LKB1-AMPK signaling in muscle from obese insulin-resistant Zucker rats and effects of training

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Sriwijitkamol, Apiradee, John L. Ivy, Christine Christ-Roberts, Ralph A. DeFronzo, Lawrence J. Mandarino, and Nicolas Musi. LKB1-AMPK signaling in muscle from obese insulin-resistant Zucker rats and effects of training. Am J Physiol Endocrinol Metab 290: E925–E932, 2006. First published December 13, 2005; doi:10.1152/ajpendo.00429.2005.—AMPK is a key regulator of fat and carbohydrate metabolism. It has been postulated that defects in AMPK signaling could be responsible for some of the metabolic abnormalities of type 2 diabetes. In this study, we examined whether insulin-resistant obese Zucker rats have abnormalities in the AMPK pathway. We compared AMPK and ACC phosphorylation and the protein content of the upstream AMPK kinase LKB1 and the AMPK-regulated transcriptional coactivator PPAR in gastrocnemius of sedentary obese Zucker rats and sedentary lean Zucker rats. We also examined whether 7 wk of exercise training on a treadmill reversed abnormalities in the AMPK pathway in obese Zucker rats. In the obese rats, AMPK phosphorylation was reduced by 45% compared with lean rats. Protein expression of the AMPK kinase LKB1 was also reduced in the muscle from obese rats by 43%. In obese rats, phosphorylation of ACC and protein expression of PGC-1α, two AMPK-regulated proteins, tended to be reduced by 50 (P = 0.07) and 35% (P = 0.1), respectively. There were no differences in AMPKα1-,-2, -β1,-β2, and -γ3 protein content between lean and obese rats. Training caused a 1.5-fold increase in AMPKα1 protein content in the obese rats, although there was no effect of training on AMPK phosphorylation and the other AMPK isoforms. Furthermore, training also significantly increased LKB1 and PGC-1α protein content 2.8- and 2.5-fold, respectively, in the obese rats. LKB1 protein strongly correlated with hexokinase II activity (r = 0.75, P = 0.001), citrate synthase activity (r = 0.54, P = 0.02), and PGC-1α protein content (r = 0.81, P < 0.001). In summary, obese insulin-resistant rodents have abnormalities in the LKB1-AMPK-PGC-1 pathway in muscle, and these abnormalities can be restored by training.

Acetyl-CoA carboxylase; peroxisome proliferator-activated receptor-γ coactivator-1ε; exercise

AMP-activated protein kinase (AMPK) is an energy-sensing enzyme that is activated by increases in AMP-to-ATP ratios through mechanisms involving allosteric modification and phosphorylation by one or more upstream AMPK kinases (AMPKKs) (11, 25). AMPK is a heterotrimer that is formed by a catalytic α-subunit and the regulatory β- and γ-subunits. There are two isoforms of the α- and β-subunits and three isoforms of the γ-subunit (11, 25). Upon activation, AMPK switches on catabolic pathways that generate ATP, such as glucose transport and fatty acid oxidation, coupled with the inhibition of ATP-consuming (anabolic) pathways that include triglyceride and cholesterol synthesis. It has become apparent in recent years that AMPK plays a key role in mediating the acute and long-term effects of exercise on carbohydrate and fat metabolism in skeletal muscle. AMPK mediates, at least in part, exercise-stimulated increases in muscle glucose transport (3, 15, 34, 35) and fatty acid oxidation (53, 57). The stimulatory effect of AMPK on fatty acid oxidation results from the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) by AMPK (10, 53, 59). There is evidence that AMPK may also be implicated in regulating some of the adaptations that take place during physical training in muscle. Chronic treatment with the AMPK activator 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR) increases the content of the mitochondrial enzymes cytochrome c and δ-aminolevulinic acid synthase and the activity of citrate synthase, succinate dehydrogenase, and malate dehydrogenase (58). The signaling mechanism by which chemical AMPK activation leads to this training-like effect is not known, but some studies suggest that it involves increased expression of peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1γ), a master mediator of mitochondrial biogenesis and function. In rats, swimming exercise stimulates PGC-1 gene expression in muscle, and this effect is reproduced by incubating isolated muscles with AICAR (51). Treatment of mice for 8 wk with β-guadinopropionic acid, a compound that stimulates AMPK by depleting cellular energy, causes an increase in PGC-1 expression and mitochondrial density in muscle, but these effects are blocked in mice overexpressing dominant-negative (inactivating) AMPKα subunit mutant (64). Activation of AMPK with AICAR also mimics other effects of training, such as increasing GLUT4 protein content and hexokinase activity (17, 40) and enhancing GLUT4 and hexokinase II gene transcription rates in muscle (48). Furthermore, in cultured muscle cells, adenovirus-mediated expression of PGC-1 induces GLUT4 gene expression (31). Collectively, these findings suggest that AMPK may induce exercise-stimulated mitochondrial biogenesis and GLUT4 expression through PGC-1 and give further support to the hypothesis that AMPK is involved in the cellular adaptations to exercise training.

It has been known for several years that activation of AMPK involves phosphorylation by one or more upstream kinases (13), yet the identity of these kinases has been elusive. LKB1 has now been identified as a major AMPKK (12, 45, 61). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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LKB1 is a tumor suppressor, and LKB1 germ line mutations cause Peutz-Jeghers syndrome, a dominantly inherited disease characterized by predisposition to hamartomatous intestinal polyposis, mucocutaneous melanin pigmentation, and various neoplasms. LKB1 is constitutively active and phosphorylates AMPK at site Thr172 of the α-subunit (12, 45, 61). In mice, genetic deletion of LKB1 significantly inhibits AMPK activity and blunts the increases in glucose transport caused by electrically-stimulated contraction and the AMPK-activating compounds AICAR and phenformin (44). Because AMPK has been identified as a potential target for the treatment of insulin resistance and type 2 diabetes (36, 55, 56), a central issue in the AMPK field has been to determine whether type 2 diabetes and other insulin-resistant states are associated with abnormalities in LKB1-AMPK-PGC-1 signaling. Thus the first goal of this study was to examine whether obese insulin-resistant Zucker rats have abnormalities at different steps of the LKB1-AMPK-PGC-1 pathway. For this purpose, we compared AMPK and ACC phosphorylation as well as the protein content of the upstream AMPKK, LKB1, and the AMPK-regulated transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) in skeletal muscle of sedentary obese (insulin resistant) Zucker rats and sedentary lean (insulin sensitive) Zucker rats. A second goal of the study was to examine whether physical training would reverse the abnormalities in LKB1-AMPK-PGC-1 signaling found in the obese rats.

METHODS

Experimental animals. We studied three groups of animals: I) sedentary obese (insulin-resistant) Zucker (fa/fa) rats (n = 5); 2) sedentary lean (insulin-sensitive) littermates (n = 5); and 3) trained obese Zucker rats (n = 7). All of the rats from the three groups were 14 wk old at the time of the hindlimb perfusion experiments and when the animals were killed. All of the rats were provided laboratory chow and water ad libitum. The temperature of the animal room was maintained at 21°C, and an artificial 12:12-h light-dark cycle was set. All of the procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Training protocol. The obese Zucker rats were trained for 5 days/wk for 7 wk on a motorized treadmill beginning at 7 wk of age. Training began with the rats running at 15 m/min for 10 min on an 8% grade and was gradually increased during the next 6 wk until the rats were running for 90 min continuously at 22 m/min on an 8% grade. Animals were 14 wk of age at the end of training.

Hindlimb perfusion. To measure skeletal muscle insulin sensitivity, a hindlimb perfusion system was used with an infusion of 2-deoxy-D-[3H]glucose (5). Food was withdrawn 12–14 h before hindlimb perfusion. The trained animals were studied 24 h after their last exercise bout. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body wt). The surgical procedure for hindlimb perfusion of the rats and the perfusion apparatus used were similar to those described previously (21). After completion of the surgical preparations, the left gastrocnemius (mixed) was removed and clamp frozen. Then the right hindlimb was prepared for perfusion. Once the cannulas were inserted, the right hindlimb was washed out with 35 ml of Krebs-Henseleit buffer. The rat was then killed by an intracardiac injection of pentobarbital sodium. Immediately thereafter, the cannulas were placed in line with the perfusion system, and the hindlimb was allowed to stabilize during a 10-min noncirculating washout period. The perfusion medium consisted of Krebs-Henseleit buffer (pH 7.4) containing 4.5% dialyzed bovine serum albumin, 20% washed time-expired human red blood cells, 1 mmol/l glucose, 10 μU/ml Humulin, and 0.2 mM pyruvate. Perfusion flow rate during the washout period was 5 ml/min. After the washout period, the arterial line was switched to a perfusate with 10 mU/ml Humulin, 6 mmol/l glucose, 0.2 mmol/l pyruvate, 2 mmol/l mannitol, 0.2 μCi/ml 2-deoxy-D-[3H]glucose (2-[[3H]DG], 0.15 μCi/ml [14C]mannitol, and the same concentration of human red blood cells and BSA as was used during the washout period. Perfusion was performed at 37°C and continued for a total of 22 min, at which time the right gastrocnemius was excised, split, and clamp frozen. Muscle samples were stored at −80°C until analysis.

Blood chemistry. Blood samples (2 ml) were drawn from the abdominal vena cava before catheterization of the rats. The samples were collected in vials containing ethylenediaminetetraacetic acid (EDTA, 24 mg/ml, pH 7.4) and placed on ice until centrifuged (1,000 g for 15 min), and the plasma was recovered. The plasma samples were stored at −20°C until analyzed for glucose, insulin, triglycerides, and free fatty acids (FFA). We determined plasma glucose with a YSI 23A glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) and plasma insulin by radioimmunoassay using a double-antibody procedure (Linco, St. Louis, MO). Plasma FFA were determined according to Miles et al. (32), and plasma triglycerides were enzymatically determined with the use of a commercially available kit (Sigma Diagnostics, St. Louis, MO).

Determination of 2-[[3H]DG uptake. Muscle samples used to determine glucose uptake were weighed, homogenized in 1 ml of 10% TCA at 4°C, and centrifuged in a microcentrifuge for 10 min. Duplicate 0.3-ml samples of the supernatant were transferred to 20-ml scintillation vials containing 10 ml of Scintiverse E (Fisher Scientific, Pittsburgh, PA) and vortexed. Samples were counted for radioactivity in an LS-350 liquid scintillation spectrophotometer (Beckman, Fullerton, CA). Efficiency and channel crossover were determined by counting 3H and 14C standards of known activities. The accumulation of intracellular 2-[[3H]DG was calculated by subtracting the concentration of 2-[[3H]DG in the extracellular space from the total muscle 2-[[3H]DG concentration. The 2-[[3H]DG in the extracellular space was quantified by measuring the concentration of [14C]mannitol in the muscle homogenate.

Glycogen concentrations. Muscle samples were homogenized in 1 mol/l HClO4 at −10°C. An aliquot of muscle homogenate was hydrolyzed in 2 N HCl at 100°C for 2 h followed by neutralization with 2 N NaOH, and glycogen content was measured by the hexokinase enzymatic method (29).

Citrate synthase activity. To verify that there was a training response in muscle, citrate synthase activity was assayed by homogenizing weighed muscle samples in HES buffer (20 mmol/l HEPES, 1 mmol/l EDTA, and 250 mmol/l sucrose, pH 7.4, 1:20 wt/vol) on ice with three consecutive 15-s bursts of a Virtshear homogenizer (Virtshear, Gardiner, NY) set at its highest speed. A 300-μl aliquot was further diluted 1:2 in HES buffer and 1:10 in 0.1 mol/l Tris and 0.4% Triton X-100, pH 8.1. Citrate synthase activity was determined spectrophotometrically (47).

Hexokinase II activity. A portion of the muscle biopsy sample was used to measure hexokinase II activity, as previously described (30).

Western blotting. Muscle samples were weighed while still frozen and homogenized in ice-cold lysis buffer (1:10, wt/vol) containing 20 mmol/l Tris (pH 7.5), 10 mmol/l sodium pyrophosphate, 100 mmol/l sodium fluoride, 2 mmol/l sodium orthovanadate, 5 mmol/l EDTA (pH 8.0), 1% Nonidet P-40, 1 mmol/l PMSF, 3 mmol/l benzamidine, 10 μg/ml leupeptin, and 10 μg/ml aprolin (43). Homogenates were rotated for 1 h at 4°C and then centrifuged at 14,000 g for 10 min at 4°C. The supernatants were collected and protein concentrations measured by the Bradford assay. Muscle lysate proteins were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. After blocking in Tris-buffered saline with 5% nonfat dry milk, the membranes were incubated overnight at 4°C with the primary antibody against phospho-AMPK (Thr172; Cell Signaling, Beverly, MA), LKB1 (Cell Signaling), phospho-ACC (Ser79; Cell Signaling), PGC-1α (Calbiochem, San Diego, CA), AMPKα1 and -α2 (35), AMPKβ1 and -β2 (Upstate Biotechnology, Lake Placid, NY), and
AMPK\(\alpha\)3 (63). ACC was detected with the use of streptavidin (in Tris-buffered saline with 5% nonfat dry milk; Pierce Biotech, Rockford, IL) because it is a biotin-containing enzyme (28, 60). Bound antibodies were detected with anti-rabbit immunoglobulin-g (Santa Cruz Biotechnology, Santa Cruz, CA) because it is a biotin-containing enzyme (28, 60). The membranes were exposed to film, and band intensity was quantified using Image Tool Software (University of Texas Health Science Center at San Antonio).

**Statistical Analysis.** All data are expressed as means ± SE. Statistical differences among groups were determined by using one-way repeated-measures ANOVA and Fisher’s post hoc tests. Correlation analysis was performed by the Pearson product-moment method. For all analyses, a value of \(P < 0.05\) was considered to be statistically significant.

**RESULTS**

**Animal characteristics.** The animal characteristics are summarized in Table 1. The body weight of the obese Zucker rats was 82% greater than that of the lean Zucker rats (\(P < 0.05\)). Plasma triglycerides and free fatty acid concentrations were also higher in the obese rats (\(P < 0.05\)). Fasting plasma glucose concentrations were similar between groups. There was a 7% decrease in body weight in the obese Zucker rats that underwent training. There was a tendency for a reduction in plasma triglyceride (\(P = 0.07\)) and insulin (\(P = 0.1\)) concentrations with training.

**Muscle glucose uptake and muscle glycogen concentrations.** Insulin-stimulated glucose uptake in the skeletal muscle from obese rats was reduced by 60% compared with the lean rats (\(P < 0.05\); Table 1). In the obese insulin-resistant rats, training significantly increased insulin-stimulated glucose uptake by 120% (\(P < 0.05\)) to a value comparable to that of the lean rats. Muscle glycogen was not different between the lean and obese rats, and training had no effect on muscle glycogen content.

**Muscle enzyme activities.** To determine whether the exercise protocol induced adaptive changes in the skeletal muscle, we measured citrate synthase and hexokinase II activity. Citrate synthase activity in sedentary obese rats was 30% (\(P < 0.05\)) higher than in lean rats, whereas hexokinase II activity was not different between the lean and obese rats (Table 1). Exercise training increased citrate synthase activity by 62% (\(P < 0.05\)) and hexokinase II activity by 125% (\(P < 0.05\)) in the obese rats. Thus this exercise protocol resulted in the classical adaptations that occur after training.

**LKB1-AMPK-PGC-1 signaling in insulin-resistant animals.** In the obese rats, basal AMPK phosphorylation was reduced by 45% compared with the lean rats (\(P < 0.05\); Fig. 1A). The content of the AMPKK, LKB1, was also significantly reduced in the muscle from the obese rats by 43% (\(P < 0.05\); Fig. 1B). Consistent with the reduction in AMPK phosphorylation and LKB1 content, in the obese rats phosphorylation of ACC and the protein expression of PGC-1\(\alpha\), two AMPK regulated proteins, tended to be reduced by 50 (\(P = 0.07\)) and 35% (\(P = 0.1\)), respectively (Fig. 2. A and B). There were no differences in AMPK\(\alpha\)1, -\(\alpha\)2, -\(\beta\)1, -\(\beta\)2, and -\(\gamma\)3 protein content between the lean and obese Zucker rats (Fig. 3).

**Effects of training on LKB1-AMPK-PGC-1 signaling.** We examined whether 7 wk of exercise training on a treadmill...
reversed abnormalities in the LKB1-AMPK-PGC-1 pathway in the obese Zucker rats. After 7 wk of training, there was no change in AMPK phosphorylation in the obese rats (Fig. 1A).

Similar to findings from other studies (8, 38), training resulted in a 60% increase in the protein content of AMPKα1 in the obese Zucker rats (P < 0.05) but had no effect on AMPKα2, β1, β2, and γ3 (Fig. 3). Furthermore, training significantly increased LKB1 and PGC-1 protein content 2.8- and 2.5-fold (both P < 0.05), respectively, in the obese rats (Figs. 1B and 2B). Consistent with these changes, training tended to increase ACC phosphorylation (P = 0.07; Fig. 2A) without affecting ACC protein. Importantly, LKB1 protein content strongly correlated with PGC-1α protein content (r = 0.81, P < 0.05), hexokinase II activity (r = 0.75, P < 0.05), and citrate synthase activity (r = 0.54, P < 0.05; Fig. 4, A, B, and C, respectively).

**DISCUSSION**

Acute activation of AMPK with AICAR leads to increases in muscle glucose transport (3, 15, 35) and fatty acid oxidation (53, 57). Long-term AICAR treatment results in increases in GLUT-4 protein content, mitochondrial number, and mitochondrial enzyme activity (17, 58). Because increases in muscle glucose transport and fatty acid oxidation, as well as increases in mitochondrial number and function, improve insulin sensitivity, acute and long-term AMPK activation likely contribute to the insulin-sensitizing effect of training. In view of the beneficial effects resulting from AMPK activation, this enzyme has become a potential target for the treatment of type 2 diabetes. Studies done in type 2 diabetic subjects with body mass indexes (BMIs) ranging from 26 to 29 kg/m² have not shown abnormalities in muscle AMPK protein content or activity (16, 26, 35), further strengthening the notion that these subjects have a normally functioning AMPK pathway, which is therefore amenable to pharmacological intervention. A novel finding from the present study is that severely insulin-resistant rats have reductions at several steps of the AMPK pathway, spanning from the upstream AMPKK LKB1 to the downstream transcriptional coactivator PGC-1. In addition to our findings, there are other reports indicating that insulin-resistant animals...
have abnormal AMPK signaling. For example, Barnes et al. (1) showed that in muscle from obese Zucker rats, contraction failed to increase AMPK activity, whereas in lean rats contraction caused a 2.3-fold increase in AMPK activity. Also, in obese Zucker rats AMPK activation with AICAR did not stimulate whole body glucose disposal, whereas in lean littermates AICAR increased whole body glucose disposal more than twofold (2). Even though the AMPK system is considered an insulin-independent axis (14, 15), taken collectively the results from these studies suggest that the AMPK pathway may function abnormally in phenotypes of severe obesity and insulin resistance. This finding is relevant in regards to the pharmacological treatment of diabetes, because a large number of type 2 diabetic patients have a BMI >30 kg/m². Thus, it will be important to determine in future studies whether type 2 diabetic subjects with moderate-to-severe obesity (BMI >30 kg/m²) have impaired function of the LKB1-AMPK-PGC-1 axis and whether AMPK-activating compounds can stimulate muscle glucose transport and improve glycemia in these subjects.

A critical finding from this study is that obese insulin-resistant Zucker rats have decreased LKB1 muscle content. The finding that this reduction in LKB1 content is reversed by exercise training, in concert with an increase in potential AMPK targets such as PGC-1, underscores the importance of exercise to improve the molecular defects that characterize obesity, type 2 diabetes, and other insulin-resistant states. Moreover, we observed that increases in LKB1 muscle content strongly correlated with increases in citrate synthase and hexokinase activities, which is consistent with previous studies done in normal rats (49, 50). This suggests that LKB1 plays an important role in regulating the adaptations to training through a mechanism that involves AMPK. Our results also support a role for PGC-1 in mediating the effects of LKB1-AMPK signaling on insulin sensitivity. Consistent with the hypothesis that AMPK regulates PGC-1 expression, inactivation (18) and deletion (24) of AMPK was found to reduce AICAR-induced increases in PGC-1 (24) and GLUT4 (18, 24) mRNA expression in muscle. Yet deletion of AMPKα1 or AMPKα2 did not affect the increases in muscle PGC-1 mRNA expression seen after acute exercise (24). These findings indicate that
AMPK activation leads to higher PGC-1 expression, yet AMPK is not indispensable for exercise-induced increases in PGC-1 expression. These studies, however, do not exclude an action of AMPK to increase PGC-1. AMPK complexes containing the remaining α-isoform could have compensated for the isoform that was inactivated/deleted. It is also possible that exercise-induced increases in PGC-1 are mediated by redundant pathways in addition to AMPK. For example, calcium/calmodulin-dependent protein kinase signaling has also been implicated in the regulation of PGC-1 expression and the adaptive changes to training in muscle (39, 62).

In the obese rats, training resulted in increases in LKB1 and several potential targets of AMPK, including PGC-1, hexokinase, and citrate synthase. Nonetheless, basal AMPK phosphorylation was not increased after training. This probably occurred because muscles were removed 24 h after the last exercise bout, and in rats AMPK activity returns to baseline within 30 min of rest (exercise bout, and in rats AMPK activity returns to baseline). This probably implicates in the regulation of PGC-1 expression and the LKB1-AMPK tumor suppressor, STRADalpha/beta and MO25alpha/beta are upstream kinases. Other than LKB1, in response to training (19). To be active, LKB1 needs to exist as a complex with two accessory subunits, STRADalpha/beta and MO25alpha/beta (4, 12). An imbalance between the three subunits could also lead to increased LKB1 content without a change in AMPK phosphorylation. Indeed, Taylor et al. (49) found that in Sprague-Dawley rats, training increased LKB1 and MO25 content but had no effect on STRAD. LKB1 is a master kinase that activates at least 13 other kinases of the AMPK subfamily in addition to AMPK (27). Thus the reduction in LKB1 content in the obese Zucker rats could lead to decreased activity of these AMPK-related kinases. If one or more of these kinases regulates PGC-1 expression, this could result in lower basal PGC-1 content and would help explain increases in PGC-1 caused by training. Nonetheless, it remains to be determined whether AMPK-related kinases regulate PGC-1 expression.

Research suggests that abnormal mitochondrial function can impair insulin action and result in insulin resistance in human muscle (33, 41, 42). There is also evidence that the mitochondria of the obese Zucker rat are dysfunctional. For example, these rats have a low metabolic rate and decreased reliance on fat as an energy source at rest and during exercise (6, 21, 22, 52). In addition, the obese Zucker rat has extremely high intramuscular triglyceride content, which suggests that its muscle is incapable of normal fat oxidation (9). Consistent with our present findings, we (21) and others (54) have reported previously that citrate synthase activity is higher in muscle from obese Zucker rats compared with lean littermates. Citrate synthase activity is a marker of mitochondrial concentration. Thus the slight increase in mitochondrial density (i.e., basal citrate synthase activity) in the obese rats likely represents a compensatory mechanism for an inefficient mitochondrial system to provide sufficient aerobic energy. The present results suggest that insulin-resistant states associated with mitochondrial dysfunction could possibly be ameliorated by activation of the LKB1-AMPK-PGC-1 axis and overexpression of the mitochondria (Fig. 5). Finally, not all of the effects of AMPK on insulin sensitivity appear to be mediated by PGC-1. Acute treatment with AICAR enhances insulin-stimulated GLUT4 translocation (23) and glucose transport in muscle (7, 23). Smith et al. (46) showed that hyperosmotic stress, a known AMPK-activating stimulus, enhances insulin sensitivity in C2C12 myotubes, and this increase is blocked with a specific AMPK inhibitor. Furthermore, in insulin-resistant rats a single dose of AICAR improves peripheral insulin sensitivity (20).

In summary, insulin-resistant obese rats have abnormalities at different steps of the LKB1-AMPK-PGC-1 axis that could explain some of their metabolic derangements. The data from this study indicate that training can reverse these abnormalities in LKB1-AMPK-PGC-1 signaling and that this effect may contribute to the beneficial effects of training. Future studies will help to determine whether insulin-resistant obese subjects also have these abnormalities and whether they can be reversed by training.

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