Potentiation of insulin-stimulated glucose transport by the AMP-activated protein kinase

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Ju, Jeong-Sun, Michael A. Gitcho, Carter A. Casmaer, Pankaj B. Patil, Dae-Gyue Han, Susan A. Spencer, and Jonathan S. Fisher. Potentiation of insulin-stimulated glucose transport by the AMP-activated protein kinase. Am J Physiol Cell Physiol 292: C564–C572, 2007. First published July 26, 2006; doi:10.1152/ajpcell.00269.2006.—Data from the use of activators and inhibitors of the AMP-activated protein kinase (AMPK) suggest that AMPK increases sensitivity of glucose transport to stimulation by insulin in muscle cells. We assayed insulin action after adenoviral (Ad) transduction of constitutively active (CA; a truncated form of AMPKα) and dominant-negative (DN; which depletes endogenous AMPKα) forms of AMPKα (Ad-AMPKα-CA and Ad-AMPKα-DN, respectively) into C2C12 myotubes. Compared with control (Ad-green fluorescent protein, Ad-GFP), Ad-AMPK-CA increased the ability of insulin to stimulate glucose transport. The increased insulin action in cells expressing AMPK-CA was suppressed by compound C (an AMPK inhibitor). Exposure of cells to 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (an AMPK activator) increased insulin action in uninfected myotubes and myotubes transduced with green fluorescent protein but not in Ad-AMPK-DN-infected myotubes. In Ad-AMPK-CA-transduced cells, serine phosphorylation of insulin receptor substrate 1 was decreased at a mammalian target of rapamycin (or p70 S6 kinase) target site that has been reported to be associated with insulin resistance. These data suggest that, in myotubes, activated AMPKα is sufficient to increase insulin action and that the presence of functional AMPKα is required for 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside-related increases in insulin action.

compound C; AMPK; insulin sensitivity; Akt; mTOR

FOR NEARLY A DECADE, there has been intense scrutiny of the role of the AMP-activated protein kinase (AMPK) in the insulin-independent stimulation of glucose transport in skeletal muscle (7, 13, 24, 32). In contrast, there has been relatively little examination of the possibility that AMPK activation acutely affects insulin responsiveness (glucose transport stimulated by a maximally effective concentration of insulin) in isolated skeletal muscle (26, 38). However, whether insulin sensitivity was decreased (i.e., a rightward shift in the insulin dose-response curve, with an increase in the insulin concentration required to achieve a given level of glucose transport) in AMPKα-deficient muscle or cells has not been addressed. The distinction between insulin responsiveness and insulin sensitivity in regard to potential effects of AMPK is important, because exercise, which has been suggested to act through AMPK to increase insulin sensitivity (5), does not necessarily affect insulin responsiveness (9, 10). Additionally, to determine whether AMPK knockout or depletion has effects on insulin sensitivity, it would be necessary to assess insulin sensitivity after an AMPK-activating treatment.

Recent studies have demonstrated a link between the AMPK and mammalian target of rapamycin (mTOR) signaling pathways (20, 22, 29, 37). For example, incubation with AICAR inhibits p70 S6 kinase (S6K, an mTOR effector) activity in mammalian cells (21). Once activated, AMPK phosphorylates tuberous sclerosis complex 2, leading to suppression of mTOR/S6K signaling (17, 18). When activated, mTOR/S6K represses insulin receptor substrate (IRS)-1-related signaling via a transcriptional repression of IRS-1 gene expression mediated by S6K and direct phosphorylation of IRS-1 protein by mTOR or S6K (14).

We hypothesized that AMPK regulates insulin action in C2C12 myotubes. We assayed insulin-stimulated glucose transport after use of adenovirus (Ad)-mediated gene transfer to express constitutively active (CA) and dominant-negative (DN) forms of AMPKα (Ad-AMPKα-CA and Ad-AMPKα-DN, respectively) or green fluorescent protein (Ad-GFP) as a control. In addition, we examined whether the regulation of insulin signaling by AMPK was associated with mTOR signaling.

METHODS

Materials. A polyclonal antibody specific for the GLUT4 glucose transporter was the generous gift of Dr. Mike Mueckler (Washington University). Horseradish peroxidase-conjugated goat anti-rabbit IgG was purchased from Pierce Biotechnology (Rockford, IL). An antibody against GAPDH was obtained from Novus Biologicals (Littleton, CO). Isoform-specific AMPKα antibodies were purchased from Upstate (Charlottesville, VA). Antibodies against phosphorylated (Ser79), acetyl-CoA carboxylase (ACC), mTOR, phosphorylated (Thr389), p70 S6K, p70 S6K, phosphorylated (Ser447 and Ser451) IRS-1, IRS-1, phosphorylated (Ser637 and Thr648) Akt, Akt, phosphorylated AMPK, AMPK, and myc epitope were purchased from Cell Signaling.

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Technology (Danvers, MA). Phosphospecificity of antibodies was determined by the manufacturer (Cell Signaling Technology) with use of induction systems (e.g., treatment with insulin) and by treatment of samples with calf intestinal phosphatase for phosphorylated AMPK, phosphorylated (Thr^308) Akt, and phosphorylated (Ser^638/639) IRS-1. Treatment of samples with λ-phosphatase (for phosphorylated ACC), or use of an Akt S473A mutant, which is not detectable with the antibody against phosphorylated (Ser^778) Akt. 2-Deoxy-D-[1,2-^3H (N)]glucose (2-DG) was purchased from American Radiolabeled Chemicals (St. Louis, MO), purified porcine insulin from Eli Lilly (Indianapolis, IN), and AICAR from Toronto Research Chemicals (North York, ON, Canada). Compound C was generously provided by Merck (Rahway, NJ). All other reagents were obtained from Sigma Chemical (St. Louis, MO).

Cell culture and adenovirus infection. The mouse myoblast cell line C2C12 (American Type Culture Collection, Rockville, MD) was maintained at 37°C in 5% CO₂ in low-glucose DMEM (Washington University School of Medicine Tissue Culture Support Center, St. Louis, MO) supplemented with 10% fetal bovine serum, 50 μg/ml penicillin, and 50 μg/ml streptomycin. Cultures were induced to differentiate in DMEM containing 2% horse serum. The AMPKA-CA adenoviral vector (a generous gift from Dr. Kenneth Walsh, Boston University School of Medicine, Boston, MA) was constructed from a rat cDNA encoding residues 1-312 of AMPKα1 and bearing a mutation of Thr^172 into aspartic acid (T172D), as described previously (39). The AMPK-DN adenoviral vector (constructed by Morris Birnbaum, University of Pennsylvania, Philadelphia, PA) was constructed from AMPKα2 bearing a mutation of Lys^45 to arginine (K45R), as described previously (26, 27, 41). Adenoviruses were amplified in 293 cells and purified using cesium chloride ultracentrifugation. The experiments were performed 2 days after infection.

2-DG transport. Glucose uptake into C2C12 myotubes was measured using radiolabeled 2-DG, as described previously (35). Before glucose transport assays, cells were washed twice with HEPES-buffered saline. For assay of glucose transport, cells were incubated with HEPES-buffered saline containing radiolabeled 2-DG (3 μCi/ml) and unlabeled 10 μM 2-DG at room temperature. If cells had previously been exposed to insulin (see below), insulin was included in the glucose uptake assay medium. After 10 min, 2-DG uptake was terminated by three quick washes with ice-cold 0.9% saline. Cells were lysed in 0.2 N NaOH containing 0.2% SDS, and then 1H was counted by a liquid scintillation spectrophotometer. Data are presented as picomoles of 2-DG per milligram protein per minute.

To determine the effect of Ad-AMPK-CA on 2-DG transport, on the day of the experiment, myotubes were serum starved for 3 h and then incubated for 20 min with 0, 10, or 100 nM insulin before 2-DG transport assays (see above). In our hands, 10 nM is near the threshold insulin concentration for stimulation of glucose transport in C2C12 myotubes (35, 36). To examine the effects of Ad-AMPK-CA and compound C [an AMPK inhibitor (40)] on glucose uptake, myotubes were preincubated for 3 h in the absence or presence of 20 μM compound C in serum-free DMEM. Glucose transport assays were performed after a 20-min preincubation of myotubes in the absence or presence of 10 or 100 nM insulin. Compound C was intentionally omitted from the glucose transport medium, with the expectation that 3 h of incubation with compound C (not the presence of compound C during glucose transport) would be sufficient to prevent the potentiating effect of AMPK-CA on insulin-stimulated glucose transport. Compound C was excluded from the rinses before glucose transport or during glucose transport assays to prevent inhibition of the insulin-independent effects of AMPK-CA on glucose transport.

To assess the effects of Ad-AMPK-DN expression and AICAR stimulation on glucose uptake, myotubes (uninfected or transduced with GFP or AMPK-DN) were pretreated with 0.5 mM AICAR for 1 h in medium containing 2% horse serum. Then myotubes were allowed to recover for 4 h in serum-free medium. After recovery incubations, myotubes were preincubated in the absence or presence of 10 nM insulin. Then 2-DG uptake was measured in myotubes (see above). AICAR was deliberately excluded from the recovery medium after the 1-h incubation with AICAR to allow the immediate effects of AICAR to decrease over the recovery period. We based this model of AICAR treatment followed by recovery before exposure to insulin on knowledge about exercise-induced increases in insulin action. The insulin-sensitizing effect of exercise is robust a few hours after exercise (5, 10, 25), but not necessarily during or immediately after exercise (28).

Immunofluorescence and confocal microscopy. C2C12 cells plated on glass coverslips were fixed with 4% paraformaldehyde for 10 min at room temperature. Then the cells were permeabilized with 0.1% Triton X-100-PBS for 7 min at room temperature. After nonspecific binding sites were blocked in 3% BSA-PBS for 1 h at room temperature, the cells were incubated with GLUT4 antibody at a 1:100 dilution in buffer A (PBS containing 0.5% Tween 20 and 1% BSA) for 1 h at room temperature. After they were washed with PBS, the cells were incubated with Cy3-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at a 1:200 dilution in buffer A for 1 h at room temperature. Subsequently, the cells were extensively washed with buffer A and mounted onto glass slides. The specimens were examined by confocal microscopy (LSM 5 Pascal, Carl Zeiss, Jena, Germany) for fluorescence of GFP and/or Cy3.

Western blot analysis. C2C12 cells grown on six-well plates were washed with ice-cold PBS and then scraped into homogenizing buffer containing 50 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EDTA, 10 mM Na₃PO₄, 100 mM NaF, 2 mM Na₂VO₃, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. Lysates were centrifuged at 14,000 rpm for 10 min. Soluble proteins were quantitated using the bicinchoninic acid kit (Pierce Chemical, Rockford, IL) with BSA as a standard and adjusted to 1 μg/μl. Aliquots of homogenate were solubilized in Laemmli sample buffer, and 50 μg of protein were subjected to SDS-PAGE (10% resolving gel). Proteins were transferred to nitrocellulose membranes (Hybond-ECL, Amersham Biosciences). Membranes were blocked in a solution of Tris-buffered saline containing 5% nonfat dry milk. The myc epitope was detected by incubation of blots with a 1:5,000 dilution of myc-tagged antibody, and followed by horseradish peroxidase-conjugated anti-mouse IgG. Protein was visualized using enhanced chemiluminescence. Western blots for phosphorylated (Ser^792) ACC, phosphorylated (Thr^389) p70 S6K, phosphorylated (Ser^638/639) IRS-1, IRS-1, phophorylated (Ser^778) and (Thr^308) Akt, and Akt were performed by a method analogous to that described above. GAPDH content of myotubes was also determined. GLUT4 content was detected by incubation of blots with a 1:5,000 dilution of rabbit polyclonal antiserum raised against the COOH terminus of GLUT4 followed by horseradish peroxidase-conjugated anti-rabbit IgG.

Assay of endogenous AMPKα. Because DN-AMPK and endogenous AMPKα migrated to overlapping positions on our minigels, we performed an immunodepletion of DN-AMPK (a myc-tagged construct) before assessing the levels of remaining (i.e., endogenous) AMPKα1 and AMPKα2. Antibodies against the myc tag (~14 μg sample) were incubated with protein A-Sepharose, washed several times with PBS, and then incubated with myotube lysates. Beads were pelleted by centrifugation, and residual AMPKα and myc-tagged proteins in immunodepleted supernatants were assessed by Western blot analysis. For the Western blot against the myc tag, a nonimmunodepleted sample from Ad-AMPK-DN cells (loaded with the same amount of protein as immunodepleted samples) was used as a positive control.
Analyses were performed by ANOVA followed by Fisher’s least significant difference post hoc comparisons only when ANOVAs yielded $P < 0.05$. For Western blots of AMPK, phosphorylated ACC, mTOR, S6K, phosphorylated S6K, IRS-1, phosphorylated IRS-1, Akt, and phosphorylated Akt, there were 16 samples per group. For GLUT4 Western blots, there were 12 samples per group.

**RESULTS**

Expression of Ad-GFP, AD-AMPK-CA, and AD-AMPK-DN in C2C12 myotubes. Confocal microscopic examination demonstrated that control Ad-GFP was uniformly expressed within 48 h after incubation of C2C12 myotubes with the adenoviral...
vector (Fig. 1A). Western blot analysis of lysates from myotubes expressing 
myc-tagged Ad-AMPK-CA and Ad-AMPK-DN (Fig. 1B), with anti-
myc-tagged antibody used as a
probe, showed bands at ~30 kDa (Ad-AMPK-CA, a truncated
form of AMPK) and ~65 kDa (Ad-AMPK-DN). Expression
of AMPK protein was determined by immunoblotting with
AMPK antibody (Fig. 1B). Endogenous AMPK and Ad-
AMPK-DN were detected at ~60 kDa, and there was also a
band at ~30 kDa from cells transduced with Ad-AMPK-CA.
After immunodepletion of myc-tagged AMPKα, it was possible
to assess the amount of endogenous AMPKα: AMPKα2 was not present in myotubes transduced with Ad-AMPK-DN, and AMPKα1 content was drastically reduced (Fig. 1C). It appeared that the immunoprecipitation completely immuno-
depleted myc-tagged protein from the samples, because no
AMPK2 (the myc-tagged construct) remained in immuno-
depleted samples and there was no signal against the myc tag for
immunodepleted samples in Western blots (Fig. 1C). There
was no difference in AMPK phosphorylation between unin-
fected and infected myotubes (Fig. 1D). (Molecular sizes in
Figs. 1 and 5–8 represent the expected molecular sizes of the
products, not the molecular sizes of specific molecular size
markers.) All proteins shown in blots ran to positions corre-
sponding to their known sizes.

Infection with Ad-AMPK-CA resulted in a 70% increase of
phosphorylation of an AMPK target, ACC (Fig. 1E). Phos-
phorylation of ACC was reduced in Ad-AMPK-DN-infected
cells.

In summary, these data demonstrate that AMPKα-CA and
AMPKα-DN were expressed and caused the expected changes
in ACC phosphorylation.

**Regulation of insulin action by AMPK.** Expression of Ad-
AMPK-CA increased 2-DG uptake by ~50% compared with
the Ad-GFP controls (Fig. 2). Expression of Ad-AMPK-CA

Fig. 2. Effect of Ad-AMPK-CA expression on glucose [2-deoxyglucose (2-
DG)] uptake in C2C12 myotubes transduced with Ad-GFP and Ad-AMPK-CA
(100 pfu/cell, 2 days) in the absence or presence of 10 or 100 nM insulin (INS).
Values are means ± SE; n = 6–12/group. *, **, and ***P < 0.05 vs.
corresponding Ad-GFP control. †P < 0.05; ‡P < 0.001 vs. corresponding
control without insulin.

Fig. 3. Effects of Ad-AMPK-DN expression and
5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR) on glucose uptake. Cells not infected
with virus or cells infected with Ad-GFP or Ad-AMPK-DN were incubated with or without 0.5 mM
AICAR for 1 h. After 4 h of recovery, 2-DG uptake
was assayed in the absence or presence of 10 nM
insulin. Values are means ± SE; n = 6/group. *P <
0.05 vs. no virus and no virus + insulin. **P < 0.05
vs. no virus, no virus + insulin, and no virus +
AICAR. ***P < 0.05 vs. Ad-GFP. ****P < 0.05 vs.
Ad-GFP + insulin. #P < 0.05 vs. Ad-GFP + AICAR.
##P < 0.05 vs. Ad-GFP + AICAR + insulin. &P <
0.05 vs. Ad-GFP + AICAR.
resulted in an ~60% increase of glucose transport in the presence of 10 nM insulin and an ~70% increase in the presence of 100 nM insulin. Thus it appears that the presence of active AMPK is sufficient to increase insulin action.

One hour of treatment with AICAR (followed by 4-h recovery) had a permissive effect on stimulation of glucose transport by insulin (10 nM) in uninfected myotubes and myotubes expressing Ad-GFP, but not in the Ad-AMPK-DN group (Fig. 3), such that prior AICAR treatment and exposure to insulin tended to have greater than additive effects on glucose transport.

The increase in insulin action caused by expression of Ad-AMPK-CA was suppressed after 3 h of treatment with compound C (Fig. 4), an AMPK inhibitor (40). In contrast, compound C did not affect insulin action in cells transduced with Ad-GFP (Fig. 4B). Glucose transport was increased by 10 and 100 nM insulin in myotubes expressing AMPK-CA (Fig. 4A). In contrast, 100 nM (but not 10 nM) insulin increased glucose transport in cells transduced with Ad-GFP.

**Phosphorylation of S6K, IRS-1, and Akt.** In the Ad-AMPK-DN-transduced C2C12 myotubes, S6K phosphorylation at Thr389 (an mTOR target site) was significantly elevated by 63% ($P < 0.05$) compared with C2C12 cells transfected with Ad-GFP (Fig. 5). Ad-AMPK-DN and Ad-AMPK-CA did not affect mTOR and S6K expression (Fig. 5A). Myotube content of GAPDH was not different among groups infected with the different viruses (Fig. 5C). These data suggest a posttranslational role for AMPK in mTOR regulation and are consistent with the previously described role of AMPK as a negative regulator of mTOR (20).

Transfection with Ad-AMPK-CA decreased IRS-1 phosphorylation at Ser636/639 (Fig. 6), the mTOR and/or S6K target site that is associated with insulin resistance (22, 29, 37). In contrast, transfection with Ad-AMPK-DN caused a 3.5-fold increase in IRS-1 phosphorylation at Ser636/639 (normalized to IRS-1 content) compared with Ad-GFP-transfected C2C12 cells. IRS-1 protein expression was reduced in cells transduced with Ad-AMPK-DN, although expression of AMPK-CA did not significantly increase IRS-1 protein content.

Compared with Ad-GFP controls, there was a twofold increase in Akt phosphorylation at Thr308 (Fig. 7) in Ad-AMPK-CA.
CA-transfected cells and a 65% decrease in Akt phosphorylation at Thr\(^{308}\) in Ad-AMPK-DN-transfected cells. Phosphorylation of Akt at Ser\(^{473}\) was decreased by \(\frac{1}{2}\)Akt in Ad-AMPK-DN-transfected C2C12 myotubes vs. the control group, although phosphorylation of Akt at Ser\(^{473}\) was unaltered by transduction with Ad-AMPK-CA.

AMPK-related changes in glucose transport are not paralleled by changes in GLUT4 content. Subcellular localization of GLUT4 was evaluated by confocal fluorescence microscopy. GLUT4 displayed punctate, intracellular localization in myotubes transfected with Ad-GFP (Fig. 8A) and Ad-AMPK-DN (Fig. 8C). In contrast, localization of GLUT4 was apparent at the plasma membrane in cells expressing AMPK-CA. Western blotting showed that levels of GLUT4 did not differ among the three groups (Fig. 8, D and E). This suggests that AMPK is involved in potentiation of insulin action, even in the absence of changes in GLUT4 content in these C2C12 myotubes. Thus the impact of AMPK-CA and AMPK-DN on glucose transport shown in Fig. 2–4 is likely to consist of posttranslational signaling events (which is consistent with the short time course for potentiation of insulin action by AICAR) or the compound C-related suppression of insulin action in cells expressing AMPK-CA), rather than effects mediated by changes in gene expression.

**DISCUSSION**

In the present study, we used adenoviral-mediated expression of AMPK-CA and AMPK-DN mutant forms to investigate the role of AMPK in regulating insulin-stimulated glucose uptake by C\(_2\)C\(_12\) myotubes. Consistent with previous suggestive findings (5, 16, 35), it appears that AMPK activation positively regulates insulin action. Our findings indicate that activated AMPK\(_{\alpha}\) (which is known to be activated by multiple models of cellular stress, including muscle contractions, AICAR, and mitochondrial uncoupling (8, 19)) was sufficient to increase insulin action. Additionally, incubation with an AMPK inhibitor suppressed the increased insulin action associated with expression of AMPK-CA. Furthermore, an inactive form of AMPK that depleted endogenous AMPK\(_{\alpha}\) prevented potentiation of insulin action by AICAR.

AICAR is not necessarily specific for activation of AMPK, because it activates AMPK after the intracellular conversion of AICAR to ZMP, an AMP analog. Thus any pathway that is affected by AMP could theoretically also be affected by AICAR. Although reported actions of compound C are consistent with inhibition of AMPK (see discussion in Ref. 35), selectivity of compound C has only been screened against a...
GLUT4 protein expression. Values are means ± SE; n = 12 in each group.

Though the effect is not dependent on protein synthesis (5). Similarly, insulin action is not increased immediately after incubation of myotubes with hyperosmotic medium (an AMPK-activating treatment) but, rather, is apparent after a few hours of recovery after incubation in hyperosmotic medium (35).

The present data complement and extend previous findings from studies involving animals. For example, AICAR increases insulin-stimulated glucose transport in rat skeletal muscle (5, 16), suggesting a possible role for AMPK in regulation of insulin action. On the other hand, insulin responsiveness (with ~2 mU/ml insulin) of isolated skeletal muscle is not dampened in mice expressing AMPK-CA in skeletal muscle (which completely depletes endogenous active AMPK) or in whole body AMPKα2-knockout mice (38). Additionally, insulin sensitivity (60 μU/ml insulin) and responsiveness (2 mU/ml insulin) were normal in a rested AMPKγ-knockout mouse; insulin responsiveness (~2 mU/ml insulin) was the same after exercise for the AMPKγ-knockout and wild-type animals (1, 2). However, if, as has been suggested, AMPK is responsible for exercise-related increases in insulin action, it may (similar to exercise) have effects on insulin sensitivity, but not necessarily on insulin responsiveness (9, 10) [although exercise sometimes also increases effects of extremely high insulin concentrations (31)].

In our hands, compound C did not reduce insulin stimulation of glucose transport. Thus it appears that although activation of AMPK potentiates insulin action, inhibition of basal AMPK activity does not reduce insulin action. This is analogous to the role of AMPK in stimulation of insulin-independent glucose transport: although activation of AMPK increases glucose transport and decreases basal AMPK activity (as a result of AMPKα knockout or inactive forms of AMPKα) reportedly does not decrease basal glucose transport (8, 19, 26).

It is important to the interpretation of the increased insulin action in myotubes transduced with AMPK-CA that GLUT4 content of myotubes was not altered by AMPK-CA. In this regard, the increased insulin action related to AMPK-CA is similar to exercise-related increases in insulin sensitivity that occur before GLUT4 has increased after exercise (12) and even when postexercise protein synthesis is inhibited (5). Long-term (5 days) expression of constitutively active AMPK in myotubes reportedly increases GLUT4 (6), so perhaps AMPK-CA was not expressed long enough in our short-term study (2 days) for GLUT4 to be increased. Interestingly, there is reportedly no alteration in GLUT4 content in mice expressing AMPK-CA (the same construct used in the present study) in skeletal muscle (26). Similarly, GLUT4 expression is not dampened in muscles of AMPKα1- or AMPKα2-knockout mice or in mice expressing inactive AMPKα (8, 19).

Recent studies suggest a linkage between AMPK and insulin signaling pathways and the possibility that the mTOR/S6K pathway may play an important role in regulation of insulin signaling (20). The present data showing a role for AMPK in regulation of serine phosphorylation of IRS-1 (at an mTOR or S6K target site) are consistent with the postulated role for AMPK in acute regulation of insulin action (5, 16). mTOR exists in two distinct complexes: one that contains mTOR, GβL, and raptor (23) and another that contains mTOR, GβL, and rictor (23, 33). The raptor-containing complex is sensitive to the drug rapamycin and phosphorylates S6K (3), and the
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ritor-containing complex does not appear to be sensitive to rapamycin (33). It has been demonstrated that mTOR-rictor directly phosphorylates Akt on Ser473; mTOR-riceptor is the Akt Ser473 kinase that is associated with insulin signaling in adipocytes, and a reduction in rictor or mTOR expression inhibits Akt (15, 34). Although there is an absence of data in regard to regulation of mTOR-riceptor, it is tempting to hypothesize that activation of AMPK could have differential effects on mTOR-riceptor (an Akt Ser473 kinase) and mTOR-riaptor (which mediates serine phosphorylation of IRS-1).

It has been known for about 20 years that exercise increases insulin sensitivity in skeletal muscle in rats (31), and similar findings have just been reported for mice (11). In humans, insulin action is increased a few hours after exercise (25) and remains elevated for up to 2 days (25, 30). On the basis of the present findings, it seems possible that activation of AMPK contributes to at least part of the increase in insulin action after exercise, especially the acute increase in insulin sensitivity (25) that occurs even in the absence of protein synthesis (5).

In summary, the data presented here suggest that, in myotubes, AMPK regulates insulin action and also suggest that changes in insulin action caused by AMPK in myotubes may occur through decreased serine phosphorylation of IRS-1 by mTOR/S6K. These findings suggest that similar studies should be undertaken to determine whether activation of AMPK affects insulin action in animal muscle.

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