D., Kawano, F., Matsuoka, Y., Fukunaga, K., Terada, M., Sudouh, M., Ishihara, A., and Ohira, Y. Mechanical load-dependent regulation of satellite cell and fiber size in rat soleus muscle. Am. J. Physiol. Cell Physiol. 290: C981-C989, 2005.
21. Terada, M., Kawano, F., Lan, Y.B., Matsuoka, Y., Wang, X.D. and Ohira, Y. Mechanical and/or neural activity-dependent regulation of soleus muscle fibers of mdx mice. J. Gravit. Physiol., volume 12, Number 1, p135-136, 2005.
22. Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Umemoto, S., Kawabe, N., Wang, X.D., Shinoda, Y., Lan, Y.B., Fukuda, H., and Ohira, Y. Role of afferent input and/or mechanical load in size regulation of rat soleus muscle. J. Gravit. Physiol., volume 12, Number 1, p133-134, 2005.
23. Wang, X.D., Kawano, F., Terada, M., Matsuoka, Y., Shinoda, Y., Ishihara, A., and Ohira, Y. Different responses in soleus muscle fibers of Wistar and Wistar Hannover rats to hindlimb unloading. J. Gravit. Physiol., volume 12, Number 1, p131-132, 2005.
24. Matsuoka, Y., Wang, X.D., Kawano, F., Terada, M., Shinoda, Y., Lan, Y.B., Goto,

K., Ogura, A., and Ohira, Y. How does the nucleolar number involve in muscle fiber atrophy? Response to β-guanidinopropionic acid supplementation. J. Gravit. Physiol., volume 12, Number 1, p147-148, 2005.

原著論文 (和文)

1. 大平充宣、王暁東、寺田昌弘、河野史倫、松岡由和、肥後葉子、大平宇志、小島敦、後藤勝正、吉岡利忠。微小重力環境における筋萎縮機構および防止策の検討 : 機械的負荷または衛星細胞の役割。第 1 回札幌冬季宇宙医学国際シンポジウム 2007 報告書「市場原理が動かす宇宙開発の動向を探る」、p94-96、2007。

学会発表（国際）

1. Terada, M., Kawano, F., Ohira, T., Oke, Y., Nakai, N., Ohira, Y. Effects of mechanical overloading on the properties of soleus muscle fibers, with or without damage, in *mdx* and wild type mice. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meetnig, ELGRA Symposium, P3.033. Angers, France, 22-27 June, 2008. (poster)
2. Ohira, T., Wang, X.D., Terada, M., Kawano, F., Higo, Y., Nakai, N., Ochiai, T., Gyotoku, J., Nishimoto, N., Ogura, A., Ohira, Y. Responses of electromyogram activity in adductor logus muscle of rats to the altered gravity levels. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meetnig, ELGRA Symposium, P3.032. Angers, France, 22-27 June, 2008. (poster)
3. Kawano, F., Wang, X.D., Nakai, N., Higo, Y., Terada, M., Ohira, T., Nonaka, I., Ohira, Y. Gravitational loading stimulates adhesion of satellite cells and

myonuclear accretion during fiber growth in rat soleus muscle. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meetnig, ELGRA Symposium, P3.023. Angers, France, 22-27 June, 2008. (poster)

4. Ohira, Y., Kawano, F., Goto, K., Terada, M., Ohira, T., Nakai, N., Higo, Y., Yohioka, T. Role(s) of gravitational loading on the growth-related transformation of fiber phenotype in rat soleus. 10th ESA Life Sciences Symposium, 29th Annual ISGP Meeting, 24th Annual ASGSB Meetnig, ELGRA Symposium, p115. Angers, France, 22-27 June, 2008. (oral)
5. Kawano, F., Terada, M., Nakai, N., Ohira, T., Higo, Y., Nishimoto, N., and Ohira, Y. Responses of protein expression to cardiotoxin-induced muscle injury. American Society for Gravitational and Space Biology, 23th Annual Meeting, p35. Moffett Field, CA, USA, 25-28 Oct, 2007. (poster)
6. Nakajima, Y., Terada, M., Kawano, F., Sumi, H., Shimizu, Y., Ohira, T., Higo, Y., Wang, X.D., Nakai, N., and Ohira, Y. Responses of body composition and hematological and muscular properties of *Meriones Unguiculatus* to gravitational unloading. American Society for Gravitational and Space Biology, 23th Annual Meeting, p12. Moffett Field, CA, USA, 25-28 Oct, 2007. (poster)
7. Terada, M., Kawano, F., Kume, H., Ohira, T., Higo, Y., Nakajima, Y., Wang, X.D., Nakai, N., Hinds, W.E., and Ohira, Y. Responses of back muscle fibers in Sprague-Dawley rats to spaceflight. American Society for Gravitational and Space Biology, 23th Annual Meeting, p9. Moffett Field, CA, USA, 25-28 Oct, 2007. (poster)

   Award：Travel award ($ 250).
8. Ohira, T., Wang, X.D., Terada, M., Kawano, F., Higo, Y., Nakai, N., Ochiai, J.,

Gyotoku, N., NIishimoto, N., and Ohira, Y. Are the responses of adductor longus muscle to gravitational unloading in Wistar Honnover rats region-specific? American Society for Gravitational and Space Biology, 23th Annual Meeting, p8. Moffett Field, CA, USA, 25-28 Oct, 2007. (poster)

9. Nakai, N., Kawano, F., Terada, M., Ohira, Y. Peroxisome proliferators-activated receptor α (PPARα) agonist, WY-14,643, increased leucine-induced activation of translation in C2C12 cells. The 12th International Congress of the World Muscle Society, G.P.19.11, Naxos, Italia, 17 - 21 Oct, 2007. (poster)
10. Terada, M., Kawano, F., Higo, Y., Ohira, T., Wang, X., Nakai, N., Ohira, Y. The characters of soleus muscle fibers of *mdx* mice with different distribution of myonuclei. The 12th International Congress of the World Muscle Society, G.P.6.16, Naxos, Italia, 17 - 21 Oct, 2007. (poster)
11. Ohira, Y., Wang, X.D., Terada, M., Kawano, F., Matsuoka, Y., Higo, Y., Ohira, T., Kojima, A., Goto, K., and Yoshioka, T. Investigation of the mechanism responsible for muscle atrophy and countermeasures in microgravity environment: Role(s) of mechanical load and/or satellite cells. The 1st International Space Medicine Symposium, p91-93. Sapporo, Japan, 3-4 Feb, 2007. (oral)
12. Terada, M., Lyubaeva, E., Ohira, T., Netreba, A., Vinogradova, O., and Ohira, Y. Responses of Vastus Lateralis Muscle Fibers to concentric and/or eccentric training. Biochemistry of exercise, 13th International conference, p159. Seoul, Korea, 20-23 Oct, 2006. (poster)
13. Higo, Y., Terada, M., Umemoto, S., Kawabe, N., Kawano, F., Ohira, Y. Effects of tenotomy and/or denervation on the properties of M. Plantaris of *mdx* and wild type mice. Biochemistry of exercise, 13th International conference, p181. Seoul, Korea,

20-23 Oct, 2006. (poster)

14. Morikuni, S., Ohira, T., Wang, X.D., Terada, M., Kawano, F., Matsuoka, Y., Lan, Y.B., Yamamoto, H., Ito, T., Goto, K., Seki, T., and Ohira, Y. Responses of plantaris muscle fibers in *OP/OP* mice to gravitational unloading. Biochemistry of exercise, 13th International conference, p43. Seoul, Korea, 20-23 Oct, 2006. (poster)
15. Ohira, T., Morikuni, S., Wang, X, D., Terada, M., Kawano, F., Matsuoka, Y., Higo, Y., Lan, Y, B., Seki, T., Ohira, Y. Effects of hindlimb suspension on the properties of M. Adductor longus in Wistar Hannover rats. Biochemistry of exercise, 13th International conference, p46. Seoul, Korea, 20-23 Oct, 2006. (poster)
16. Wang, X.D., Kawano, F., Matsuoka, Y., Terada, M., Ohira, T., Yamamoto, H., Ito, T., Goto, K., Lan, Y.B., and Ohira, Y. Effects of chronic unloading and reloading on soleus muscle fibers in *OP/OP* mice. Biochemistry of exercise, 13th International conference, p47. Seoul, Korea, 20-23 Oct, 2006. (poster)
17. Matsuoka, Y., Kawano, F., Wang, X.D., Terada, M., Higo, Y., Lan, Y.B., Ohira, T., Ogura, A., Nishimoto, N., and Ohira, Y. Inhibition of interleukin-6 receptor promotes muscle cell differentiation. Biochemistry of exercise, 13th International conference, p65. Seoul, Korea, 20-23 Oct, 2006. (poster)
18. Kawano, F., Matsuoka, Y., Terada, M., Higo, Y., Nakai, N., and Ohira, Y. Effects of functional overload and/or afferent input on fiber size and protein synthesis in rat soleus muscle. Biochemistry of exercise, 13th International conference, p143. Seoul, Korea, 20-23 Oct, 2006. (poster)
19. Terada, M., Kawano, F., Wang, X.D., Matsuoka, Y., Ochiai, T., Inada, H., and Ohira, Y. Effects of acute exposure to microgravity or hyper–gravity environment on

motor control of arm in human. 27th Annual International Gravitational Physiology Meeting, PS2.13, Osaka, Japan, 23-28 April, 2006. (poster)

20. Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Wang, X.D., Lan, Y.B., Nakai, N., and Ohira Y. Mechanical stress-dependent transformation of nucleoli in myonuclei of rat soleus muscle fibers. 27th Annual International Gravitational Physiology Meeting, PS2.11, Osaka, Japan, 23-28 April, 2006. (poster)
21. Wang, X.D., Kawano, F., Matsuoka, Y., Terada, M., Yamamoto, H., Ito, T., Goto, K., Lan, Y.B., and Ohira, Y. Role of macrophage function in regeneration of soleus muscle fibers in *op/op* mice. 27th Annual International Gravitational Physiology Meeting, PS2.11, Osaka, Japan, 23-28 April, 2006. (poster)
22. Matsuoka, Y., Kawano, F., Wang, X.D., Terada, M., Higo, Y., Lan, Y.B., Ogura, A., Nishimoto, N., Adachi, Y., and Ohira, Y. Blockade of interleukin-6 signaling pathway promotes muscle cell differentiation. 27th Annual International Gravitational Physiology Meeting, PS2.10, Osaka, Japan, 23-28 April, 2006. (poster)
23. Higo, Y., Terada, M., Umemoto, S., Kawabe, N., Kawano, F., and Ohira, Y. Effects of tenotomy and/or denervation on the characteristics of plantaris muscle fibers of *mdx* mice. 27th Annual International Gravitational Physiology Meeting, Osaka, Japan, 23-28 April, 2006. (poster)
24. Ohira, T., Morikuni, S., Wang, X, D., Terada, M., Kawano, F., Matsuoka, Y., Higo, Y., Lan, Y, B., Seki, T., Ohira, Y. Responses of adductor longus muscle to hindlimb suspension in wistar hannover rats. 27th Annual International Gravitational Physiology Meeting, PS2.5, Osaka, Japan, 23-28 April, 2006. (poster)
25. Morikuni, S., Ohira, T., Wang, X.D., Terada, M., Kawano, F., Matsuoka, Y., Lan,

Y.B., Yamamoto, H., Ito, T., Goto, K., Seki, T., and Ohira, Y. Role(s) of macrophage in the plasticity of plantaris muscle fibers in *op/op* mice. 27th Annual International Gravitational Physiology Meeting, PS2.9, Osaka, Japan, 23-28 April, 2006. (poster)

26. Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Umemoto, S., Kawabe, N., Wang, X.D., Shinoda, Y., Fukuda, H., Ohmi, S., and Ohira, Y. Effects of deafferentation on translational activity in rat soleus muscle. The 10th International Congress of the World Muscle Society, G.P.14.09, Iguassu falls, Brazil, 28 Sep-1 Oct, 2005. (poster)
27. Ohira, Y., Wang, X.D., Kawano, F., Matsuoka, Y., Terada, M., Yamamoto, H., Ito, T., and Goto, K. Failure of regeneration from unloading-induced atrophy in *op/op* mouse soleus muscle. The 10th International Congress of the World Muscle Society, G.P.14.08, Iguassu falls, Brazil, 28 Sep - 1 Oct, 2005. (poster)
28. Terada, M., Kawano, F., Lan, Y.B., Matsuoka, Y., Wang, X.D. and Ohira, Y. Mechanical and/or neural activity-dependent regulation of soleus muscle fibers of *mdx* mice. 26th Annual International Gravitational Physiology Meeting, PS2.19, Cologne, Germany, 26 June - 1 July, 2005. (poster)

    Award：Special Student Poster Presentation and Young Researcher Sponsorship (Euro 350).
29. Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Umemoto, S., Kawabe, N., Wang, X.D., Shinoda, Y., Lan, Y.B., Fukuda, H., and Ohira, Y. Role of afferent input and/or mechanical load in size regulation of rat soleus muscle. 26th Annual International Gravitational Physiology Meeting, PS2.4, Cologne, Germany, 26 June - 1 July, 2005. (poster)

30. Wang, X.D., Kawano, F., Terada, M., Matsuoka, Y., Shinoda, Y., Ishihara, A., and Ohira, Y. Different responses in soleus muscle fibers of Wistar and Wistar Hannover rats to hindlimb unloading. 25th Annual International Gravitational Physiology Meeting, PS2.23, Cologne, Germany, 26 June - 1 July, 2005. (poster)
31. Matsuoka, Y., Wang, X.D., Kawano, F., Terada, M., Shinoda, Y., Lan, Y.B., Goto, K., Ogura, A., and Ohira, Y. How does the nucleolar number involve in muscle fiber atrophy? Response to β-guanidinopropionic acid supplementation. 26th Annual International Gravitational Physiology Meeting, PS2.21, Cologne, Germany, 26 June - 1 July, 2005. (poster)
32. Ohira, Y., Terada, M., Lan, Y.B., Matsuoka, Y., Wang, X.D., Goto, K., and Kawano, F. Neural and/or mechanical influence on the shift of myonuclei from peripheral to central region of soleus muscle fiber in *mdx* mice. The 35th Congress of the International Union of Physiological Sciences, 112.9, San Diego, U.S.A., 31 March-5 April, 2005. (poster)
33. Ohira, Y., Lan, Y.B., Terada, M., Kawano, F., Matsuoka, Y., Wang, X.D., Yoshii, N., Goto, K., and Takeda, S. Activity-dependent changes in the myonuclear distribution of soleus muscle fibers of *mdx* mice. The 4th Annual Scientific Meeting of Asian & Oceanian Myology Center (Abstract, p. 31), A-O-001, Kaohsiung, Taiwan, 3-4 March, 2005. (oral)

学会発表（国内）

1. 寺田昌弘、河野史倫、大平宇志、大平充宣。*mdx*マウスヒラメ筋における過負荷の影響。第16回日本運動生理学会大会、奈良、2008年8月2～3日（poster）。
Award：Young Investigator Award 研究奨励賞

2. 大平宇志、河野史倫、寺田昌弘、大平充宣。抗重力活動除去および再負荷がラット長内転筋の特性に及ぼす影響。第 16 回日本運動生理学会大会、奈良、2008 年 8 月 2～3 日（poster）。
3. 河野史倫、寺田昌弘、大平宇志、大平充宣。骨格筋萎縮・肥大時における HSP27 リン酸化動態の変化。第 16 回日本運動生理学会大会、奈良、2008 年 8 月 2～3 日（口頭）。
4. 寺田昌弘、河野史倫、大平宇志、肥後葉子、中井直也、大平充宣。*mdx*マウス骨格筋の再生・崩壊機構における筋線維の形態的特性。日本宇宙生物化学会第 21 回大会、東京、2007 年 9 月 27～28 日 (poster)。
5. 河野史倫、中井直也、寺田昌弘、大平宇志、肥後葉子、大平充宣。後肢懸垂およびその後の回復に対するラットヒラメ筋 27kDa heat shock proteinの応答。日本宇宙生物化学会第 21 回大会、東京、2007 年 9 月 27～28 日 (口頭)。
6. 大平宇志、寺田昌弘、河野史倫、王暁東、肥後葉子、落合俊昌、大平充宣。パラボリックフライト中のウィスターハノーバーラット長内転筋活動の変化。日本宇宙生物化学会第 21 回大会、東京、2007 年 9 月 27～28 日 (poster)。
7. 寺田昌弘、松岡由和、河野史倫、王暁東、藍勇波、肥後葉子、大平充宣。IL-6 レセプター阻害剤(MR16-1)による*mdx*マウス骨格筋への影響。日本航空環境医学会第 52 回大会、鳥取、2006 年 11 月 9～11 日 (口頭)。
8. 王暁東、河野史倫、寺田昌弘、大平宇志、後藤勝正、大平充宣。後肢懸垂とその後の回復に対する*op/op*マウスヒラメ筋と足底筋の反応の違い。日本航空環境医学会第 52 回大会、鳥取、2006 年 11 月 9～11 日 (口頭)。
9. 河野史倫、寺田昌弘、大平充宣。後肢懸垂によるラットヒラメ筋萎縮からの回復時におけるHSP27 リン酸化阻害の影響。日本航空環境医学会第 52 回大会、鳥取、2006 年 11 月 9～11 日 (口頭)。

10. 大平宇志、王暁東、寺田昌弘、河野史倫、松岡由和、大平充宣。ラット長内転筋における抗重力筋活動抑制の影響。日本航空環境医学会第 52 回大会、鳥取、2006 年 11 月 9～11 日 (口頭)。
11. 大平宇志、森国さやか、王暁東、寺田昌弘、河野史倫、松岡由和、肥後葉子、藍勇波、関隆晴、大平充宣。後肢懸垂がウィスターハノーバーラット長内転筋の特性に及ぼす影響。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
12. 王暁東、河野史倫、松岡由和、寺田昌弘、大平宇志、藍勇波、後藤勝正、大平充宣。後肢懸垂語の重力負荷が*op/op*マウスヒラメ筋線維に及ぼす影響。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
13. 河野史倫、松岡由和、寺田昌弘、肥後葉子、中井直也、大平充宣。過負荷および感覚神経切除がラットヒラメ筋線維のサイズ調節に及ぼす影響。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
14. 藍勇波、寺田昌弘、河野史倫、肥後葉子、松岡由和、王暁東、大平充宣。*mdx*マウス骨格筋の可塑機構における機械的・神経的刺激の影響。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
15. 寺田昌弘、Lyubaeva, E., 大平宇志、Vinogradova, O., 大平充宣。エクセントリック又はコンセントリック運動が人外側広筋に及ぼす効果。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
16. 肥後葉子、寺田昌弘、梅本詩織、川部直子、河野史倫、大平充宣。張力発揮又は電気的活動抑制に対するマウス足底筋の反応。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
17. 森国さやか、大平宇志、王暁東、寺田昌弘、河野史倫、肥後葉子、松岡由和、藍勇波、関隆晴、大平充宣。筋活動抑制に対するマウス足底筋の反応におけ

るマクロファージの役割。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。

18. 松岡由和、河野史倫、王暁東、寺田昌弘、肥後葉子、藍勇波、大平宇志、小倉明彦、西本憲弘、大平充宣。インターロイキン-6 シグナル伝達経路の阻害は筋細胞の分化を促す。日本宇宙生物科学会第 20 回大会、大阪、2006 年 9 月 28～29 日 (poster)。
19. 寺田昌弘、河野史倫、藍勇波、肥後葉子、松岡由和、王暁東、大平充宣。張力発揮または神経活動が*mdx*マウス骨格筋の再生機構における役割。日本航空環境医学会第 51 回大会、横浜、2005 年 11 月 10～12 日 (口頭)。
20. 河野史倫、松岡由和、寺田昌弘、肥後葉子、王暁東、大平充宣。感覚神経がラットヒラメ筋における細胞内シグナル伝達に及ぼす影響。日本航空環境医学会第 51 回大会、横浜、2005 年 11 月 10～12 日 (口頭)。
21. 王暁東、河野史倫、松岡由和、寺田昌弘、藍勇波、後藤勝正、大平充宣。10 日間の後肢懸垂とその後の回復が *op/op* マウスヒラメ筋線維特性に及ぼす影響。日本航空環境医学会第 51 回大会、横浜、2005 年 11 月 10～12 日 (口頭)。
22. 松岡由和、河野史倫、肥後葉子、王暁東、寺田昌弘、藍勇波、大平充宣。エネルギー状態の変化が筋核小体数に及ぼす影響。日本航空環境医学会第 51 回大会、横浜、2005 年 11 月 10～12 日 (口頭)。
23. 王暁東、河野史倫、藍勇波、松岡由和、寺田昌弘、石原昭彦、大平充宣。後肢懸垂に対するWistarとWistar Hannoverラットヒラメ筋の反応の違い。日本航空環境医学会第 51 回大会、横浜、2005 年 11 月 10～12 日 (口頭)。

講演

1. Terada, M., Lyubaeva, E., Ohira, T., Netreba, A., Kawano, F., Okabe, H., Imaizumi, K., Vinogradova, O., and Ohira, Y. Responses of vastus lateralis muscle fibers to cycling training with different loading parameter in human. 日本航空環境医学会第 54 回大会、東京、2008 年 11 月 13～15 日 (第 11 回研究奨励賞受賞講演)。

受賞

1. 第 11 回研究奨励賞、日本航空環境医学会第 54 回大会、2008 年 11 月 14 日。
2. Young Investigator Award 研究奨励賞、第 16 回日本運動生理学会大会、2008 年 8 月 3 日。
3. Travel award ($ 250), American Society for Gravitational and Space Biology, 23th Annual Meeting, 25-28 Oct, 2007.
4. Special Student Poster Presentation and Young Researcher Sponsorship (Euro 350), 26th Annual International Gravitational Physiology Meeting, 26 June - 1 July, 2005.