

# Isoform-Specific Regulation of 5' AMP-Activated Protein Kinase in Skeletal Muscle From Obese Zucker (*fa/fa*) Rats in Response to Contraction

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Glucose transport can be activated in skeletal muscle in response to insulin via activation of phosphoinositide (PI) 3-kinase and in response to contractions or hypoxia, presumably via activation of 5' AMP-activated protein kinase (AMPK). We determined the effects of insulin and muscle contraction/hypoxia on PI 3-kinase, AMPK, and glucose transport activity in epitrochlearis skeletal muscle from insulin-resistant Zucker (*fa/fa*) rats. Insulin-stimulated glucose transport in isolated skeletal muscle was reduced 47% in obese versus lean rats, with a parallel 42% reduction in tyrosine-associated PI 3-kinase activity. Contraction and hypoxia elicited normal responses for glucose transport in skeletal muscle from insulin-resistant obese rats. Isoform-specific AMPK activity was measured in skeletal muscle in response to insulin, contraction, or hypoxia. Contraction increased AMPK $\alpha$ 1 activity 2.3-fold in lean rats, whereas no effect was noted in obese rats. Hypoxia increased AMPK $\alpha$ 1 activity to a similar extent (more than sixfold) in lean and obese rats. Regardless of genotype, contraction, and hypoxia, each increased AMPK $\alpha$ 2 activity more than fivefold, whereas insulin did not alter either AMPK $\alpha$ 1 or  $\alpha$ 2 activity in skeletal muscle. In conclusion, obesity-related insulin resistance is associated with an isoform-specific impairment in AMPK $\alpha$ 1 in response to contraction. However, this impairment does not appear to affect contraction-stimulated glucose transport. Activation of AMPK $\alpha$ 2 in response to muscle contraction/exercise is associated with a parallel and normal increase in glucose transport in insulin-resistant skeletal muscle. *Diabetes* 51: 2703–2708, 2002

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AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; DTT, dithiothreitol; KHBB, Krebs-Henseleit bicarbonate buffer; PI, phosphoinositide; PMSF, phenylmethylsulfonyl fluoride.

**D**uring recent years, major advances have been made in the understanding of the molecular mechanisms of insulin action (1), bringing the goal of identifying defects in peripheral tissues that lead to insulin resistance closer. In people with type 2 diabetes, impaired whole-body insulin-mediated glucose uptake is associated with defects in glucose transport in skeletal muscle (2–6). This most likely occurs from defects in postreceptor insulin signal transduction at the level of insulin receptor substrate-1 and phosphoinositide (PI) 3-kinase (6–9) as well as defects in trafficking and/or function of GLUT4 (10,11), the major insulin-regulated GLUT isoform expressed in skeletal muscle (12). In skeletal muscle from insulin-resistant diabetic rodents, glucose transport defects are not observed in response to contraction, a stimulus known to increase glucose transport by an insulin-independent mechanism (13–15). This implies that a greater understanding of mechanisms involved in the regulation of insulin-independent glucose transport may lead to the identification of molecular targets for pharmacological therapy to improve glucose homeostasis. Activation of this insulin-independent pathway may circumvent defects in insulin action on glucose transport that are observed in skeletal muscle from people with type 2 diabetes.

AMP-activated protein kinase (AMPK) has emerged as one potential therapeutic molecular target that plays a role in mediating insulin-independent glucose transport (16–18). Much of the evidence supporting the role of AMPK in the regulation of glucose transport comes from studies using 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), a cell-permeable activator of AMPK. Treatment of rat skeletal muscle with AICAR is associated with parallel increases in AMPK activity and glucose transport via a PI 3-kinase-independent pathway (19,20). Consistent with this, activation of AMPK in response to multiple stress stimuli, including hypoxia, osmotic shock, and mitochondrial uncoupling, is correlated with a parallel increase in glucose transport activity in skeletal muscle (21). Direct evidence for a role of AMPK in the regulation of glucose transport in skeletal muscle has emerged from studies in transgenic mice (17). Transgenic overexpression of a dominant-inhibitory mutant of AMPK in skeletal muscle completely blocked the ability of hypoxia or AICAR to activate glucose uptake while only partially

reducing contraction-stimulated glucose uptake (17). Furthermore, adenoviral-mediated expression of a dominant-negative form of the catalytic ( $\alpha$ ) subunit of AMPK in a skeletal muscle cell line blocked the stimulation of glucose transport by both AICAR and hyperosmotic stress, but was without effect on either insulin- or phorbol-ester-stimulated transport (18). Thus, AMPK-dependent and -independent pathways contribute to the regulation of glucose uptake in skeletal muscle.

Deficiencies in the AMPK signaling pathway have been proposed to result in insulin insensitivity and type 2 diabetes (22) and obesity (23). Activation of AMPK by AICAR may be efficacious in the regulation of glucose transport in skeletal muscle from obese insulin-resistant Zucker rats or *ob/ob* mice (24,25). To fully validate AMPK as a target for pharmacological intervention to improve glucose homeostasis in obese insulin-resistant or type 2 diabetic subjects, additional studies are warranted. For example, isoform-specific responses of AMPK to more common metabolic stressors have not been fully established in skeletal muscle from animal models of type 2 diabetes. Furthermore, because contraction and hypoxia recruit AMPK-dependent and -independent pathways in the regulation of glucose transport (17), it is not clear whether either of these stimuli elicit a normal response toward AMPK in insulin-resistant models, where it would be advantageous to increase glucose uptake by insulin-independent mechanisms. Thus, the aim of this study was to examine the effects of contraction or hypoxia on isoform-specific activation of AMPK in skeletal muscle from Zucker (*fa/fa*) rats, an obese animal model of insulin resistance.

## RESEARCH DESIGN AND METHODS

**Animals.** Obese male Zucker (*fa/fa*) rats (aged 11–12 weeks) and lean littermates were purchased from Charles River (Uppsala, Sweden) and housed at the animal facility at the Karolinska Institute for 4 weeks before use. Rats were maintained on a 12-h light-dark cycle and given free access to standard rodent chow and water. All studies we performed on rats in the overnight-fasted state. The regional animal ethical committee approved all experimental procedures.

**Glucose tolerance test.** Glucose (2 g/kg body wt) was administered by intraperitoneal injection. Blood samples were obtained via the tail vein before and 15, 30, 60, and 120 min after glucose injection. Blood glucose levels were measured using a One Touch Basic glucose meter (Lifescan, Milpitas, CA).

**Muscle incubations.** All incubation media were prepared from a stock solution of Krebs-Henseleit bicarbonate buffer (KHBB) supplemented with 5 mmol/l HEPES and 0.1% BSA (radioimmunoassay grade). Rats were anesthetized with sodium pentobarbital (60 mg/kg body wt), and epitrochlearis muscles were carefully isolated for *in vitro* incubation. The viability of the incubated muscle preparation from large rats has been previously established by measuring high-energy phosphate levels of epitrochlearis muscle from adult rats that are of similar weight to the animals used in our study (26). Muscles were preincubated at 30°C for 40 min in KHBB containing 5 mmol/l glucose and 15 mmol/l mannitol, without (basal) or with insulin (60 nmol/l) for either 6 min (PI 3-kinase activity) or 40 min (glucose transport activity). Unless otherwise stated, all media were continuously gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>. After preincubation, muscles were immediately frozen between aluminum tongs cooled to the temperature of liquid nitrogen or further incubated to assess glucose transport activity.

**In vitro muscle contraction.** Epitrochlearis muscles were placed in a temperature-controlled (30°C) incubation chamber and immersed in 4 ml preincubation media without or with insulin as described above. Muscles were positioned between two platinum electrodes. The distal tendon was mounted to the bottom of the incubation chamber. The proximal tendon was connected to an isometric force transducer (Harvard Apparatus, South Natick, MA), and resting tension was adjusted to 0.5 g. Electrical stimulation (contraction group only) was applied during the final 10 min of the 40-min preincubation period. Basal and insulin-stimulated muscles were treated as

described above minus the application of electrical stimulation. Muscle contraction was induced via electrical stimulation. Muscles were stimulated at 100 Hz (0.2-ms pulse duration, 20 V), at a rate of one 0.2-s contraction every 2 s for 10 min, as previously described (27). Muscles were frozen immediately for AMPK or PI 3-kinase activity measurements or further incubated for the assessment of glucose transport activity.

**Hypoxia incubations.** Epitrochlearis muscles were preincubated at 30°C for 80 min in KHBB containing 5 mmol/l glucose and 15 mmol/l mannitol, under a gas phase of 95% O<sub>2</sub>/5% CO<sub>2</sub> (basal) or 95% N<sub>2</sub>/5% CO<sub>2</sub> (hypoxia) (28). Muscles were frozen immediately for AMPK activity or further incubated for the assessment of glucose transport activity.

**Glucose transport activity.** After preincubation, muscles were incubated at 30°C for 10 min in glucose-free KHBB containing 20 mmol/l mannitol. When effects of hypoxia were studied, muscles were incubated for 15 min in KHBB containing 2 mmol/l pyruvate and 18 mmol/l mannitol under a gas phase of 95% O<sub>2</sub>/5% CO<sub>2</sub>. Thereafter, muscles were transferred to vials containing 1 mmol/l 2-deoxy-[1,2-<sup>3</sup>H]glucose (2.5  $\mu$ Ci/ml) and 19 mmol/l [<sup>14</sup>C]mannitol (0.7  $\mu$ Ci/ml) without or with insulin and incubated for 15 min. The rate of 2-deoxyglucose uptake is linear between 5 and 120 min in rat epitrochlearis muscle (29). Glucose transport activity is expressed as micromoles per milliliter of intracellular water per hour (29).

**AMPK activity.** Muscles were homogenized in ice-cold lysis buffer containing 50 mmol/l Tris-HCl (pH 7.5), 1 mmol/l EDTA, 1 mmol/l diethiothreitol (DTT), 10% (vol/vol) glycerol, 50 mmol/l NaF, 5 mmol/l Na pyrophosphate, 1 mmol/l benzamide, 0.1 mmol/l phenylmethylsulfonyl fluoride (PMSF), and 1% (vol/vol) Triton X-100. Muscle homogenates were subjected to centrifugation at 14,000g for 10 min at 4°C. Supernatants were removed and used for determination of protein content using a commercially available kit based on the Bradford method (Bio-Rad, Hercules, CA). Aliquots (400  $\mu$ g protein) were sequentially immunoprecipitated for 2 h at 4°C with sheep AMPK $\alpha$ 1 and - $\alpha$ 2 antibodies precoupled to protein G-Agarose (5 mg/sample). The antibodies were raised against peptides predicted from the rat AMPK $\alpha$ 1 and - $\alpha$ 2 amino acid sequences, as previously described (30). Immunoprecipitates were washed three times with lysis buffer and twice with 50 mmol/l HEPES (pH 7.5), 10% (vol/vol) glycerol, 1 mmol/l EDTA, and 1 mmol/l DTT. AMPK activity in the immune complex was determined by *in vitro* phosphorylation of the SAMS (full sequence: HMRSAMSGHLVKKRR) synthetic peptide substrate (30), as previously described (18). Kinase reactions performed in reaction buffer (40 mmol/l HEPES buffer, pH 7.0, 0.2 mmol/l SAMS peptide, 0.2 mmol/l AMP, 80 mmol/l NaCl<sub>2</sub>, 0.8 mmol/l DTT, 5 mmol/l MgCl<sub>2</sub>, and 0.2 mmol/l ATP (containing 2  $\mu$ Ci [ $\gamma$ -<sup>32</sup>P]ATP). Reactions were incubated on a vibrating platform for 60 min at 30°C and terminated by centrifugation (9,000g for 30 s). Immediately thereafter, aliquots were spotted onto phosphocellulose units (Pierce, Rockford, IL). Phosphocellulose units were washed with 1% phosphoric acid according to the manufacturer specifications. Incorporation of [<sup>32</sup>P] into peptide substrate was measured by liquid scintillation counting.

**PI 3-kinase activity.** Epitrochlearis skeletal muscle was homogenized in ice-cold lysis buffer (20 mmol/l Tris, pH 8.0, 137 mmol/l NaCl, 2.7 mmol/l KCl, 1 mmol/l MgCl<sub>2</sub>, 0.5 mmol/l Na<sub>3</sub>VO<sub>4</sub>, 1% Triton X-100, 10% vol/vol glycerol, 10  $\mu$ g/ml leupeptin, 0.2 mmol/l PMSF, 10 mmol/l NaF, and 10  $\mu$ g/ml aprotinin). Homogenates were subjected to centrifugation at 8,000g for 10 min at 4°C, and protein content was determined as described above. Supernatants (800  $\mu$ g) were subjected to immunoprecipitation with a polyclonal anti-phosphotyrosine antibody (Signal Transduction Laboratories, Lexington, KY) coupled to protein A-Sepharose. PI 3-kinase activity was assayed for 20 min at room temperature as described (31). Reaction products were separated by thin-layer chromatography, and quantification of [<sup>32</sup>P] incorporation into PI was determined using a phosphorimager (Bio-Rad Laboratories, Hercules, CA).

**Statistical analyses.** Differences between two groups were determined by unpaired *t* test. Differences between more than two groups were determined by one-way ANOVA followed by the Fisher's least significant difference post hoc analysis. Significance was accepted at  $P < 0.05$ .

## RESULTS

**Animal characteristics.** The body weight of 11-week-old obese Zucker rats was significantly greater than that of age-matched lean rats ( $393 \pm 7$  vs.  $267 \pm 3$  g;  $P < 0.05$ ). Epitrochlearis muscle weights were reduced in obese Zucker rats ( $42.2 \pm 1.6$  vs.  $59.4 \pm 2.1$  mg, obese versus lean rats;  $P < 0.05$ ). Obese Zucker rats displayed marked glucose intolerance, with blood glucose levels significantly elevated versus lean rats at all time points measured ( $P < 0.05$ ) (Fig. 1).

**Skeletal muscle glucose uptake.** Skeletal muscle glu-

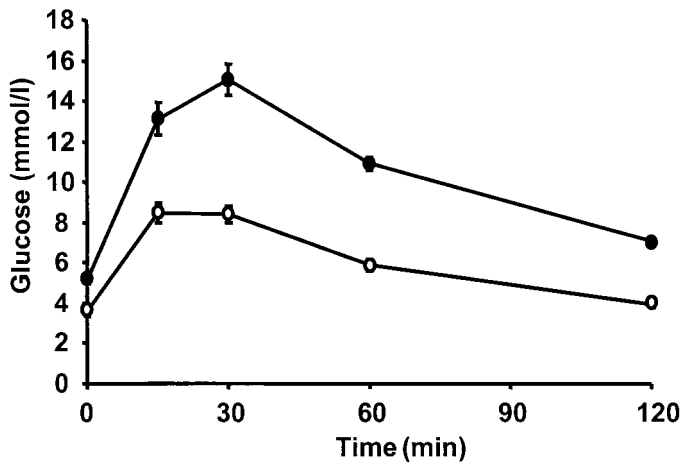


FIG. 1. Glucose tolerance in lean or obese Zucker rats after an intraperitoneal injection of glucose. Blood glucose was measured at 0, 15, 30, 60, and 120 min. Results are means  $\pm$  SE for lean ( $\circ$ ;  $n = 16$ ) and obese ( $\bullet$ ;  $n = 17$ ) rats.  $P < 0.05$  vs. lean rats at all time points.

cose uptake was determined in response to insulin, contraction, or hypoxia. Insulin increased 2-deoxyglucose transport 3.4-fold in isolated skeletal muscle from lean rats ( $P < 0.05$ ) (Fig. 2). Obese rats exhibited marked skeletal muscle insulin resistance. The absolute rate of insulin-stimulated glucose transport in obese rats was reduced 47% compared with lean controls ( $1.27 \pm 0.08$  vs.  $0.68 \pm 0.06 \mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ ;  $P < 0.05$ ). Rates of contraction- and hypoxia-stimulated glucose transport in skeletal muscle were similar between lean and obese rats (Fig. 2). In lean rats, contraction- and hypoxia-stimulated glucose transport was increased 3.1-fold ( $P < 0.05$ ) and 3.4-fold ( $P < 0.05$ ), respectively. In insulin-resistant obese Zucker rats, contraction- and hypoxia-stimulated glucose transport was increased 3.9-fold ( $P < 0.05$ ) and 4.1-fold ( $P < 0.05$ ), respectively.

**PI 3-kinase activity.** Phosphotyrosine-associated PI 3-kinase activity was determined in epitrochlearis skeletal muscle from lean and obese rats, in response to insulin or contraction. Insulin increased phosphotyrosine-associated PI 3-kinase activity 2.7-fold in lean rats ( $P < 0.05$ ) (Fig. 3), with a 42% reduction noted in skeletal muscle from obese

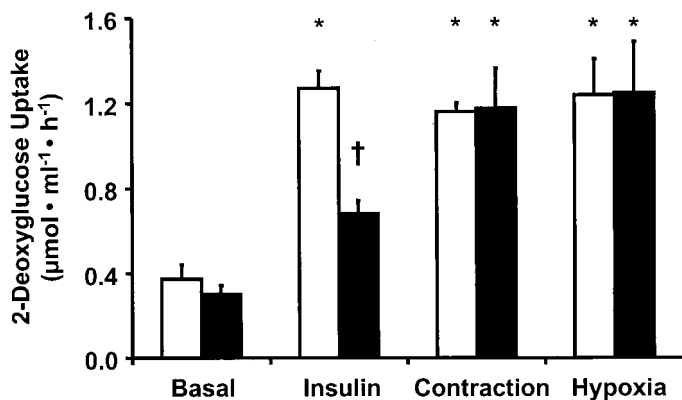


FIG. 2. 2-Deoxyglucose transport activity in epitrochlearis muscle from lean ( $\square$ ) and obese ( $\blacksquare$ ) Zucker rats incubated in the absence (basal) or presence of insulin, after exposure to in vitro muscle contraction, or after exposure to hypoxia. Values are means  $\pm$  SE for 6–11 muscles per group.  $*P < 0.05$  vs. basal;  $\dagger P < 0.05$  vs. lean rats.

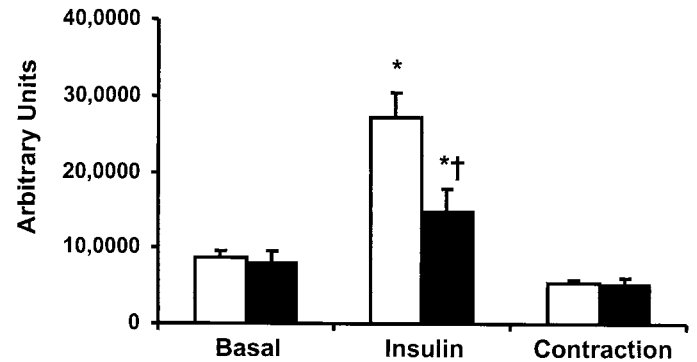


FIG. 3. PI 3-kinase activity in epitrochlearis muscles from lean ( $\square$ ) or obese ( $\blacksquare$ ) Zucker rats incubated in the absence (basal) or presence of insulin or after in vitro muscle contraction. Results are expressed as means  $\pm$  SE (arbitrary phosphoimager units for five to six muscles per group).  $*P < 0.05$  vs. basal;  $\dagger P < 0.05$  vs. lean rats.

rats ( $P < 0.05$  vs. lean). PI 3-kinase activity was not increased after muscle contraction.

**Isoform-specific AMPK activity.** Basal AMPK $\alpha$ 1 (Fig. 4A) and AMPK $\alpha$ 2 (Fig. 4B) activities were similar in skeletal muscle between lean and obese rats during experiments involving insulin and muscle contraction. Regardless of genetic background, insulin was without effect on AMPK $\alpha$ 1 or  $\alpha$ 2 activity. Contraction elicited an increase in activation of both the  $\alpha$ 1 and  $\alpha$ 2 isoforms in skeletal muscle. In lean rats, AMPK $\alpha$ 1 and  $\alpha$ 2 activity was increased 2.3- and 4.8-fold, respectively ( $P < 0.05$ ). Interestingly, in skeletal muscle from obese rats, an isoform-specific effect of contraction on AMPK was noted:

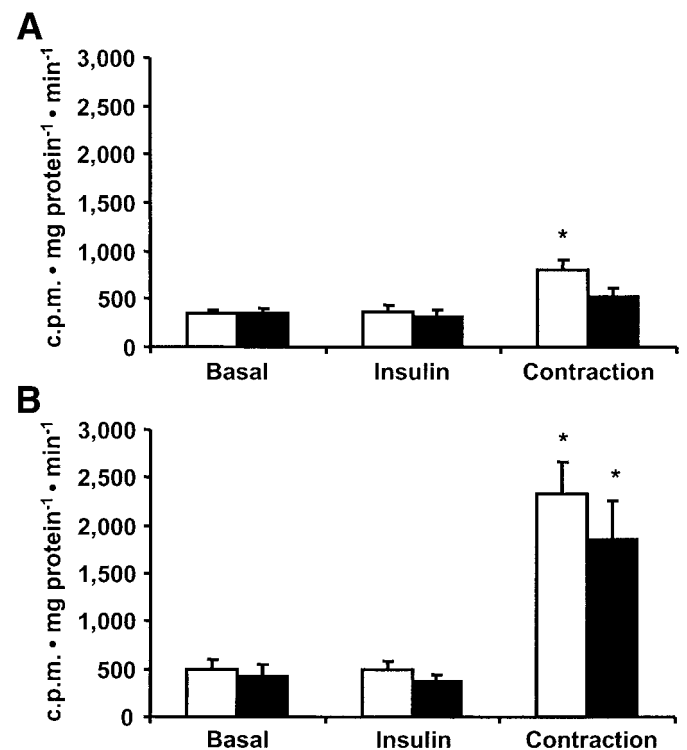
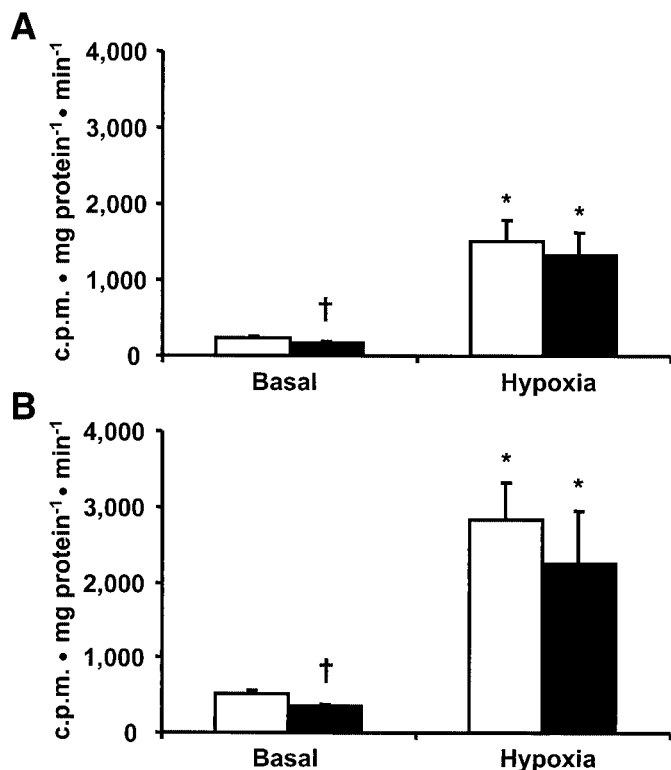


FIG. 4. Insulin- and contraction-stimulated AMPK activity in epitrochlearis muscles from lean ( $\square$ ) or obese ( $\blacksquare$ ) Zucker rats incubated in the absence (basal) or presence of insulin or after in vitro muscle contraction. AMPK $\alpha$ 1 (A) and AMPK $\alpha$ 2 (B) activity is expressed as means  $\pm$  SE (cpm  $\cdot$  mg protein $^{-1} \cdot$  min $^{-1}$  for six muscles per group).  $*P < 0.05$  vs. basal.





**FIG. 5.** Hypoxia-stimulated AMPK activity in epitrochlearis muscles from lean (□) or obese (■) Zucker rats. Muscles were incubated under basal (95% O<sub>2</sub>/5% CO<sub>2</sub>) or hypoxic (95% N<sub>2</sub>/5% CO<sub>2</sub>) conditions. AMPKα1 (A) and AMPKα2 (B) activity is expressed as means ± SE (cpm · mg protein<sup>-1</sup> · min<sup>-1</sup> for five to six muscles per group. \**P* < 0.05 vs. basal; †*P* < 0.05 vs. lean rats.

AMPKα1 activity was not altered (*P* = 0.10), whereas AMPKα2 activity was increased 4.3-fold (*P* < 0.05). Furthermore, when comparing responses between both genotypes, the contraction-mediated response of AMPKα1 tended to be reduced in obese rats (*P* < 0.08). In contrast, contraction-mediated AMPKα2 activity was comparable between lean and obese rats. In response to hypoxia, AMPKα1 (Fig. 5A) and -α2 (Fig. 5B) activity in lean rats was increased 6.6-fold (*P* < 0.05) and 5.5-fold (*P* < 0.05), respectively. In obese rats, hypoxia-stimulated AMPK was preserved, with increases in AMPKα1 and -α2 activity 8.4-fold (*P* < 0.05) and 6.5-fold (*P* < 0.05) over basal values, respectively. Basal AMPKα1 and -α2 activity in obese rats was slightly reduced (*P* < 0.05).

## DISCUSSION

In skeletal muscle, separate and distinct signaling pathways can activate glucose transport. Insulin increases glucose transport via activation of PI 3-kinase (13,14) and possibly via activation of the CAP/TC10 pathway (32,33). However, the latter pathway has not been validated in skeletal muscle. Exercise (34), muscle contraction (35,36), and hypoxia (28,37) increase glucose transport via insulin-independent pathways (13–15), presumably via activation of AMPK (17,18). In type 2 diabetic patients, defects in insulin-mediated whole-body glucose uptake are coupled to impairments in glucose transport in skeletal muscle (4,10), which arise from aberrant signal transduction at the level of insulin receptor substrate-1, PI 3-kinase (6,8,9), and GLUT4 translocation (10,38). Since muscle contrac-

tion and hypoxia increase glucose transport via an alternative mechanism that bypasses defective insulin signaling (37,39,40), strategies to identify and characterize components of this insulin-independent pathway will potentially reveal novel entry points that can be targeted to increase glucose uptake in insulin-resistant muscle. Recent interest has focused on AMPK, as it is the most distal signaling molecule identified in the insulin-independent regulation of glucose transport. AMPK mediates contraction- and hypoxia-regulated glucose transport in skeletal muscle (17) and has been proposed as a promising target for treatment of altered glucose homeostasis in type 2 diabetes (41). Contraction-stimulated glucose transport in skeletal muscle is normal in severely insulin-resistant obese Zucker rats (39,40); however, effects of contraction and hypoxia on isoform-specific activation of AMPK in skeletal muscle from this animal model have not been determined.

Consistent with earlier studies (39,40), insulin resistance at the level of PI 3-kinase and glucose transport was observed in skeletal muscle from obese Zucker rats, whereas stimulation of AMPKα2 activity and glucose transport in response to muscle contraction or hypoxia was normal. These findings are also consistent with observations in humans, whereby moderate aerobic exercise elicits an appropriate increase in AMPKα2 in skeletal muscle from type 2 diabetic subjects (42). Thus, contraction and hypoxia increase AMPKα2 activity and glucose transport in insulin-resistant skeletal muscle. These studies provide evidence to suggest that exercise- and hypoxia-induced AMPK activity is not impaired in insulin-resistant skeletal muscle. Furthermore, they support the recent observation in cultured skeletal muscle, indicating direct activation of AMPK alone is sufficient for stimulation of glucose uptake via an increase in cell surface expression of GLUTs (18).

An isoform-specific reduction in AMPKα1 activity in skeletal muscle from obese Zucker rats was observed in response to contraction, but not hypoxia. Contraction increased AMPKα1 activity in epitrochlearis muscle from lean rats, whereas no effect was observed in obese rats. In contrast, hypoxia-stimulated AMPKα1 activity was similar between lean and obese rats; however, basal AMPKα1 activity was slightly reduced. Interestingly, basal AMPKα1, although not significant, tended to be lower in skeletal muscle from type 2 diabetic subjects (42). A previous study provides evidence that total AMPK activity in calf muscles in response to AICAR infusion was similar between lean and obese Zucker rats (24). However, since total rather than isoform-specific AMPK activity was measured (24), subtle defects in AMPKα1 activity may have been masked.

An explanation for the differential response of AMPKα1 in response to contraction versus hypoxia in the present study may be related to the amount of cellular stress applied to the muscle. The hypoxia stimulus is likely to have elicited a greater degree of stress on the muscle (21). Thus, the AMPKα1 defect may represent reduced sensitivity of kinase to cellular stress. Physiological evidence for this comes from human studies in which AMPKα1 activity is increased in response to anaerobic (sprint) (43), but not aerobic (endurance) (44,45), cycle ergometry. The contraction protocol used in the present study is more closely

related to anaerobic (high-intensity) rather than aerobic (low-intensity) exercise. An alternative possibility is that the lack of a contraction effect on AMPK $\alpha$ 1 in obese muscle may be due to the fact that the contraction protocol was not intense enough, although it did stimulate glucose transport to the same extent. Despite the isoform-specific defect in AMPK $\alpha$ 1 activity in response to contraction, normal glucose transport was achieved in skeletal muscle from insulin-resistant Zucker rats.

Hypoxia increases AMPK activity to a greater degree than in vitro muscle contraction (21). Since only subtle defects in contraction-induced AMPK $\alpha$ 1 activity were observed in obese rats in the present investigation, the observed defect is not likely to have a major impact on the rate of glucose transport in response to muscle contraction. Importantly, full activation of AMPK may not be necessary for complete activation of exercise/contraction-stimulated glucose transport. Transgenic overexpression of a dominant inhibitory mutant of AMPK in skeletal muscle completely blocks the ability of hypoxia or AICAR to activate glucose uptake while only partially (30–40%) reducing contraction-stimulated glucose uptake (17). Although these results (17) confirm a role of AMPK in contraction-induced glucose transport, they also demonstrate that AMPK is only partially responsible for this effect. Thus, AMPK-dependent and -independent pathways contribute to the regulation of glucose uptake in skeletal muscle in response to exercise (17). Consistent with this hypothesis, glucose transport in slow-twitch muscle can be markedly activated in response to contraction, without measurable changes in AMPK activity (46). Collectively, these studies illustrate the complexity in identifying the precise role of AMPK signaling in the regulation of metabolic events and they strongly suggest that additional factors contribute to the regulation of exercise-mediated glucose uptake.

In summary, obesity-related insulin resistance in skeletal muscle is associated with reduced contraction-stimulated AMPK $\alpha$ 1 activity, whereas AMPK $\alpha$ 2 activity is normal. However, when muscles were challenged by cellular hypoxia, activation of both AMPK $\alpha$ 1 and  $\alpha$ 2 were normal. In skeletal muscle from lean animals, hypoxia was more effective than contraction in stimulating AMPK $\alpha$ 1 activity. Thus, the impairment in AMPK $\alpha$ 1 in insulin-resistant muscle may be related to a reduced sensitivity of the enzyme in response to cellular stress. Importantly, contraction-mediated glucose transport is normal, despite impaired AMPK $\alpha$ 1 activity in muscles from obese Zucker rats. Thus, AMPK $\alpha$ 1 does not appear to play a major role in stimulation of glucose transport by muscle contraction. Our results are consistent with the hypothesis that activation of AMPK $\alpha$ 2 in response to muscle contraction/exercise is associated with a parallel and normal increase in glucose transport in insulin-resistant skeletal muscle.

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