Skeletal Muscle Adaptation to Exercise Training: AMP-Activated Protein Kinase Mediates Muscle Fiber Type Shift

Received for publication 21 February 2007 and accepted in revised form 1 May 2007.

Katja S.C. Röckl, MD*, Michael F. Hirshman, BS*, Josef Brandauer, PhD*, Nobuharu Fujii, PhD*, Lee A. Witters, MD§, Laurie J. Goodyear, PhD*

*Research Division, Joslin Diabetes Center and Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston MA; §Dartmouth Medical School, Hanover NH

Running title: AMPK Mediates Muscle Fiber Type Shift

Address for correspondence:
Laurie J. Goodyear, Ph.D.; Senior Investigator and Head, Section on Metabolism; Joslin Diabetes Center; One Joslin Place; Boston, MA 02215
Laurie.Goodyear@joslin.harvard.edu

word count: 3986
number of tables: 2
number of figures: 6
ABSTRACT

Regular endurance exercise has profound benefits on overall health, including the prevention of obesity, cardiovascular disease, and diabetes.

**Objective.** To determine whether AMPK mediates commonly observed adaptive responses to exercise training in skeletal muscle.

**Research Design/Methods and Results.** Six weeks of voluntary wheel running induced a significant ($p < 0.05$) fiber type IIb to IIa/x shift in triceps muscle of wild type mice. Despite similar wheel running capacities, this training-induced shift was reduced by ~40% in transgenic mice expressing a muscle-specific AMPKα2 inactive subunit. Sedentary mice carrying an AMPK-activating mutation (γ1TG) showed a 2.6-fold increase in type IIa/x fibers but no further increase with training. To determine if AMPK is involved in concomitant metabolic adaptations to training, we measured markers of mitochondria (citrate synthase, succinate dehydrogenase) and glucose uptake capacity (GLUT4, hexokinase II). Mitochondrial markers increased similarly in wild type and AMPKα2 inactive mice. Sedentary γ1TG mice showed a ~25% increase in citrate synthase activity, but no further increase with training. GLUT4 protein expression was not different in either line of transgenic mice compared to wild type, and tended to increase with training, although this was not statistically significant. Training induced a ~65% increase in hexokinase II protein in wild type, but not in AMPKα2 inactive mice. Hexokinase II was significantly elevated in sedentary γ1TG mice, without an additional increase with training.

**Conclusions.** AMPK is not necessary for exercise training-induced increases in mitochondrial markers, but is essential for fiber type IIb to IIa/x transformation and increases in hexokinase II protein.
The beneficial effects of regular physical activity on a variety of pathological conditions such as obesity, cardiovascular disease, or diabetes are undisputed (1). These positive effects are most likely related to a number of adaptations that occur in skeletal muscle as a response to exercise training. They allow the muscle to more efficiently utilize substrates for ATP production and thus become more resistant to fatigue. Adaptations of skeletal muscle fibers to exercise training occur, for example, by the expression of specific contractile proteins (myosin heavy chain (MHC) isoforms) and by an increase in activity and content of mitochondria, also referred to as "oxidative capacity" (2-4). The exact molecular mechanisms that underlie these adaptations remain elusive. A better understanding of the signaling pathways mediating skeletal muscle adaptations to exercise training may help reveal new approaches for treating metabolic or cardiovascular diseases, which are on a rapid rise in our increasingly sedentary society.

Muscle fibers have traditionally been classified according to their expression of myosin heavy chain isoforms as fast twitch fibers (type IIb (not expressed in humans (5)), IIx, and IIa), and slow twitch fibers (type I) (5;6). Type IIb and type IIx fibers mainly depend on glycolytic pathways for ATP production, while type IIa and type I fibers rely predominantly on oxidative pathways (5). Endurance exercise training has been shown to increase mitochondria (2-4). Exercise training also has well established effects to change MHC isoform expression, thus provoking a fiber type switch from type IIb to IIx and IIa, and in rare cases also to type I muscle fibers (7;8). Importantly, mitochondrial biogenesis and fiber type changes can occur independently from each other, suggesting distinct signaling pathways for both types of adaptive responses (3).

Known as the “fuel gauge” of the cell, AMP-activated protein kinase (AMPK) plays a key metabolic role during exercise. In order to restore ATP levels, AMPK has been proposed to increase muscle glucose uptake and to be involved in fatty acid and carbohydrate metabolism (9-12). AMPK has previously been suggested as a possible mediator of muscle fiber plasticity (13); however, a role of AMPK in training-induced fiber type transformation has not yet been investigated. AMPK activation by 5-aminoimidazole-4-carboxamide 1-ß-D-ribofuranoside (AICAR) has also been shown to activate transcription factors (12) as well as initiate mitochondrial biogenesis (14). Yet, reports on exercise-induced increases in mitochondria through AMPK signaling have been inconsistent (15;16).

Peroxisome-proliferator-activated receptor-γ co-activator-1 (PGC-1α) is a major regulator of mitochondrial biogenesis (17). In addition, overexpression of PGC-1α in transgenic mice promotes muscle fiber type II to I transformation (13). Interestingly, studies in which rats were treated with AICAR have shown an induction of PGC-1α mRNA expression in skeletal muscle (12), which creates a possible link between AMPK and PGC-1α signaling.

The interest in exploring the role of AMPK in muscle adaptations to exercise training lies particularly in its clinical relevance. Individuals with insulin resistance or type 2 diabetes tend to have more glycolytic type IIx (formerly misclassified as type IIb) skeletal muscle fibers than healthy individuals, and a number of studies have shown that fiber type distribution correlates with insulin resistance, as reviewed in Zierath & Hawley, 2004 (18). This implies that muscle fiber plasticity may play an essential role in the pathogenesis of diabetes. AMPK activation, which occurs during exercise (10;19), may be involved in metabolic changes such as increased skeletal muscle glucose uptake (10;20) and increased fatty acid oxidation (9;12). Moreover, experiments with animal models of type 2 diabetes have provided evidence that AICAR activation of AMPK can reverse many of the metabolic defects of these animals in vivo (11;21). Finally, the AMPK system is the probable target of the antidiabetic drugs metformin and thiazolidinediones (11;22). Given
the significance of AMPK in exercise-induced metabolic signal transduction, it is likely that its repeated activation plays an important role in provoking skeletal muscle adaptations to exercise training.

The overall purpose of our study was to investigate whether AMPK is necessary for adaptations of skeletal muscle to endurance training. In particular, we studied transformations of muscle fiber types, increases in mitochondrial markers, and increases in proteins involved in skeletal muscle glucose uptake. Using transgenic mouse models of attenuated AMPKα2 or chronically increased total AMPK activity, we found that AMPK is an important mediator of muscle fiber type changes and increases in hexokinase II protein following exercise training, whereas increases in mitochondrial markers are not dependent on AMPK activity.

RESEARCH DESIGN AND METHODS

Animals. Muscle specific transgenic mice expressing the inactivating D157A mutation in their α2 subunit of AMPK (α2iTG) were generated on an FVB background as described previously (23). Basal and contraction-stimulated AMPKα2 activity of α2iTG mice is almost abolished, whereas basal AMPKα1 activity is normal, and contraction stimulated AMPK α1 activity is about 50% decreased compared to their wild type littermates (23); (Fujii et al.; Diabetes Research & Clinical Practice 2007; in press). The α2iTG mice used for this study were heterozygote for the inactivating mutation. Muscle specific R70Qγ1 transgenic mice (γ1TG) expressing the activating R70Q mutation in their γ1 subunit of AMPK were generated on an FVB background as described previously (24). Basal total AMPK activity in γ1TG mice is approximately 3-fold higher than in wild type littermates (24). The γ1TG mice used for this study were heterozygote for the activating mutation. Physiological and behavioral characterization using the Comprehensive Lab Animal Monitoring System in both transgenic animal models showed that the respective transgene did not significantly alter locomotive activity, oxygen consumption, carbon dioxide generation, heat generation or food and water consumption (23); (Hirshman, Witters & Goodyear, unpublished data). Mice were housed at a constant temperature (20-22 °C) with a 12 h:12 h light-dark cycle and received LabDiet® rodent chow (Purina Mills, St. Louis, MO) and water ad libitum.

Training protocol. Seven-week old female α2iTG and γ1TG mice and corresponding wild type littermates were randomly assigned to housing in individual cages with or without running wheels (Nalgene, Rochester, NY) for a total of six weeks. Completed wheel revolutions and time spent running were continuously monitored. On day 42, all mice were removed from their cages 3-4 h prior to being sacrificed by cervical dislocation. Preliminary experiments from our laboratory have shown that wheel cage running has the most pronounced training effects (as determined by increases in citrate synthase activity) in triceps brachii. We therefore chose to use this muscle for our studies. After sacrificing the mice, triceps muscles from both forelegs were immediately removed. One triceps of each animal was snap frozen in liquid nitrogen and stored at -80 °C. The other triceps was embedded in Tissue-Tek® O.C.T. compound (Sakura Finetek, Torrance, CA), quick frozen in isopentane cooled by liquid nitrogen, and stored at -80 °C until cryosections were prepared for histology. All experiments were in accordance with the Institutional Animal Care and Use Committee of the Joslin Diabetes Center and the National Institutes of Health.

Blood glucose, body weight, and food consumption. At weeks 0, 2, 4, and 6, blood glucose, body weight, and food consumption were recorded. Blood glucose measurements were performed between 3PM and 4PM by obtaining blood samples from the tail of fully conscious mice, using a One Touch Ultra portable
glucometer (Lifescan Inc., Mipitas, CA). Body weight and food consumption were determined using a digital scale (Model CS200, Ohaus Corporation, USA).

**Immunoblot analysis.** Frozen muscles were pulverized and homogenized as described previously (25), and protein concentrations were determined via the Bradford assay. 40 µg of skeletal muscle proteins were resolved by SDS-PAGE for Western blot analysis (26). Antibody-bound proteins were visualized on film using chemiluminescence detection reagents (Perkin-Elmer Life Sciences, Boston, MA). Protein bands were scanned by ImageScanner (Amersham Biosciences) and quantitated by densitometry. Bands were identified according to their migration characteristics as described previously (28). In triceps muscle, electrophoresis resulted in two distinct bands. The upper band corresponded to combined MHC IIa and IIx (referred to as MHC IIa/x), and the lower band to MHC IIb. In soleus muscle, an additional lower band was identified as MHC I, and as expected, this band was not present in triceps, which is a mainly glycolytic muscle as determined by ATPase staining.

**Succinate Dehydrogenase Staining.** Mid-belly cross-sections of triceps muscle were cut at 8 µm in a cryostat (-20 °C). After drying for 5 min at room temperature, the sections were incubated for 30 min at 37 °C in the incubation solution (pH 7.6) containing 6.5 mM sodium phosphate monobasic, 43.5 mM sodium phosphate biphasic, 0.6 mM nitroblue tetrazolium (Sigma N6876), and 50 mM sodium succinate. The sections were then rinsed for 3x30 sec in physiological saline and for 5 min in 15% ethanol, and coverslipped with Faramount aqueous mounting medium (DakoCytomation) (29).

**Citrate Synthase Activity.** Citrate synthase activity was determined in muscle lysates spectrophotometrically at room temperature (412nm) to detect the transfer of sulfhydryl groups to 5,5'-dithiobis-2-nitrobenzoate (DTNB) using saturating concentrations of substrates and co-factors: 80 mM Tris-HCl, 0.1 mM DTNB, 0.4 mM acetyl-CoA, and 0.6 mM oxalacetate (30). Citrate synthase activity was calculated as µmol/min/g protein.
Statistical analysis. Data are means ± standard errors of the means (SEM). All data were compared using two-way analysis of variance and Tukey's post hoc analysis. Pearson’s correlation coefficients were used to describe the linear association between variables. The differences between groups were considered significant when p < 0.05.

RESULTS
Baseline parameters and exercise training performance. Body weight and blood glucose concentrations were not different between the study groups throughout the training period (Table 1). All training groups showed a tendency of an increase in food intake compared to the sedentary control groups, which was statistically significant in the trained α2iTG and γ1TG mice. Running distances and running speeds did not differ between transgenic mice and their wild type littermates and were maintained throughout the training period (Table 1 and Fig. 1A and 2A).

Training-induced fiber type shift from IIb to IIa/x is reduced in α2iTG mice. We hypothesized that one of the responses of skeletal muscle to chronic intermittent (exercise training) or chronic sustained (γ1TG mice) AMPK activation may be the mediation of muscle fiber type transformation. In order to study fiber type changes, we performed electrophoretic separation of MHC isoforms in triceps muscle, a mainly glycolytic muscle with very few type I fibers, as determined by ATPase staining. Electrophoretic separation of MHC isoforms resulted in two distinct bands that were identified according to their migration characteristics as previously described (28). The lower band corresponded to MHC IIb, the upper one corresponded to a combination of MHC IIa and IIx (Fig. 1B). Further separation of MHC IIa and IIx was not achieved, a difficulty which has been reported earlier (31). To signify that the highest migrating band may contain both MHC IIa and IIx isoforms, we refer to this band as IIa/x.

In wild type mice, exercise training significantly increased the relative proportion of type IIa/x fibers (Fig. 1B). Exercise training also induced an increase of type IIa/x fibers in α2iTG mice. However, the proportion of type IIa/x fibers in trained α2iTG mice was significantly lower than in their trained wild type littermates. Some of the variability in fiber type adaptations in trained wild type mice can be explained by the differences in total running distance. Fig. 1C illustrates that running distance correlated strongly with the relative proportion of type IIa/x fibers in wild type mice. In α2iTG mice, this correlation was absent.

Increase of type IIa/x muscle fibers in γ1TG mice. In a second approach to investigate the role of AMPK activity in fiber type transformation, we performed muscle fiber type analysis in triceps of sedentary and trained γ1TG mice, a genetic model of chronic AMPK activation (24) (Fig. 2B). Training significantly increased type IIa/x fibers in wild type mice. Sedentary γ1TG mice had a ~2.6-fold higher proportion of type IIa/x fibers compared to their sedentary wild type littermates, and exercise training did not further increase type IIa/x fibers in the transgenic animals. Fig. 2C illustrates total running distances and corresponding type IIa/x fibers in wild type and γ1TG mice. There was no significant correlation between running distance and type IIa/x fibers in these training groups.

Decreased mitochondrial markers in α2iTG mice but normal adaptive response to exercise training. Exercise training induces a variety of metabolic adaptations which lead to improvements in fatty acid oxidation (32) and glucose metabolism (33). In order to study whether AMPK mediates exercise training-induced increases in oxidative capacity, we assessed mitochondrial markers. Citrate synthase activity was ~14% lower (p < 0.05, main effect) in triceps of α2iTG mice compared to wild type littermates (Fig. 3A). Exercise training, however, induced a similar increase in citrate synthase
activity in α2iTG and wild type mice. Succinate dehydrogenase staining of triceps mid-belly cross sections support these results (Fig. 3B); The stain was more intense in muscle sections from all trained animals, suggesting that ablation of AMPKα2 activity has little effect on the increase in mitochondria in response to exercise training.

Increased mitochondrial markers in γ1TG mice. In sedentary γ1TG mice, citrate synthase activity was ~13% higher compared to their wild type littermates (Fig. 4A). Similarly, succinate dehydrogenase staining suggested an increase of mitochondria in sedentary γ1TG mice compared to their wild type littermates as shown in representative micrographs (Fig. 4B). We did not observe any further increase in mitochondrial markers due to exercise training in γ1TG mice.

PGC-1α and PGC-1β protein expression. The transcriptional co-activator PGC-1α has previously been linked to fiber type II to I transformation and mitochondrial biogenesis (13,17). The PGC-1α homologue, PGC-1β, has recently been proposed to drive muscle fiber type IIx formation (34). In our study, PGC-1β protein was not altered in either line of transgenic mice compared to wild type animals or following exercise training (data not shown). In contrast, protein expression of PGC-1α was influenced by genotype as well as training. Ablation of AMPKα2 activity in α2iTG mice was associated with a 32% decrease in PGC-1α protein expression (p < 0.05, main effect, Fig. 5A). Exercise training, however, increased PGC-1α in α2iTG mice to a similar extent as in wild type mice (54% vs. 55%). Sedentary γ1TG mice expressed elevated levels of PGC-1α protein compared to their corresponding wild type littermates (Fig. 5B), and there was a tendency for training to increase PGC-1α in γ1TG mice (28%, p = 0.06).

GLUT4 and hexokinase II protein expression. Muscle glucose uptake is controlled by glucose transport across the cell membrane, mainly by GLUT4, as well as intracellular phosphorylation by hexokinase II (35,36). Both GLUT4 and hexokinase II have previously been reported to be up-regulated by exercise training (37-39). In the current study, training-induced increases in GLUT4 protein expression did not reach statistical significance (main effect: p = 0.06 (Fig. 6A) and 0.12 (Fig. 6B)), and no differences were observed between genotypes. Hexokinase II protein expression was not different in sedentary α2iTG and wild type mice (Fig. 6C). However, while exercise training induced a ~65% increase in hexokinase II in wild type mice, there was no significant change in α2iTG mice. γ1TG mice showed an overall higher expression of hexokinase II than their wild type littermates with no significant additional increase after exercise training (Fig. 6D).

DISCUSSION
We investigated the role of AMPK in skeletal muscle adaptations to exercise training. For this purpose, we used transgenic mouse models with either attenuated (α2iTG mice) or increased (γ1TG mice) AMPK activity and studied long-term effects of endurance training. Our main finding was that AMPKα2 is an important mediator of training-induced muscle fiber type changes as shown by decreased fiber type transformation in trained α2iTG mice. The fiber type profile of γ1TG mice with increases in type IIa/x muscle fibers further emphasizes the significance of AMPK activity in the determination of muscle fiber types. Previous studies show that AMPK activity in γ1TG mice is not further increased by acute bouts of exercise (24). Hence, exercise training does not result in repeated periods of (additional) AMPK activation in these mice, which may explain the absence of a training-induced increase in type IIa/x muscle fibers in γ1TG mice.

Although inactive AMPKα2 in α2iTG mice was associated with a significant reduction in training-induced fiber type transformation, the fiber type IIb to IIa/x shift was not completely
inhibited. These data suggest that AMPKα2 is not solely responsible for this skeletal muscle adaptation. Preserved AMPKα1 activity may account for the residual response. However, data from a variety of studies suggest that AMPK activity in skeletal muscle is almost exclusively a function of the α2 isoform (20;40). Hence, it is more likely that alternative fiber type-regulating pathways remain responsive in α2iTG mice and account for the residual fiber type adaptation. In particular, the Ca2+/calmodulin-dependent protein phosphatase calcineurin has been proposed as an important regulator of fiber type II to I transformation (41).

It seems possible that AMPKα2 and calcineurin signaling are parallel pathways in muscle fiber type adaptations, possibly being differentially regulated by certain stimuli, such as innervation patterns or exercise intensity. Our transgenic mouse model with chronically active AMPK (γ1TG) has a marked increase in type IIa/x fibers but no increase in type I fibers in triceps muscle. This closely imitates the physiological response to endurance exercise training which typically leads to muscle fiber type IIb to IIa transformations (7;8). Conversely, expression of constitutively active calcineurin mainly induces slow twitch type I fibers (41). This kind of fiber type switch has been seen following powerful stimuli such as very rigorous exercise training programs in humans (8) and electrical stimulation or cross-innervation in animal studies (42). Thus, calcineurin may be the predominant mediator fiber type II to I transformations, whereas AMPKα2 appears particularly important for mediating type IIb to IIa/x transformations as seen in response to moderate intensity exercise training. It will be intriguing to further investigate Ca2+-dependent regulators of fiber type changes in combination with the AMPK pathway and their distinct responses to various stimuli.

AMPK activation has previously been reported to induce mitochondrial biogenesis (14;16). We did not directly assess mitochondrial content in the present study. However, the reduction in citrate synthase activity in α2iTG mice and its increase in γ1TG mice support the role of AMPK for baseline mitochondrial content. Thus, we were intrigued to find that training-induced increases in mitochondrial markers (citrate synthase activity and succinate dehydrogenase) occurred normally in a2iTG mice. These data suggest that AMPKα2 activity is not necessary for training-induced increases in mitochondria. Alternative pathways, such as the above mentioned calcineurin signaling pathway (43), may play a critical role mediating exercise training-induced increases in mitochondria. However, recent reports have questioned the involvement of calcineurin in these training adaptations (44;45). Clearly, more studies are needed to comprehensively understand the mechanisms leading to exercise training-induced mitochondrial biogenesis.

The transcriptional co-activator PGC-1α is known as a principal factor in muscle fiber plasticity by inducing mitochondrial biogenesis and fast-to-slow fiber type transformation (13). PGC-1α has been described to be downstream of AMPK signaling (12). In the present study, consistent with the proposed AMPK/PGC-1α signaling pathway, α2iTG mice showed decreased levels of PGC-1α protein, whereas γ1TG mice had increased PGC-1α expression. Interestingly, exercise training increased PGC-1α protein even when AMPKα2 activity was inhibited (α2iTG mice). Hence, in addition to AMPK, there must be other pathways that regulate PGC-1α protein expression during exercise training. Ca2+/calcineurin signaling as well as the p38/MAPK pathway are possible candidates that have been reported upstream of PGC-1α (13;46;47). Thus, PGC-1α may represent a point of convergence of various signaling pathways in skeletal muscle plasticity. The PGC-1α homologue, PGC-1β, has recently been shown to mediate the formation of type IIx muscle fibers (34). In contrast to PGC-1α, however, PGC-1β protein expression was unchanged in either line of transgenic mice and
following exercise training. Hence, the present study provides no evidence for a role of PGC-1β in training-induced muscle fiber type transformations.

In states of impaired glucose metabolism, endurance training leads to improvements in glucose tolerance (48). Two major regulators of glucose uptake into the muscle cell are the glucose transporter GLUT4 and hexokinase II. While training-induced increases in GLUT4 expression have been described in rats and humans (49) only few studies have examined GLUT4 in trained mice. In our study, GLUT4 protein expression resulted in a non-significant increase in wild type triceps muscle by 24% and 28%. These mild changes are comparable to previously published training effects (17%-26% increases) in gastrocnemius muscle of wild type and AMPKα2 knockout mice (16). The minimal changes in GLUT4 expression in trained mouse muscle are consistent with previous reports that adaptations of skeletal muscle to exercise training are more subtle in mice than in rats or humans, which may be due to the higher basal metabolism in mice (50).

Hexokinase II phosphorylates glucose upon entering the muscle cell and may be the major rate-limiting factor for glucose uptake in the insulin- or exercise stimulated state (36). AMPK and exercise training have been suggested as playing a role in the regulation of hexokinase (16;39). In our study, hexokinase II protein expression was substantially increased in association with exercise training and chronically activated AMPK. Interestingly, in triceps muscle of α2iTG mice, exercise training did not up-regulate hexokinase II. This conflicts with data from Jorgensen et al. showing normal increases in hexokinase II protein expression in trained gastrocnemius muscle of whole body AMPKα2 knockout mice (16). The reason for this discrepancy is not entirely clear, since both AMPKα2 knockout and α2iTG mice lack AMPKα2 activity. Although the different mouse lines might yield conflicting results, it is more likely that there are muscle specific differences between triceps and gastrocnemius in responsiveness to AMPK activity. The exercise-induced increase of hexokinase II suggests increased capacities for glucose phosphorylation as an adaptation to endurance training. Our results imply that this effect requires AMPKα2 activity, thus extending the importance of this enzyme for exercise training adaptations in skeletal muscle.

There is strong epidemiological evidence that regular physical activity improves insulin resistance (1). Furthermore, individuals with diabetes appear to have a distinct skeletal muscle phenotype with a prevalence of type IIx fibers and impaired oxidative capacity (18;51). These observations suggest that skeletal muscle adaptations to exercise training may play an important role in the prevention of diabetes. The underlying molecular mechanisms, however, are only partly understood. We here demonstrate that AMPK plays a critical role in training adaptations of skeletal muscle. While our results suggest that the increase in oxidative capacity is mediated through AMPK-independent pathways, we show the significance of AMPK signaling for transforming skeletal muscle fiber type IIb to IIa/x as well as for increasing hexokinase II protein expression. In the long run, a better understanding of the molecular mechanisms that link exercise training to improvement or prevention of type 2 diabetes should reveal new targets for treatment and provide fundamental knowledge of the complex physiological processes in this disease.

ACKNOWLEDGEMENTS
This work was supported by grants to L.J. Goodyear (National Institutes of Health R01DK068626 and R01AR45670), to L.A. Witters (National Institutes of Health DK35712), to K.S.C. Röckl (fellowship within the Postdoc-Program of the German Academic Exchange Service, DAAD), to J. Brandauer (National Institutes of Health Training Grant T32DK07260-29), and a Diabetes Endocrinology Research Grant (National Institutes of Health DK36836).
REFERENCES

5. Smerdu, V, Karsch-Mizrachi, I, Campione, M, Leinwand, L, Schiaffino, S: Type IIx myosin heavy chain transcripts are expressed in type IIb fibers of human skeletal muscle. Am.J.Physiol 267:C1723-C1728, 1994


**Table 1. Baseline parameters of sedentary and trained wild type and α2iTG mice.**

<table>
<thead>
<tr>
<th></th>
<th>wild type sedentary</th>
<th>wild type trained</th>
<th>α2iTG sedentary</th>
<th>α2iTG trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>20.67 ± 0.58</td>
<td>19.37 ± 0.72</td>
<td>20.22 ± 0.76</td>
<td>20.51 ± 0.38</td>
</tr>
<tr>
<td>total food intake (g/g body weight)</td>
<td>8.79 ± 0.51</td>
<td>9.44 ± 0.36</td>
<td>7.61 ± 0.20 *</td>
<td>9.01 ± 0.31 *</td>
</tr>
<tr>
<td>blood glucose (mg/dl)</td>
<td>170 ± 5.0</td>
<td>167 ± 4.5</td>
<td>162 ± 4.8</td>
<td>169 ± 3.3</td>
</tr>
<tr>
<td>distance (km/day)</td>
<td>N/A</td>
<td>4.56 ± 0.66</td>
<td>N/A</td>
<td>4.56 ± 0.58</td>
</tr>
<tr>
<td>speed (m/min)</td>
<td>N/A</td>
<td>24.06 ± 0.79</td>
<td>N/A</td>
<td>24.36 ± 0.55</td>
</tr>
</tbody>
</table>

The data are represented as means ± SEM. * p < 0.05 (versus sedentary), # p < 0.05 (versus wild type for respective condition).
Table 2. Baseline parameters of sedentary and trained wild type and γ1TG mice.

<table>
<thead>
<tr>
<th></th>
<th>wild type sedentary</th>
<th>wild type trained</th>
<th>γ1TG sedentary</th>
<th>γ1TG trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>20.71 ± 0.43</td>
<td>20.60 ± 0.34</td>
<td>20.59 ± 0.45</td>
<td>21.14 ± 0.78</td>
</tr>
<tr>
<td>total food intake (g/g body weight)</td>
<td>8.00 ± 0.31</td>
<td>8.63 ± 0.28</td>
<td>7.50 ± 0.25</td>
<td>8.92 ± 0.39 *</td>
</tr>
<tr>
<td>blood glucose (mg/dl)</td>
<td>148 ± 3.8</td>
<td>157 ± 2.6</td>
<td>154 ± 5.6</td>
<td>156 ± 3.1</td>
</tr>
<tr>
<td>distance (km/day)</td>
<td>N/A</td>
<td>2.07 ± 0.24</td>
<td>N/A</td>
<td>2.26 ± 0.31</td>
</tr>
<tr>
<td>speed (m/min)</td>
<td>N/A</td>
<td>22.45 ± 0.50</td>
<td>N/A</td>
<td>22.34 ± 0.59</td>
</tr>
</tbody>
</table>

The data are represented as means ± SEM. * p < 0.05 (versus sedentary).
FIGURE LEGENDS

Figure 1. Myosin heavy chain profile in sedentary and trained wild type and α2iTG muscle. α2iTG mice and their wild type littermates performed six weeks of voluntary wheel cage running or were kept as sedentary controls. (A) Average daily running distances were calculated from continuous recordings. Triceps muscles were harvested and processed for electrophoresis. (B) Myosin heavy chain (MHC) isoforms were resolved by SDS-PAGE electrophoresis and visualized by Coomassie Blue staining, resulting in two distinct bands for MHC IIa/x (upper band) and MHC IIb (lower band). The relative proportion of MHC IIa/x isoform in sedentary and trained wild type and α2iTG mice was compared by two way analysis of variance. The data are represented as means ± SEM. * p < 0.05 (versus sedentary); ** p < 0.01 (versus sedentary); # p < 0.05 (versus wild type for respective condition). (C) The correlation between total running distance and percentage of type IIa/x fibers of each study animal is shown for wild type (left graph) and α2iTG mice (right graph). sed, sedentary.

Figure 2. Myosin heavy chain profile in wild type and γ1TG muscle. The same experimental procedures as described for Figure 1 were carried out using γ1TG mice and their wild type littermates. The data are represented as means ± SEM. * p < 0.05 (versus sedentary); ## p < 0.01 (versus wild type for respective condition). sed, sedentary.

Figure 3. Mitochondrial markers in sedentary and trained wild type and α2iTG triceps muscle. Citrate synthase activity assays as well as succinate dehydrogenase staining were performed in order to assess differences in mitochondrial content between sedentary and trained wild type and α2iTG muscle. (A) Citrate synthase activity is expressed relative to wild type sedentary (1.0 activity unit equals 487 ± 23 µmol/min/g protein). Data are represented as means ± SEM. ** p < 0.01 (versus sedentary); # p < 0.05 (versus wild type). (B) Mid-belly cross sections of triceps muscle were obtained and stained for succinate dehydrogenase activity. Note the marked difference in stain intensity between sedentary and trained muscle in both wild type and α2iTG mice. sed, sedentary.

Figure 4. Mitochondrial markers in sedentary and trained wild type and γ1TG muscle. (A) Citrate synthase activity is expressed relative to wild type sedentary (1.0 activity unit equals 373 ± 22 µmol/min/g protein). The data are represented as means ± SEM. ** p < 0.01 (versus sedentary); # p < 0.05 (versus wild type); ### p < 0.01 (versus wild type for respective condition). (B) Representative micrographs of mid-belly cross sections of triceps muscle stained for succinate dehydrogenase activity. sed, sedentary.

Figure 5. PGC-1α protein expression. After six weeks of voluntary wheel cage running, triceps muscle of α2iTG (A) and γ1TG mice (B) was studied for PGC-1α protein expression by immunoblotting with anti-PGC-1α antibody. Data are means ± SEM. * p < 0.05; ** p < 0.01 (versus sedentary); # p < 0.05 (versus wild type for respective condition). sed, sedentary; tr, trained; a.u., arbitrary units.

Figure 6. GLUT4 and hexokinase II protein expression. After six weeks of voluntary wheel cage running, triceps muscle of α2iTG (A and C) and γ1TG mice (B and D) was studied for GLUT4 (A and B) hexokinase (C and D) protein expression by immunoblotting with anti-hexokinase II and anti-GLUT4 antibody. Data are means ± SEM. ** p < 0.01 (versus sedentary); # p < 0.05; ## p < 0.01 (versus wild type). HXKII, hexokinase II; sed, sedentary; tr, trained; a.u., arbitrary units.
Figure 1

A

![Graph showing distance (km/day) vs. week](image)

- **wild type**
- **α2iTG**

B

![Bar graph showing type IIa/x fibers (%)](image)

- **sed**
- **trained**
- **wild type**
- **α2iTG**

C

- **wild type**
  - $R = 0.80$
  - $p < 0.01$

- **α2iTG**
  - $R = 0.38$
  - $p = 0.28$
Figure 2

A

![Graph showing distance (km/day) over weeks for wild type and γ1TG mice.]

B

![Bar graph showing type IIa/x fiber percentages for wild type and γ1TG mice.]

C

![Scatter plots showing type IIa/x fiber percentages vs. total distance (km) for wild type and γ1TG mice.]

Legend:
- Wild type
- γ1TG

Statistical Values:
- R = 0.42, p = 0.35 for wild type
- R = 0.47, p = 0.24 for γ1TG
Figure 3

A

![Graph showing citrate synthase activity.]

B

![Images comparing wild type sed vs. trained, and α2ITG sed vs. trained.]
Figure 4

A

![Graph showing citrate synthase activity for wild type and y1TG sed and trained groups.](image)

B

![Images showing tissue samples for wild type sed and trained, y1TG sed and trained groups.](image)
Figure 5

**A**

- Wild type
- α2TG
  - sed
  - tr

**B**

- Wild type
- γ1TG
  - sed
  - tr

PGC-1α expression (a.u.)
Figure 6