5-Amino-Imidazole Carboxamide Riboside Increases Glucose Transport and Cell-Surface GLUT4 Content in Skeletal Muscle From Subjects With Type 2 Diabetes

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AMP-activated protein kinase (AMPK) activation by AICAR (5-amino-imidazole carboxamide riboside) is correlated with increased glucose transport in rodent skeletal muscle via an insulin-independent pathway. We determined in vitro effects of insulin and/or AICAR exposure on glucose transport and cell-surface GLUT4 content in skeletal muscle from nondiabetic men and men with type 2 diabetes. AICAR increased glucose transport in a dose-dependent manner in healthy subjects. Insulin and AICAR increased glucose transport and cell-surface GLUT4 content to a similar extent in control subjects. In contrast, insulin- and AICAR-stimulated responses on glucose transport and cell-surface GLUT4 content were impaired in subjects with type 2 diabetes. Importantly, exposure of type 2 diabetic skeletal muscle to a combination of insulin and AICAR increased glucose transport and cell-surface GLUT4 content to levels achieved in control subjects. AICAR increased AMPK and acetyl-CoA carboxylase phosphorylation to a similar extent in skeletal muscle from subjects with type 2 diabetes and nondiabetic subjects. Our studies highlight the potential importance of AMPK-dependent pathways in the regulation of GLUT4 and glucose transport activity in insulin-resistant skeletal muscle. Activation of AMPK is an attractive strategy to enhance glucose transport through increased cell surface GLUT4 content in insulin-resistant skeletal muscle. *Diabetes* 52:1066–1072, 2003

Skeletal muscle is the principal site of glucose uptake under insulin-stimulated conditions, accounting for ~75% of glucose disposal after glucose infusion (1,2). Insulin-mediated glucose uptake and utilization is markedly impaired in patients with type 2 diabetes (2–4). Although the primary cause for type 2 diabetes is still unknown, insulin resistance is an early defect in the pathogenesis of this metabolic disorder, as it is observed already in normal glucose-tolerant relatives of patients with type 2 diabetes (5–7). In vivo studies using a nuclear magnetic resonance technique provide evidence that the major rate-limiting impairment in the regulation of insulin-stimulated glycogen synthesis in skeletal muscle from patients with type 2 diabetes is localized at the level of glucose transport (8). Moreover, in vitro studies have directly shown that insulin-stimulated glucose transport is markedly reduced in isolated skeletal muscle from patients with type 2 diabetes (4,9,10) and insulin-resistant morbidly obese (BMI ~50 kg/m^2) subjects with or without type 2 diabetes (11), as well as in women with gestational diabetes (12). Thus, reduced skeletal muscle glucose transport may be among the early factors accounting for increased risk of developing type 2 diabetes.

In skeletal muscle, glucose transport can be activated by distinct and separate pathways mediated by insulin, insulin-mimicking agents, and insulin-like growth factors, as well as by muscle contraction (exercise), cellular stress, and hypoxia (13). PI 3-kinase is involved in insulin-, but not contraction-stimulated, glucose transport and GLUT4 translocation (14,15). PI 3-kinase activation is necessary but not sufficient for metabolic actions of insulin (16,17), suggesting that novel cascades contribute to the regulation of insulin-stimulated glucose transport. Activation of 5′-AMP-activated kinase (AMPK) has been suggested to contribute to the regulation of insulin-independent glucose transport in skeletal muscle in response to exercise/contraction or factors that induce metabolic stress (18). AMPK has been proposed as a major metabolic switch that phosphorylates key target proteins along diverse metabolic pathways, including hepatic lipid metabolism, adipocyte lipolysis, and skeletal muscle fatty acid oxidation and glucose transport (19,20). AMPK is a heterotrimeric enzyme consisting of catalytic α- and regulatory β- and γ-subunits and is activated by both allosteric and covalent mechanisms, which are sensitive to an increase in cellular AMP to ATP and creatine to phosphocreatine ratio (19). The nucleoside analogue 5-aminoimidazole-4-carboxamide 1-β-D-ribonucleoside (AICAR) is one pharmacological means to activate AMPK (21,22). 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

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ACC, acetyl-CoA carboxylase; AICAR, 5-amino-imidazole carboxamide riboside; AMPK, AMP-activated protein kinase; KHB, Krebs-Henseleit buffer; MAPK, mitogen-activated protein kinase; Vo_{2max}, maximal oxygen uptake.

TABLE 1
Subject characteristics

	Nondiabetic (n = 9)	Type 2 diabetic (n = 10)
Age (years)	55 ± 3	59 ± 1
BMI (kg/m^2)	26.6 ± 0.6	27.7 ± 0.8
Body fat (%)	22.9 ± 1.9	25.4 ± 1.9
Waist (cm)	96 ± 2	102 ± 2
Hip (cm)	100 ± 2	101 ± 1
Waist-to-hip ratio	0.96 ± 0.02	1.0 ± 0.01
Vo_{2max} (ml · kg fat free mass^{-1} · min^{-1})	46.2 ± 2.2	39.2 ± 2.7
HbA_{1c} (%)	4.6 ± 0.1	5.9 ± 0.4†
GIR (μmol · kg^{-1} · min^{-1})	34.3 ± 3.1	16.7 ± 2.6*
Glucose (mmol/l)	5.6 ± 0.1	9.0 ± 0.5*
Insulin (mU/l)	5 ± 1	10 ± 1†
C-peptide (nmol/l)	0.62 ± 0.05	0.99 ± 0.11‡
Cholesterol (mmol/l)	5.3 ± 0.3	4.6 ± 0.2
Triglyceride (mmol/l)	1.4 ± 0.3	1.5 ± 0.2
HDL (mmol/l)	1.22 ± 0.06	1.12 ± 0.07
LDL (mmol/l)	3.4 ± 0.3	2.8 ± 0.1
Duration of diabetes (years)	—	5 ± 1

GIR, glucose infusion rate during 120–180 min of the euglycemic-hyperinsulinemic clamp. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.02$ versus nondiabetic subjects.

contraction-stimulated glucose uptake (18). Thus, direct evidence is emerging that AMPK-dependent and AMPK-independent pathways contribute to the regulation of glucose uptake in skeletal muscle.

Treatment of isolated rodent muscle with AICAR is associated with an increase in glucose transport by an insulin-independent mechanism (23), and this is associated with increased translocation of the insulin-sensitive glucose transporter GLUT4 from intracellular microvesicles to sarcolemmal membranes in skeletal muscle (24). Importantly, whereas insulin-stimulated glucose transport is reduced in skeletal muscle from insulin-resistant *ob/ob* mice, AICAR-stimulated glucose transport is normal (25), suggesting that therapies that activate AMPK may be useful to increase glucose uptake in insulin-resistant skeletal muscle. Thus, we determined the effect of AICAR on glucose transport and cell-surface GLUT4 content in skeletal muscle from control subjects and type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

The study protocol was reviewed and approved by the institutional ethical committee of the Karolinska Institute, and informed consent was received from all subjects before participation. Subjects were recruited on the basis of a normal resting electrocardiogram; normal blood count; and normal kidney, liver, and thyroid function. Nondiabetic subjects with impaired glucose tolerance, as determined by 2-h plasma glucose concentration ≥7.8 mmol/l following the standard 75-g oral glucose tolerance test (26), cigarette smokers, or subjects who were using antihypertensive medication (β-blocking agent, angiotensin-converting enzyme inhibitors, Ca^{2+} inhibitors, or diuretics) were excluded. Physiological characteristics of the study participants (9 nondiabetic men and 10 men with type 2 diabetes) are presented in Table 1. Five of the nondiabetic subjects had no family history of diabetes, whereas four nondiabetic subjects had one or more first- or second-degree relatives with type 2 diabetes. The subjects with diabetes were treated with diet (n = 1); glibenclamide (n = 5); metformin (n = 1); a combination of glibenclamide, metformin, acarbose, and insulin (n = 1); a combination of glipizide and metformin (n = 1); or insulin only (n = 1). The mean duration of diabetes was 5 years (range, 2–11 years). All subjects were instructed to avoid strenuous exercise for 72 h before the muscle biopsy or euglycemic-hyperinsulinemic clamp studies. The subjects reported to the laboratory after an overnight fast and, in the case of diabetic patients, before administration of antidiabetic medication.

Euglycemic-hyperinsulinemic clamp. Whole-body insulin-stimulated glucose disposal was determined using the euglycemic-hyperinsulinemic clamp technique (27). A catheter was inserted into the antecubital vein for glucose and insulin infusion and into the brachial artery for blood sampling. After baseline blood samples were taken, insulin was infused at a rate of 40 mU · m^{-2} · min^{-1} for 180 min. Plasma glucose concentration was kept constant by a variable 20% glucose infusion that was adjusted after plasma glucose measurements every 5 min. Blood samples were obtained every 30 min for analysis of plasma-free insulin concentration. Plasma free insulin concentrations during the clamp were comparable between control subjects (56 ± 3 mU/l) and subjects with type 2 diabetes (63 ± 3 mU/l). For correcting the fasting hyperglycemia in patients with type 2 diabetes, insulin was infused for 64 ± 10 min (range, 15–132 min) before the glucose infusion was started. During 120–180 min, a similar steady state in plasma glucose concentration was achieved between control subjects (5.5 ± 0.03 mmol/l) and subjects with type 2 diabetes (5.5 ± 0.08 mmol/l), with a comparable coefficient of variation of plasma glucose concentration of 4.6 ± 0.6% in control subjects and 3.7 ± 0.4% in subjects with type 2 diabetes. The glucose infusion rate required to maintain euglycemia during the last hour (120–180 min) of the clamp was used as a measure of whole-body insulin sensitivity.

Blood chemistry. Plasma glucose concentration was determined using a glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma free insulin and C-peptide concentrations were determined using a commercially available radioimmunoassay (Pharmacia, Uppsala, Sweden). HbA_{1c} was determined using an immunological method.

Maximal oxygen uptake and body composition determinations. Maximal oxygen uptake (Vo_{2max}) was determined on a bicycle ergometer. Vo_{2max} was measured continuously with a breath-by-breath data collection technique (Erich Jaeger, Hoechberg, Germany). Regional analysis of lean body mass, body fat, and bone mineral content was performed by dual-energy X-ray absorptiometry (Lunar, Madison, WI).

Open biopsy and in vitro incubation of skeletal muscle. Open muscle biopsies were taken from vastus lateralis muscle, under local anesthesia (mepivacaine chloride 5 mg/ml), as previously described (28). A 4-cm incision was made 15 cm above the proximal border of patella, and muscle fascia was exposed. A skeletal muscle biopsy (~2 g of muscle) was excised and placed in oxygenated Krebs-Henseleit buffer (KHB) containing 5 mmol/l HEPES and 0.1% BSA (RIA Grade; Sigma, St. Louis, MO). Smaller muscle strips were dissected, mounted on Plexiglas clamps, and incubated in shaking water bath at 35°C for 30 min in pregassed (95% O_2/5% CO_2) KHB containing 5 mmol/l glucose and 15 mmol/l mannitol. Thereafter, muscle specimens were incubated for 30 min in the absence (basal) or presence of insulin (120 nmol/l), AICAR (0.1, 1.0, or 2.0 mmol/l), or a combination of AICAR (1 mmol/l) and insulin (120 nmol/l). The concentrations of insulin and AICAR were maintained throughout all remaining incubation steps. Muscle specimens were next incubated for 10 min at 35°C in glucose-free KHB. Osmolarity was maintained by addition of 20 mmol/l mannitol to the media. Muscle specimens were incubated as described below for measurement of glucose transport activity or cell-surface GLUT4 content.

Glucose transport. Muscle specimens were incubated for 20 min in KHB containing 5 mmol/l 3-O-[^{3}H]methyl-D-glucose (800 μCi/mmol) and 15 mmol/l [^{14}C]mannitol (53 μCi/mmol). After incubation, muscles were blotted of excess fluid, frozen in liquid nitrogen, and stored at −80°C until analysis. Glucose transport was analyzed as accumulation of intracellular 3-O-[^{3}H]methyl-D-glucose, as described previously (29).

Cell-surface GLUT4-labeling procedure. Muscle specimens were incubated for 8 min in the presence of 400 μmol/l Bio-LC-ATB-BGPA (4,4-O-[2-[2-[2-[2-[2-[6-(biotinylamino)hexanoyl]amino]ethoxy]exoxy]ethoxy]-4–1-azi-2,2,2-trifluoroethyl)benzoyl]amino-1,3-propanediyl]bis-D-glucose). Muscles were then irradiated twice for 3 min in a Rayonet photochemical reactor (Southern New England Ultraviolet, Branford, CT) using 300-nm lamps. Muscle specimens were frozen directly in liquid nitrogen and stored at −80°C until analysis. Cell surface GLUT4 was determined as previously described (30).

Phosphorylation of AMPK and acetyl-CoA carboxylase. Sufficient sample was available from four nondiabetic subjects and six subjects with type 2 diabetes to assess phosphorylation of AMPK and acetyl-CoA carboxylase (ACC). Muscles were lysed as described (10), and 40 μg of total protein was separated on 7.5% SDS-PAGE. Phosphorylation of AMPK was determined using an antibody specific for phospho-AMPK pan α-subunit (Thr^{172}; Cell Signaling Technology, Beverly, MA). Phosphorylation of the AMPK substrate ACC was detected with a phosphospecific antibody raised against a peptide corresponding to the sequence in rat ACCα containing the Ser^{79} phosphory-

lation site that also recognizes human ACCβ phosphorylated at Ser^{221} (Upstate Biotechnology, Lake Placid, NY).

Statistical analysis. Data are presented as means ± SE. Normal distribution of the variables was verified by Kolmogorov-Smirnov's test. Unless otherwise noted, Student's paired and unpaired *t* tests were used in the analysis of paired and unpaired data, respectively. All statistical analysis was performed with SPSS statistical package (version 11.0; SPSS Inc, Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Subject characteristics. Subjects with type 2 diabetes and nondiabetic subjects were matched for age, BMI, percentage of body fat, and waist-to-hip ratio (Table 1). Vo_{2max} was slightly higher in nondiabetic subjects, but this difference was not statistically significant ($P = 0.07$). Total, HDL, and LDL cholesterol and serum triglyceride concentrations were comparable between nondiabetic subjects and subjects with type 2 diabetes. Systolic blood pressure was higher in subjects with type 2 diabetes (147 ± 4 vs. 129 ± 5 mmHg for type 2 diabetic versus nondiabetic subjects, respectively; $P < 0.02$), whereas diastolic blood pressure was comparable between the two groups (85 ± 2 vs. 85 ± 3 mmHg for type 2 diabetic versus nondiabetic subjects, respectively). Fasting plasma glucose was higher in subjects with type 2 diabetes ($P < 0.001$). HbA_{1c} level was only slightly elevated in the subjects with type 2 diabetes, suggestive of good metabolic control. During steady-state conditions of 120–180 min of the clamp, the glucose infusion rate needed to maintain euglycemia was 50% lower in subjects with type 2 diabetes ($P < 0.001$; Table 1).

Effects of AICAR on in vitro glucose transport in isolated skeletal muscle. The dose-response effect of AICAR on glucose transport was determined in skeletal muscle strips from five nondiabetic men (Fig. 1). Glucose transport increased 2.6-fold ($P < 0.05$; Wilcoxon's test) in response to 1 mmol/l AICAR, whereas the increase after exposure to 0.1 and 2.0 mmol/l AICAR did not reach statistical significance. Therefore, in subsequent studies to determine effects of AICAR on glucose transport in skeletal muscle from control subjects and subjects with type 2 diabetes, muscle specimens were exposed to 1 mmol/l AICAR.

Glucose transport in isolated skeletal muscle. Basal glucose transport was not different between control subjects and subjects with type 2 diabetes (Fig. 2*A*). Insulin increased glucose transport 2.5-fold ($P < 0.001$) in control subjects and 1.9-fold ($P < 0.005$) in subjects with type 2 diabetes (Fig. 2*A*). The absolute change in glucose transport response to insulin was 34% lower in subjects with type 2 diabetes ($P = 0.06$; Fig. 2*B*). Consistent with our in vivo measurements of whole-body glucose uptake, where a 50% reduction in whole-body insulin-mediated glucose uptake was noted, insulin-stimulated glucose transport in vitro was impaired in skeletal muscle from men with type 2 diabetes. AICAR increased glucose transport 2.9-fold ($P < 0.01$) in control subjects and 1.8-fold ($P < 0.02$) in subjects with type 2 diabetes (Fig. 2*A*). The absolute change in glucose transport in response to AICAR stimulation was 52% lower in subjects with type 2 diabetes ($P = 0.12$; Fig. 2*B*). A combined exposure of insulin and AICAR increased glucose transport 3.3-fold ($P < 0.001$) in control subjects and 2.3-fold ($P < 0.005$) in subjects with type 2 diabetes. In subjects with type 2 diabetes, the additive effect of AICAR and insulin on glucose transport was 24% greater ($P < 0.02$) than observed in response to exposure to insulin alone (Fig. 2*A*) and similar to rates achieved in control subjects (Fig. 2*B*).

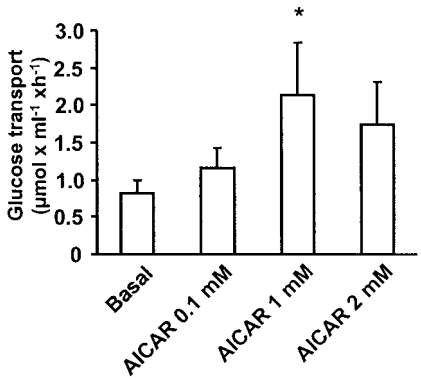

FIG. 1. Effects of AICAR on glucose transport. Glucose transport was determined in muscle strips from five nondiabetic subjects. Muscle strips were obtained and isolated as described in RESEARCH DESIGN AND METHODS and incubated in the absence or presence of 0.1, 1.0, or 2.0 mmol/l AICAR. Data are means ± SE; *$P < 0.05$ versus basal (Wilcoxon's test).

Cell-surface GLUT4 content. Cell-surface GLUT4 content was determined using an exofacial bis-glucose photolabeling reagent in six nondiabetic subjects and six subjects with type 2 diabetes (Fig. 3*A*). Insulin increased cell-surface GLUT4 content 2.1-fold in skeletal muscle from nondiabetic subjects ($P < 0.03$), but not in skeletal muscle from subjects with type 2 diabetes. Exposure of isolated skeletal muscle to AICAR led to a 2.5-fold increase in cell-surface GLUT4 content in nondiabetic subjects ($P = 0.16$) and a 1.7-fold increase in cell-surface GLUT4 content in subjects with type 2 diabetes ($P < 0.05$). A combined exposure to AICAR and insulin increased cell-surface GLUT4 content 2.6-fold over basal ($P < 0.05$) in skeletal muscle from men with type 2 diabetes, but in nondiabetic men, this was not statistically significant ($P = 0.10$). The absolute change in cell-surface GLUT4 content (Fig. 3*B*) in response to insulin or AICAR followed a similar profile as reported for glucose transport (Fig. 2*B*).

AMPK and ACC phosphorylation. We used a phosphospecific antibody to determine AMPK and ACC phosphorylation in skeletal muscle from four nondiabetic subjects and six subjects with type 2 diabetes incubated for 60 min in the absence or presence of 1 mmol/l AICAR. AMPK phosphorylation (Fig. 4*A*) was increased to a similar extent (twofold; $P < 0.05$) in control subjects and subjects with type 2 diabetes. Consistent with this, ACC phosphorylation (Fig. 4*B*) was increased to a similar extent between control subjects and subjects with type 2 diabetes ($P < 0.05$).

DISCUSSION

Insulin-stimulated glucose transport in skeletal muscle is impaired in subjects with type 2 diabetes (4,9–11,30). We tested the hypothesis that glucose transport could be restored in skeletal muscle from patients with type 2 diabetes after incubation with AICAR, a pharmacological agent that activates AMPK (21,22) and increases glucose transport in rodent skeletal muscle by an insulin-independent mechanism (23). Control subjects and subjects with

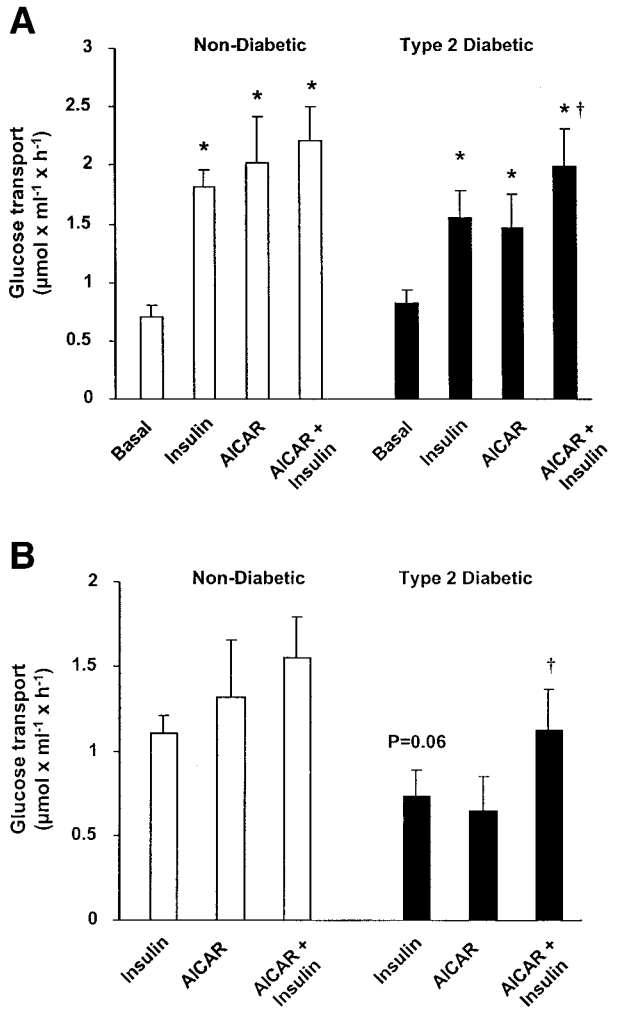

FIG. 2. Effects of insulin or AICAR on glucose transport in skeletal muscle from nondiabetic subjects (n = 9) and subjects with type 2 diabetes (n = 8). *A*: Muscle strips were exposed and incubated in the absence or presence of 120 nmol/l insulin, 1 mmol/l AICAR, or a combination of 120 nmol/l insulin and 1 mmol/l AICAR as described in RESEARCH DESIGN AND METHODS. For a combined exposure to both AICAR and insulin, muscle strips were available from eight nondiabetic subjects and seven subjects with type 2 diabetes. *B*: The individual change in glucose transport rate in response to insulin or AICAR. Data were calculated by subtracting respective rate of basal glucose transport from the insulin or AICAR response. Data are means ± SE; *P < 0.05 versus basal; †P < 0.05 versus corresponding insulin-stimulated value.

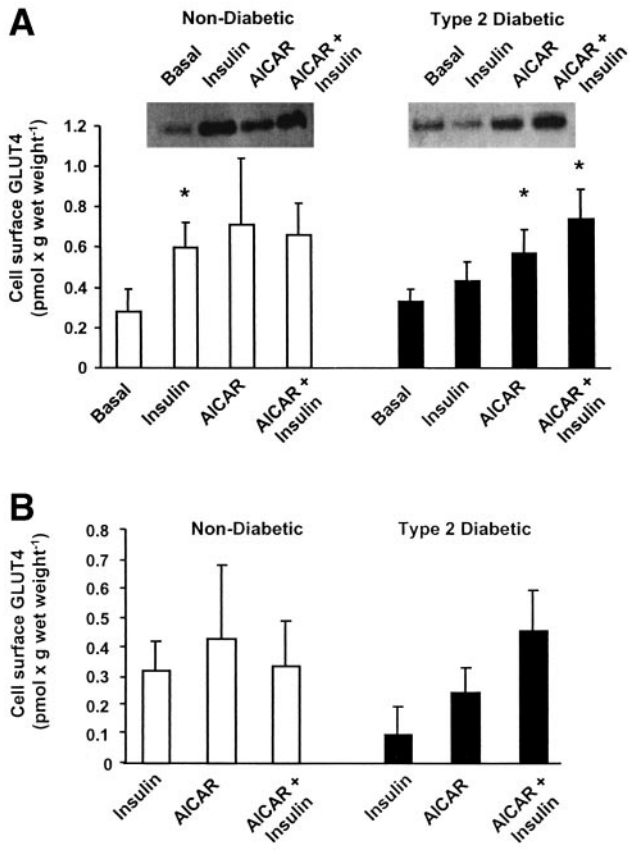

FIG. 3. Effects of insulin or AICAR on cell-surface GLUT4 content in skeletal muscle from nondiabetic subjects (n = 6) and subjects with type 2 diabetes (n = 6). For the combined treatment with insulin and AICAR, five nondiabetic subjects and five subjects with type 2 diabetes were studied. *A*: Skeletal muscle was incubated as described in Fig. 2. Cell-surface GLUT4 content was determined using a bis-glucose photolabel as described in RESEARCH DESIGN AND METHODS. *B*: The individual change in cell-surface GLUT4 content in response to insulin or AICAR. Data were calculated by subtracting respective basal from the insulin or AICAR response. Data are means ± SE; *P < 0.05 versus basal.

type 2 diabetes were matched with respect to age, adiposity, and physical fitness. Skeletal muscle was obtained and exposed to a pharmacological insulin concentration and/or AICAR. In accordance with studies in rodents (23,24), AICAR increases glucose transport in human skeletal muscle, and this effect is associated with an increase in cell-surface GLUT4 content. Importantly, in skeletal muscle from subjects with type 2 diabetes, AICAR increased glucose transport and cell-surface GLUT4 content, providing evidence that the AMPK pathway can be evoked in insulin-resistant muscle. This provides further validation of AMPK as an entry point for therapeutic strategies to restore metabolic balance in patients with type 2 diabetes.

AICAR increased glucose transport in a dose-dependent manner in skeletal muscle from healthy subjects, with effects comparable to those achieved in response to a maximal concentration of insulin. Incubation of human skeletal muscle with AICAR increases AMPK phosphorylation at Thr^{172}, a site implicated in the covalent activation of AMPK (19,20). Moreover, phosphorylation of AMPK is associated with increased phosphorylation of ACC, a downstream target of AMPK (19,20), as further validated here in human skeletal muscle. In rodents, AICAR-mediated glucose transport in skeletal muscle is AMPK-dependent (18,31). Thus, we provide evidence that AICAR activates AMPK in human muscle, as demonstrated indirectly by two known biological consequences of AMPK activation: an increase in glucose transport rate and an increase in the phosphorylation of ACC. Importantly, on the basis of our preliminary results in the subgroup of subjects studied, AICAR increased AMPK and ACC phosphorylation to a similar extent in skeletal muscle from subjects with type 2 diabetes and nondiabetic subjects and provides evidence that this pathway can be evoked in insulin-resistant skeletal muscle.

Insulin-dependent and AMPK-dependent pathways of glucose metabolism seem to be impaired in skeletal muscle from patients with type 2 diabetes. Consistent with earlier observations (4,9–11,30), insulin-stimulated glucose transport in skeletal muscle is blunted in subjects

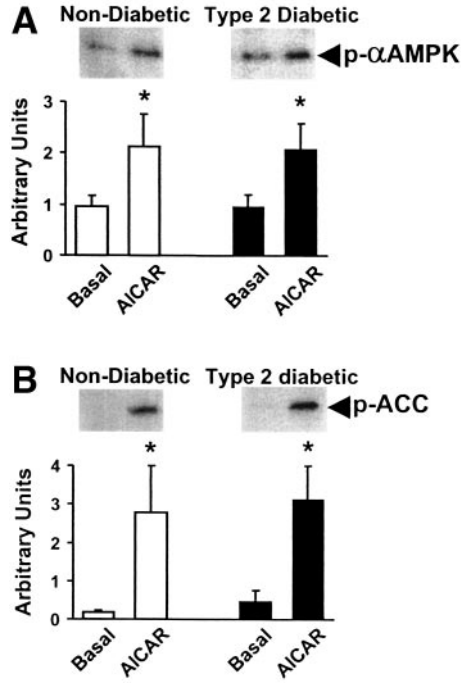

FIG. 4. Exposure of human muscle to AICAR increases phosphorylation of αAMPK and ACC. Phosphorylation of AMPK (*A*) and ACC (*B*) was detected using an antibody specific for phospho-AMPK and phospho-ACC as described in RESEARCH DESIGN AND METHODS. Data are means ± SE arbitrary units; *$P < 0.05$ versus basal (Wilcoxon's test).

with type 2 diabetes versus control subjects. It is interesting that a trend for reduced glucose transport in response to AICAR was noted in skeletal muscle from subjects with type 2 diabetes, a finding consistent with previous studies reporting reduced hypoxia-stimulated glucose transport in subjects with type 2 diabetes (30,32). Moreover, in skeletal muscle from subjects with type 2 diabetes, there was a marked reduction in cell-surface GLUT4 content in response to hypoxia (30). As both hypoxia and AICAR are believed to increases glucose transport in skeletal muscle by an insulin-independent pathway requiring functionally active AMPK (18), our findings provide further evidence to suggest that glucose transport defects in patients with type 2 diabetes are not limited to the insulin-signaling pathway. Consistent with these results, a tendency for reduced (~30%) plasma membrane GLUT4 content has been observed in subjects with type 2 diabetes versus control subjects both at rest and after 45–60 min of cycle exercise at 60–70% Vo_{2max} (33). Because activation of AMPK by AICAR (present study) or exercise/muscle contraction is normal in skeletal muscle from patients with type 2 diabetes (34) and insulin-resistant Zucker rats (35), the impairment in glucose transport in type 2 diabetic muscle is likely to reflect defects in converging pathways downstream of AMPK and the classical insulin-signaling steps.

The downstream signaling steps linking activation of AMPK to glucose transport in human skeletal muscle are unknown. Studies in rodents and cultured cells suggest that the proline-rich tyrosine kinase-2, the extracellular regulated kinase pathway, phospholipase D, and members of the atypical protein kinase C family may link AMPK to glucose transport (36). Moreover, AICAR-stimulated activation of p38 mitogen-activated protein kinase (MAPK) by AMPK may also link AMPK to glucose transport (37). Activation of p38 MAPK has been suggested to increase the intrinsic activity of glucose transporters, as inhibition of p38 attenuates glucose transport but does not affect the translocation of glucose transporters to cell surface (38). Future studies to determine whether insulin or AICAR activates p38 MAPK in human skeletal muscle are warranted to resolve the possible role of this pathway in the activation of glucose transport.

Insulin stimulates glucose transport in skeletal muscle primarily by eliciting translocation of insulin-sensitive glucose transporter GLUT4 from an intracellular pool to the plasma membrane (39,40). In humans, insulin-stimulated glucose transport rate and cell-surface GLUT4 content are closely correlated (41). Insulin induces a profound increase in cell-surface GLUT4 content and glucose transport in control subjects, whereas in subjects with type 2 diabetes, this effect is severely blunted. Thus, we confirm previous observations (30,42,43) showing that insulin-stimulated GLUT4 translocation is impaired in skeletal muscle from subjects with type 2 diabetes. Defects in GLUT4 trafficking are likely to contribute to the insulin resistance in skeletal muscle from patients with type 2 diabetes, as total GLUT4 protein expression in skeletal muscle is normal (44,45). In addition, insulin-signaling defects in skeletal muscle of patients with type 2 diabetes (10,13) are likely to contribute to a blunted GLUT4 translocation in response to insulin stimulation.

AICAR and insulin have additive effects on glucose transport in skeletal muscle from subjects with type 2 diabetes. This finding is consistent with the reported twofold greater increase in insulin-stimulated glucose transport in rat epitrochlearis muscles pretreated for 3.5 h with either hypoxia or AICAR (46). When skeletal muscle from subjects with type 2 diabetes were exposed to a co-incubation of AICAR and insulin, glucose transport was 24% higher than observed in response to insulin alone. Importantly, the absolute increase over basal in response to a combined stimulus was of the same magnitude as observed in nondiabetic subjects, suggesting that synergistic activation of AMPK and insulin-signaling pathways can elicit a greater response on glucose uptake than activation of either pathway alone. It is interesting that AICAR and insulin did not have additive effects on glucose transport in nondiabetic subjects, suggesting that the full biological response is reached with an exposure to either agent. Whether this is due to AICAR and insulin acting on the same terminal-signaling pathway is unknown. Nevertheless, our present data are consistent with our earlier observations (9,47), showing that metformin, a recently described activator of AMPK (48), does not have an effect on basal or insulin-stimulated glucose transport in skeletal muscle from control subjects, whereas it potentiates insulin-stimulated glucose transport in skeletal muscle from insulin-resistant subjects, with or without type 2 diabetes.

In conclusion, AICAR increases cell-surface GLUT4 content and glucose transport activity in skeletal muscle from subjects with type 2 diabetes. Our studies highlight the potential importance of AMPK-dependent pathways in the regulation of GLUT4 and glucose transport activity in insulin-resistant skeletal muscle. Furthermore, AICAR and insulin have a partial additive effect to increase cell-

surface GLUT4 content and glucose transport in skeletal muscle from subjects with type 2 diabetes. Thus, therapies that target insulin-independent pathways in the regulation of GLUT4 and glucose transport are likely to bypass defects in insulin signaling. Activation of AMPK is an attractive strategy to increase glucose transport and improve glucose homeostasis in patients with type 2 diabetes.

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