AICAR and hyperosmotic stress increase insulin-stimulated glucose transport

Jill L. Smith,* Pankaj B. Patil,*, and Jonathan S. Fisher

Department of Biology, Saint Louis University, St. Louis, Missouri
Submitted 17 November 2004; accepted in final form 27 April 2005

Smith, Jill L., Pankaj B. Patil, and Jonathan S. Fisher. AICAR and hyperosmotic stress increase insulin-stimulated glucose transport. J Appl Physiol 99: 877–883, 2005. First published April 28, 2005; doi:10.1152/japplphysiol.01297.2004.—Sensitivity of glucose transport to stimulation by insulin has been shown to occur concomitant with activation of the AMP-activated protein kinase (AMPK) in skeletal muscle, suggesting a role of AMPK in regulation of insulin action. The purpose of the present study was to evaluate a possible role of AMPK in potentiation of insulin action in muscle cells. The experimental model involved insulin-responsive C2C12 myotubes that exhibit a twofold increase in glucose transport in the presence of insulin. Treatment of myotubes with the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), followed by a 2-h recovery, augmented the ability of insulin to stimulate glucose transport. Similarly, incubation in hyperosmotic medium, another AMPK-activating treatment, acted synergistically with insulin to stimulate glucose transport. Furthermore, the increase in insulin action caused by hyperosmotic stress was prevented by inclusion of compound C, an AMPK inhibitor, in hyperosmotic medium. In addition, iodotubercidin, a general kinase inhibitor that is effective against AMPK, also prevented the combined effects of insulin and hyperosmotic stress on glucose transport. The new information provided by these data is that previously reported AICAR effects on insulin action are generalizable to myotubes, hyperosmotic stress and AMPK appears to mediate potentiation of insulin action.

indinavir; adenosine 5′-monophosphate-activated protein kinase; acetyl coenzyme A carboxylase; C2C12 myotubes; wortmannin

MUSCLE CONTRACTIONS, exercise, treatment with the adenosine analog 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), and exposure to hypoxic conditions all increase the sensitivity of skeletal muscle glucose transport to stimulation by insulin (2, 7, 11, 16, 22, 36). A factor shared by all of these insulin-sensitizing treatments is that they activate AMPK. We further hypothesized that pretreatment of cells with AICAR or hyperosmotic stress and injection of AICAR would increase insulin-stimulated glucose transport concomitantly with increased phosphorylation of AMPK. We further hypothesized that inhibition of AMPK would prevent development of insulin sensitivity after exposure of cells to hyperosmotic stress. We selected the hyperosmotic stress treatment, because it, unlike AICAR incubation, is a suitable model for use with the AMPK inhibitor compound C (9).

MATERIALS AND METHODS

Materials. FBS and antibiotic/antimycotic solution (10,000 U penicillin/ml, 10 mg streptomycin/ml, 0.025 mg fungizone/ml) were from Gemini Bio-Products (Woodland, CA). Cell culture dishes were from Fisher Scientific (Hanover Park, IL). Purified porcine insulin was from Eli Lilly (Indianapolis, IN). 3H-labeled 2-deoxyglucose (2DG) was from American Radiolabeled Chemicals (St. Louis, MO). Sorbitol, wortmannin, and cytochalasin B were obtained from Sigma Chemical (St. Louis, MO), and AICAR was purchased from Toronto Research Chemicals (North York, ON). Indinavir and compound C were generously provided by Merck (Rahway, NJ). Iodotubercidin was purchased from EMD Biosciences (La Jolla, CA). Antibodies against AMPK, phosphorylated AMPK (pAMPK), and phosphorylated acetyl-CoA carboxylase (pACC) were from Cell Signaling Technology (Beverly, MA). The pAMPK antibody is directed toward Thr172 of the catalytic subunit of AMPK, and phosphorylation of AMPK at this site activates the kinase (5, 14). Antibodies against β-tubulin that were used to demonstrate equal loading among lanes for pACC blots were from Zymed Laboratories (San Francisco, CA). C2C12 culture. Mouse C2C12 myoblasts were purchased from the American Type Culture Collection (Rockville, MD). Cells were cultured (39) in 100-mm polystyrene culture dishes in a α-MEM supplemented with 10% FBS and 1% antibiotic antimycotic mixture and passaged by trypsinization using 0.25% trypsin. Cells were maintained in a humidified incubator under an atmosphere of 5% CO2 at 37°C. Fresh medium was supplied every 24 h. For differentiation of myoblasts into myotubes, cells were seeded ~2 × 10^4 cells/cm2 in 24-well plates for 2DG uptake assays and in 6-well or 12-well plates for Western blotting. Differentiating cells were fed every 4 h for 3 days with α-MEM containing 2% horse serum, 1% antibiotic antimycotic mixture, and 10-7 M insulin. Thereafter, differentiation continued with α-MEM containing 2% horse serum and 1% antibiotic antimycotic mixture (but no insulin) for the next 3 days before experiments were conducted. Insulin was included in the initial differentiation medium, because it has been reported that insulin stimulates myogenesis in L6 and C2C12 myoblasts (26, 41). In our

* J. L. Smith and P. B. Patil contributed equally to this work.

Address for reprint requests and other correspondence: J. S. Fisher, Dept. of Biology, Saint Louis Univ., 3507 Laclede Ave., St. Louis, MO 63103 (E-mail: fisherjs@slu.edu).

DOI: 10.1152/japplphysiol.01297.2004

http://www.jap.org
8750-7587/05 $8.00 Copyright © 2005 the American Physiological Society

877

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
hands, C2C12 myotubes that have been differentiated in media lacking insulin are not insulin responsive.

**Effects of wortmannin and indinavir on insulin-stimulated glucose transport.** For initial experiments, myotubes were serum starved for 12 h before glucose transport assays. 3H-labeled 2DG uptake was measured in myotubes as described by Somwar et al. (39). Timelines for experimental protocols are included in Figs. 1–6. Incubations were performed in HEPES-buffered saline (HBS) solution (20 mM HEPES, pH 7.4, 140 mM NaCl, 5 mM KCl, 2.5 mM MgSO4, 1 mM CaCl2) containing 5 mM glucose (40). In some experiments, cells were incubated for 20 min in HBS with 100 nM wortmannin, a phosphatidylinositol-3-kinase (PI3K) inhibitor, in 0.1% DMSO or with vehicle before a 20-min exposure to 0 or 100 nM insulin. Glucose transport assays in the absence or presence of 100 nM insulin were performed as described below. Some cells were incubated for 10 min with 100 μM indinavir (37) after initial exposure to insulin but before glucose transport assays were performed. Indinavir is a human immunodeficiency virus (HIV) protease inhibitor that also inhibits glucose transport through GLUT4 and thus is a tool to assess the functional contribution of GLUT4 to insulin-dependent glucose influx (28, 29, 37).

**Determination of 2DG transport rates.** Before glucose transport assays, cells were rinsed twice with glucose-free HBS. Following the rinses, cells were incubated at room temperature for 10 min in 200 μl of glucose-free HBS containing 1H-labeled 2DG (3 μCi/ml), 10 μM 2DG, and insulin or inhibitors if they had been present for the previous steps. Nonspecific 2DG uptake was determined by quantitation of cell-associated radioactivity in the presence of 10 μM cytochalasin B, which blocks transporter-mediated glucose uptake. After incubation with the transport medium, the medium was aspirated rapidly, and the cells were immediately washed three times with ice-cold 0.9% saline before lysis in 0.2 N NaOH containing 0.2% SDS. Aliquots of cell lysates were saved for the determination of protein content by the bicinchoninic acid assay (Pierce, Rockford, IL). Samples were neutralized and then were counted for 1H in a Packard TopCount liquid scintillation plate reader (PerkinElmer, Boston, MA).

**Insulin action after treatment with AICAR.** For insulin sensitivity experiments, myotubes were deliberately not serum starved, because serum starvation itself is a means of increasing insulin sensitivity of myotubes (24). Additionally, 2% horse serum was present during AICAR treatments, because it has been found that the presence of serum during AICAR incubations, hypoxia, or muscle contractions is essential to the development of insulin sensitivity (7, 11). In some experiments, cells were pretreated with 2 mM AICAR for 1 h before they were allowed to recover for 2 h in medium containing 2% horse serum. In a second set of experiments, cells were allowed to recover in serum-free medium after AICAR treatments. After the recovery incubations, glucose transport assays in the presence of 0, 10, or 100 nM insulin were performed as described above.

**Insulin action after exposure to hyperosmotic stress.** To assess the effects of hyperosmotic stress on insulin action, myotubes were incubated for 1 h in standard medium containing 2% horse serum (as described above) or the same medium made hyperosmotic by the addition of 0.6 M sorbitol. Incubation of mouse myotubes in hyperosmotic media has been shown to activate AMPK (9). After the 1-h incubation, cells were allowed to recover for 2 h in serum-free medium before glucose transport assays in the absence or presence of 10 nM insulin, as described above. For cells exposed to insulin after incubation in normal or hyperosmotic medium, inhibition of insulin-stimulated glucose transport by indinavir was determined, as described above.

To examine a role for AMPK in mediation of the increased insulin action after hyperosmotic stress, some myotubes were incubated with 20 μM compound C before, during, and after incubation in hyperosmotic media. Compound C is a fairly specific inhibitor of AMPK (3, 9, 45). Parallel experiments were performed with 50 μM iotubercidin, which has been demonstrated to inhibit AMPK in vitro (15) and prevent activation of AMPK by cyanide in cardiac myocytes (38).

**Immediate effects of hyperosmotic medium on glucose transport.** Myotubes were serum starved for 12 h before incubation in the absence or presence of 0.6 M sorbitol in serum-free α-MEM for 30 min. Myotubes (±0.6 M sorbitol) were then immediately preincubated for 20 min in the absence or presence of 10 nM insulin before glucose transport assays (±0.6 M sorbitol, ±insulin), as described above. Additional glucose transport assays (±0.6 M sorbitol, ±insulin) were performed without serum starvation and for which all media contained 2% horse serum.

**Western blot analysis.** Myotubes were incubated for 30 min in the absence or presence of 2 mM AICAR or 600 mM sorbitol. Additional myotubes were incubated for 30 min in the absence or presence of kinase inhibitors (20 μM compound C or 10 μM iotubercidin) and then for 30 min in medium ±kinase inhibitors and ±0.6 M sorbitol. Cells were rinsed with HBS, scraped in ice-cold buffer containing phosphatase and protease inhibitors (50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EDTA, 10 mM sodium pyrophosphate, 100 mM NaF, 2 mM Na2VO4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.5 μg/ml pepstatin, and 2 mM PMSF), homogenized in Kontes ground glass tubes, and centrifuged for 10 min at 14,000 g. Supernatants were subjected to Western blot analysis of AMPK, pAMPK, pACC, or β-tubulin (to demonstrate equal loading among lanes for pACC blots).

**Statistical analysis.** One- or two-way analyses of variance were performed. Post hoc comparisons were performed with Fisher’s protected least significance difference tests. For Western blot data, t-tests were used to compare values for sorbitol- and AICAR-treated cells with values for control cells. A level of P < 0.05 was set for significance for all tests, and all values are expressed as means ± SE. Statistics were performed with SPSS (Chicago, IL) software, version 10.0.

**RESULTS**

**Insulin-stimulated glucose uptake in C2C12 myotubes.** Insulin (100 nM) increased glucose uptake up to twofold in C2C12 myotubes (Fig. 1). This insulin-stimulated increase in glucose transport was likely to be mediated by GLUT4 (Fig. 1A), as demonstrated by prevention of insulin action in myotubes in the presence of indinavir, a HIV protease inhibitor that also prevents glucose transport mediated by GLUT4, but not GLUT1 (37). As shown in Fig. 1B, wortmannin prevented stimulation of glucose transport by insulin, suggesting that the increase in 2DG uptake is mediated by PI3K activation. These data are important in establishing that, under the culture conditions described, C2C12 myotubes are insulin responsive, and the increased glucose transport stimulated by insulin appears to be dependent on GLUT4. Others have reported that insulin does not stimulate glucose transport in C2C12 myotubes and that C2C12 myotubes might not be an appropriate model for GLUT4-mediated glucose uptake (42). However, these issues do not appear to be applicable under the culture conditions of the present study.

**Insulin action after pretreatment with AICAR.** Figure 2A shows that treatment with AICAR followed by a 2-h recovery in the presence of serum increased glucose transport in the presence of 10 nM insulin (P < 0.05). Also shown in Fig. 2A, AICAR pretreatment increased glucose transport in the presence of 100 nM insulin by about threefold (P < 0.05) over myotubes that were treated with AICAR but not insulin. AICAR pretreatment by itself raised the rate of 2DG uptake above baseline by ~35%. Figure 2B shows that AICAR...
pretreatment followed by a 2-h recovery in serum-free media and exposure to 10 nM insulin increased glucose transport >100% above basal transport ($P < 0.05$), despite no separate effect of 10 nM insulin on glucose transport.

**Fig. 1.** Insulin-stimulated glucose transport in C2C12 myotubes is dependent on GLUT4 and phosphatidylinositol 3-kinase. 

---

**A**

- Indinavir
- + Indinavir

2 DG Transport, pmol/min

0 5 10 15 20 25 30 35 40

Control 100 nM Insulin

**B**

- Wortmannin
- + Wortmannin

2 DG Transport, pmol/min

0 5 10 15 20 25 30 35

Control 100 nM Insulin

---

**Fig. 2.** 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) increases insulin sensitivity. Myotubes were incubated for 1 h in incubation media containing 2% horse serum and in the absence or presence of 2 mM AICAR. 

---

**A**

- AICAR
- + AICAR

2DG transport

0 10 20 30 40 50 60

60 min 2 h recovery serum present

20 min glucose transport

Control 10 nM Insulin 100 nM Insulin

---

**B**

- AICAR
- + AICAR

2DG transport

0 5 10 15 20 25 30

60 min 2 h recovery serum absent

20 min glucose transport

Control 10 nM Insulin

---

Insulin action after incubation in hyperosmotic medium. Hyperosmotic stress (Fig. 3) caused by inclusion of 0.6 M sorbitol in the incubation medium acted synergistically with 10 nM insulin to promote glucose transport ($P < 0.001$). There were no separate (i.e., unrelated to synergistic action) effects of either 10 nM insulin or pretreatment in hyperosmotic medium on glucose transport. The stimulation of glucose transport caused by 10 nM insulin after treatment with hyperosmotic medium was abolished by 20 µM compound C, an AMPK inhibitor, suggesting that AMPK plays a role in potentiation of insulin action. Indinavir prevented the synergistic effects of
AMPK POTENTIATES INSULIN ACTION


ingested to AMPK) was approximately two- to threefold greater compared with control (Fig. 5A). AMPK content of cells did not differ by treatment (data not shown). As shown in Fig. 5A, hyperosmotic stress caused an increase in phosphorylation of the AMPK target ACC, and this effect was prevented by compound C. Similarly, iodotubercidin prevented an increase in ACC phosphorylation for cells incubated in hyperosmotic media (Fig. 5C).

Immediate effects of hyperosmotic medium on glucose transport. Glucose transport was stimulated when cells were in hyperosmotic medium (Fig. 6), as has previously been described for myotubes (8). However, insulin action was not increased in hyperosmotic medium. The lack of immediate (i.e., in the absence of a recovery period) additive and synergistic effects of hyperosmotic stress on insulin action is not surprising, as insulin action has previously been reported to be impaired during hyperosmotic stress (13). Data shown in Fig. 5 are for experiments with serum-free medium. Results for media containing 2% horse serum were not qualitatively different (data not shown).

DISCUSSION

The new information provided by this study is that cultured myotubes appear to be an appropriate model for the study of AICAR-related insulin sensitivity, hyperosmotic stress acts synergistically with insulin to promote glucose transport, and inhibition of AMPK prevents potentiation of insulin action after exposure of myotubes to hyperosmotic stress.

As reported by Richter et al. (36) and Garetto et al. (12), exercise causes sensitivity of glucose transport to stimulation by insulin in skeletal muscle. It has been demonstrated in rat skeletal muscle that contractions, hypoxia, and treatment with AICAR increase insulin sensitivity and increase AMPK activity, suggesting that an increase in insulin sensitivity of muscle glucose transport is mediated by activation of AMPK (7, 16).

AMPK is activated by AICAR following uptake of AICAR into skeletal muscle and conversion of AICAR to the AMP analog AICAR 5′-monophosphate (ZMP) (4). However, ZMP effects cannot be specifically attributed to activation of AMPK, as opposed to regulation of some other AMP-sensitive pathway. In the present study, we have used compound C and iodotubercidin to provide evidence for involvement of AMPK in potentiation of insulin action by hyperosmotic stress. The hyperosmotic stress model provides valuable information that cannot be obtained from the use of compound C or iodotubercidin.

Increased glucose transport in response to insulin action is reduced by treatment with indinavir, a human immunodeficiency virus protease inhibitor that also prevents GLUT4-mediated glucose transport. Values are means ± SE. *Significantly different from all other groups, \( P < 0.001 \).
cidin with AICAR treatments. For example, in addition to their inhibition of AMPK, compound C prevents AICAR uptake into cells (9), and iodo-tubercidin prevents both adenosine uptake and adenosine phosphorylation (33). Either compound would thus prevent activation by AICAR of any signaling pathways regulated by ZMP. The hyperosmotic stress model would not be subject to such nonspecificity of compound C or iodo-tubercidin action (9). However, comparison of AICAR and hyperosmotic stress treatments could provide mechanistic information important for future studies. For example, AICAR increases insulin action to a greater extent than hyperosmotic stress. This might be attributable by the different means of AMPK activation by AICAR (through phosphorylation and allosterically) and hyperosmotic stress (through phosphorylation but without allosteric activation) (6, 8–10, 34).

Compound C is competitive with ATP at the AMPK catalytic site and does not inhibit kinases related to AMPK, including Syk, PKC-θ, PKA, or JAK3 (45). Compound C does not inhibit p38-α or p38-β (9), and it reportedly does not interfere with activity of LKB1 (3), the upstream kinase of AMPK. However, compound C does prevent cellular actions of LKB1, which is consistent with inhibition of AMPK by the compound (3). Although there appears to be no information on the specificity of compound C against the two isoforms of the catalytic subunit of AMPK, compound C blocks AMPK actions (including phosphorylation of ACC) under conditions that activate AMPK in a variety of cell types (3, 21, 25, 44). Actions of compound C are consistent with the effects of transfection of cells with dominant-negative forms of either AMPK-α1 or AMPK-α2 (17, 23, 25). While the specificity of compound C for AMPK inhibition has not been well characterized, and iodo-tubercidin inhibits other kinases (27) in addition to AMPK (15), prevention of hyperosmotic stress-related...
insulin sensitivity by these two unrelated compounds provides
evidence for involvement of AMPK.

As described above, incubation of rat skeletal muscle with
AICAR in the presence of serum increases the ability of insulin
to stimulate glucose transport (7). In an attempt to replicate this
finding for human myotubes, Al-Khalili et al. (1) treated differ-
entiated primary muscle cells in the absence or presence of 10 and
100% serum and 2 mM AICAR followed by glucose transport
assays in the presence of 0, 1.2, and 18 nM insulin. They found
that AICAR-treated cells displayed increased phosphorylation
of AMPK and its substrate ACC. On the other hand, regardless
of the insulin concentrations used, AICAR did not cause a
significant increase in insulin-stimulated glucose transport. The
authors (1) suggested the possibility that low levels of GLUT4
in the human myotubes could have limited the ability of
AICAR to potentiate glucose transport (1). The present study
describes culture conditions under which AICAR-related insu-
lin sensitivity is apparent in C2C12 myotubes, and it seems
possible that the culture conditions could be applicable to the
study of AMPK-related potentiation of insulin action in other
muscle cell lines or in primary muscle cells.

AMPK has been identified as a potential modulator of insulin
signaling through phosphorylation of insulin receptor substrate
1 (IRS-1) on Ser789 (18). It has been demonstrated that treat-
ment with AICAR rapidly leads to phosphorylation of IRS-1
on Ser789 in C2C12 myotubes, and this phosphorylation is
linked to a 65% increase in insulin-stimulated IRS-1-associated
PI3K activity (18). Even though the effects of AICAR on
insulin-stimulated glucose transport were not assessed in that
study (18), the results illustrate an interaction between AMPK
and early insulin signaling that may underlie the effects of
AMPK activation on insulin action.

In the present study, prevention of insulin-stimulated glu-
cose transport in the C2C12 myotubes in the presence of the
wortmannin, a PI3K inhibitor (40), suggests that insulin stimu-
lation of glucose transport in these cells involves classical
insulin-signaling pathways. Furthermore, the insulin-stimu-
lated increase in glucose transport was prevented by indinavir,
A protease inhibitor that also blocks glucose transport
mediated by GLUT4 (29, 37). This suggests that, in the C2C12
myotubes in this study, GLUT4 (and not some other trans-
porter, such as GLUT1) is responsible for insulin-stimulated
increases in glucose uptake. It has been previously reported
that insulin does not stimulate glucose transport in C2C12
myotubes and that GLUT4 appears unresponsive to insulin
(42). However, in the present study, C2C12 myotubes are
responsive to insulin in what appears to be a GLUT4-depen-
dent fashion.

In conclusion, we have shown that AICAR and hyperos-
motic stress increase insulin action in myotubes concomitant
with phosphorylation of AMPK. Through the use of the AMPK
inhibitor compound C, we have demonstrated involvement
of AMPK in potentiation of insulin action by hyperosmotic stress,
and we have presented a cultured myotube model suitable for
characterization of potential pathways that contribute to sensi-
tivity of glucose transport to stimulation by insulin.

ACKNOWLEDGMENTS

We thank Dr. Philip Bilan (The Hospital for Sick Children, Toronto, ON)
and Dr. Barrie Bode (Saint Louis University) for advice concerning cell growth
and glucose transport assay procedures.

J Appl Physiol • VOL 99 • SEPTEMBER 2005 • www.jap.org

REFERENCES

1. Al-Khalili L, Krook A, Zierath JR, and Cartee GD. Prior serum and
AICAR-induced AMPK activation in primary human myocytes does not
lead to subsequent increase in insulin-stimulated glucose uptake. Am J

2. Cartee GD, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson
H, and Holloszy JO. Prolonged increase in insulin-stimulated glucose
transport in muscle after exercise. Am J Physiol Endocrinol Metab 256:

3. Corradetti MN, Inoki K, Bardeesy N, DePinho RA, and Guan KL.
Regulation of the TSC pathway by LKB1: evidence of a molecular link
between tuberous sclerosis complex and Peutz-Jeghers syndrome. Genes

4. Corton JM, Gillespie JG, Hawley SA, and Hardie DG. 5-Aminoiwim-
dazole-4-carboxamide ribonucleoside. A specific method for activating

5. Crute BE, Seeffeld K, Gamble J, Kemp BE, and Witters LA. Functional
domains of the α catalytic subunit of the AMP-activated protein kinase.

6. Davies SP, Helps NR, Cohen PT, and Hardie DG. 5'-AMP inhibits
dephosphorylation, as well as promoting phosphorylation, of the AMP-
activated protein kinase. Studies using bacterially expressed human pro-
tein phosphatase-2C alpha and native bovine protein phosphatase-2AC.

7. Fisher JS, Guo J, Han DH, Holloszy JO, and Nolte LA. Activation of
AMP kinase enhances sensitivity of muscle glucose transport to insulin.

D. Characterization of the role of the AMP-activated protein kinase
in the stimulation of glucose transport in skeletal muscle cells. Biochem J

9. Fryer LG, Parbu-Patel A, and Carling D. Protein kinase inhibitors
block the stimulation of the AMP-activated protein kinase by 5-aminour-

rosiglitazone and metformin stimulate AMP-activated protein kinase
through distinct signaling pathways. J Biol Chem 277: 25226–25232,
2002.

11. Gao J, Gulye EA, and Holloszy JO. Contraction-induced increase in
muscle insulin sensitivity: requirement for a serum factor. Am J Physiol

muscle glucose metabolism after exercise in the rat: the two phases. Am J

13. Gual P, Marchand-Brustel Y, and Tanti J. Positive and negative regula-
tion of glucose uptake by hyperosmotic stress. Diabetes Metab 29:

14. Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D,
and Hardie DG. Characterization of the AMP-activated protein kinase
kinase from rat liver and identification of threonine 172 as the major site at
which it phosphorylates AMP-activated protein kinase. J Biol Chem

15. Henin N, Vincent MF, and Van den Bergh G. Stimulation of rat liver
AMP-activated protein kinase by AMP analogues. Biochim Biophys Acta

16. Iglesias MA, Ye JM, Frangioudakis G, Saha AK, Tomas E, Ruderman
NB, Cooney GJ, and Kraegen EW. AICAR administration causes an
apparent enhancement of muscle and liver insulin action in insulin-

17. Inoki K, Zhu T, and Guan KL. TSC2 mediates cellular energy response

characterization of potential pathways that contribute to sensitivity
of glucose transport to insulin action by AMPK.

This work was funded by National Institute of Diabetes and Digestive and
Kidney Diseases Grant K01 DK-66330.


