AMP Kinase–Induced Skeletal Muscle Glucose But Not Long-Chain Fatty Acid Uptake Is Dependent on Nitric Oxide

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The purpose of this study was to examine the effects of AMP kinase (AMPK) activation on in vivo glucose and long-chain fatty acid (LCFA) uptake in skeletal muscle and to examine the nitric oxide (NO) dependence of any putative effects. Catheters were chronically implanted in the carotid artery and jugular vein of male Sprague-Dawley rats. After 4 days of recovery, rats were given either water or water containing 1 mg/ml nitro-L-arginine methyl ester (L-NAME) for 2.5 days. After an overnight fast, rats underwent one of five protocols: saline, 5-aminoimidazole-4-carboxamide-1-B-D-ribofuranoside (AICAR) (10 mg · kg–1 · min–1), L-NAME, AICAR + L-NAME, or AICAR + Intralipid (20%, 0.02 ml · kg–1 · min–1). Glucose was clamped at ~6.5 mmol/l in all groups, and an intravenous bolus of 2-deoxy[3H]glucose and [125I]-15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid was administered to obtain indexes of glucose (Kg) and LCFA (Kf) uptake and clearance. At 150 min, soleus, gastrocnemius, and superficial vastus lateralis were excised for tracer determination. Both Kg and Kf increased with AICAR in all muscles studied. Kg decreased with increasing muscle composition of type 1 slow-twitch fibers, whereas Kf increased. In addition, AICAR-induced increases in Kg but not Kf were abolished by l-NAME in the majority of muscles examined. This shows that the mechanisms by which AMPK stimulates glucose and LCFA uptake are distinct. Diabetes 53:1429–1435, 2004

Type 2 diabetes and obesity are metabolic disease states characterized by impairments in glucose and fatty acid metabolism (1,2). As such, the discovery and understanding of signaling pathways involved in the uptake, oxidation, and storage of these substrates is of importance. A protein key to metabolic regulation is AMP kinase (AMPK), which functions as an intracellular fuel gauge responding to alterations in the energy status of the cell. Activation of AMPK results in glucose uptake, fatty acid oxidation, and a decline of fatty acid storage in skeletal muscle (3–5). Furthermore, the oral hypoglycemic agents rosiglitazone and metformin, are known to act in part through AMPK-mediated pathways (6–9).

Despite our increased knowledge of AMPK and its role in tissue-specific and whole-body metabolic regulation, the mechanism by which it exerts its actions are unclear. Recent studies indicate that its effects are in part mediated through nitric oxide (NO) or an NO-mediated pathway (10,11). NO is produced and released from skeletal muscle in response to exercise-stimulated skeletal muscle glucose uptake responses (13–15). Elevation of NO through the NO donor sodium nitroprusside (SNP) results in increased glucose transport and plasma membrane content of GLUT-4 (13,16). Correspondingly, blockade of NO by administration of NG-nitro-L-arginine methyl ester (l-NAME) or NG-monomethyl-L-arginine (l-NMMA) during hyperinsulinemic-euglycemic clamps results in decreased blood flow and glucose disposal in vivo (14,17). To determine the dependence of AMPK on NO, a number of studies have activated AMPK in the presence or absence of NO inhibitors. In cell culture, the combination of l-NMMA and the AMPK activator 5-aminoimidazole-4-carboxamide-1-B-D-ribofuranoside (AICAR) results in the abolishment of AICAR-stimulated glucose uptake, suggesting that AMPK increases glucose uptake by an NO–dependent pathway (11). Additional evidence is found in a study by Morrow et al. (10), where activation of AMPK by AICAR in human aortic endothelial cells resulted in an increase in NO synthase (NOS) phosphorylation and NO production. When a mutant AMPK was transfected into the endothelial cells via adenovirus, AMPK-activated NO production was diminished, showing a clear mechanistic link between AMPK and NOS. Taken together,
these in vitro studies provide strong evidence that AMPK acts via an NO pathway; however, in vivo data are lacking.

It is clear from the above discussion that AMPK activation stimulates muscle glucose uptake and intracellular pathways for fatty acid oxidation (3,5,18). However, it is unknown whether the increased fatty acid oxidation translates into an increased fatty acid uptake from the blood in vivo and whether such an effect is, like glucose uptake, reliant on the formation of NO. The purpose of the present study was to examine the effects of AMPK stimulation on skeletal muscle glucose and long-chain fatty acid (LCFA) uptake and clearance and to determine the dependence of any effects on NO. Skeletal muscle fiber type is a major determinant of the metabolic response to a number of stimuli. Therefore, an additional objective was to examine the magnitude and mechanism for AICAR-induced skeletal muscle glucose and LCFA uptake in muscle comprised of different fiber types. Experiments were performed in chronically catheterized conscious rats, where isotopic analogs of glucose and LCFA were administered to quantify tissue-specific substrate influx. It was hypothesized that if AMPK’s effects on NO occurred by a common pathway, then proportional reductions in tissue-specific glucose and fatty acid uptake would occur.

**RESEARCH DESIGN AND METHODS**

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were housed individually and maintained at 23°C on a 0600–1800 light cycle. Rats were fed chow (Purina Nestlé, St. Louis, MO) ad libitum and given free access to water. The rats were housed under these conditions for ~1 week, by which time their weights had reached ~300 g. After weight gain, rats were randomly divided into each of the experimental groups (n = 7 per group). All procedures were approved by the Vanderbilt University Animal Care and Use Subcommittee and followed the National Institutes of Health guidelines for the care and use of laboratory animals.

**Surgical procedures.** Surgical procedures were performed as previously described for arterial and venous catheterizations (19). Briefly, animals were anesthetized with a 50:5:1 vol/vol/vol mixture of ketamine, rompun, and acepromazine, and the left common carotid artery and right jugular vein were catheterized with PE50. Catheters were exteriorized and secured at the back of the neck, filled with heparinized saline (150 units/ml), and sealed with a stainless steel plug. Immediately postsurgery, each animal received 75 mg/kg stenal and 150 units/ml heparin. Surgical procedures were performed as previously described (19,20). Briefly, anesthetized rats were placed in a supine position, and a cannula (20G) was inserted into the right femoral vein. After the cannula was flushed with heparinized saline (150 units/ml), and connected to PE50 and silastic tubing for infusions and sampling. Throughout the experimental protocol rats were conscious and unrestrained. The experimental protocol consisted of continuous infusions of saline, AICAR (10 mg·kg⁻¹·h⁻¹), or AICAR + Intralipid 20% (Intralipid: 0.02 ml · kg⁻¹ · min⁻¹, containing 100 units/ml heparin; Pharmacia & Upjohn, Uppsala, Sweden). All infusions lasted for 150 min. In those rats receiving AICAR, glucose (50 ml) was clamped to levels found in saline and L-NAME groups (~6.5 mmol/l). Since the administration of AICAR resulted in a decline in nonesterified fatty acid (NEFA), Intralipid was infused in one protocol to ensure NEFA levels were maintained throughout the experimental period to ensure the accuracy of LCFA uptake (R₂) was not concentration dependent. In total this resulted in five experimental groups: saline (n = 7), AICAR (n = 7), L-NAME (n = 7), AICAR + L-NAME (n = 7), and AICAR + Intralipid (n = 7). All infusions were equilibrated for 90 min in which small arterial blood samples (20 µl) were obtained every 10 min throughout the experiment for the measurement of glucose, insulin, NEFA, and plasma glucose. This provided feedback that was used to adjust glucose infusion rates needed to maintain glycemia. After the equilibration period, rats received an intravenous bolus of [²-H]DG and [¹²⁵I]-BMIPP for the measurement of glucose and LCFA uptakes, respectively (t = 95 min). The time period from 90 to 120 min is referred to as the “experimental period” in the results section. At time 0 and 90 min, large arterial blood samples (150 µl) were withdrawn for the measurement of insulin, glucose, and NEFA. In addition, small samples (50 µl) were obtained at 97, 100, 110, 120, 130, 140, and 150 min for the measurement of [²-H]DG, [¹²⁵I]-BMIPP, NEFA, and insulin. To prevent declines in hematocrit, the erythrocytes taken before the isotopic analog infusion were washed in saline and reinfused shortly after each sample was taken. At t = 150 min, rats were anesthetized with pentobarbital sodium, and their soleus (89% type I, 11% type IIa, 0% type IIb) (24), gastrocnemius (6% type I, 23% type IIa, 71% type IIb) (24), and superficial vastus lateralis (SVL) (0% type I, 18% type IIa, 90% type IIb) (24) were excised and rapidly freeze-clamped in liquid nitrogen.

**Plasma analyses**

Metabolites. Plasma glucose concentrations were measured by the glucose oxidase method using an automated glucose analyzer (Beckman Instruments, Fullerton, CA), and immunoreactive insulin was measured in samples obtained at t = 0, 90, 120, and 150 min using a double-antibody method (25). NEFA concentrations were measured at t = 0, 90, 110, 120, and 150 min spectrophotometrically using a kit obtained from Wako Chemicals (NEFA-C, Richmond, VA) in the saline, AICAR, L-NAME, and AICAR + L-NAME groups. In the AICAR + Intralipid group, administration of heparin would have resulted in increased in vitro lipolysis due to the presence of lipoprotein lipase. As such, samples used for NEFA analysis in this group were treated with 5 mol/l NaCl as previously described (26,27) before analysis.

Isotopic analogs. [¹²⁵I]-BMIPP and [²-H]DG were measured in the same plasma samples (20 µl) as previously described (28). Briefly, 20 µl plasma was counted for [¹²⁵I]-BMIPP using a Beckman Gamma 5500 counter (Beckman, Fullerton, CA). After this, the plasma sample was deproteinated in 100 µl of Ba(OH)₂ and 100 µl of ZnSO₄ and subsequently centrifuged. Supernatants (100 µl) were then diluted in 900 µl of H₂O. [³H]radioactivity was counted after addition of flour (10 ml; Ultimate Gold, Packard Bioscience, Boston, MA) using a Packard Tri-Carb 2900TR Liquid Scintillation Analyzer (PerkinElmer, Boston, MA). The relationship between γ-radioactivity and β-emissions has been established in our laboratory for that specific counter. This relationship was used to correct [³H]radioactivity for β-emissions originating from [¹²⁵I] radioactivity (28).

Tissue analyses

**Isotopic analogs.** Tissues were analyzed for accumulation of free ([²-H]DG) and phosphorylated deoxy-[²-H]glucose ([²-H]DGDP) as previously described (29). Briefly, muscle [¹²⁵I]-BMIPP and [²-H]DG were determined on 50 µg of soleus and 100 µg of gastrocnemius and SVL. After the determination of tissue [¹²⁵I] radioactivity, tissue was homogenized in 2 ml of 0.5% perchloric acid and centrifuged for 20 min. Supernatants (1.5 ml) were then neutralized with 5 mmol/l KOH, and 250 µl was counted after the addition of flour. This fraction provided total radioactive [²-H]DG (free + phosphorylated [²-H]DG). In addition, 500 µl of supernatant was treated with 250 µl of Ba(OH)₂ and 250 µl of ZnSO₄ and centrifuged. After this, 500 µl of supernatant was diluted to 1 ml in distilled H₂O before flour (10 µl) was added and samples were counted. Treatment of Ba(OH)₂ and ZnSO₄ removed all but free [²-H]DG (30). Therefore, [²-H]DG is calculated as the difference in radioactivity without (total) and with (free [²-H]DG) Ba(OH)₂ and ZnSO₄. This analytical approach allows the separation of [²-H]DG from the fraction of [²-H]DG that is incorporated into glycogen, inclusion of which results in the underestimation of its release in response to agonist activation (31,32). Sample [³H]-specific counts were corrected for β-emissions originating from [¹²⁵I] radioactivity (28).

**Immunoblotting.** To determine the effects of AICAR on the phosphorylation of AMPK, acetyl-CoA carboxylase (ACC), and NOS, gastrocnemius muscles were homogenized in a solution containing 10% glycerol, 20 mmol/l Na-pyrophosphate, 150 mmol/l NaCl, 50 mmol/l Hepes (pH 7.5), 1% NP-40, 20 mmol/l NaF, 2 mmol/l EDTA, 2 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l CaCl₂, 1 mmol/l MgCl₂, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 2 mmol/l NaVO₄, and 3 mmol/l benzamidine. After

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**AMPK, NO, AND METABOLIC REGULATION**

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centrifugation (1 h at 4,500g) the pellets were discarded while supernatants retained for protein determination using a Pierce BCA protein assay kit (Rockford, IL). Proteins (30 μg) were separated on a SDS-PAGE gel and then transferred to a polyvinylidene fluoride membrane. Membranes were blotted with rabbit phospho-AMPK α (Thr172), phosphor-acetyl CoA carboxylase (Ser79), or phospho–endothelial NOS (Ser1177) (1:1,000) (Cell Signaling, Beverly, MA) and then incubated with a rabbit–horseradish peroxidase (Pierce, Rockford, IL). Densitometry was performed using Lab Image software (Kaplan).

Calculations. Indexes of glucose and LCFA uptake and clearance: tissue glucose clearance (Kg) and metabolic (Rg) indexes were calculated from the accumulation of [2-3H]DGP in muscle and the integral of the plasma [2-3H]DG concentration after a [2-3H]DG bolus (33,34). The relationships are defined as:

$$K_g = \frac{([2-3H]DGP)(t)}{\int ([2-3H]DG)dt}$$

$$R_g = K_g \times (G)_{pl}$$

where the subscript “p” refers to mean arterial plasma from t = 90 to 120 min. The measurement of Kg has been described earlier (33,34). In an analogous manner, LCFA clearance (Kf) and metabolic (Rf) indexes were calculated after a [125I]BMIPP bolus from the accumulation of [125I]BMIPP in muscle and the integral of the plasma [125I]BMIPP concentration after the bolus.

$$K_f = \frac{([125I]BMIPP)(t)}{\int ([125I]BMIPP)dt}$$

$$R_f = K_f \times [LCFA]_{pl}$$

[125I]BMIPPPpl is the [125I]BMIPP in the cell, [125I]BMIPPPpl is the [125I]BMIPP in plasma. The measurement of Rf and Kg has been described earlier (28,35).

Statistical analyses. To compare differences, ANOVA was performed. To establish differences within the ANOVA, a Student’s Newman Keuls post hoc test was used. Significance levels of P > 0.05 were used, and data are reported as means ± SEM.

RESULTS

Animal characteristics. Preexperimental values for animal weight, arterial plasma glucose, insulin, and NEFA concentrations are reported in Table 1. Rats in the AICAR + L-NAME group were lighter compared with the AICAR group with mean values of 281 ± 7 g vs. 317 ± 10 g. This was not due to a treatment effect but rather lower presurgery weights. No differences were apparent between groups for plasma glucose, insulin, and NEFA. Hematocrit values were not different between groups before (44 ± 0.4%, t = 0) or after (42 ± 0.7%, t = 150 min) the experimental protocol.

Glucose, glucose infusion rates, insulin, and NEFA. Mean arterial plasma values during the experimental period (t = 90–120 min) are reported in Table 2. No differences in plasma glucose were apparent between groups with time or treatment. In addition, no differences were seen for glucose infusion rates between AICAR, AICAR + L-NAME, and AICAR + Intralipid. Plasma insulin levels were not significantly different among the saliné, AICAR, L-NAME, and AICAR + L-NAME groups during the experimental period. In AICAR + Intralipid, plasma insulin levels were higher compared with all other groups. This was not unexpected, as previous studies have shown Intralipid infusion to increase plasma insulin levels due to a decrease in insulin clearance (36). Plasma NEFAs in the AICAR and AICAR + L-NAME groups were significantly lower at all time points in the experimental period compared with baseline values (t = 0). In contrast, NEFAs were stable, and no differences were apparent with time or treatment for saline and L-NAME. In AICAR + Intralipid, baseline NEFAs (t = 0) were lower compared with 150 min; however, no differences were present among saline, L-NAME, AICAR + Intralipid treatments. NEFA values were lower in AICAR and AICAR + L-NAME compared with saline, L-NAME, and AICAR + Intralipid in the experimental period at all time points measured.

Table 1

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Baseline characteristics</th>
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<tbody>
<tr>
<td>n</td>
<td>Weight (g)</td>
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<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>Saline</td>
<td>7</td>
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<tr>
<td>AICAR</td>
<td>7</td>
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<tr>
<td>L-NAME</td>
<td>7</td>
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<td>AICAR + L-NAME</td>
<td>7</td>
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<td>AICAR + LIPID</td>
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Data are means ± SEM. *Significant difference (P < 0.05) from AICAR.

Table 2

<table>
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<th>TABLE 2</th>
<th>Arterial plasma glucose, insulin, NEFA, and glucose infusion rates during the experimental period</th>
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<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>Insulin (μU/ml)</td>
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<tr>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Saline</td>
<td>6.2 ± 0.2</td>
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<tr>
<td>AICAR</td>
<td>6.9 ± 0.2</td>
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<tr>
<td>L-NAME</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>AICAR + L-NAME</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>AICAR + LIPID</td>
<td>6.9 ± 0.2</td>
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</table>

Data are means ± SEM. Data with different symbols within a measurement indicate a significant difference (P < 0.05).
uptake in the gastrocnemius was not as dependent on NO activation as the soleus and SVL. AICAR/H11001 Intralipid had a significantly greater \( K_f \) and \( R_f \) than AICAR in gastrocnemius, but not in soleus and SVL.

**Immunoblotting.** The effects of AICAR on AMPK phosphorylation (AMPK Thr\(^{172} \)), ACC phosphorylation (ACC Ser\(^{79} \)), and endothelial NOS phosphorylation (NOS Ser\(^{1177} \)) in gastrocnemius muscle are shown in Fig. 5. AMPK Thr\(^{172} \) and ACC Ser\(^{79} \) phosphorylation was increased in all groups treated with AICAR, demonstrating that the dosage (10 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) min\(^{-1} \)) activated AMPK. NOS Ser\(^{1177} \) phosphorylation was increased in AICAR but not AICAR/NAME, L-NAME, or AICAR/H11001 Intralipid compared with saline.

**Muscle specificity.** As previously mentioned, AICAR resulted in increases in \( K_g \) and \( K_f \) in all muscles compared with saline (Figs. 2 and 4). When percentage increases in AICAR-induced \( K_g \) and \( K_f \) were compared between muscle fiber types, results showed AICAR-induced glucose uptake to be greatest in fast-twitch muscles, while AICAR-induced LCFA uptake was greater in muscles comprised of slow-twitch muscle fibers.

**DISCUSSION**

The primary objectives of the present study were to determine the fiber type–specific effects of AMPK activation on muscle LCFA and glucose uptake in vivo and the NO dependence of the observed increases. Using isotopic analogs to measure indexes of glucose and LCFA uptake in the presence or absence of the NO synthase inhibitor L-NAME and the AMPK activator AICAR, we found that AMPK activation increased glucose and LCFA clearance. Only muscle glucose uptake was inhibited by L-NAME. This demonstrates that the mechanism by which AMPK increases glucose uptake is NO dependent, while AMPK-induced LCFA uptake occurs independent of this pathway. In addition, we demonstrate that in vivo, the magnitude of the AICAR activation of both glucose and LCFA uptake is fiber-type specific. Soleus that has the greatest percentage of slow-twitch fibers exhibits the greatest increase in LCFA uptake and the smallest increase in glucose uptake.

The finding that L-NAME impedes AMPK-induced glucose uptake is consistent with the hypothesis that NO may act as signal that couples tissue energy demands to glucose delivery. This has been clearly demonstrated for both insulin- and contraction-stimulated glucose uptake. Studies using hyperinsulinemic-euglycemic clamps have shown L-NAME to reduce whole-body glucose disposal by \( 15 \% \)–\( 30 \% \) (14,17). Similarly, moderate exercise in humans in the presence of L-NMMA reduces skeletal muscle glu-

**FIG. 1.** Index of glucose uptake (\( R_g \)) in the soleus (A), gastrocnemius (B), and SVL (C). AICAR + LIPID represents treatment with AICAR + Intralipid. Different letters above bars indicate significant differences between treatments (\( P < 0.05 \)). All data are presented as means \( \pm \) SEM.

**FIG. 2.** Glucose clearance (\( K_g \)) for soleus (A), gastrocnemius (B), and SVL (C). AICAR + LIPID represents treatment with AICAR + Intralipid. Different letters above bars indicate significant differences between treatments (\( P < 0.05 \)). All data are presented as means \( \pm \) SEM.
cose uptake by ~30% in normal subjects and ~75% in subjects with type 2 diabetes (15). Taken together, these data imply that a percentage of both insulin- and exercise-stimulated glucose uptake can be attributed to NO. Since L-NAME reduced basal and AICAR-stimulated glucose uptake with no effect on fatty acid uptake, the mechanism by which NO regulates glucose uptake must be to some extent distinct from that of LCFA. Indeed, there is in vitro evidence that NO directly regulates glucose transport. Isolated muscle preparations treated with SNP have elevated glucose transport and GLUT-4 translocation via a phosphatidylinositol 3-kinase–independent mechanism (13,37,38). This is thought to occur by an NO-activated guanosine 3’-5’-cyclic monophosphate (cGMP) pathway, although the exact signaling pathway of cGMP-induced glucose trafficking remains to be established (39). Alternative NO targets that could potentially influence glucose uptake include creatine kinase, cytochrome-c oxidase, sacroplasmic reticulum Ca^2+ ATPase, and glyceraldehyde-3-phosphate (39).

To assess the activation of AMPK and its effects on NOS, immunoblotting for AMK Thr^{172}, ACC Ser^{79}, and NOS Ser^{1177} was performed on gastrocnemius muscle. Results demonstrated that the AICAR dose (10 mg · kg^{-1} · min^{-1}) was sufficient to activate both AMK Thr^{172} and ACC Ser^{79} activity. However, results of NOS Ser^{1177} are not as clearly defined. Corroborating previous findings, we show that AMPK stimulation increases NOS Ser^{1177} compared with saline (40,41). However, no increase in NOS Ser^{1177} was observed in either the AICAR + L-NAME or the AICAR + Intralipid groups. This finding reflects the complex nature of NOS regulation. Numerous kinases, including protein kinase G, protein kinase A, protein kinase B (Akt), and/or mitogen-activated protein kinases are known to act at this site (40,42–46). In addition, AMPK can also phosphorylate an alternate inhibitory site on the NOS protein (Thr^{495}) (40). Therefore, a lack of effect of AICAR on NOS Ser^{1177} in these groups may reflect phosphorylation of ACC Thr^{495} or alterations in the ability of AMPK to phosphorylate this site in the presence of L-NAME or Intralipid.

In the present study, lack of effect of L-NAME on basal and AMPK-stimulated LCFA clearance indicates that the coupling of LCFA delivery to uptake is not dependent on NO and must, therefore, be mediated by an alternative signaling intermediate. The finding that AICAR-induced increases in $K_f$ were sustained, even in the presence of L-NAME, was unexpected considering that NO-dependent vascular perfusion was likely to be considerably reduced. To ensure that the increase in $K_f$ was not caused by...
zyme that deactivates malonyl-CoA, malonyl-CoA decarboxylase, and also phosphorylates and activates the enzyme ACC, the rate-limiting enzyme in malonyl-CoA synthesis, and also inhibits ACC, the rate-limiting enzyme in malonyl-CoA synthesis, and also inhibits ACC, the rate-limiting enzyme in malonyl-CoA synthesis, and also inhibits ACC, the rate-limiting enzyme in malonyl-CoA synthesis, and also inhibits ACC, the rate-limiting enzyme in malonyl-CoA synthesis, and also inhibits ACC, the rate-limiting enzyme in malonyl-CoA synthesis, and also inhibits ACC, the rate-limiting enzyme in malonyl-CoA

In addition to determining the NO dependence of AMPK’s actions, this study demonstrates that both AMPK-induced glucose and LCFA uptake are dependent on muscle fiber-type composition. Our results demonstrate that muscles comprised of a greater percentage of fast-twitch fibers are more responsive to AMPK-stimulated glucose uptake than muscles comprised of more slow-twitch fibers. This finding is consistent with previous in vitro studies showing AMPK protein content and AMPK activity to be greater in fast-twitch versus slow-twitch muscle fibers (50,51). As previously mentioned, AICAR treatment also increased LCFA clearance in a muscle-specific manner, although the fiber-type dependency was opposite that seen for glucose. The effect of AICAR on LCFA uptake was actually greater in muscle with lower AMPK activity. LCFA clearance was greatest in muscle comprised of slow-twitch fibers compared with fast-twitch fibers, a finding that is likely explained by increased LCFA transporter number and LCFA oxidative capacity of slow-twitch muscle (52).

In summary, we found that AMPK activation simultaneously increased glucose and LCFA clearance in vivo. Only muscle glucose uptake, however, was inhibited by L-NAME. This demonstrates that the mechanism by which AMPK increases glucose uptake is NO dependent, while AMPK-induced LCFA uptake occurs independent of this pathway in the majority of muscles studied. In addition, the magnitude of AICAR activation of both glucose and LCFA uptake in vivo is fiber-type specific. Soleus, which has the greatest percentage of slow-twitch fibers, exhibits the greatest increase in LCFA uptake and the smallest increase in glucose uptake. Taken together, our findings provide insight into the signaling mechanisms by which AMPK results in increased glucose and LCFA uptake in skeletal muscle.

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