JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2010, 61, 5, 559-563
www.jpp.krakow.pl

M. MACHIDA, T. TAKEMASA

IBUPROFEN ADMINISTRATION DURING ENDURANCE TRAINING CANCELS RUNNING-DISTANCE-DEPENDENT ADAPTATIONS OF SKELETAL MUSCLE IN MICE

Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-0006, Japan

Exercise training induces many adaptations in skeletal muscle, representative examples of which include an increase in the IIa myofibre and an increase in the capillary-to-fibre ratio (C:F ratio). Moreover, these phenomena are thought to be dependent on running distance. Ibuprofen is one non-steroidal anti-inflammatory drug that is often used as an analgesic, but its effect on skeletal muscle adaptation during endurance training is unclear. In the present study, therefore, we administered ibuprofen to mice during running wheel exercise for four weeks, and examined its effects on the increase in the IIa myofibre and the C:F ratio in skeletal muscle. We observed a significant increase of the IIa myofibre and C:F ratio even in the presence of ibuprofen. Moreover, in untreated mice, there was a significant positive and strong correlation between these parameters and running distance. These results indicate that the increase in the IIa myofibre and the C:F ratio in skeletal muscle usually depend on running distance. Interestingly, we observed no significant correlation between these parameters and running distance in ibuprofen-administered mice. Moreover, we found no significant increase of these parameters when the running distance was significantly increased, in comparison with untreated mice. These results indicate that ibuprofen administration during endurance training cancels running-distance-dependent adaptations in skeletal muscle. This suggests that even if ibuprofen administration facilitates longer-distance running, no further effects of training on skeletal muscle can be expected.

Key words: *endurance exercise, ibuprofen, skeletal muscle, body weight, muscle adaptation, cyclooxygenase*

INTRODUCTION

Exercise training is thought to be a beneficial method for improving physiological parameters such as skeletal muscle mass, percentage body fat, and body weight. Skeletal muscle is a mosaic organ composed of several types of myofibre. Fast myofibre has an ability to contract faster than slow one, conversely slow myofibre has an ability to contract for a longer time than fast one. Slow myofibre is composed by myosin heavy-chain (MHC), type I. And, fast myofibres are composed by three (IIa, IId/x, and IIb) MHCs (1). Especially, fast myofibre composed by IIa is thought to be able to contract for a longer time than other fast myofibres composed by IId/x or IIb. Endurance training, such as periodic voluntary wheel-running or treadmill running, is thought to increase the percentage of IIa myofiber in plantaris muscle (2, 3).

The capillary is a basic vascular network component that permits diffusive exchange between the inside and outside of the vascular wall. Thus, an increase in the capillary-to-fibre ratio (C:F ratio) improves the efficiency of diffusive exchange between the vascular space and the intracellular volume of myofibres (4). Periodic voluntary wheel-running also induces an increase of the C:F ratio in mouse skeletal muscle (2). Therefore, these increases in the percentage of IIa myofiber and C:F ratio are thought to be representative adaptations to endurance exercise.

Ibuprofen is a non-steroidal anti-inflammatory drug that is often used as an analgesic and antipyretic (5). Previous studies have demonstrated an inhibitory effect of ibuprofen on skeletal muscle hypertrophy induced by functional overload, suggesting that ibuprofen administration during resistance training inhibits training-induced skeletal muscle hypertrophy (6, 7). On the other hand, the effect of ibuprofen on skeletal muscle adaptations induced by periodic endurance training is still unclear. In order to elucidate the effect of ibuprofen administration on endurance training-induced skeletal muscle adaptations, we housed mice in a cage with a running wheel for four weeks, and subsequently examined the effects of ibuprofen administration on the change of the percentage of I and IIa myofiber and C:F ratio.

MATERIALS AND METHODS

Experimental approval

Animal experiments were carried out in a human manner after receiving approval from the Institutional Animal Experiment Committee of the University of Tsukuba, and in accordance with the Regulations for Animal Experiments at the University, and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Animals and animal care

Eight-week-old (body weight 35-40 g) male ICR mice (Kiwa Laboratory Animals Co., Ltd., Japan) were housed in the animal facility under a 12-12 hour light-dark cycle at room temperature (21±2°C) and 50±5% humidity, and fed a rodent chow with free access to water. These mice were divided into four groups (each n=8): sedentary-D.W. (SED-D.W.), sedentary-ibuprofen (SED-IBU), exercise-D.W. (EXE-D.W.) and exercise-ibuprofen (EXE-IBU). The mice in the EXE-D.W. or EXE-IBU group were housed individually in a cage with a running wheel. The mice in the SED-IBU or EXE-IBU group were given ibuprofen (50 mg/l) in drinking water. The running distance of each mouse was monitored using a device we have reported previously (8).

Immunohistochemistry

Immediately after a four-week experiment period, all the mice were sacrificed by cervical dislocation, and the plantaris muscle was dissected out quickly from each mouse, and then frozen in isopentane cooled with liquid nitrogen. Transverse sections several micrometers thick were made with a cryostat (CRYO-STAR HM560, Microm, Germany) cooled to less than -16°C, and then stored at -80°C until staining. The sections were dried in air for 20 minutes, and then fixed with 4% paraformaldehyde for 10 minutes in room temperature. To inhibit endogenous peroxidase, the sections were incubated in 0.3% hydrogen peroxide diluted in methanol for 10 minutes at room temperature. Anti-CD31 (Pharmingen, USA) or anti-myosin (N2.261, human neonatal slow and fast IIa fibers, Iowa University, USA) antibody was used as the primary antibody, and biotin-conjugated anti-rat or anti-mouse antibody (Pharmingen, USA) as the secondary antibody. After incubation with avidin-biotin peroxidase complex (Nakarai Tesque, Japan) for 30 minutes, visualization of antibody staining was achieved using 3,3'-diaminobenzidine tetrahydrochloride (WAKO, Japan) as the chromogen. Serial number was entered in each slide to blind the condition to investigator quantifying the number of each antibody positive stain. The stained sections were finally visualised by microscopy (BX51, Olympus, Japan) and evaluated using a specific software package (OpenLab, Improvision, USA).

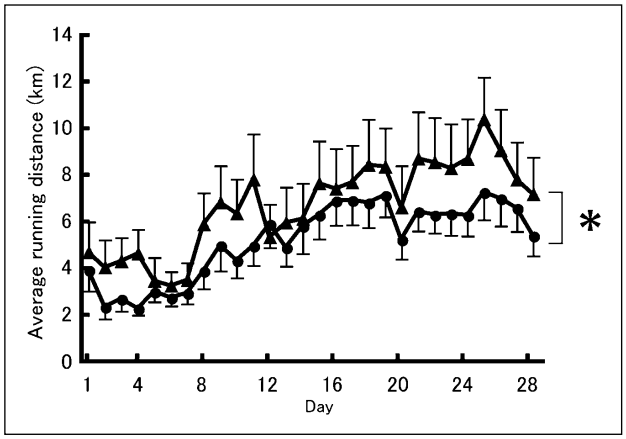

Fig. 1. Average daily running-distance control group and ibuprofen-administered group. This figure shows the average distance run per day. Solid circles indicate the EXE-D.W. group, and solid triangles indicate the EXE-IBU group. All data represent the standard error of the mean (S.E.M.). Asterisk indicates significant difference between the two groups at P<0.01.

Statistical analysis

The significance of differences in daily running distance between the EXE-D.W. and EXE-IBU groups was measured by two-way analysis of variance (ANOVA). The effects of ibuprofen or wheel-running on the percentage of I and IIa myofiber and C:F ratio were analyzed using the Tukey multiple comparison test following two-way ANOVA. Pearson correlation coefficients were used to measure associations between average daily running-distance and various parameters, including the percentage of I and IIa myofibre and C:F ratio. Differences at P<0.05 were considered to be statistically significant.

RESULTS

Water intake, body weight, muscle mass and running distance

The daily running distance in the two groups gradually increased throughout the experimental period for four weeks (*Fig. 1*). Two-way ANOVA revealed that there was a significant difference in the average daily running distance between the two groups (P<0.001). *Table 1* shows the average daily water intake (with or without ibuprofen), body weight, and muscle mass in each group. Two-way ANOVA revealed that wheel running led to a decrease of body weight (P<0.001), whereas it increased the average daily amount of water drunk (P<0.01). However, there was no significant difference in these parameters between the groups with and without ibuprofen administration. Wet weight of plantaris muscle was not influenced by both wheel running and ibuprofen administration (*Table 1*).

Lack of effect of ibuprofen administration on endurance training-induced adaptation in skeletal muscle

Next, we measured the percentage of I and IIa myofiber and C:F ratio in each of the four groups. Tukey multiple comparison test following two-way ANOVA revealed that wheel-running for four weeks increased both the percentage of I and IIa myofiber (P<0.01) and the C:F ratio (P<0.01). However, ibuprofen administration had no effect on either of these parameters (*Fig. 2*).

Abrogation of running distance-dependent adaptation to wheel training by ibuprofen administration

It is well known that endurance training increases the percentage of I and IIa myofiber in proportion to the running

Table 1. Influence of ibuprofen administration during wheel running to body weight, plantaris muscle mass and daily water intake

	SED-D.W.	SED-IBU	EXE-D.W.	EXE-IBU
Body weight (g)	41.67 (±0.83)	43.69 (±0.77)	39.22 (±0.64)	39.73 (±0.41) * (SED vs EXE)
Absolute plantaris muscle mass (mg)	21.58 (±1.06)	22.23 (±1.40)	23.40 (±1.21)	25.19 (±1.45)
Average daily drink water (g)	6.55 (±0.26)	7.04 (±0.81)	8.42 (±0.45)	8.52 (±0.40) * (SED vs EXE)

This table shows body weight, absolute muscle mass of plantaris, and average daily water intake. SED, sedentary; EXE, exercise; D.W., Distiled Water; IBU, ibuprofen. Asterisks indicate significant difference between SED and EXE. All data are indicated as S.E.M.

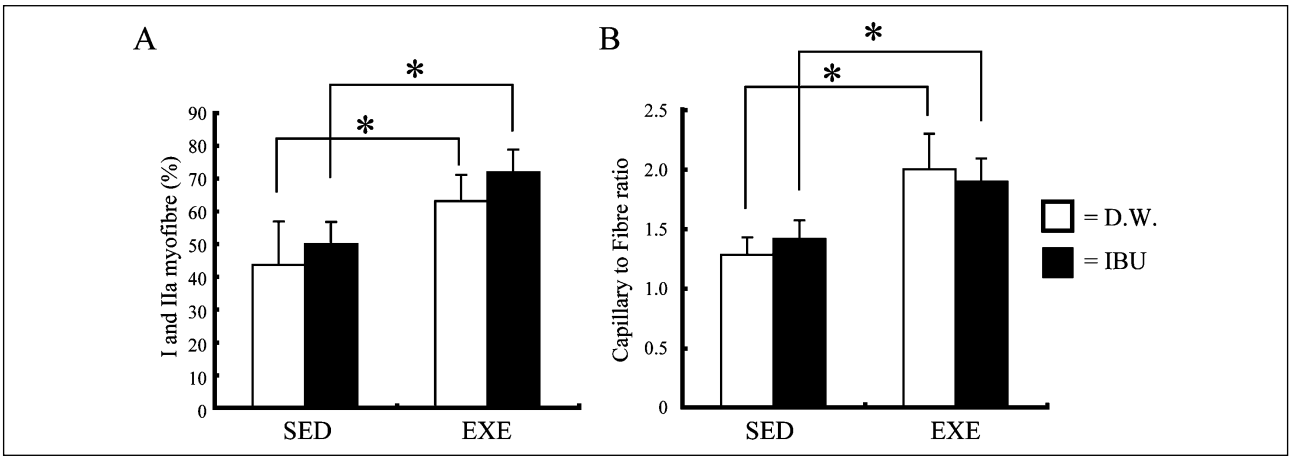

Fig. 2. Effect of ibuprofen administration during endurance training on the percentage of I and IIa myofibre and the capillary to fibre ratio. These figures show the influence of ibuprofen administration on skeletal muscle adaptations induced by wheel running for four weeks. The percentage of I and IIa myofibre (A), and the capillary to fibre ratio (B) are indicated. Empty bars indicate each D.W. groups, and solid bars indicate each IBU groups. All data represent S.E.M. Asterisks indicate significant differences between groups at $P<0.01$.

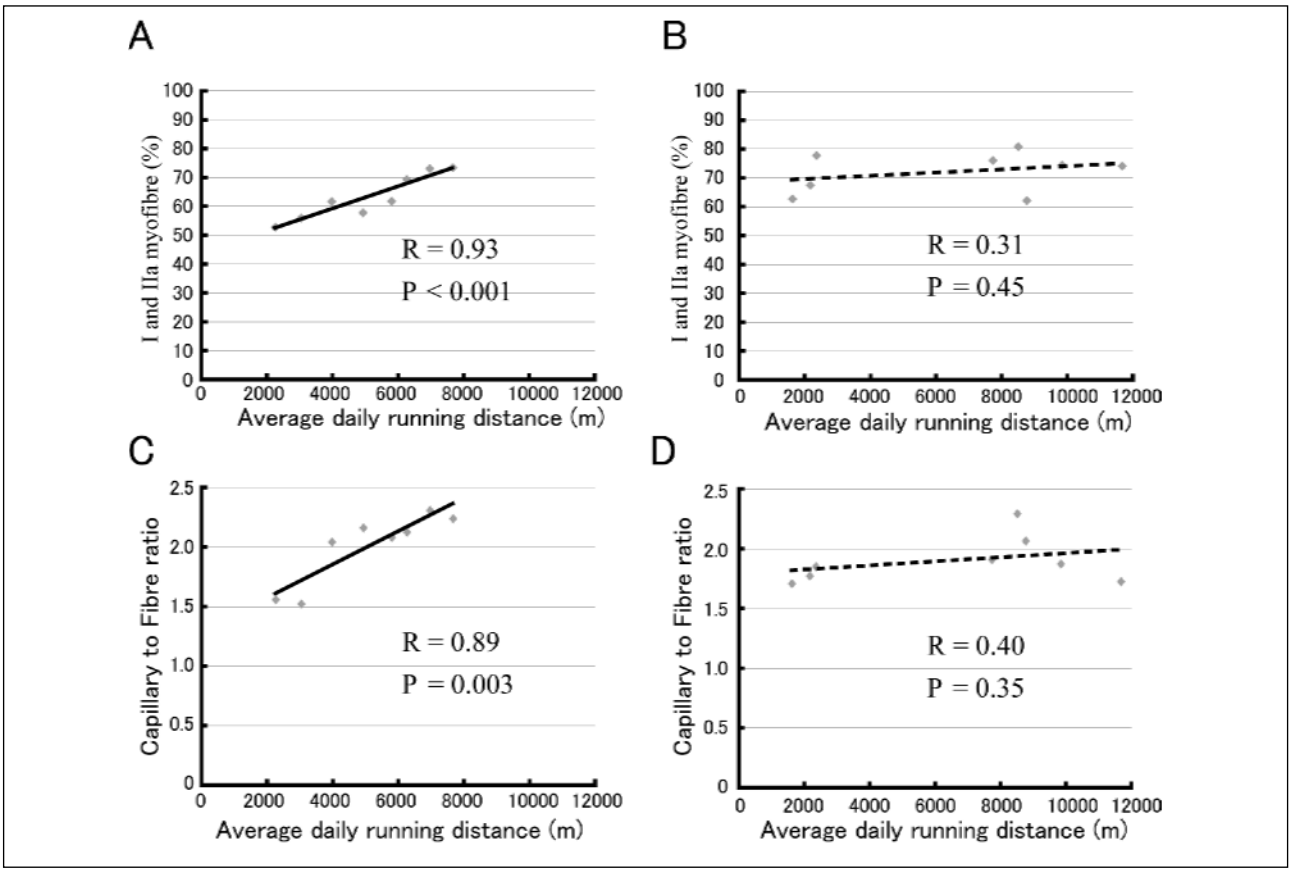

Fig. 3. Relationship between average daily running distance and signs of skeletal muscle adaptation. These figures show the correlation between average daily running distance and skeletal muscle adaptation with (B and D) and without (A and C) the influence of ibuprofen administration. The percentage of I and IIa myofibre (A and B) and the C:F ratio (C and D). Solid lines indicate a significant and strong correlation (A and C), and dotted lines indicate non-significant correlation (B and D).

distance. Therefore, the myofibre composition and C:F ratio were each compared in terms of the average daily running distance using Pearson correlation coefficients. This revealed that without ibuprofen administration, the percentage of I and IIa myofiber and the C:F ratio were directly correlated with the average daily running distance (*Fig. 3A* and *C*), whereas this was not the case in mice administered ibuprofen (*Fig. 3B* and *D*).

DISCUSSION

Skeletal muscle has an ability to fit to its workload-type. For instance, it is well known that periodic resistance training induces skeletal muscle hypertrophy (9), whereas endurance training induces an increase in IIa myofibre and C:F ratio (2, 3). In fact, it was revealed that endurance training did not increase

maximal voluntary isometric contraction, but it increased time to fatigue at 50% of maximal voluntary isometric contraction in human skeletal muscle (10). Moreover, it is also well known that exercise increased oxidative capacity and capillary density in only muscle which contracted during exercise (11). Because ibuprofen is often used as an analgesic and antipyretic, we examined its effect on skeletal muscle adaptation induced by endurance training in mice. Although we had hypothesized that ibuprofen administration during endurance training would inhibit endurance training-induced adaptation, no such effect was observed. However, we observed that ibuprofen administration during voluntary wheel-running abrogated running-distance dependent percentage of I and IIa myofibre and C:F ratio (*Fig. 3*).

At first, the cause of this interesting result may be that mice administered ibuprofen ran a longer distance than those without ibuprofen (*Fig. 1*). Ibuprofen is a non-specific inhibitor of cyclooxygenases (COX) I and II, which produce prostaglandins, known to have an analgesic effect (5). It was reported that ibuprofen reduced muscle soreness after exercise (12), which indicated that exercise-induced muscle soreness is caused by prostaglandins catalyzed by COX. Pizza *et al*. reported that passive stretching, isometric contraction, and lengthening contraction without severe injury in skeletal muscle increased the recruitment of leukocytes, which are known to secrete prostaglandins (13). We also observed an increase of leukocytes on a day after a single bout of endurance training without severe injury in mouse skeletal muscle (14). It was possible that voluntary running also infiltrated leukocyte-secreting prostaglandins in skeletal muscle as well as previous study (14), and muscle soreness caused by this infiltration might limit voluntary running in mice without ibuprofen administration (*Fig. 1*). However, in current study, we did not confirm leukocyte infiltration and some inflammatory response in skeletal muscle. In the future study, relationship between voluntary running and inflammatory response should be studied.

Inflammation is thought to have a close relationship to angiogenesis (15). Co-cultivation of cancer cells with macrophages in the presence of inflammatory stimulation synergistically enhances the migration of vascular endothelial cells *in vitro*, and macrophage depletion by clodronate liposomes reduces angiogenesis *in vivo* (16). Macrophage depletion by clodronate reduces both tumour angiogenesis and subsequent tumour growth in lung cancers (17). COX-2 is a representative mediator of inflammatory responses, and its expression is highly influenced by inflammatory stimulation. COX-2 also plays a key role in tumour angiogenesis by catalyzing the production of prostanoids, including prostaglandin E_2 and thromboxane A_2 (18, 19). In mouse corneal angiogenesis induced by interleukin-1 beta, either administration of COX-2 inhibitors or COX-2 knockdown almost completely attenuates angiogenesis (20). There are considerable reports indicating that inflammation facilitates tumour angiogenesis (17-20). Previous studies reported that exercise increased inflammation-related cytokines such as interleukin-1 (21), interleukin-6 (22) and transforming growth factor-beta1 (23). We previously observed vascular endothelial growth factor-secreting leukocyte in mouse skeletal muscle on a day after exercise (14). These results indicated that exercise induces inflammation in skeletal muscle and suggested that exercise-induced angiogenesis also related to inflammation. In current study, we observed that running-distance dependent C:F ratio in D.W.-EXE group was abrogated in IBU-EXE group (*Fig. 3*). We have hypothesized the second reason of this abrogation was that exercise-induced angiogenesis was inhibited by ibuprofen administration in longer running mice in IBU-EXE group.

The percentage of I and IIa myofibre was increased by voluntary wheel running (*Fig. 2*). Moreover, this parameter was dependent to running distance in D.W.-EXE group (*Fig. 3*). Interestingly, this positive correlation between running distance and the percentage of I and IIa myofibre as well as C:F ratio was abrogated by ibuprofen administration (*Fig. 3*). Although the longer running induced by ibuprofen administration might be the cause of the abrogation, there is no sufficient evidence to support the effect of ibuprofen administration inhibited the increase of IIa myofibre as well as C:F ratio. However, it was reported that immuno-suppressing drug such as FK506 reduced fibre-type transition from fast to slow (24). It is possible that one immune-system factor related to inflammation has a relationship to fibre-type transition.

Soltow *et al*. showed that skeletal muscle hypertrophy was inhibited by ibuprofen (6), and Novak *et al*. demonstrated that the specific COX-2 inhibitor, NS-398, reduced skeletal muscle hypertrophy in the mouse (25). Moreover, DiPasquale *et al*. reported that macrophage depletion by clodronate delayed skeletal muscle hypertrophy (26). These results indicate that inflammation is an important factor for skeletal muscle hypertrophy, and also that administration of anti-inflammatory drugs (including ibuprofen) inhibits skeletal muscle hypertrophy. Taken together, the present and previous findings indicate that inhibition of inflammation may reduce the skeletal muscle adaptations induced by exercise training. In the future, however, it will be necessary to investigate the effects of administration of anti-inflammatory drugs on the I and IIa myofibre and C:F ratio using a treadmill, which will make it possible to control the running distance and intensity.

In conclusion, the present study has demonstrated that ibuprofen administration during endurance training facilitates a longer distance run, but this is not associated with effects that were originally considered to depend on running distance. On the basis of these results, we propose that administration of anti-inflammatory drugs may diminish proper effects of training that are correlated with the degree of training labor.

Acknowledgements: This work was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#21240062).

The anti-myosin antibody was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242.

Conflict of interests: None declared.

REFERENCES

1. Schiaffino S, Reggiani C. Myosin isoforms in mammalian skeletal muscle. *J Appl Physiol* 1994; 77(2): 493-501.
2. Waters RE, Rotevatn S, Li P, Annex BH, Yan Z. Voluntary running induces fiber type-specific angiogenesis in mouse skeletal muscle. *Am J Physiol Cell Physiol* 2004; 287(5): C1342-C1348.
3. Demirel HA, Powers SK, Naito H, Hughes M, Coombes JS. Exercise-induced alterations in skeletal muscle myosin heavy chain phenotype: dose-response relationship. *J Appl Physiol* 1999; 86(3): 1002-1008.
4. Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? *J Appl Physiol* 2004; 97(3): 1119-1128.
5. Hasson SM, Daniels JC, Divine JG, *et al*. Effect of ibuprofen use on muscle soreness, damage, and performance: a preliminary investigation. *Med Sci Sports Exerc* 1993; 25(1): 9-17.

6. Soltow QA, Betters JL, Sellman JE, Lira VA, Long JH, Criswell DS. Ibuprofen inhibits skeletal muscle hypertrophy in rats. *Med Sci Sports Exerc* 2006; 38(5): 840-846.
7. Peterson JM, Trappe TA, Mylona E, *et al*. Ibuprofen and acetaminophen: effect on muscle inflammation after eccentric exercise. *Med Sci Sports Exerc* 2003; 35(6): 892-896.
8. Ikeda S, Kawamoto H, Kasaoka K, *et al*. Muscle type-specific response of PGC-1 alpha and oxidative enzymes during voluntary wheel running in mouse skeletal muscle. *Acta Physiol (Oxf)* 2006; 188(3-4): 217-223.
9. Goto K, Nagasawa M, Yanagisawa O, Kizuka T, Ishii N, Takamatsu K. Muscular adaptations to combinations of high- and low-intensity resistance exercises. *J Strength Cond Res* 2004; 18(4): 730-737.
10. Grandys M, Majerczak J, Duda K, Zapart-Bukowska J, Sztefko K, Zoladz JA. The effect of endurance training on muscle strength in young, healthy men in relation to hormonal status. *J Physiol Pharmacol* 2008; 59(Suppl 7): 89-103.
11. Laughlin MH, Roseguini B. Mechanisms for exercise training-induced increases in skeletal muscle blood flow capacity: differences with interval sprint training versus aerobic endurance training. *J Physiol Pharmacol* 2008; 59(Suppl 7): 71-88.
12. Rahnama N, Rahmani-Nia F, Ebrahim K. The isolated and combined effects of selected physical activity and ibuprofen on delayed-onset muscle soreness. *J Sports Sci* 2005; 23(8): 843-850.
13. Pizza FX, Koh TJ, McGregor SJ, Brooks SV. Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *J Appl Physiol* 2002; 92(5): 1873-1878.
14. Machida M, Takemasa T. Increased macrophages following endurance exercise without severe injury play a role in angiogenesis in skeletal muscle. *Adv Exerc Sports Physiol* 2009; 15(2): 39-44.
15. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest* 2007; 117(5): 1175-1183.
16. Kimura YN, Watari K, Fotovati A, *et al*. Inflammatory stimuli from macrophages and cancer cells synergistically promote tumor growth and angiogenesis. *Cancer Sci* 2007; 98(12): 2009-2018.
17. Ono M. Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. *Cancer Sci* 2008; 99(8): 1501-1506.
18. Sawaoka H, Tsuji S, Tsujii M, *et al*. Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. *Lab Invest* 1999; 79(12): 1469-1477.
19. Masferrer JL, Koki A, Seibert K. COX-2 inhibitors. A new class of antiangiogenic agents. *Ann N Y Acad Sci* 1999; 889: 84-86.
20. Kuwano T, Nakao S, Yamamoto H, *et al*. Cyclooxygenase 2 is a key enzyme for inflammatory cytokine-induced angiogenesis. *FASED J* 2004; 18(2): 300-310.
21. Cannon JG, Fielding RA, Fiatarone MA, Orencole SF, Dinarello CA, Evans WJ. Increased interleukin 1 beta in human skeletal muscle after exercise. *Am J Physiol* 1989; 257(2): R451-R455.
22. Febbraio MA, Steensberg A, Starkie RL, McConell GK, Kingwell BA. Skeletal muscle interleukin-6 and tumor necrosis factor-alpha release in healthy subjects and patients with type 2 diabetes at rest and during exercise. *Metabolism* 2003; 52(7): 939-944.
23. Czarkowska-Paczek B, Zendzian-Piotrowska M, Bartlomiejczyk I, Przybylski J, Gorski J. The effect of acute and prolonged endurance exercise on transforming growth factor-beta1 generation in rat skeletal and heart muscle. *J Physiol Pharmacol* 2009; 60(4): 157-162.
24. Miyazaki M, Hitomi Y, Kizaki T, Ohno H, Haga S, Takemasa T. Contribution of the calcineurin signaling pathway to overload-induced skeletal muscle fiber-type transition. *J Physiol Pharmacol* 2004; 55(4): 751-764.
25. Novak ML, Billich W, Smith SM, *et al*. COX-2 inhibitor reduces skeletal muscle hypertrophy in mice. *Am J Physiol Regul Integr Comp Physiol* 2009; 296(4): R1132-R1139.
26. DiPasquale DM, Cheng M, Billich W, *et al*. Urokinase-type plasminogen activator and macrophages are required for skeletal muscle hypertrophy in mice. *Am J Physiol Cell Physiol* 2007; 293(4): C1278-C1285.

Received: February 1, 2010
Accepted: September 24, 2010

Author's address: Dr. Tohru Takemasa, Physical Education, Health and Sport Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ten-noudai 1-1-1, Tsukuba-shi, Ibaraki 305-0006, Japan; Phone & Fax: +81-(0)29-853-2622; E-mail: takemasa@taiiku.tsukuba.ac.jp