Endurance training increases LKB1 and MO25 protein but not AMP-activated protein kinase kinase activity in skeletal muscle

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Taylor, E. B., D. Hurst, L. J. Greenwood, J. D. Lamb, T. D. Cline, S. N. Sudweeks, and W. W. Winder. Endurance training increases LKB1 and MO25 protein but not AMP-activated protein kinase kinase activity in skeletal muscle. Am J Physiol Endocrinol Metab 287:E1082–E1089, 2004. First published August 3, 2004; doi:10.1152/ajpendo.00179.2004.—LKB1 complexed with MO25 and STRAD has been identified as an AMP-activated protein kinase kinase (AMPKK). We measured relative LKB1 protein abundance and AMPKK activity in liver (LV), heart (HT), soleus (SO), red quadriceps (RQ), and white quadriceps (WQ) from sedentary and endurance-trained rats. We examined trained RQ for altered levels of MO25 protein and LKB1, STRAD, and MO25 mRNA. LKB1 protein levels normalized to HT (1 ± 0.03) were LV (0.50 ± 0.03), SO (0.28 ± 0.02), RQ (0.32 ± 0.01), and WQ (0.12 ± 0.03). AMPKK activities in nanomoles per gram per minute were HT (79 ± 6), LV (220 ± 9), SO (22 ± 2), RQ (29 ± 2), and WQ (42 ± 4). Training increased LKB1 protein in SO, RQ, and WQ (P < 0.05). LKB1 protein levels after training (%controls) were SO (158 ± 17), RQ (316 ± 17), WQ (191 ± 27), HT (106 ± 2), and LV (104 ± 7). MO25 protein after training (%controls) was 595 ± 71. Training did not affect AMPKK activity. MO25 but not LKB1 or STRAD mRNA increased with training (P < 0.05). Trained values (%controls) were MO25 (164 ± 22), LKB1 (120 ± 16), and STRAD (112 ± 17). LKB1 protein content strongly correlated (r = 0.93) with citrate synthase activity in skeletal muscle (P < 0.05). In conclusion, endurance training markedly increased skeletal muscle LKB1 and MO25 protein without increasing AMPKK activity. LKB1 may be playing multiple roles in skeletal muscle adaptation to endurance training.

adenosine 5’-monophosphate-activated protein kinase; diabetes; serine-threonine kinase-11; Ste20-related adaptor protein

LKB1 (serine-threonine kinase-11; STK11) in complex with the regulatory proteins MO25 and Ste20-related adaptor protein (STRAD) has recently been identified as a major upstream kinase for the AMP-activated protein kinase (AMPK) (16, 42, 49). LKB1 requires association with STRAD for catalytic activity that is enhanced by binding to MO25 (1, 16). MO25 stabilizes the interaction between LKB1 and STRAD and is thought to act as a scaffolding protein (5, 30). AMPK is a master metabolic regulator responsible for modulating cellular responses to an energy challenge (15, 38, 47). These responses include increased glucose uptake (3, 18, 22, 29), increased fatty acid oxidation (29, 46), decreased protein synthesis (4, 9, 20), and induction of mitochondrial biogenesis (2, 48). AMPK has been the subject of intense investigation because of its potential as a therapeutic target for antidiabetic drugs (25, 32, 37). Full activation of AMPK requires phosphorylation of its activation loop at Thr172 by an AMPK kinase (AMPKK) (17, 44). Thus the LKB1-STRAD-MO25 AMPKK complex may be a key regulator of the downstream effects of AMPK, including the regulation of exercise-induced glucose uptake.

LKB1 is a tumor suppressor protein regulating cell proliferation and polarity (7). Inactivating mutations in LKB1 were discovered as the causative agent for Peutz-Jeghers syndrome (PJS) in 1998 (19). PJS is an autosomal dominant disorder characterized by the development of hamartomatous polyps in the gastrointestinal tract and the pigmentation of mucous membranes. PJS is also associated with a considerable increase in risk for the development of cancer of the intestine, stomach, pancreas, breast, cervix, lung, ovary, testis, and uterus (7). Whereas LKB1 is predominantly a nuclear protein, association with STRAD, which is essential for AMPKK activity and LKB1-mediated G1 cell cycle arrest, anchors it in the cytoplasm (1, 16, 45).

STRAD is a pseudokinase that lacks several amino acid residues essential for kinase activity. LKB1 binding with STRAD results in the activation of LKB1, the phosphorylation of both LKB1 and STRAD, and the translocation of the LKB1-STRAD complex from the nucleus to the cytoplasm (1). The recently resolved crystal structure of the MO25-STRAD complex reveals that MO25 binds directly to the COOH terminus of STRAD (30). The authors of that study propose that MO25 is a potential scaffold protein for other regions of STRAD-LKB1, LKB1 substrates, and LKB1 regulatory components.

The effects of exercise on AMPK signaling in skeletal muscle have been studied extensively (15). Endurance training reduces the activation of AMPK in response to exercise in both rats and humans (12, 33, 50). Endurance training has also been shown to alter the expression of AMPK subunits in both rats and humans (12, 13, 33). The most recent of these studies found an increase in basal AMPK activity with training (13). The attenuated AMPK activation and increased basal AMPK activity with endurance training may be a result of altered AMPKK signaling. However, in contrast to AMPK, neither the role nor regulation of AMPKK in skeletal muscle is well understood.

The LKB1 protein content and AMPKK activity in different types of skeletal muscle and their regulation by endurance training are not well characterized. Investigating the role and regulation of AMPKK by endurance training may yield new insight into the regulation of glucose transport and fatty acid oxidation by exercise. Such information may be important for developing new treatments and prevention strategies for type 2 diabetes and the metabolic syndrome (37). Additionally, be-
cause LKB1 is a tumor suppressor and regulator of cell growth, it may serve as an etiological intersection between type 2 diabetes and some cancers (23, 28, 31, 34). Here, we present the results of our investigation on the effects of endurance training on AMPKK activity and LKB1 protein content in skeletal muscle, heart, and liver.

MATERIALS AND METHODS

Animal care and training protocol. 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 assigned to either a training or control group. Training rats were fed standard rat chow (Harlan Teklad Rodent Diet; Madison, WI) and water ad libitum. Control rats were food restricted to maintain body weight similar to that of trained rats. Body weights at the end of the study were 356 ± 5 g for trained rats and 353 ± 4 g for control rats. Rats were trained on a motor-driven rodent treadmill 5 days/wk for 10–11 wk on a 15% grade in a room maintained at 16–17°C. Initially, rats completed two 45-min exercise bouts per day at 16 m/min. Training progressed to a single 120-min exercise bout at 32 m/min with 30-s sprints at 53 m/min every 10 min for the final 4–5 wk. Rats were anesthetized 18–24 h after the last training bout by intraperitoneal injection of pentobarbital sodium (48 mg/kg body wt). Food consumption was measured by 10.220.33.5 on April 13, 2017 http://ajpendo.physiology.org/ Downloaded from

Preparation of α1132. AMPKα1 mRNA was isolated from homogenized rat gastrocnemius muscle, using the Qiagen RNeasy kit (Valencia, CA). cDNA coding for the first 312 amino acids of the α1-subunit was obtained (35 cycles of 94°C for 20 s, 54°C for 30 s, and 72°C for 1 min) with the SuperScript One Step RT-PCR System (GIBCO/Invitrogen, Carlsbad, CA). cDNA was further amplified (35 cycles of 94°C for 15 s, 55°C for 30 s, and 69°C for 1 min) with Platinum pfX high-fidelity DNA polymerase (GIBCO/Invitrogen). Cut sites for the restriction enzymes NcoI and XhoI were included in the primers, which were the following: forward primer 5′-AGC-TGCCATGGCAGAGACAGGAAGCA-3′ and reverse primer 5′-AGCTGCTGATTTAGTACGCAGGACG-3′. AMPKα1132 cDNA was cloned into the pET-30a vector with a 6x-His-Tag using the restriction sites NcoI (NH2 terminal) and XhoI (COOH terminal). The insert was ligated to the vector after treatment with calf intestinal alkaline phosphatase (Promega, Madison, WI) by use of the Roche Rapid DNA Ligation Kit (Nutley, NJ). The ligated vector and insert were first introduced into nonexpression host NovaBlue cells and then into expression host BL21(DE3) cells using heat shock (Novagen, Madison, WI). Single transformed colonies were used to create glycerol (80%) stocks. Standard Luria-Bertani (LB) broth with kana-mycin (30 μg/ml) was inoculated with transformed bacteria from glycerol stocks. Bacteria were cultured at 30°C, and isopropyl-β-D-thiogalactopyranoside (IPTG; 1 mM) was added to induce production of α1132 when optical density (OD)600 reached 0.6. Cells were incubated an additional 2.5 h after induction with IPTG. After incubation, cells were plated on ice for 5 min and then centrifuged for 5 min at 5000 g at 4°C. Pellets were resuspended in 5 ml of 1× BugBuster/g cells (Novagen), 1 mM PMSF, 0.2 g/ml lysozyme, and 0.1 μg/ml benzoylbenzyl benzene phosphate. After resuspension, cells were incubated at room temperature for 20 min with slow shaking. Resuspended cells were centrifuged at 16,000 g for 20 min at 4°C. Supernatant was applied to, and AMPKα1132 eluted from, a manual His-Bind column according to the manufacturer’s instructions (Novagen). Eluates were concentrated with Centricon Plus-20 Centrifugal Filter Devices (Millipore, Billerica, MA), diluted in AMPKα1132 storage buffer, concentrated again, and then frozen at −95°C.

AMPK activity assay. The AMPK activity of tissue homogenates was assayed by the activation of a standardized preparation of bacterially expressed AMPKα1132 (14). Activation of α1132 was measured by 32P incorporation from [γ-32P]ATP into AMAR peptide (16). For AMPK assays, tissue homogenates were diluted (vol/vol) in tissue homogenization buffer as follows: SO 1:1; RQ 1:1, WQ 1:1, HT 1:3, and LV 1:5. Diluted tissue homogenates (2 μl) were incubated with AMPKK assay buffer (4 μl) and AMPKα1132 in storage buffer (μl) for 15 min. Phosphorylation buffer (15 μl) was added, and the reaction was stopped by spotting 1-cm2 pieces of P81 filter paper (Whatman, Tewksbury, MA) with the final reaction mixture (15 μl) after 12 min. Filter papers were washed 6 × 5 min in 100 ml of 1% phosphoric acid, rinsed with acetone, dried, and counted for 10 min in 3 ml of Ecolite scintillation fluid (ICN, Irvine, CA).

Western blots. Homogenates were diluted in 4X Laemmli’s buffer and then separated by SDS-PAGE at 200 V for 35 min in 7.5% Tris-HCl, 50 μl well Ready Gels (Bio-Rad, Hercules, CA). Proteins were transferred from the gels to nitrocellulose membranes at 100 V for 30 min. Membranes were blocked in PBST and 5% blotting-grade blocker nonfat dry milk (Bio-Rad) for 1 h. Membranes were incubated overnight at 4°C in PBST, 5% blocking milk, and anti-LKB1 antibody (1:2,500). Antibodies for LKB1 and heat shock protein 90 (HS90) were obtained from Cell Signaling Technology (Beverly, MA). Antibody for MO25 from rabbit was made against the peptide MPFPFGKSHKSPAD(C) and obtained from Af-

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finity BioReagents (Golden, CO). Human MO25 was cloned into a pET14 vector for expression with a his-tag by MCLAB (San Francisco, CA). This recombinant MO25 was used as a standard and for competition studies in Western blotting for MO25. After incubation with the first antibody, membranes were washed twice with PBST for 10 min and twice with PBS for 5 min. Membranes were then incubated for 1 h at room temperature in PBST, 3% blocking milk, and anti-rabbit immunoglobulin horseradish peroxidase-linked whole antibody from donkey (1:1,500; Amersham Pharmacia, Piscataway, NJ). After incubation with the second antibody, membranes were washed twice with PBST for 10 min and twice with PBS for 5 min. Membranes were covered with enhanced chemiluminescence (ECL) Western blotting detection reagent (Amersham Pharmacia), enclosed in plastic wrap, and visualized on Hyperfilm ECL high-performance chemiluminescence film (Amersham Pharmacia). Relative amounts of LKB1 were quantified by measuring spot size and intensity with AlphaEaseFC software (Alpha Innotech, San Leandro, CA).

Citrate synthase assay. Aliquots from raw tissue homogenates of SO, 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 (43).

Real-time PCR. Relative changes in mRNA with training were examined by real-time PCR. Total mRNA was isolated from 30 mg of tissue from trained and control RQ muscle with the RNeasy fibrous tissue kit (Qiagen) according to the manufacturer’s instructions. Samples were homogenized with an Ultra-Turrax T8 (IKA, Wilmington, NC). cDNA libraries for each sample were generated using the SuperScript III first-strand synthesis kit (Invitrogen) according to the manufacturer’s instructions. Real-time PCR using gene-specific primers for LKB1, MO25, and STRAD mRNA and 18S rRNA was performed, using the Platinum SYBR Green qPCR Supermix UDG kit (Invitrogen) with an ABI-7000 real-time PCR System. Primers (Invitrogen) were the following: LKB1 forward, AGAGGAAGTGGGT-CAGAATGGA; LKB1 reverse, CCGCGCCTTGGCCTTCA; MO25 forward, GGTTCGATGAAAGAAAAACTCTGTG; MO25 reverse, AGCTGTGCTACGGCCTCTGT; STRAD forward, TCCAGGC-TTTACCTGAGTGT; STRAD reverse, AGACTGCGTGGCCTTC-GA; 18S forward, GTGCAATGGCCGTTCTAGTTG; 18S reverse, GCCACTTTGCTCCCTCTAAGAAGTTG. Samples were amplified in triplicate. Amplification protocol was 50°C for 2 min and 95°C for 10 min and then 60 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 30 s. For postamplification dissociation to generate melting curves, temperature was raised from 60°C to 95°C. Cycle threshold (Ct) was calculated by fitting the fluorescence curves (∆ΔRn) to a sigmoidal function and then obtaining the second derivative of the function using Graph Pad Prism software. Relative fold expression was calculated using the 2−∆∆Ct method after normalizing to 18S RNA (26).

Statistics. Two-sample Student’s t-tests were used to assess the training effect on relative LKB1 protein content and AMPKK activity for a given tissue. Comparisons of LKB1 protein content and AMPKK activity across tissues were done by one-way analysis of variance (ANOVA). When main effects reached significance, the Fisher least significant difference (LSD) multiple comparison test was used to determine the location. For all tests, statistical significance was set at $P < 0.05$. All statistical procedures were performed with the NCSS statistical program (Kaysville, Utah). All data are reported as means ± SE.

RESULTS

Table 1. AMPKK activities from trained and control rat homogenates of SO, RQ, WQ, HT, and LV

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control, nmol·min⁻¹·g⁻¹</th>
<th>Trained, nmol·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO†</td>
<td>22 ± 2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>RQ</td>
<td>29 ± 2</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>WQ†</td>
<td>42 ± 4</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>HT*</td>
<td>79 ± 6</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>LV*</td>
<td>220 ± 9</td>
<td>210 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7–8. SO, soleus; RQ, red quadriceps; WQ, white quadriceps; HT, heart; LV, liver. *HT and LV activities were significantly different from each other and SO, RQ, and WQ (P < 0.05). †SO and WQ were significantly different from each other but not RQ. Training did not significantly change AMP-activated protein kinase kinase (AMPKK) activities.

In contrast to AMPKK activity, LKB1 protein content increased significantly (P < 0.05) in trained SO, RQ, and WQ but not HT or LV. Figure 3 shows relative LKB1 protein levels in tissues from trained rats as percentage of LKB1 protein levels in controls. LKB1 protein in trained RQ was 316% of LKB1 protein in control RQ. Trained WQ and SO were 191% and 159% of control values, respectively. To ensure that diet restriction did not affect AMPKK activity and LKB1 protein content, we compared AMPKK activity and LKB1 protein content in tissues from control rats with tissues from rats fed ad libitum. No differences were observed (data not shown). Because nuclear LKB1 might have been removed with the centrifugation at 700 g to remove connective tissue, we compared raw, uncentrifuged muscle homogenates from trained and control RQ for LKB1 protein levels. LKB1 protein content was markedly greater in raw homogenates from trained RQ compared with control RQ (data not shown).

Fig. 1. Relative LKB1 protein abundance in soleus (SO), red quadriceps (RQ), white quadriceps (WQ), heart (HT), and liver (LV) from control rats determined by Western blotting. Values are means ± SE; n = 7–8. *HT, LV, and WQ were significantly different from each other, SO, and RQ. SO and RQ were not significantly different from each other (P < 0.05).
Although LKB1 protein content did not correlate ($r = 0.27$) with AMPKK activity, LKB1 protein levels strongly correlated with citrate synthase activity in trained and control tissues at $r = 0.93$ ($P < 0.05$). Figure 4 shows a scatter plot and regression line for LKB1 protein content and citrate synthase activity.

In addition to LKB1 protein, MO25 protein increased substantially with training. MO25 protein in trained RQ was 595% of MO25 in control RQ (Fig. 5). For unknown reasons, Western blots for MO25 indicate that it has a molecular mass of ~55 kDa in rat skeletal muscle. MO25 in rat LV was found at ~39 kDa. MO25 antibody strongly detected recombinant MO25 protein when used as a standard during Western blotting. Both the antigen peptide and recombinant MO25 protein effectively blocked binding of MO25 antibody in Western blot competition studies. We were unable to obtain antibody for STRAD. Western blots comparing levels of HSP90 from trained and control RQ indicated a trend for an increase in HSP90 with training ($P = 0.09$). Normalized HSP90 values in control and trained RQ were $100 \pm 23\%$ and $188 \pm 56\%$, respectively.

Relative levels of LKB1 (Fig. 6A), STRAD (Fig. 6B), and MO25 (Fig. 6C) mRNA were measured for increases with training in RQ, the tissue showing the greatest increase in LKB1 protein. Relative levels of LKB1, STRAD, and MO25 mRNA were not compared. In contrast to LKB1 protein, LKB1 mRNA did not increase with training. STRAD mRNA also did not increase. Conversely, MO25 mRNA did increase with training and was 164% of MO25 mRNA in control RQ ($P < 0.05$).

AMPKK activities in homogenates and resuspended PEG precipitates from trained and control RQ were compared (Fig. 7). No training effect was found. Resuspended PEG precipitates had significantly more AMPKK activity than homogenates per milligram of protein (Fisher LSD, $P < 0.05$). Overall, AMPKK activity in PEG precipitates was 5.8-fold the AMPKK activity in homogenates.

Figure 8 shows that resuspended PEG precipitates from trained and control RQ did not differ in LKB1 protein content ($n = 8$). Note that although LKB1 protein levels were not different, the thin top LKB1 band present in trained RQ and HT persisted through the PEG precipitation.

DISCUSSION

To our knowledge, this is the first study investigating the effects of endurance training on LKB1 and MO25 protein content and AMPKK activity.
abundance, the distribution of LKB1 between different types of skeletal muscle, and the AMPKK activities found in different types of skeletal muscle. A previous study examined AMPKK activity in human skeletal muscle and reported that AMPKK activity increased with increasing exercise intensity and duration (10). The increase in AMPKK activity paralleled the increase in AMPK activity but was much less than the increase in AMPK activity. A recent study found that LKB1 in skeletal muscle was not activated by electrical stimulation-induced in situ contraction, 5-aminoimidazole-4-carboxamide 1-D-riboside (AICAR), or phenformin (39).

In the present study, we found that endurance training did not affect AMPKK activity, but AMPKK activity is different across tissues (Table 1). LV, HT, and skeletal muscle had distinct activity levels, indicating that AMPK signaling may be regulated differently in these tissues. There was a tendency for AMPKK activity in different types of skeletal muscle to positively correlate with the contractile speed of the dominant fiber type. WQ, which is composed mostly of type IIb fibers, had the greatest AMPKK activity, whereas SO, which is composed mostly of type I fibers, had the lowest AMPKK activity. Although RQ AMPKK activity was not significantly different from WQ or SO AMPKK activity, it was intermediate between them. This relationship held both before and after training. A previous study demonstrated that treatment of rats with AICAR resulted in an increase in phospho-AMPK that was positively related to the contractile speed of the fibers composing them. Phospho-AMPK increased the most in epitrochlearis (65% IIb, 20% IIa, and 15% I), followed by extensor digitorum longus (59% IIa, 38% IIb, and 3% type I) and then SO (84% type I and 16% IIa) (21). Previous work in our lab showed a greater AICAR-induced AMPK activation in WQ compared with SO muscle (48). In HeLa and mouse embryo fibroblast cells, LKB1 is necessary for the AICAR-induced activation of AMPK (16). Thus the greater phospho-AMPK response in faster muscle to AICAR may be related to the greater basal activity of AMPKK we observed in WQ compared with SO. Because basal AMPKK activity did not change with training, this may indicate that muscles composed of faster fibers are more sensitive to an energy challenge independent of their oxidative capacity.

Endurance training had a pronounced effect on LKB1 protein levels in the three types of skeletal muscle studied. A marked change occurred in RQ muscle, where LKB1 protein abundance increased by 10.220.33.5 on April 13, 2017 http://ajpendo.physiology.org/ Downloaded from
content was more than three times as high compared with controls. Interestingly, relative LKB1 protein content was strongly and positively correlated with citrate synthase activities in skeletal muscle before and after training. The blots for LKB1 (Fig. 3) in HT, which is extremely rich in mitochondria, show a thinner LKB1 band immediately above the dominant LKB1 band. Training induced the appearance of a thin higher band in RQ (Fig. 3). This top band is present in untrained RQ but requires very long exposure times to visualize.

The distinguishing characteristic between the top and bottom bands is presently unknown but may be associated with the high oxidative capacity of trained RQ and HT. LKB1 is subject to many posttranslational modifications that could induce a band shift, including phosphorylation and farnesylation (11, 40, 41). LV, which had the highest AMPKK activity, had LKB1 levels intermediate between HT and skeletal muscle, but no doublet could be detected. Higher and lower LKB1 bands have previously been observed in crude AMPKK purifications from rat LV, but the separating characteristics for these two bands have not been reported (16). In our study, the bottom LKB1 band also increased with training and was the greatest in HT. Thus total LKB1 protein content in muscle is strongly associated with mitochondrial content rather than AMPKK activity.

We also compared trained and control RQ for relative abundance of MO25 and found an even greater increase in MO25 than in LKB1 with training. MO25 levels in trained RQ were nearly 600% of control values (Fig. 5). Although basal AMPKK activity did not change with training, the increases in LKB1 and MO25 may still be modulating AMPK signaling but not under resting conditions. The previously described activation of AMPKK with exercise did not occur early during the exercise bout at a low intensity but later, at a higher intensity (10).

We measured LKB1 mRNA in RQ to see whether the increase in LKB1 protein was related to an increase in LKB1 mRNA abundance. We found no difference in LKB1 mRNA between trained and control RQ. However, rats were killed 18–24 h after their last exercise bout. Training could have induced transient postexercise increases in LKB1 mRNA sufficient to increase LKB1 protein levels. Thus endurance training does not induce a stable increase in LKB1 mRNA.

Alternatively, endurance training may be increasing the stability of LKB1 protein. A previous study found that the chaperone protein HSP90, along with the cell division cycle (Cdc)37 kinase-specific targeting subunit for HSP90, stabilizes LKB1 by preventing its degradation by the proteasome (6). Later it was shown that treating cells with the HSP90-inhibiting antibiotics geldanamycin and novobiocin destabilized LKB1. Geldanamycin treatment resulted in the ubiquitination of LKB1 and its rapid degradation (35). One possible mechanism for the higher LKB1 in trained rat muscle could be an increase in HSP90 protein. Western blots showed that the mean value for HSP90 in trained RQ was almost double the control value, but the difference fell slightly short of statistical significance (P = 0.09) because of high variation. In principle, the studies on the stabilization of LKB1 by HSP90 (6, 35) demonstrate that LKB1 protein abundance can be regulated by protein-to-protein interactions. An increase in LKB1 protein is therefore not dependent on an increase in LKB1 mRNA.

Training may induce an increase in HSP90 or another protein(s) that contributes to the increase in LKB1 with training.

We also measured RQ STRAD (LYK5) and MO25 mRNA. Like LKB1, we found no stable increase in STRAD mRNA. However, consistent with the increase in MO25 protein, we did find a 64% increase in MO25 mRNA, indicating that endurance training induces a stable increase in MO25 mRNA. The increase in MO25 mRNA may be part of the mechanism responsible for increasing MO25 protein. If MO25 and LKB1 are increasing, but STRAD is not, AMPKK activity might remain the same because LKB1 absolutely requires association with STRAD for AMPKK activity (16).

Resuspended PEG precipitates from trained and control RQ did not have different AMPKK activity levels and, in contrast to muscle homogenates, did not have different levels of LKB1 protein. This indicates that the PEG precipitation process, which enriched AMPKK activity 5.8-fold, had a differential effect on LKB1 between trained and control tissues. For this to occur, the state of the LKB1 in the trained and control tissues must somehow be different. Perhaps the strong doublet on the blots from the trained tissue reflects this difference. LKB1 could also be complexed to different binding partners in trained tissue compared with controls, thereby affecting its precipitation properties. LKB1 has been found to bind to many proteins, including Brg-1, SMARCC1, STRAD, polyplody-associated protein kinase (PAPK), FK506-binding protein (FKBP)51, particularly interesting new cysteine-histidine rich protein (PINCH), ubiquitin-specific protease (USP)11, Cdc37, HSP90-α, HSP90-β, and KIF23 (5, 8, 27). However, LKB1 interaction with binding partners in skeletal muscle has not been specifically studied.

The functional significance of the increase in LKB1 and MO25 with training is unknown but may have potentially profound consequences on cell function. Most recently, the LKB1-STRAD-MO25 complex was found to phosphorylate eleven of the twelve AMPK-related kinases (42). NUA1, NUA2, BRSK1, BRSK2, quiescence-induced kinase (QIK), QSK, salt-inducible kinase (SIK), microtubule affinity-regulating kinase (MARK)1, MARK2, MARK3, and MARK4 were all phosphorylated and activated, whereas maternal embryonic leucine zipper kinase (MELK) was not. Phosphorylation of the AMPK-related kinases resulted in a >50-fold increase in their activity. The authors of that study propose that these kinases may mediate many of the physiological effects of LKB1, including its tumor-suppressive effects. An increase in LKB1 could affect any or all of the signaling pathways mediated by these AMPK-related kinases. Contraction, phenformin, and AICAR were found not to activate the AMPK-related kinases QSK, QIK, MARK2/3, and MARK4 in skeletal muscle (39). However, chronic contraction may regulate these proteins over time through an increase in LKB1 and/or MO25.

It is unlikely that the major role for the increase in LKB1 with training is one of tumor suppression, because Peutz-Jeghers patients are not characterized by skeletal muscle tumors. However, the same mechanism that induces an increase in skeletal muscle LKB1 with endurance training may induce an increase in LKB1 in metabolically stressed growing tumors. LKB1 has been shown to be elevated in some tumors; this increase in LKB1 may serve as a check against uncontrolled proliferation (36).
In conclusion, we found that endurance training markedly increased LKB1 and MO25 protein abundance in skeletal muscle but had no effect on AMPK activity. Additionally, among different types of skeletal muscle, WQ had the highest AMPKK activity but the lowest LKB1 protein level. These results indicate that factors other than LKB1 protein abundance are important for AMPK activity, and LKB1 may be playing an additional role(s) in the adaptation to metabolic stress. Because LKB1 is a master kinase for AMPK and the AMPK-related kinases and a tumor suppressor, the increase in LKB1 could have implications for glucose homeostasis, control of cell growth, and other unidentified cellular functions. Identification of the mechanism and effect of endurance training-induced increases in LKB1 may yield insight useful for the treatment of type 2 diabetes and certain cancers.

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