The change in thyroid hormone signaling by altered training intensity in male rat skeletal muscle

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Abstract. Aerobic (sub lactate threshold; sub-LT) exercise training facilitates oxidative phosphorylation and glycolysis of skeletal muscle. Thyroid hormone (TH) also facilitates such metabolic events. Thus, we studied whether TH signaling pathway is activated by treadmill training. Male adult rats received 30 min/day treadmill training with different exercise intensity for 12 days. Then plasma lactate and thyrotropin (TSH) levels were measured. By lactate levels, rats were divided into stationary control (SC, 0 m/min), sub-LT (15 m/min) and supra lactate threshold (supra-LT; 25 m/min) training groups. Immediately after the last training, the soleus muscles were dissected out to measure TH receptor (TR) mRNA and protein expressions. Other rats received intraperitoneal injection of T3, 24 h after the last training and sacrificed 6 h after the injection to measure TH target gene expression. TSH level was suppressed in both sub-LT and supra-LT groups during the exercise. TRβ1 mRNA and protein levels were increased in sub-LT group. Sensitivity to T3 was altered in several TH-target genes by training. Particularly, induction of Na+/K+-ATPase β1 expression by T3 was significantly augmented in sub-LT group. These results indicate that sub-LT training alters TH signaling at least in part by increasing TRβ1 expression. Such TH signaling alteration may contribute metabolic adaptation in skeletal muscle during physical training.

Key words: Exercise intensity, Thyrotropin, Thyroid hormone receptor, Na+/K+-ATPase

EXERCISE training affects metabolism of skeletal muscle. Exercise enhances insulin sensitivity by activating atypical protein kinase C (aPKC), which then activates translocation of glucose transporter 4 (GLUT4) to the muscle cell membrane to increase glucose uptake [1]. Training also enhances glycolysis-oxidative phosphorylation through activation of oxidative enzyme such as citrate synthase (CS) both in rat and human [2, 3]. Insulin-like growth factor (IGF)-1-mediated signaling pathway is also activated by training through induction of IGF binding protein-1 (IGFBP-1) [4]. Exercise training also activates oxidative phosphorylation in skeletal muscle. Pinho et al. [5] have reported that exercise can increase activity of cytochrome c oxidase I, II, III and IV. Endurance training increases mitochondrial number and density in human skeletal muscle [3]. Through these alterations, skeletal muscle can adapt increased metabolic demands by repeated exercise/training. Such adaptation can contribute to maintain the physical fitness. In spite of these findings, mechanisms of such metabolic alteration by training have not yet been fully understood.

Thyroid hormone (TH) affects metabolism of skeletal muscle. TH stimulates oxidative phosphorylation via inducing citrate synthase protein levels and its activities [6]. TH also increases basal and insulin-stimulated glucose transport in skeletal muscle through a rapid post-transcriptional effect on GLUT4 mRNA polyadenylation [7, 8]. TH also increases oxidative phosphorylation of skeletal muscle through activation of cytochrome c oxidase I and IV activities [6]. Furthermore, Na+/K+-ATPase expression is altered by TH [9]. The physiological importance of the Na+/K+-ATPase sodium pump is emphasized by the fact...
that up to 45% of the cell’s metabolic energy, in the form of ATP, is utilized by sodium pump action [10]. In the intact cell, the respiratory response may be a consequence of simultaneous effects on the permeability of the plasma membrane to Na⁺ and K⁺, activation of Na⁺/K⁺-ATPase activity, and a shift in the sensitivity of the mitochondrial system to ATP/ADP levels [11]. In responsive tissues, T3 can up-regulate the metabolic rate in part by increasing ATP, which is then consumed by Na⁺/K⁺-ATPase [12]. Interestingly, T3 also increases Na⁺/K⁺-ATPase α and β subunit mRNA abundance in rat kidney [13].

Considering these similarities between exercise training and TH action, metabolic alteration of skeletal muscle by training, particularly aerobic (sub-lactate threshold; sub-LT) training, may be at least in part exerted through TH system. However, influence of endurance training on TH action has not yet been clarified [14].

TH may be involved in controlling metabolism of skeletal muscle during training. A series of reports support this idea that TH was altered during training. Maximal aerobic exercise affects greatly the level of circulating TH [15]. Serum TH level was increased after acute exercise but not after most chronic exercise [16]. Acute exercise for 20 min with 75% maximal oxygen consumption increased T3, T4, and TSH levels [17]. However, some reports showed that TH might not show significant alteration during training. Smallridge et al. [18] have reported that thyroid stimulating hormone (TSH) secretion and serum TH levels were unaltered by treadmill exercise in human. Other group has reported that exercise did not change level of free T4 and thyroglobulin, although they observed a little increase in T3 level in the plasm due to haemocencentration after exercise [19].

It is generally accepted that most TH actions are exerted by binding to nuclear TH receptor (TR), which then activates or suppresses transcription of target genes. All major TR isoforms such as TRα1, TRα2, TRβ1 and TRβ2 are expressed in the skeletal muscle [20], although a controversy exists on relative abundance [20-25]. TRα1 and TRβ1 may play an important but distinct role on development and functional maintenance of skeletal muscle [26]. However, the change in TR levels during exercise training has not yet been clarified. Furthermore, the change in TH action by exercise in skeletal muscle has not yet been studied.

In the present study, to examine the relationship between TH action and exercise, we examined the effect of different intensity of exercise training on TR mRNAs and protein expressions, and TH-target gene responsiveness to T3 in rat skeletal muscle.

**Materials and Methods**

**Animals**

The animal experiment protocol in the present study was approved by the Animal Care and Experimentation Committee, Gunma University. Male Wistar rats at the age of 8 weeks (body weight 230 ± 10 g, n=105) used in the present study were purchased from SLC (Hamamatsu, Japan). Animals were bred in the animal facility of Gunma University Graduate School of Medicine, and were kept in standard individual cages with food and water available ad libitum. The room temperature was kept at 24°C under a 12 h light -12 h dark cycle (light on: 0600-1800 h).

**Treadmill habituation protocol**

Treadmill habituation protocol was adopted from a previous study [27] with several modifications to minimize the stress. All rats were habituated for 5 days with weak exercise using flatbed treadmill machine (KN. 73 Tread-Mill; Natsume Seisakusho Co., Ltd., Tokyo, Japan). Habituation was started with putting rats in the machine for 60 min/day at day 1, followed by 2 m/min for 20 min at day 2 and the training speed was gradually increased to 11 m/min until day 5. At the end of habituation, rats were selected based on Dishman Score [28] and only rats showing a good Dishman score, and ability to run with targeted speed without any electrical stimulation, were included into the experiment.

**Determination of exercise intensity**

After habituation process, a sylastic cannula (0.037 inch in outer diameter and 0.020 inch in inner diameter) was inserted into the right atrium via the right jugular vein for blood sampling, under anesthesia with ketamine 10 mg/(200 g body weight; bw) in combination with xylazine 1 mg/(200 g bw). Cannula was externalized at the cervical dorsum. Patency was maintained by flushing with 0.2 mL saline solution containing heparin (20 units/mL), penicillin (100 units/mL), and streptomycin (100 µg/mL). Post-operated animals were kept in special clean and solitary cages for 3 days. At the time of serial blood sampling, the cannula was attached to an extension tube that exited from the top of
the enclosed chamber of treadmill, allowing animal to move freely. Rats ran on treadmill at a speed of 15, 20 or 25 m/min. Blood samples (0.2 mL each) were withdrawn at 0, 10, 20 and 30 min during running. Blood lactate levels were measured by Lactate Pro Test Meter (ARKRAY, Inc., Miami, FL). According to the lactate level, training at 15 m/min for 30 min was determined as sub lactate threshold (sub-LT), whereas training at 25 m/min for 30 min as supra lactate threshold (supra-LT) training (see Results section for detail).

**Measurement of TSH concentration**

Plasma TSH level was analyzed using ELISA kit (Endocrine Technology Inc., Newark, CA). All experimental procedures were performed according to manufacturer’s instruction. ELISA result was measured by microplate reader (Versamax, Molecular Devices, Silicon Valley, CA) at 450 nm optical density. Standard curve was generated with R = 0.99.

**Semi-quantitative RT-PCR**

Total RNA was extracted from the soleus muscle using RNeasy Kit (Qiagen, Hilden, Germany). Complementary DNA was reverse-transcribed from 500 ng total RNA as described in the PrimeScript RT reagent Kit (Takara Bio Inc., Saga, Japan), using the oligo(dT) and random primers. In one reaction, 2.5 µL of reverse-transcribed cDNA, 0.5 µM sense and antisense primers, 200 µM dNTPs and 0.125 µL Taq polymerase (Roche, Mannheim, Germany) were added in a final volume of 25 µL. All experiments were repeated at least three times to confirm the consistency of results. All specific primers including TRα1, α2, β1, β2, CS, Na+/K+-ATPase β, G6PD, MHC I, IGF-1, IGF-1R, MyoD and GAPDH with condition to define the linear range for PCR amplification, and the optimal cycle numbers of different groups are shown in Table 1. All PCR products were detected and analyzed by electrophoresis. Documentation and analysis of ethidium bromide staining was performed using Kodak 290 (Kodak, Norwalk, CT) and Image J software (Ver.1.41, NIH, Bethesda, MD). The PCR result of each sample was normalized by GAPDH mRNA level as an internal control.

**Western blot**

Western blot analysis for TRβ1 was performed using the soleus muscle. The dissected soleus muscles were weighted, homogenized in the protein extraction reagent (RIPA Lysis buffer: 20 mM Tris-HCl (pH 7.5); 150 mM NaCl; 1 mM Na2EDTA; 1 mM EGTA; 1% NP-40; 1% sodium deoxycholate; 2.5 mM sodium pyrophosphate; 1 mM β-glycerophosphate; 1 mM Na3VO4; 1 µg/mL leupeptin) with protease inhibitors and 1 mM sodium orthovanadate (a phosphatase inhibitor). The tissue lysates were separated by centrifugation for 15 min at 15,000 xg at 4°C and protein concentrations in the supernatant were measured (Bradford protein assay, Bio-Rad, Hercules, CA). Equal amounts of protein were resolved by SDS-PAGE, transferred to a nitrocellulose membrane and immunoblotted with anti-TRβ1 antibody (1:1,000;
Results

Change in blood lactate levels under different exercise intensity

After habituation, rats were subjected to treadmill running for 30 min with different speed (15, 20 and 25 m/min) to measure lactate levels in blood (Fig. 1). At a speed of 15 m/min, lactate level did not change significantly for 30 min. At a speed of 20 m/min, lactate level started to increase 20 min after the onset of exercise, indicating that it is the velocity at the lactate threshold. This is consistent with previous study showing that 20 m/min running on a treadmill was the speed at the lactate threshold of rat [27]. At a speed of 25 m/min, lactate level increased approximately 2.5 folds from basal level 10 min after the onset of exercise. Thus, we trained rats at 15 m/min for sub lactate threshold (sub-LT) and 25 m/min for supra lactate threshold (supra-LT) training in the following experiments.

Table 1  Primer sequences, annealing temperatures and amplification cycles for RT-PCR

| Primers | Sequences (5’-3’) | Upper strand : sense | Lower strand : antisense | Products (bp) | Annealing (°C) | Cycle | References |
|---------|-------------------|----------------------|--------------------------|--------------|----------------|-------|------------|
| TRα1    | AGAGGCTGTCGGAAGCTGT | 136                  | 29                       |              |                |       |            |
| TRα2    | ACCCGGAACGCAGCGTTT | 265                  | 57                       |              |                | 29    |            |
| TRβ1    | CGCCGCTGTGGTACCAAGT | 284                  | 59                       |              |                | 29    |            |
| TRβ2    | GCTTGGCCGAGCAGCAT | 183                  | 59                       |              |                | 29    |            |
| CS      | CCGTGTCTCATGGACTTGCG | 176                  | 60                       | 23           |                |       | (Siu et al. 2003) |
| IGF-1   | AAGCCTACAAAGTCAGCTGC | 166                  | 58                       |              |                | 31    | (Buehlmeyer et al. 2007) |
| IGF-1R  | AAAACATCGATTCGAGCG | 199                  | 60                       |              |                |       | (Buehlmeyer et al. 2007) |
| G6PD    | ACTGACAACGTGTCCCTTG | 383                  | 57                       | 26           |                |       | (Lane et al. 1998) |
| MHC 1   | GAAAGGCAAGAAGCCCATC | 596                  | 60                       | 26           |                |       | (Kontrogianni-K et al. 2006) |
| MyoD    | ACTACAGCGCGACTCGAC | 122                  | 60                       | 30           |                |       | (Miyata, Tanaka and Tachino, 2009) |
| Na+/K+-ATPase β | AGGCAGAGTGTCGAGACTACAG | 279                  | 59                       | 24           |                |       |            |
| GAPDH   | CGAGCCGAGAATGGGAAAGCT | 184                  | 59                       | 24           |                |       |            |

Statistical analysis

Quantitative data were expressed as means ± SEM of the individual in each experimental group. Using STATCELL, data were statistically analyzed with one way-ANOVA followed by post hoc comparisons using Bonferroni’s multiple range test. The p-values < 0.05 were considered to be significant.

Affinity BioReagents, Thermoscientific, Golden, CO), and antibody Na+/K+-ATPase β1 (Millipore, Upstate, CA) overnight at 4°C. The antigen-antibody complexes were detected by chemiluminescence with an ECL system (GE Healthcare, Buckinghamshire, UK), and visualized with a Lumi-Imager imaging analyzer (Roche). The intensity of bands was quantified using an image analysis software (Lumi Analyst, Roche). Blots were reprobed with an anti-β-actin antibody (1:1,000) (Cell Signaling Technology, Danvers, MA) for monitoring the quantity and integrity of protein.
Alteration of TH signaling by training

TSH level was altered by different exercise intensity

To investigate whether TH homeostasis is affected by exercise, serum TSH levels during and after exercise were measured (Fig. 2). On day 1 in SC group, TSH level decreased significantly just by placing rat from home cage (1.67 ± 0.13) to the treadmill machine (1.14 ± 0.31). After 10 min of placing on the treadmill, TSH tended to decrease further. On day 5, in SC group, TSH levels kept increasing for the first 30 min, until the level became the same as those in the home cage. In both sub-LT and supra-LT groups, plasma TSH levels were kept at low level and there are significant differences compared to those in SC group during exercise on day 1, 5 and 12 by 30 min at the latest of exercise. On day 1 and 5, TSH levels were still lower in sub-LT and supra-LT groups at 150 min compared to SC group. On the other hand, on day 12 in sub- and supra-LT groups, TSH levels were elevated by 150 min and there were no significant differences compared to SC group. These results indicate that exercise affects the TH homeostasis via altering TSH level.

TR mRNA and protein levels were altered by different exercise intensity

To examine the effect of training on TR expression, TR mRNA and protein levels in the soleus muscle were measured after the training. The mRNA expression of 4 TR isoforms (TRα1, α2, β1 and β2) were measured using semi-quantitative RT-PCR. All 4 isoforms were detected in all groups with the same amplification
cycle (Table 1). Among 4 isoforms, TRβ1 mRNA levels were most specifically altered only in sub-LT group (Fig. 3B). TRβ1 levels did not alter significantly in both SC and supra-LT groups. In sub-LT group, TRβ1 mRNA level increased significantly at day 1 compared with those of SC group and it also showed significant gradual increase of mRNA expression following duration of training. TRβ1 mRNA level was 1.53, 1.71 and 1.83-folds higher on day 1, 5 and 12, respectively, compared to those of SC on the same day (Fig. 3B). On the other hand, TRα1 mRNA was 1.2 folds greater only on day 12 of sub-LT group (Fig. 3A). No significant alteration of TRα2 and TRβ2 mRNA levels was observed throughout the study (Supplemental Fig. 1).

Since TRβ1 mRNA levels increased significantly specifically in sub-LT group, its protein levels were also measured by Western blot analysis. As for mRNA levels, TRβ1 protein level increased significantly specifically in sub-LT group along with the increase in the days of training (Fig. 4). TRβ1 protein levels were 1.4, 3.2 and 3.1-folds higher on day 1, 5 and 12, respectively, compared to those of SC on the same day. In contrast, supra-LT training decreased protein levels by 0.8-folds on day 12 compared to the same day of SC group (Fig. 4). These results indicate that sub-LT training specifically augmented TRβ1 mRNA and protein expression.

Fig. 3  TRα1 and β1 mRNA levels after exercise
A. TRα1 and B. TRβ1 expression at day 1, 5 and day 12 with different intensity of exercise. Upper panels of A and B show representative figures of RT-PCR for TRα1 and β1 mRNA, whereas lower panels show the result of quantification of TRα1 and β1 mRNA levels, that were normalized by those of GAPDH mRNA levels. Data are expressed as mean ± SEM (n=5).
* p<0.05 was considered as statistically significant.
TH-induced Na\(^+\)/K\(^+\)-ATPase β1 expression was significantly augmented by sub-LT training

Next, to investigate whether exercise training alters the TH-induced transcription of target genes, mRNA levels of representative TH-target genes encoding CS, Na\(^+\)/K\(^+\)-ATPase β, G6PD, MHC I, IGF-1, IGF-1R and MyoD were measured (Fig. 5). The soleus muscles were dissected out 6 h after T3 intraperitoneal injection, which was performed 24 h after day 12 training. Results of fold induction by T3 in each group were summarized in Table 2. Among mRNA examined, TH-induced increase in Na\(^+\)/K\(^+\)-ATPase β1 mRNA expression was significantly augmented in sub-LT group (p<0.05). Interestingly, at mRNA expression, sub-LT group showed 1.33-fold higher response to T3 compared to SC group (Fig. 5, Table 2). Such augmentation was not observed in supra-LT group. Rather, T3 injection did not induce any increase in Na\(^+\)/K\(^+\)-ATPase β mRNA levels (Fig. 5), but induced a decrease. The mRNA induction of MyoD and MHC I showed similar tendency (Fig. 5, Table 2). These results indicate that sub-LT, but not supra-LT training increases the sensitivity to TH and augments Na\(^+\)/K\(^+\)-ATPase β1 mRNA levels.

Discussion

This study provides the first evidence that training for 12 days affects TRs expression and sensitivity to TH of TH-target genes in the skeletal muscle. Since mRNA levels are mainly affected by the change in transcription, and TRβ1 protein level was altered, TH genomic action may play a key role to alter gene expression in skeletal muscle. Such alteration may affect the metabolism and adaptation of skeletal muscle during exercise training.

In the present study, in SC group, TSH decreased 10 min after placing rats on the treadmill. Then it gradually returned to the level in the home cage at day 1 and 5 (Fig. 2A and 2B). The reason of such change has not been known. A previous study showed that stressful environment for 10 min decreases the TSH concentration [29, 30]. Thus, our findings indicate that, in spite of habituation process before the experiment, rats may still feel the stress on the treadmill on day 1 and 5. This hypothesis may be consistent with additional findings that such suppression in SC group was milder on day 12, indicating that rats were accustomed to the environment. On the other hand, such increase was not observed in sub-LT (aerobic exercise) and supra-LT (anaerobic exercise) groups during exercise. Rather, the levels tended to decrease. Although only a limited number of references are available, and the effect of exercise on TSH secretion is still controversial, previous studies have shown that TSH level was not altered by treadmill running with an intensity of lactate threshold [18, 19, 31]. In the present study, although we observed a weak decline during the exercise, such change was rather modest. It should be noted, however, we did not observe any increase in TSH level that was seen in SC group on day 1, and 5. Thus, there may be a possibility that exercise may suppress TSH secretion at least in rat model. Since previous studies did not include SC group, their conclusion stating unalteration of TSH by exercise may not correctly explain the TSH hormonal status [15, 17, 31, 32]. Based on our findings, we conclude that, regardless of the intensity, exercise may suppress TSH level. On the
Fig. 5  Change in T3-induced increase in Na⁺/K⁺-ATPase β mRNA levels on day 12

A. Schematic drawing for experiment procedure (Habituation and trainings are detailed in Supplemental Method.);
B. Na⁺/K⁺-ATPase β mRNA expression 6 h after T3 treatment, panel shows a representative result of Na⁺/K⁺-ATPase β mRNA levels, whereas C. Panel shows the result of quantification of Na⁺/K⁺-ATPase β mRNA levels, whereas D. IGF-1, E. IGF-1R, F. G6PD, G. Citrate synthase, H. MyoD, and I. MHC I mRNA levels that were normalized by those of GAPDH mRNA. Data are expressed as mean ± SEM (n=5), value of groups which were injected with NaCl compared groups which were injected with T3. Responses of each pair group were compared. * p<0.05 was considered as statistically significant. * p<0.05 compared to SC, +T3. Na⁺/K⁺-ATPase β in Sub-LT exercise showed highest sensitivity to T3 injection compared to SC and supra-LT group.
Alteration of TH signaling by training

On the other hand, on day 1 and 5 of sub- and supra-LT groups, TSH level did not return to those of SC group, even 120 min after the exercise. On day 12, however, TSH was gradually increased in both sub- and supra-LT groups and became the same level as those for SC by 150 min. These results indicate that hypothalamus-pituitary-thyroid axis has adapted the repeated training by day 12.

Although the mechanisms causing the decrease in TSH secretion by exercise and its recovery after repeated training have not yet been clarified, one possibility may be the involvement of sympathetic nervous system. The involvement of noradrenergic system in stress-induced inhibition of TSH release has been reported [33]. Since the forced exercise is a strong stressful stimulation for rats, activation of sympathetic system may have suppressed TSH level. On the other hand, physical training is associated with adaptations of cardiovascular system [34]. Such adaptation may be caused at least in part by the decreased activity of sympathetic nervous system [34] and decreased expression of β-adrenergic receptor [35]. Such decreased activity and response to sympathetic activity may have relieved exercise-induced decrease in TSH secretion in the present study. However, further study is required to clarify such possibility.

We only measured plasma TSH level without measuring T3 and T4 levels because of limited amount of plasma sample. As discussed above, studies of TH levels following exercise have shown controversial results. In many cases, however, a single strong exercise increased thyroid hormone level [15, 16, 17, 36], whereas TH level became the same level as for stationary control after the training [18, 19, 36]. Furthermore, TSH and TH levels may not always be correlated [36]. In the present study, although we applied supra-LT exercise, our training condition was not as strong as those in strong exercise studies. Thus, it is not likely that our training condition induced an increase in TH levels during first few days after the onset of training. Rather, TH may be suppressed due to decreased levels of TSH. Then, after adaptation, TH levels may return to the same or similar levels as for stationary control. Further study is definitely required, however, to confirm such hypothesis.

Table 2 Change in T3-induced increase in Na+/K+-ATPase β mRNA levels on day 12

| Gene                   | Stationary control fold induction | Sub-LT fold induction | Supra-LT fold induction |
|------------------------|----------------------------------|-----------------------|-------------------------|
| Na+/K+-ATPase β        | 1.62                             | 2.15                  | 0.96                    |
| IGF-1                  | 1.13                             | 1.07                  | 1.02                    |
| IGF-1R                 | 1.02                             | 1.18                  | 0.91                    |
| G6PD                   | 1.04                             | 1.02                  | 1.01                    |
| Citrate synthase       | 1.05                             | 1.23                  | 1.05                    |
| MyoD                   | 1.42                             | 1.55                  | 1.19                    |
| MHC I                  | 1.54                             | 2.35                  | 0.77                    |

In skeletal muscle, TH action is mainly exerted by binding to TRs, which then activate transcription of target genes. Our present study suggests that training intensity may affect such genomic action of TH via specific TR isoforms. Among TR isoforms, TRβ1 level was most differentially affected by altered training intensity (Fig. 3, Supplemental Fig. 1). Sub-LT exercise induced an increase in TRβ1 mRNA and protein levels with an increase in the day of training (Fig. 3, 4). The mechanism of such changes has not yet been clarified. To our surprise, regulation of TR expression under various conditions has not been extensively studied, and only limited number of information is available. In cardiac muscle, however, endurance training induced an increase TRβ1 protein level [37]. In the present study, we also found that training induced an increase in TRβ1 protein level in skeletal muscle. Further study may be necessary to clarify underlying mechanism of induction of TRβ1 mRNA and protein levels by training.

It has been reported that chronic treatment of TH in hypothyroid patient induces an increase in various mRNAs in human skeletal muscle [38]. Based on this study, we selected TH responsive genes that are involved in metabolic regulation of skeletal muscle, such as CS, MyoD, MHC I, G6PD, IGF-1, IGF-1R and Na+/K+-ATPase β1. Among these, we found that Na+/K+-ATPase β showed the greatest response to T3 only after sub-LT training (Fig. 5). It should be noted that, 24 h after the last training on day 12 at baseline level, Na+/K+-ATPase β1 mRNA were the same among groups and its protein level was unchanged by altered intensity (Supplemental Fig. 2). On the other hand, however, another study showed an increase in Na+/K+-ATPase β1 mRNA level was increased by 3 weeks of endurance training (20 m/min, for 20 min) but not by splint training [39]. In the study, they sacrificed animal 2 h after the last training, not 24 h. Together with our study, these results indicate that the basal (resting) levels of Na+/K+-ATPase are unaltered by training, but sub-LT exercise can activate the expression of Na+/K+-ATPase mRNA during exercise. Such finding is consistent with
our hypothesis that such activation could be induced at least in part through an increase in sensitivity to T3. Although the mechanism of such increase in sensitivity is not fully understood, one possibility is an increase in TRβ1 levels as shown in the present study.

TH is well known metabolic regulator of energy expenditure through activation of β-oxidation of fatty acids in mammals [40]. Several intracellular processes are involved in the calorigenic effects of TH. One such process is increased ATP expenditure due to increased Na⁺/K⁺-ATPase activity to maintain ion gradients in various tissues [10, 41]. TH treatment increases the aerobic metabolic capacity of rat gastrocnemius muscle, as revealed by enhanced cytochrome oxidase activity, inducing more ATP [42]. Increased Na⁺/K⁺-ATPase levels with an increase in ATP production by TH may contribute an increase in oxidative capacity of skeletal muscle. In fact, the oxidative capacity of skeletal muscles was increased after endurance training [43]. Taken together, TH may control muscle metabolism and adaptation during exercise. However the involvement of other factors may regulate the expression of Na⁺/K⁺-ATPase.

In summary, exercise training altered TSH level and showed altered homeostasis pattern at day 12. Sub-LT exercise significantly increased TRβ1 expression at both mRNA and protein levels whereas supra-LT showed suppression at both levels, suggesting that TRβ1 is a major TR isoform in skeletal muscle in response to different exercise intensity. These results indicate that sub-LT training accelerates TH-mediated signaling pathway in skeletal muscle. Such alteration may play important roles to control muscular metabolism to meet the muscle need to tolerate endurance training.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

Supplemental Fig. 1  TRα2 and β2 mRNA expression

TRα2 (A) and β2 (B) mRNA expression at day 1, 5 and 12 with different intensity of exercise. Data are expressed as mean ± SEM (n=5). * p<0.05 was considered as statistically significant compared with stationary control groups.
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