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Endurance training increases skeletal muscle LKB1 and PGC-1α protein abundance: effects of time and intensity

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Taylor, Eric B., Jeremy D. Lamb, Richard W. Hurst, David G. Chesser, William J. Ellingson, Lyle J. Greenwood, Brian B. Porter, Seth T. Herway, and William W. Winder. Endurance training increases skeletal muscle LKB1 and PGC-1α protein abundance: effects of time and intensity. *Am J Physiol Endocrinol Metab* 289: E960–E968, 2005. First published July 12, 2005; doi:10.1152/ajpendo.00237.2005.—Recent research suggests that LKB1 is the major AMP-activated protein kinase kinase (AMPKK). Peroxisome-proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a master coordinator of mitochondrial biogenesis. Previously we reported that skeletal muscle LKB1 protein increases with endurance training. The purpose of this study was to determine whether training-induced increases in skeletal muscle LKB1 and PGC-1α protein exhibit a time course and intensity-dependent response similar to that of citrate synthase. Male Sprague-Dawley rats completed endurance- and interval-training protocols. For endurance training, rats trained for 4, 11, 25, or 53 days. Interval-training rats trained identically to endurance-trained rats, except that after 25 days interval training was combined with endurance training. Time course data were collected from endurance-trained red quadriceps (RQ) after each time point. Interval training data were collected from soleus, RQ, and white quadriceps (WQ) muscle after 53 days only. Mouse protein 25 (MO25) and PGC-1α protein increased significantly after 4 days. Increased citrate synthase activity, increased LKB1 protein, and decreased AMPKK activity were found after 11 days. Maximal increases occurred after 4 days for hexokinase II, 25 days for MO25, and 53 days for citrate synthase, LKB1, and PGC-1α. In WQ, but not RQ or soleus, interval training had an additive effect to endurance training and induced significant increases in all proteins measured. These results demonstrate that LKB1 and PGC-1α protein abundances increase with endurance and interval training similarly to citrate synthase. The increase in LKB1 and PGC-1α with endurance and interval training may function to maintain the training-induced increases in mitochondrial mass.

adenosine 5′-monophosphate-activated protein kinase, AMP-activated protein kinase kinase; diabetes; MO25, Ste-20-related adaptor protein

THE INCIDENCE OF TYPE 2 DIABETES and obesity is increasing at an alarming rate. As of 2002, 8.7% of Americans over age 20 yr and 18.3% of Americans over age 60 yr were diabetic (1). Type 2 diabetes is strongly associated with obesity and is characterized primarily by a reduction in insulin-stimulated glucose uptake. Type 2 diabetes is often accompanied by a decreased skeletal muscle mitochondrial content and a higher-than-normal proportion of type IIx (IIb) muscle fibers (9, 24, 31, 37, 48). Regular exercise prevents type 2 diabetes directly by increasing insulin sensitivity and indirectly by decreasing adiposity. Physical activity also increases skeletal muscle mitochondrial mass and induces an increased expression of type IIa myosin heavy chain (MHC) and a decreased expression of type IIx (IIb) MHC (40). Training intensity regulates fiber type-specific training adaptations (2, 57). Higher-intensity training is required for the maximal recruitment and training of type IIx (IIb) fibers (16, 46). Hence, interval training might be particularly important for type 2 diabetics.

Research during the past decade has characterized some of the specific molecular signaling mechanisms that effect the classical adaptive responses in skeletal muscle. The AMP-activated protein kinase (AMPK) has been characterized as a metabolic master switch that functions as an intracellular fuel gauge (5, 15, 19, 20, 44, 62, 63). Muscle contraction results in the activation of AMPK (64). Acute activation of AMPK results in increased glucose uptake and fatty acid oxidation by skeletal muscle (23, 32, 58). Chronic activation of AMPK has also been shown to result in mitochondrial biogenesis, thereby increasing the total capacity of skeletal muscle to oxidize fats and carbohydrates (10, 65, 68). Thus AMPK has received considerable attention as a potential drug target for the treatment of type 2 diabetes (35, 61, 63).

AMPK requires phosphorylation on its activation loop at Thr^{172} by an AMPK kinase (AMPKK) for activation (22, 52, 60). Recently, the tumor suppressor kinase LKB1 (STK 11) was identified as a major AMPKK (21, 50, 66). LKB1 requires association with the regulatory proteins Ste20-related adaptor (STRAD) protein and mouse protein 25 (MO25) for full activity (12, 21). An increase in the intracellular AMP-to-ATP ratio induces a conformational change in AMPK by interacting with four cystathione β-synthase (CBS) domains on the γ-subunit (3, 49). This conformational change makes AMPK a suitable substrate for the constitutively active LKB1/STRAD/MO25 complex. A very recent report found that a conditional skeletal muscle-specific LKB1 knockout mouse exhibited no AMPKα2 phosphorylation in response to muscle contraction or 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), whereas AMPKα1 phosphorylation greatly diminished in response to AICAR (47).

The peroxisome-proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) is emerging as the master regulator of mitochondrial biogenesis (6, 42). PGC-1α was first characterized as a cold-inducible factor in murine brown fat and skeletal muscle (43). PGC-1α coordinates mitochondrial biogenesis by interacting with various nuclear genes encoding for mitochondrial proteins. PGC-1α induces nuclear respiratory factor 1 (NRF-1) NRF-2 gene expression (67). PGC-1α binds to NRF-1 and coactivates its transcriptional function on the

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mitochondrial transcription factor A (mtTFA) promoter (67). PGC-1α also binds to and coactivates PPARα and myocyte enhancer factor 2 (MEF-2) (30, 59). Inducing expression of PGC-1α in cell culture restores GLUT4 protein expression by interacting with MEF-2C (33). Increased expression of PGC-1α in type II muscle results in a fast-to-slow fiber type shift accompanied by an increase in mitochondria (30).

Mitogen-activated protein kinase (MAPK) p38, AMPK, and calcium-signaling mechanisms appear to play major roles in the regulation of PGC-1α expression and activity (38, 42). Activation of MAPK p38 increases total PGC-1α activity acutely by phosphorylation and, over time, by its increasing expression (4, 28, 41). Pharmacological activation of AMPK by AICAR in muscle results in increased PGC-1α protein content (27, 53). Incubation of L6 myotubules in caffeine without inducing contraction results in increased expression of PGC-1, NRF-1, NRF-2, and mtTFA (39).

Previously we (26, 55) demonstrated that endurance training increases LKB1 protein abundance in the skeletal muscle of rats. We also found a strong correlation between LKB1 protein abundance and citrate synthase activity across different fiber types in trained and untrained muscle (55). Rat soleus (SOL), red quadriceps (RQ), and white quadriceps (WQ) muscle are rich in type I, type IIa, and type IIb fibers, respectively. PGC-1α protein in humans and rodents has been shown to increase with endurance training (45, 56). The time course of the increase in LKB1 or PGC-1α protein abundance with endurance training has not been reported. The specific effect of interval training on LKB1 or PGC-1α abundance has also not been reported. We hypothesized that the training-induced increases in LKB1 and PGC-1α protein would follow a time course similar to that of citrate synthase. We also hypothesized that interval training would be required to elicit increased LKB1 and PGC-1α in WQ. To test this hypothesis, we treadmill-trained rats for up to 8 wk, with endurance training alone or endurance training combined with interval training, and analyzed their muscles for changes in LKB1 and PGC-1α protein abundance by Western blot.

MATERIALS AND METHODS

Animal care. All procedures were approved by the Institutional Animal Care and Use Committee of Brigham Young University. Male Sprague-Dawley (SAS:VAF) rats (Sasco, Wilmington, MA) were housed in a temperature-controlled (21–22°C) room with a 12:12-h light-dark cycle. Rats were fed standard rat chow (Harlan Teklad rodent diet) and water.

Training protocol. Rats were endurance trained, with a constant workload of 60 min twice/day, 5 days/wk for 4, 11, or 25 days, with a running speed of 27 m/min up a 15% incline ($n = 6$/group). After 25 days, remaining trained rats were divided into endurance training ($n = 7$) and interval training ($n = 7$) groups for 28 additional training days (53 days total). The endurance-training rats continued training as they had during days 1–25 without alteration. The interval-training rats continued identically to the endurance training rats except that each week, three interval-training bouts replaced three endurance-training bouts. Running speed for all intervals was 54 m/min. Rats recovered during intervals by running at 13.5 m/min for 2× the duration of the interval. Thus the total training volume and average running speed for the two groups were identical. Interval training was progressive, with rats running 16 × 30-s intervals per interval bout during *week 1*, 8 × 60-s intervals per interval bout during *week 2*, and 6 × 120-s intervals per interval bout during *weeks 3* and *4*. For all time points, each training group was paired with a control group that was diet-restricted to maintain a similar average body weight ($n = 6$/group). Control and trained rat weights were 273 ± 6 vs. 285 ± 5 g at *day 4*, 291 ± 6 vs. 286 ± 7 g at *day 11*, 318 ± 11 vs. 307 ± 11 g at *day 25*, and 346 ± 9 vs. 335 ± 9 g for distance-trained rats and 342 ± 11 g for interval-trained rats at *day 53*. Weights between control and trained groups at each time point were not different ($P < 0.05$). Rats were anesthetized 20–24 h after the last training bout by intraperitoneal injection of pentobarbital sodium (48 mg/kg body wt).

Buffers. Dithiothreitol (DTT), AMP, ATP, [γ-^{32}p]ATP, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), benzamidine, leupeptin, soybean trypsin inhibitor, and AMARA peptide (Zinsser Analytic, Maidenhead, Berkshire, UK) were added just before use when included. The following buffers were used: tissue homogenization buffer: 50 mM Tris·HCl, 250 mM mannitol, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100 with or without 10% glycerol (vol/vol), 1 mM benzamidine, 1 μg/ml soybean trypsin inhibitor, 1 mM DTT, 0.2 mM AEBSF, pH 7.4 at 4°C; AMPKK homogenization buffer: as with tissue homogenization buffer, but with 100 μM leupeptin; AMPK storage buffer: 50 mM Tris·HCl, 250 mM mannitol, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.02% (wt/vol) Brij-35, 10% (vol/vol) glycerol, pH 7.4, at 4°C; Laemmli's buffer (29); AMPKK assay buffer: 100 mM HEPES, 200 mM NaCl, 20% glycerol, 2 mM EDTA, 12.5 mM $MgCl_2$, 0.5 mM AMP, 0.5 mM ATP, 2.0 mM DTT, pH 7.0; phosphorylation buffer: 40 mM Hepes, 80 mM NaCl, 8% glycerol, 0.8 mM EDTA, 0.8 mM DTT, 5 mM $MgCl_2$, 0.2 mM AMP, 0.2 mM ATP, 0.33 mM AMARA peptide, 0.133 μCi/μl [γ-^{32}p]ATP, pH 7.0.

Tissue processing. SOL, deep lateral RQ, and superficial WQ were excised and immediately clamp frozen in liquid nitrogen. RQ and WQ were ground to powder under liquid nitrogen, using ceramic pestles and mortars, and thoroughly homogenized with a glass homogenizer. SOL was homogenized as described above but without being powdered. One part tissue was homogenized in nine parts (wt/vol) tissue homogenization buffer for Western blots and nine parts (wt/vol) AMPKK homogenization buffer for AMPKK assays. Tissue homogenates for Western blotting and AMPKK activity assays were centrifuged at 700 *g* for 10 min to remove connective tissue before blots and assays. Homogenates were stored at −95°C.

Western blots. All time course data were collected from RQ, and RQ, WQ, and SOL were analyzed for the effect of interval training. Western blots were performed as previously described (55). First antibody dilutions were: anti-hexokinase II from goat, 1:1,000 (sc-6521, Santa Cruz Biotechnology, Santa Cruz, CA); anti-LKB1 from rabbit, 1:2,500 (Cell Signaling Technology, Beverly MA); anti-MO25 from rabbit, 1:30,000 (Affinity BioReagents, Golden, CO); and anti-PGC-1 from rabbit, 1:1,000 (sc-13067, Santa Cruz Biotechnology). Second antibody dilutions were anti-rabbit horseradish peroxidase (HRP)-linked antibody from donkey, 1:1,500 (Amersham Pharmacia, Piscataway, NJ), and anti-goat HRP-linked antibody from cow, 1:30,000 (sc-2350, Santa Cruz Biotechnology). Blots were developed with ECL Western blotting detection reagents (Amersham). Blots were stripped and reprobed according to the manufacturer's instructions (ECL, Amersham).

Citrate synthase assay. Aliquots from raw tissue homogenates of SOL, RQ, and WQ were slow-frozen at −20°C overnight and subjected to two additional freeze-thaw cycles. Citrate synthase was assayed according to the method of Srere (51).

AMPKK activity assay. The AMPKK activity of tissue homogenates was assayed as previously described (54, 55), except that a standardized preparation of bacterially expressed heterotrimeric α2β2γ2 rAMPK (recombinant AMPK) was utilized as an AMPKK substrate rather than an α1-subunit truncated at the 312th residue. Activation of rAMPK was measured by ^{32}P incorporation from [γ-^{32}p]ATP into AMARA peptide (22). For AMPKK assays, tissue homogenates were diluted 1:1 (vol/vol) in AMPK storage buffer. Diluted tissue homogenates (2 μl) were incubated with AMPKK

assay buffer (4 μl) and rAMPK in storage buffer (4 μl) for 20 min. Phosphorylation buffer (15 μl) was added, and the reaction was stopped by spotting 1-cm^2 pieces of P81 filter paper (Whatman, Tewksbury, MA) with the final reaction mixture (15 μl) after 10 min. Filter papers were washed 6 × 90 s in 100 ml of 1% phosphoric acid, rinsed with acetone, dried, and counted for 1 min in 3 ml of Ecolite (ICN, Irvine, CA).

Statistics. Comparisons of relative protein abundances or enzyme activities between control and trained rats were made using one-way or two-way ANOVA. When main effects reached significance, Fisher's least significant difference multiple comparison test was used to determine the location. For all Western blots, the relative protein abundance values of *days 4*, *11*, *25*, and *53* controls were averaged for a *time 0* control value (no differences were found between control values for any protein blotted). For all tests, statistical significance was set at $P < 0.05$. All statistical procedures were performed with the Number Cruncher Statistical Systems program (NCSS, Kaysville, Utah). All data are reported as means ± SE.

RESULTS

Endurance training resulted in a time-dependent increase in the classical mitochondrial index protein citrate synthase in RQ (Fig. 1). A statistically significant increase in citrate synthase activity was found by *day 11* ($P < 0.05$). Citrate synthase activity approximately doubled with respect to controls by *day 53*. Interval training resulted in an intensity-dependent training effect in WQ but not RQ or SOL. In WQ (Fig. 2*A*), interval training, but not endurance training, produced a significant increase in citrate synthase ($P < 0.05$). In RQ (Fig. 2*B*) and SOL (Fig. 2*C*), citrate synthase was increased in both distance- and interval-trained rats but was not different between the two groups ($P < 0.05$).

Hexokinase II protein increased rapidly with a maximal and statistically significant increase of approximately sixfold by *day 4* that was maintained through *day 53* ($P < 0.05$; Fig. 3). Interval training, but not endurance training, resulted in a significant increase in hexokinase II in WQ ($P < 0.05$; Fig. 4*A*). In RQ (Fig. 4*B*) and SOL (Fig. 4*C*), hexokinase II increased in both distance- and interval-trained muscle but was not different between the two groups ($P < 0.05$). As an additional training index, total ventricular mass (right and left) was recorded after 53 days for controls and interval- and endurance-training groups. Both training protocols resulted in a similar and statistically significant increase in ventricular mass compared with controls. Total ventricular masses for control rats, distance-trained rats, and interval-trained rats were 0.87 ± 0.01, 1.11 ± 0.05, and 1.13 ± 0.05 g, respectively.

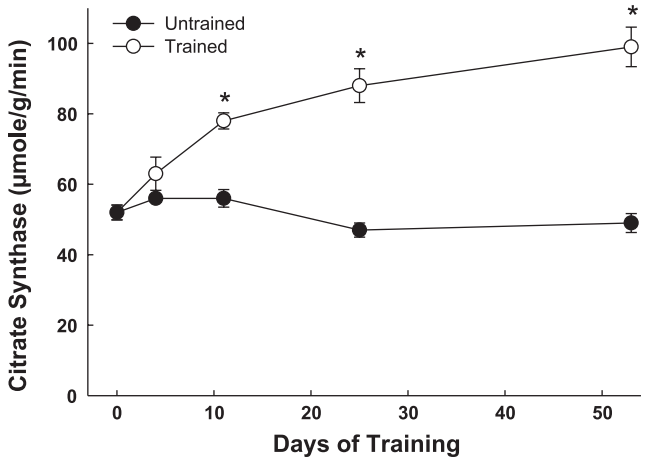

Fig. 1. Citrate synthase activity in rat red quadriceps after endurance training for 4, 11, 25, and 53 days compared with time-matched controls. Values are means ± SE ($n = 6$). *Citrate synthase values at *days 11*, *25*, and *53* were significantly different than *time 0* control value ($P < 0.05$). Control values were not different from each other ($P < 0.05$).

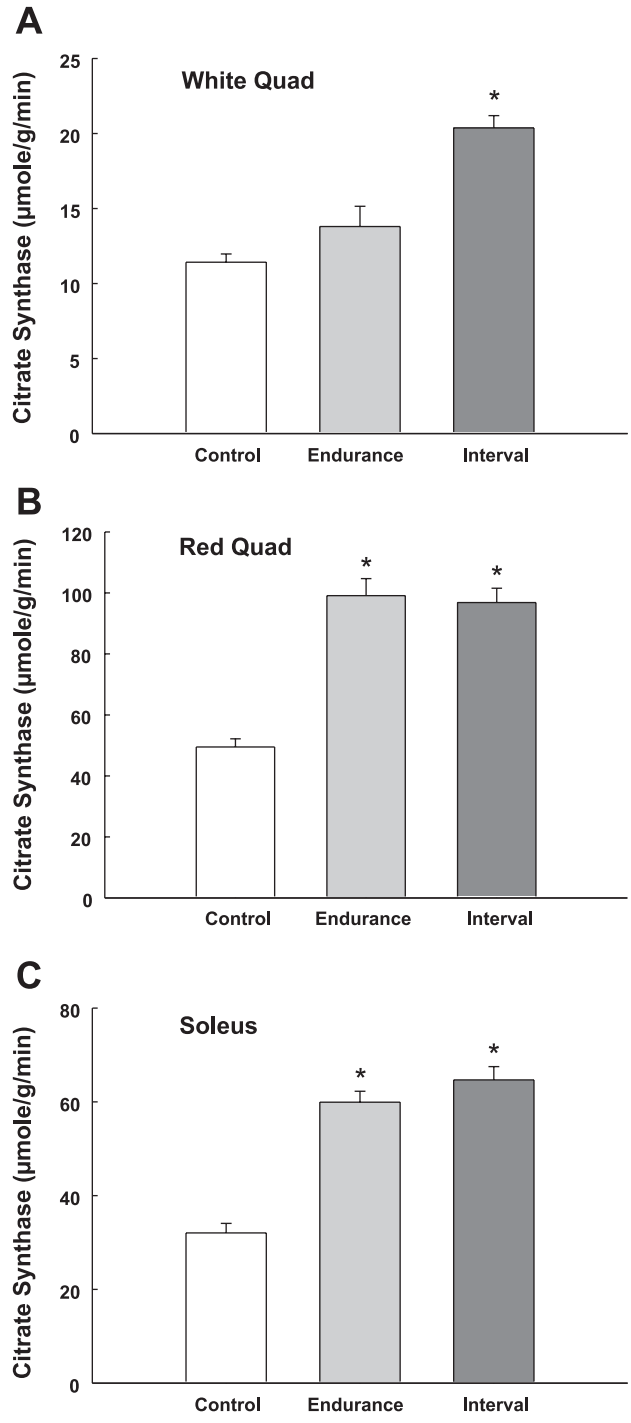

Fig. 2. Citrate synthase activity in rat white quadriceps (*A*), red quadriceps (*B*), and soleus muscle (*C*) of control, endurance-trained, and interval-trained rats. Values are means ± SE ($n = 6$). *Citrate synthase values were significantly different from *time 0* control value ($P < 0.05$).

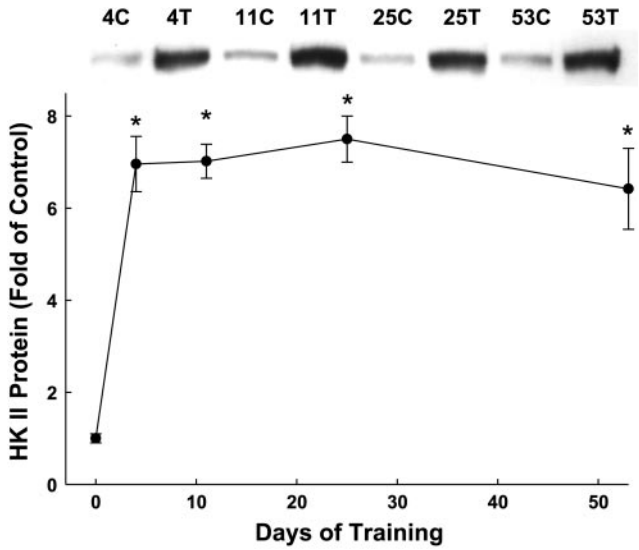

Fig. 3. Hexokinase II (HK II) protein relative abundance in rat red quadriceps after endurance training (T) for 4, 11, 25, and 53 days compared with *time 0* control (C; time-matched controls were not different). Values are means ± SE ($n = 6$). *HK II protein abundance values at *days 5*, *11*, *25*, and *53* were significantly different from *time 0* control value ($P < 0.05$).

LKB1 protein increased with time similarly to citrate synthase in RQ (Fig. 5). A statistically significant increase was found by *day 11*, with further increases through *day 53* to 1.76-fold of the control value ($P < 0.05$). Paradoxically, although LKB1 increased with time, AMPKK activity decreased with time, leading to a 57% decrease in AMPKK activity by *day 53* (Fig. 6). A significant decrease in AMPKK activity occurred by *day 11* ($P < 0.05$). Interval training resulted in an intensity-dependent increase in LKB1 in WQ (Fig. 7*A*) but not RQ (Fig. 7*B*) or SOL (Fig. 7*C*). In WQ, LKB1 was significantly increased by interval training, but not endurance training, whereas both protocols led to similar increases in LKB1 in both RQ and SOL ($P < 0.05$).

As with LKB1, training resulted in a time-dependent increase in MO25 protein (Fig. 8). However, unlike LKB1, MO25 increased maximally by *day 25*, with a statistically significant increase occurring by *day 4* ($P < 0.05$). In WQ (Fig. 9*A*), interval but not endurance training resulted in a significant increase in MO25, whereas in RQ (Fig. 9*B*) and SOL (Fig. 9*C*) both distance and interval training resulted in similar and significant increases in MO25 ($P < 0.05$). As noted previously (55), MO25 in muscle was detected at a molecular mass of ~55 kDa.

The transcriptional coactivator and regulator of mitochondrial biogenesis PGC-1α protein also displayed a time-dependent increase in response to endurance training (Fig. 10). PGC-1α was significantly increased on *day 4*, with further increases through *day 53* ($P < 0.05$). As with citrate synthase, LKB1, and MO25 in WQ (Fig. 11*A*), interval but not endurance training resulted in a significant increase in PGC-1α, whereas in RQ (Fig. 11*B*) and SOL (Fig. 11*C*), both protocols resulted in similar increases in PGC-1α ($P < 0.05$).

DISCUSSION

The overall aim of this study was to measure time and intensity-dependent changes in LKB1 and PGC-1 protein abundance with endurance and interval training. Citrate synthase activity and hexokinase II protein were measured as training indexes. Previous studies (11, 17, 25) have established that citrate synthase has a half-life of ~7 days. Similar results

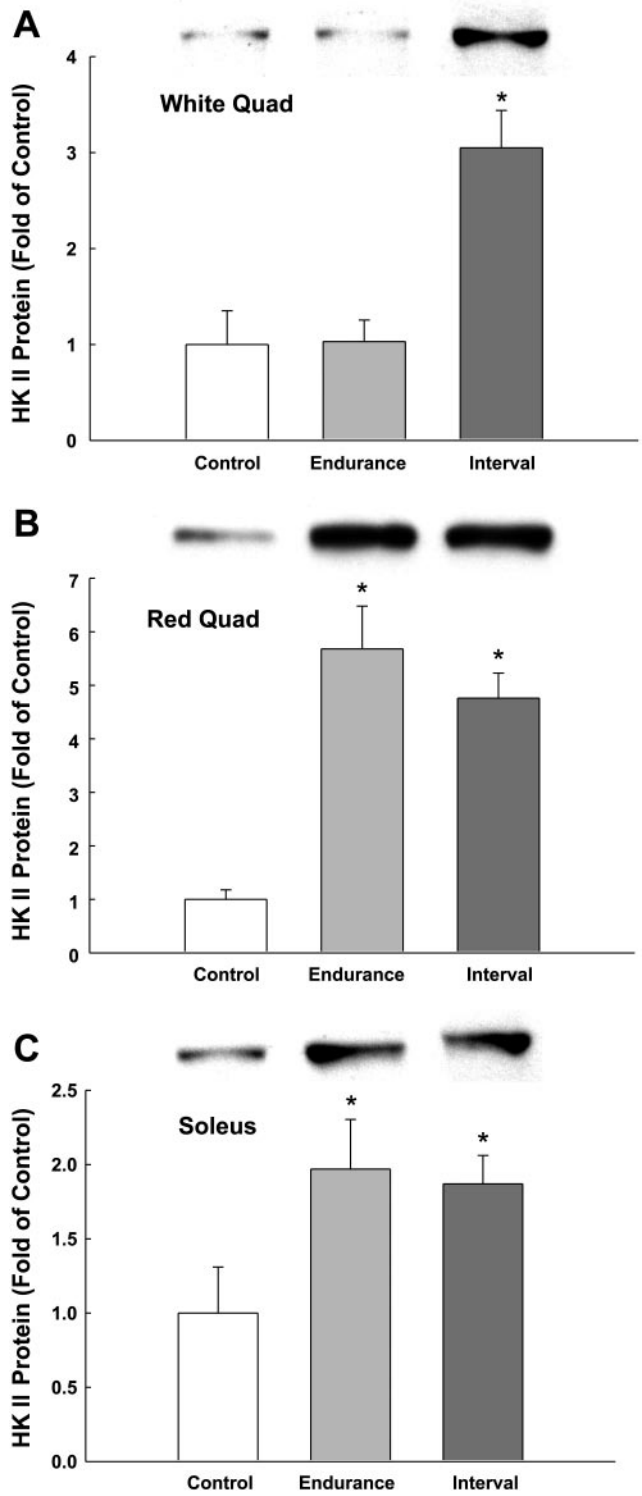

Fig. 4. HK II protein relative abundance in rat white quadriceps (*A*), red quadriceps (*B*), and soleus muscle (*C*) of control, endurance-trained, and interval-trained rats. Values are means ± SE ($n = 6$). *HK II values were significantly different from *time 0* control value ($P < 0.05$).

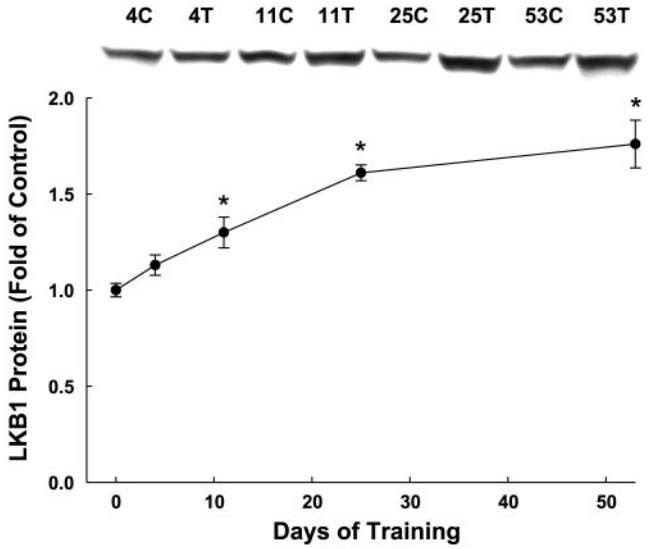

Fig. 5. LKB1 protein relative abundance in rat red quadriceps after endurance training for 4, 11, 25, and 53 days compared with *time 0* control (time-matched controls were not different). Values are means ± SE ($n = 6$). *LKB1 protein abundance values at *days 11*, *25*, and *53* were significantly different from *time 0* control value ($P < 0.05$).

were obtained in this study, indicating that the training protocol was effective. Furthermore, the rapid and maximal increase observed in hexokinase II after 4 days of training also validates the effectiveness of the training protocol, particularly the early portion. In white quadriceps, citrate synthase and hexokinase II exhibited a strong intensity-dependent response to interval training.

The effect of increased LKB1 with training is less clear. In a previous report (26), with a longer, more strenuous training protocol, we found that training increased LKB1 protein abundance in red quadricepts to approximately threefold of control levels compared with 1.76-fold of control levels in this study. Initially, we reported that although LKB1 protein abundance increased, training had no effect on AMPKK activity. However, we recently reported that AMPKK activity decreases with endurance training (26). We found that increasing the detergent Triton X-100 concentration from 0.5 to 1% results in greater extraction of AMPKK activity from untrained tissue but not

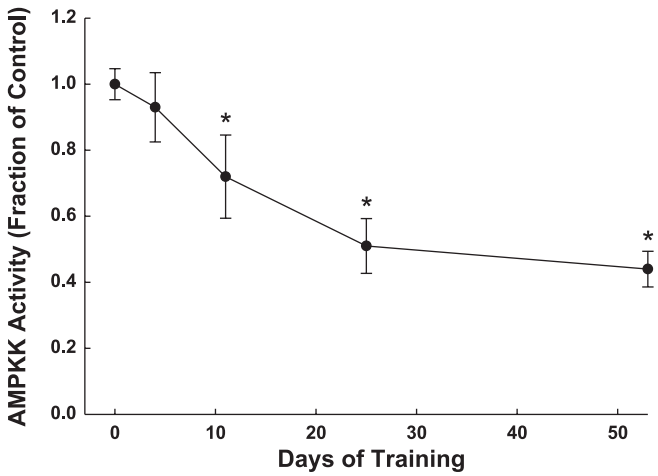

Fig. 6. AMP-activated protein kinase kinase (AMPKK) activity in rat red quadriceps after endurance training for 4, 11, 25, and 53 days compared with the *time 0* control (time matched controls were not different). Values are means ± SE ($n = 5$–6). *AMPKK activity at *days 11*, *25*, and *53* was significantly less than at *time 0* control ($P < 0.05$).

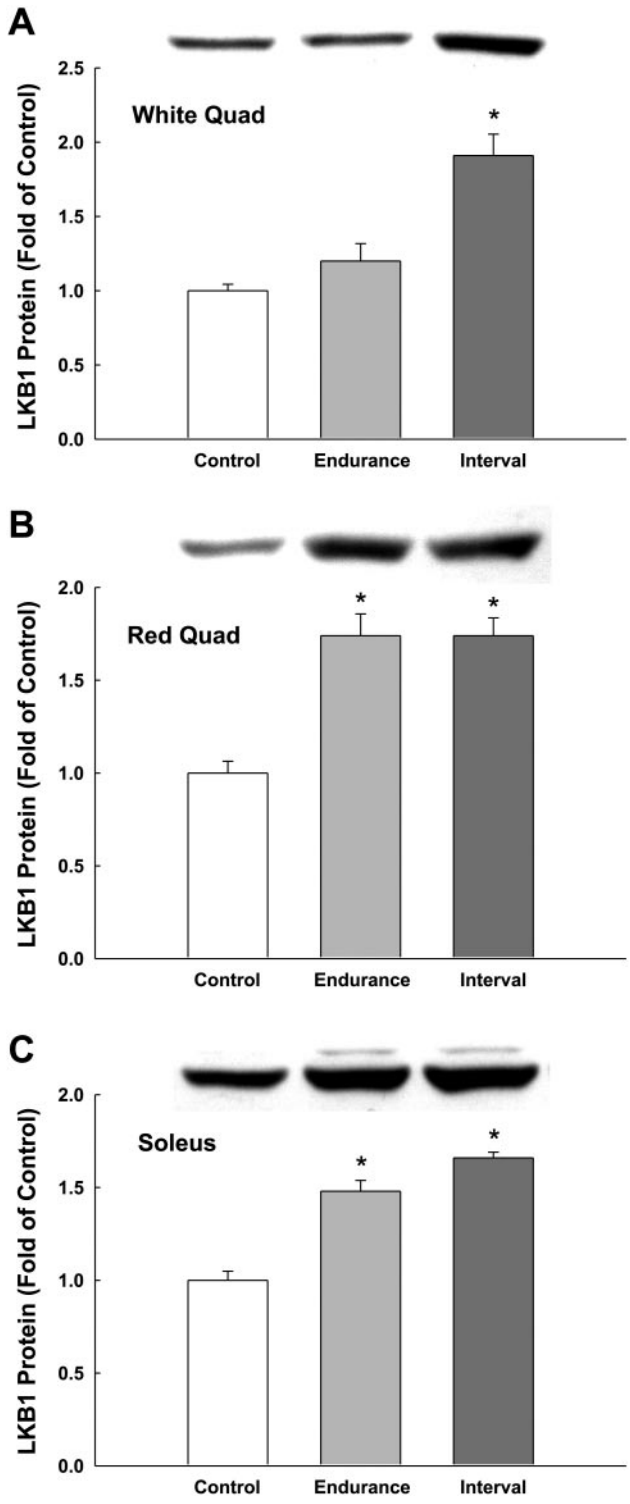

Fig. 7. LKB1 protein relative abundance in rat white quadriceps (*A*), red quadriceps (*B*), and soleus muscle (*C*) of control, endurance-trained, and interval-trained rats. Values are means ± SE ($n = 6$). *LKB1 values were significantly greater than *time 0* control value ($P < 0.05$).

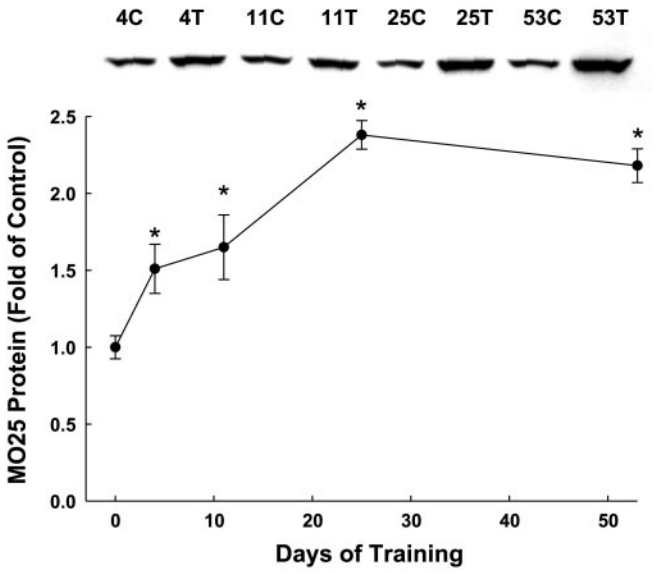

Fig. 8. MO25 protein relative abundance in rat red quadriceps after endurance training for 4, 11, 25, and 53 days compared with *time 0* control (time-matched controls were not different). Values are means ± SE ($n = 6$). *MO25 protein abundance values at *days 4*, *11*, *25*, and *53* were significantly different from *time 0* control value ($P < 0.05$).

from trained tissue. In the present study, utilizing a homogenization buffer with 1% Triton X-100, we again found that endurance training decreased AMPKK activity.

The apparent paradoxical result of increased LKB1 protein abundance with decreased AMPKK activity is reconcilable with the present research suggesting that LKB1 is the major AMPKK in skeletal muscle (47). LKB1 requires association with STRAD for activity (8, 21). The association and activity of LKB1-STRAD is stabilized by the binding of MO25 to STRAD (12). Thus the LKB1/STRAD/MO25 complex, rather than LKB1 alone, is the functional, active AMPKK. An increase in LKB1 protein abundance does not necessarily equate with an increase in the formation of the LKB1/STRAD/MO25 complex and therefore an increase in AMPKK activity. Currently, no studies have reported the effects of acute muscle contraction or chronic training on the formation of the LKB1/STRAD/MO25 complex or other LKB1 protein-protein interactions.

The increase in LKB1 protein abundance and decrease in AMPKK activity suggest that LKB1 might have important functions in the adaptation to endurance training. LKB1 is localized primarily in the nucleus but is exported to the cytoplasm upon binding to STRAD, which is imported to the nucleus by binding to MO25 (8, 12, 13). LKB1 has been demonstrated to interact with multiple proteins in addition to STRAD (14). Given the primary nuclear localization of LKB1 and the existence of numerous potential LKB1 binding partners, the increased LKB1 with training might perform a nuclear regulatory function important for skeletal muscle adaptation to exercise. This idea is made more plausible by the recent finding that contraction of LKB1-deficient skeletal muscle results in a larger-than-normal increase in AMP, characteristic of impaired metabolic capacity (47). Previously, we (26) demonstrated a strong correlation between LKB1 protein abundance and citrate synthase activity in skeletal muscle. In the present study, the time course of LKB1 increase is very similar to the citrate synthase increase. These findings demonstrate an associative relationship between LKB1 protein abundance and mitochondrial content in skeletal muscle. Future studies on the effects of muscle contraction on LKB1 subcellular localization

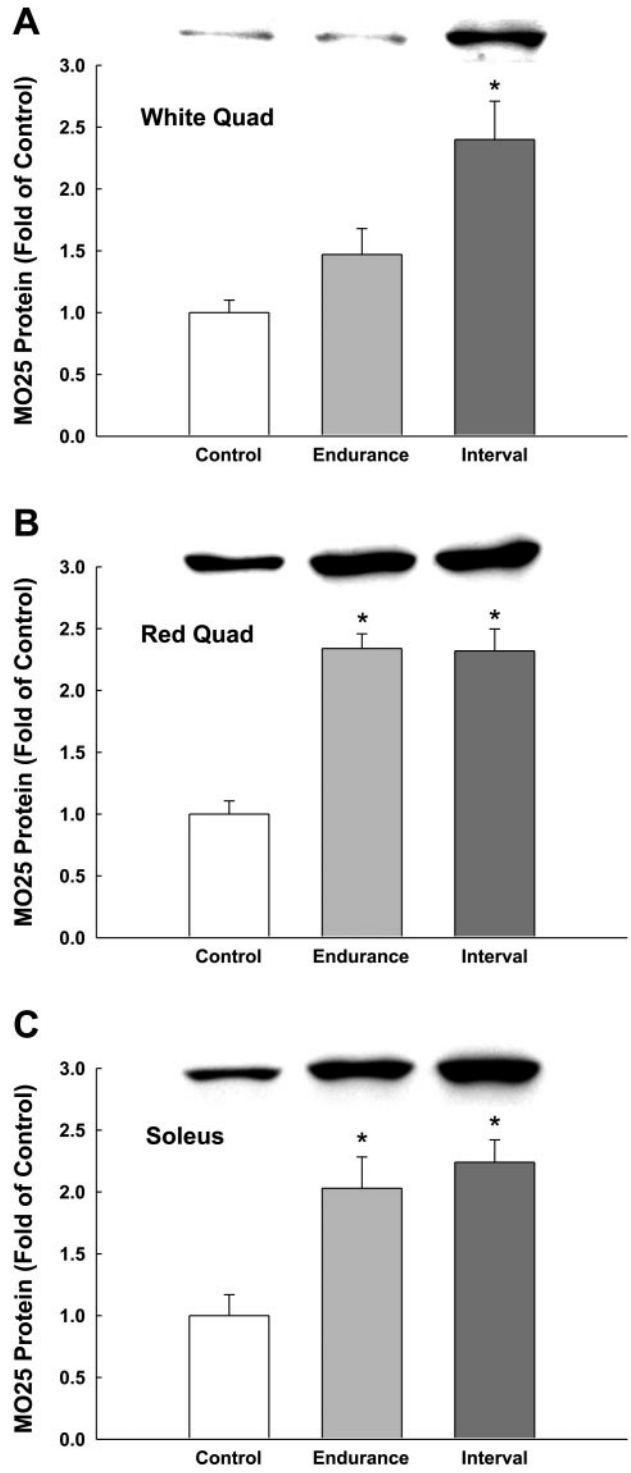

Fig. 9. MO25 protein relative abundance in rat white quadriceps (*A*), red quadriceps (*B*), and soleus muscle (*C*) of control, endurance-trained, and interval-trained rats. Values are means ± SE ($n = 6$). *MO25 values were significantly greater than *time 0* control value ($P < 0.05$).

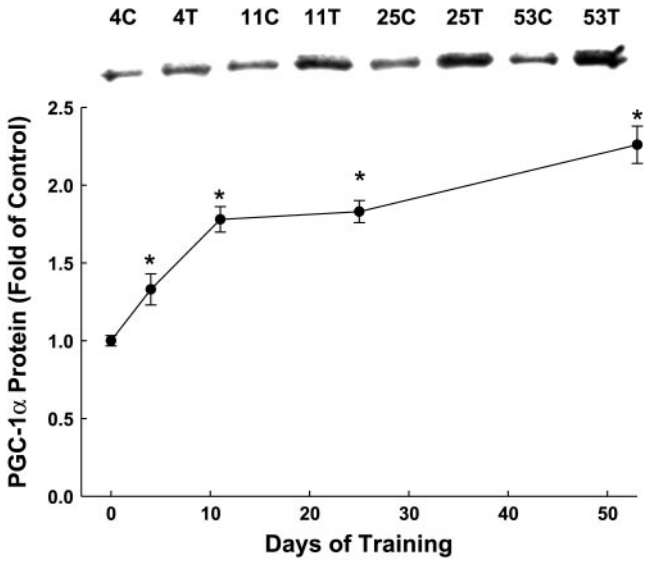

Fig. 10. Peroxisome-proliferator-activated receptor-γ coactivator-1α (PGC-1α) protein relative abundance in rat red quadriceps after endurance training for 4, 11, 25, and 53 days compared with *time 0* control (time-matched controls were not different). Values are means ± SE ($n = 6$). *PGC-1α protein abundance values at *days 4*, *11*, *25*, and *53* were significantly different from *time 0* control value ($P < 0.05$).

and LKB1 protein-protein interactions will be required to determine the physiological effects of the endurance training-induced increase in LKB1.

Previously we (55) found an approximately fivefold increase in MO25 protein abundance and a stable increase in MO25 mRNA with endurance training in rats. In the present study, MO25 protein abundance was significantly elevated after 4 days of training and increased to 2.4-fold of control levels by 25 days of training. In contrast to LKB1 and citrate synthase, MO25 protein did not increase further with additional training to *day 53*. The relative decrease in AMPKK activity is more similar in magnitude to the relative increase in MO25 protein than the relative increase in LKB1 protein. MO25 functions as a scaffold protein for the LKB1/STRAD/MO25 complex (13, 34). Increases in MO25 may alter complex formation directly or indirectly by changing stoichiometric relationships with other proteins that may participate in the LKB1 complex assembly process.

Although metabolic regulatory functions for LKB1 other than its crucial function as a component of AMPKK have not been established, multiple regulatory functions for PGC-1α have clearly been demonstrated. Previous research has shown that endurance training increases PGC-1 mRNA and protein (7, 18, 36, 45, 56). The first study investigating the effect of exercise on PGC-1α expression found PGC-1α mRNA expression in rat epitrochlearis muscle was elevated to 154 and 163% above controls levels after rats swam for 2 h/day for 3 and 7 days, respectively (18). Later, a low-intensity swimming bout of 6 h was found to increase PGC-1α mRNA up to 8-fold (56). In another study, 6 h of swimming was found to increase PGC-1α mRNA and protein approximately twofold 18 h after exercise (7). NRF-1 and NRF-2 were also increased.

To our knowledge, before the present study only one study had examined the effect of prolonged training on PGC-1α protein levels (45). After 6 wk of 3 days/wk endurance training in humans, PGC-1α protein increased 2.8-fold in type IIa fibers and 1.5-fold in type I and IIx fibers. The training time course utilized in the present study produced a significant increase in PGC-1α protein after 4 training days that increased in magnitude through *day 53* to 2.3-fold of control values. The continuous increase in PGC-1α protein during prolonged endurance training supports the hypothesis that, in addition to acutely regulating mitochondrial biogenesis by activation after a single exercise bout, accumulation of increased PGC-1α over time

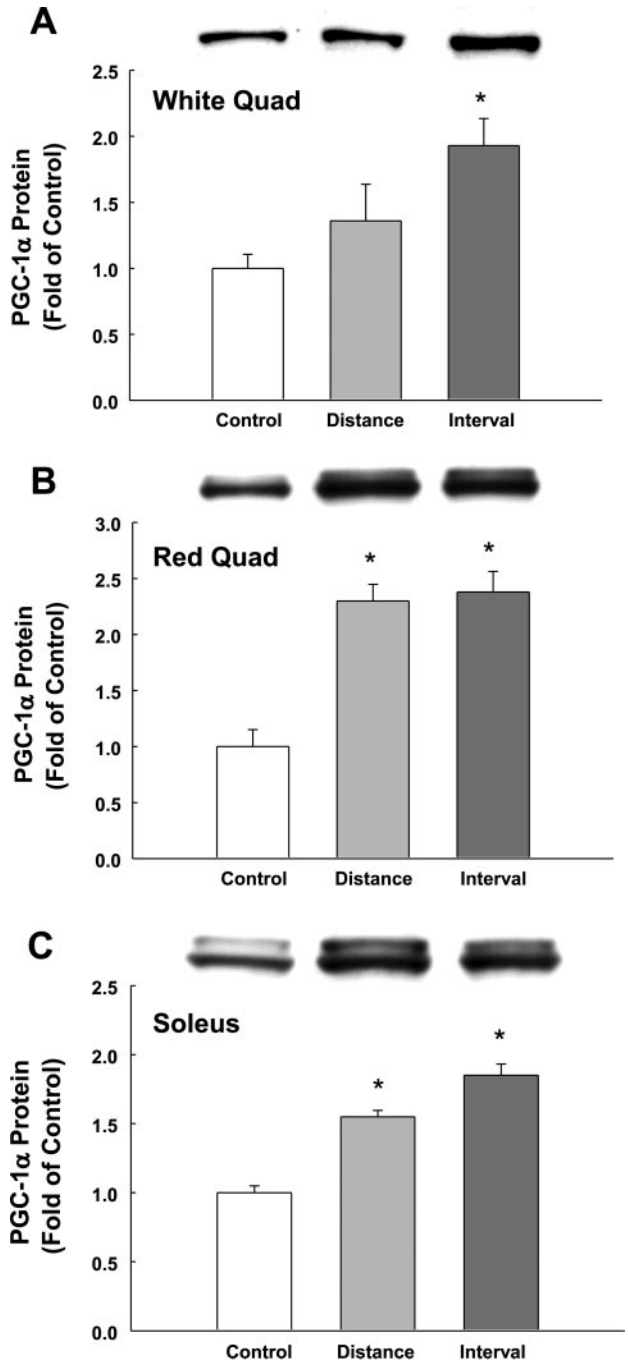

Fig. 11. PGC-1α protein relative abundance in rat white quadriceps (*A*), red quadriceps (*B*), and soleus muscle (*C*) of control, endurance-trained, and interval-trained rats. Values are means ± SE ($n = 6$). *PGC-1α values were significantly greater than *time 0* control value ($P < 0.05$).

with chronic training might maintain the training-induced increased mitochondrial mass.

The major effect of interval training was an induction of adaptation in white quadriceps. This finding is in agreement with those of the classic treatise on the training effects of time and intensity in rats by Dudley et. al (16). For all proteins measured here, interval training induced significant increases in white quadriceps that endurance training alone did not. Although red quadriceps is predominantly composed of type IIa fibers, interval training did not induce additional increases in citrate synthase activity or in the abundance of proteins we blotted for.

Utilizing interval training in combination with low-intensity exercise may be more effective than low-intensity exercise alone for type 2 diabetics seeking to optimize training-induced improvements in insulin sensitivity and glucose disposal. Compared with well-trained endurance athletes, diabetic and nondiabetic obese subjects have low maximum oxygen uptakes expressed relative to body weight. A high maximal aerobic capacity facilitates prolonged recruitment of a larger percentage of one's muscle mass, whereas a low maximal aerobic capacity prohibits this. Exercise training adaptations occur in muscle fibers that are recruited and therefore trained. Hence, interval training may be even more important for diabetics than for a well-trained athlete because *1*) they lack the aerobic capacity for sustained recruitment of a large percentage of their muscle mass and *2*) they have an elevated amount of type IIx muscle fibers (24, 31, 37).

In conclusion, we found that, in rat red quadriceps, the major metabolic regulatory molecules LKB1 and PGC-1α increase over time through 53 days of endurance training. LKB1 and PGC-1α increase with a time course similar to that of citrate synthase but distinct from hexokinase II. We also found that interval training induced an increase in LKB1 and PGC-1α protein in white quadriceps that was not found with lower-intensity training. The increases in LKB1 and PGC-1α protein with endurance training may play roles in maintaining the increased skeletal muscle mitochondrial mass resulting from endurance training.

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