Original Article

5-Aminoimidazole-4-Carboxamide 1-β-D-Ribofuranoside Acutely Stimulates Skeletal Muscle 2-Deoxyglucose Uptake in Healthy Men

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Activation of AMP-activated protein kinase (AMPK) in rodent muscle by exercise, metformin, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR), and adiponectin increases glucose uptake. The aim of this study was to determine whether AICAR stimulates muscle glucose uptake in humans. We studied 29 healthy men (aged 26 ± 8 years, BMI 25 ± 4 kg/m^2 [mean ± SD]). Rates of muscle 2-deoxyglucose (2DG) uptake were determined by measuring accumulation of total muscle 2DG (2DG and 2DG-6-phosphate) during a primed, continuous 2DG infusion. The effects of AICAR and exercise on muscle AMPK activity/phosphorylation and 2DG uptake were determined. Whole-body glucose disposal was compared before and during AICAR with the euglycemic-hyperinsulinemic clamp. Muscle 2DG uptake was linear over 9 h (R^2 = 0.88 ± 0.09). After 3 h, 2DG uptake increased 2.1 ± 0.8- and 4.7 ± 1.7-fold in response to AICAR or bicycle exercise, respectively. AMPK α_1 and α_2 activity or AMPK phosphorylation was unchanged after 20 min or 3 h of AICAR, but AMPK phosphorylation significantly increased immediately and 3 h after bicycle exercise. AICAR significantly increased phosphorylation of extracellular signal–regulated kinase 1/2, but phosphorylation of β-acetyl-CoA carboxylase, glycogen synthase, and protein kinase B or insulin receptor substrate-1 level was unchanged. Mean whole-body glucose disposal increased by 7% with AICAR from 9.3 ± 0.6 to 10 ± 0.6 mg · kg^{-1} · min^{-1} (P < 0.05). In healthy people, AICAR acutely stimulates muscle 2DG uptake with a minor effect on whole-body glucose disposal. *Diabetes* 56:2078–2084, 2007

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2DG, 2-deoxyglucose; 2DG6P, 2-deoxyglucose 6-phosphate; ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; aPKC, atypical protein kinase C; ERK, extracellular signal–regulated kinase; FDG, fluorodeoxyglucose; GS, glycogen synthase; GSK, glycogen synthase kinase; IRS-1, insulin receptor substrate-1; PKB, protein kinase B; ZMP, 5-aminoimidazole-4-carboxamide ribonucleoside.

Skeletal muscle is the major site of whole-body glucose disposal during insulin stimulation or after exercise. Muscle glucose transport is mediated through the glucose transporter proteins GLUT1 and GLUT4 (1), with most of the effects of exercise or insulin on glucose uptake being due to GLUT4 translocation. These two stimuli use distinct signaling pathways and are synergistic (2,3).

In type 2 diabetes, insulin resistance results in impaired insulin-mediated skeletal muscle glucose transport (4), due to defects in GLUT4 trafficking rather than changes in protein expression (5,6). In contrast, exercise-mediated glucose uptake is preserved (7). Hence, exercise may be used to prevent and treat type 2 diabetes (8), implicating the signaling pathways that couple exercise with glucose transport as potential therapeutic targets.

AMP-activated protein kinase (AMPK) is activated during exercise in rodent muscle (9–11) and is proposed to mediate the effects of exercise on muscle glucose uptake. AMPK may be pharmacologically activated by AICAR, which undergoes intracellular metabolism to 5-aminoimidazole-4-carboxamide ribonucleoside (ZMP), which mimics the AMP effects on AMPK signaling (12). Perfusion of rat hindlimb muscle with AICAR caused accumulation of ZMP, activating AMPK and increasing glucose uptake (13). In rat muscle AICAR increases 2-deoxyglucose (2DG) uptake because of an effect on transport rather than metabolism (14). Muscle AMPK is also activated by metformin (15), thiazolidinediones (16), and adiponectin (17), resulting in increased glucose uptake. In transgenic mice AMPK is necessary for the effect of AICAR on muscle glucose uptake. Knocking out AMPK α_2 (18), expressing a dominant negative AMPK mutant (19), or knocking out the upstream kinase LKB1 (20), led to an abolition of the stimulatory effect of AICAR on muscle glucose uptake. However, the effects of contraction on glucose uptake were more variable, with an almost complete abolition of the effect in the LKB1-deficient mice (20), no reduction in the α_2 knockouts (18), and a partial reduction in the mice expressing the dominant negative mutant (19).

In ex vivo human muscle, AICAR stimulates AMPK activity and glucose uptake twofold (21). Furthermore, in patients with type 2 diabetes, 4–10 weeks of metformin

significantly increased muscle AMPK activity, glycogen concentration, and whole-body glucose disposal (22). However, 6 months of rosiglitazone, but not of metformin, increased muscle glucose uptake during euglycemia-hyperinsulinemia and one-legged exercise (23). Although not measured, this finding suggests that muscle AMPK activity may not always correlate with glucose uptake.

AICAR is not a specific activator of AMPK. In rodent muscle and L6 myotubes, AICAR activates the extracellular signal–regulated kinase (ERK)/atypical protein kinase C (aPKC) pathway, resulting in increased glucose uptake (24). Similarly, metformin also acts through this accessory pathway, increasing aPKC basal expression and its response to insulin stimulation, which promotes skeletal muscle glucose uptake (25).

The in vivo effect of AICAR on whole-body or skeletal muscle glucose uptake has not been examined in humans. We determined whether AICAR would acutely increase muscle AMPK activity and glucose uptake or whole-body insulin sensitivity. 2DG uptake was determined as a surrogate marker of muscle glucose uptake.

RESEARCH DESIGN AND METHODS

Twenty-nine healthy men (aged 26 ± 8 years, BMI 25 ± 4 kg/m^2 [mean ± SD]) participated in the study. The subjects were habitually active at a recreational level. They adhered to their usual diet and refrained from strenuous physical activity for 2 days before the study. The study protocol was approved by the Tayside Ethics Committee and was carried out according to the Declaration of Helsinki, with subjects giving informed consent.

Experimental protocol. The subjects came to the laboratory after having fasted from 2000 h on the previous evening. A forearm vein of each arm was cannulated at the antecubital fossae for infusion of the 2DG (Sigma, Poole, U.K.) and/or AICAR (Toronto Research Chemicals, Toronto, ON, Canada), insulin (Actrapid, Novo Nordisk, Denmark), or 20% dextrose (MacoPharma, Twickenham, Middlesex, U.K.) and for blood sampling. 2DG was given as a primed, constant infusion (priming dose 10 mg/kg; infusion rate 6 mg · kg^{-1} · h^{-1}), AICAR was given at a rate of 10 mg · kg^{-1} · h^{-1}, insulin was given at 40 mU · m^{-2} · min^{-1}, and a variable 20% dextrose infusion was given to maintain plasma glucose at 5 mmol/l. Venus blood samples were taken to determine plasma 2DG, AICAR, glucose, and insulin concentrations. Quadriceps muscle biopsies were taken under 1% lignocaine anesthesia using the conchotome technique (26). All biopsies were taken through separate incisions, made from distal to proximal areas of the quadriceps.

Protocol 1: Accumulation of 2DG in skeletal muscle. To determine whether there was a linear increase in the muscle concentration of 2DG and 2-deoxyglucose 6-phosphate (2DG6P) under basal conditions, four volunteers (aged 25 ± 2 years; BMI 23 ± 1 kg/m^2) were given an infusion of 2DG for 9 h, with biopsies taken after 3, 6, and 9 h, and total 2DG accumulation was determined. No basal muscle biopsy was required, as 2DG is not naturally occurring.

Protocol 2: Effect of AICAR on skeletal muscle 2DG uptake and AMPK activity. To determine whether AICAR increases skeletal muscle glucose uptake and AMPK activity, six volunteers (aged 23 ± 3 years; BMI 25 ± 2 kg/m^2) were given an infusion of 2DG over 6 h, with the AICAR infusion starting after 3 h. Muscle biopsies were taken at 0, 3, and 6 h, with total 2DG accumulation, AMPK (total α-isoform) activity, and signaling changes measured.

Protocol 3: Effect of exercise on skeletal muscle 2DG uptake and AMPK activity. To confirm that, using our methods, we can detect increases in muscle 2DG uptake or AMPK activity or phosphorylation, 11 men (aged 29 ± 3; BMI 26 ± 1 kg/m^2) undertook 1 h of cycling at 70% of maximum heart rate, which has been shown to increase glucose uptake and AMPK phosphorylation (27). In four subjects, 2DG was infused continuously, muscle biopsies were taken after 3 h (but immediately before exercise) and 3 h after exercise, and total muscle 2DG uptake was determined. In seven subjects, muscle biopsies were taken at rest, immediately after and 3 h after exercise, and AMPK (Thr^{172}) phosphorylation measured.

Protocol 4: Time course of AMPK activity. To determine whether AICAR transiently activated AMPK, four volunteers (aged 27 ± 2 years; BMI 25 ± 1 kg/m^2) were given a 3-h AICAR infusion. Muscle biopsies were taken at 0 and 20 min and at 3 h, and AMPK (α_1 and α_2 activity) and signaling changes were measured.

Protocol 5: Euglycemic-hyperinsulinemic clamp. To determine whether AICAR increased whole-body glucose uptake, 20% glucose was infused at a variable rate to maintain plasma glucose at 5.0 mmol/l under conditions of hyperinsulinemia (40 mU · m^{-2} · min^{-1}) (28) for 3 h under basal conditions and for 3 h during an AICAR infusion. The amount of glucose metabolized (*M* value) was calculated relative to total body weight.

Analytical methods

Materials and antibodies. Except where otherwise stated, all chemicals were of the highest quality available from Sigma. Antibodies to phospho-specific and total protein kinase B (PKB) and glycogen synthase kinase (GSK) 3 were from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody to insulin receptor substrate-1 (IRS-1) (raised against 14 COOH-terminal amino acids) was purchased from Upstate Biotechnology (Lake Placid, NY), and β-actin was purchased from Sigma. Phospho-specific ERK1/2 antibody was from Cell Signaling Technology (Beverly, MA), and phospho-specific glycogen synthase (GS) was raised by the Division of Signal Transduction Therapy against a phosphopeptide based around site 3 of GS.

Plasma. Plasma was separated from whole blood by centrifugation (300*g*) immediately after collection, and the plasma was frozen until further analysis. Plasma glucose and lactate concentrations were measured with a YSI Stat2300 (Yellow Spring Instruments, Yellow Spring, OH). Plasma insulin (Invitrogen, Paisley, U.K.), cortisol (Cambridge Bioscience, Cambridge, U.K.), and glucagon (Cosmo Bio, Tokyo, Japan) were determined using commercially available enzyme-linked immunosorbent assay kits. AICAR was measured in perchloric acid extracts of plasma samples by capillary electrophoresis (20 mmol/l sodium phosphate buffer, pH 6.4) with reference to a standard curve of AICAR spiked into untreated plasma. Plasma 2DG was quantified by gas chromatography–mass spectrometry (MD800; ThermoFinnigan, Hemel Hempstead, U.K.). Briefly, 10 μl of internal standard (fluorodeoxyglucose [FDG]) was added to a 200-μl aliquot of plasma. The samples were passed through Dowex-Cl$^-$ resin and eluted with 3 ml of distilled water. The eluants were dried down and derivatized as the oxime/trimethylsilyl derivative (29). The concentrations were calculated with reference to a standard curve of 2DG spiked into untreated plasma and extracted as above.

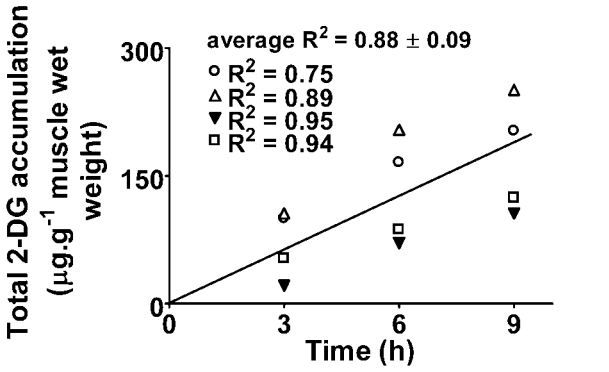
2DG and 2DG6P. Frozen muscle (30–40 mg) was ground under liquid nitrogen, and the frozen powder was transferred to 70% ethanol. The sample was vortex mixed and then centrifuged at 5,000*g* for 10 min. From this sample, the pellet containing the protein and nucleic acids was retained for glycogen analysis, and the supernatant was used for 2DG/2DG6P analysis. The supernatant was dried down under nitrogen and reconstituted in doubly distilled water (pH 9). The sample was then split into two equal fractions. The first fraction had 10 μl FDG added for determination of free 2DG concentration. The second fraction had 20 μl 100 mmol/l $MgCl_2$ and 1 unit of alkaline phosphatase (Promega, Madison, WI) added. The sample was vortex mixed and heated at 37°C for 50 min, 10 μl FDG was then added for determination of total 2DG, and both fractions were dried under nitrogen. The samples were then derivatized as above, and concentrations were determined with reference to a standard curve of known 2DG concentration spiked into untreated muscle and extracted as above. The concentration of 2DGP was calculated as the difference between total 2DG concentration and free 2DG concentration.

Muscle metabolites and glycogen. Muscle was extracted with perchloric acid and subjected to capillary electrophoresis to determine intracellular nucleotide and ZMP concentrations as described previously (30). The metabolite concentrations were determined as a ratio relative to ATP in the basal muscle sample and then normalized to known ATP concentrations (31)

The protein pellet, obtained after extraction of 2DG, was hydrolyzed in 2 N HCl at 100°C for 2 h and neutralized. The resulting free glucosyl units were assayed spectrophotometrically using a glucose oxidase/horseradish peroxidase assay (32).

AMPK α-isoform specific activity. Muscle lysates were prepared by homogenization of muscle tissue (1:20, wt/vol) in a buffer containing 50 mmol/l HEPES (pH 7.5), 150 mmol/l NaCl, 20 mmol/l sodium pyrophosphate, 20 mmol/l β-glycerophosphate, 10 mmol/l NaF, 2 mmol/l sodium orthovanadate, 2 mmol/l EDTA, 1% Nonidet P-40, 10% glycerol, 2 mmol/l phenylsulfonylmethyl fluoride, 1 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 3 mmol/l benzamidine. Lysates were obtained from the supernatant fraction after a 10-min centrifugation at 13,000*g*. AMPK α-isoform specific activity was measured in immunoprecipitate from 100 μg of muscle lysate protein by use of antibodies raised against the α_1 and α_2 subunits bound to protein G–Sepharose beads. A P81-filter paper assay, using AMARA peptide (200 μmol/l) as substrate, was used to measure AMPK activity in the presence of a saturating AMP concentration (0.2 mmol/l) (33).

Western blotting. Muscle biopsies were powdered under liquid nitrogen and homogenized in 0.5 ml of ice-cold lysis buffer (25 mmol/l Tris/HCl, pH 7.4, 50 mmol/l NaF, 100 mmol/l NaCl, 1 mmol/l sodium vanadate, 5 mmol/l EGTA, 1 mmol/l EDTA, 1% [vol/vol] Triton X-100, 10 mmol/l sodium pyrophosphate, 0.27 mol/l sucrose, complete protease inhibitor cocktail tablets [1 tablet/10



Time (h)	2DG	2DG-6-P	Total 2DG
	(μg.g^{-1} muscle wet weight)		
3	21.0 ± 10.4	49.1 ± 21.0	70.1 ± 40.0
6	49.8 ± 27.6	81.9 ± 36.4	131.7 ± 63.6
9	69.9 ± 34.1	101.0 ± 36.6	171.0 ± 68.3

FIG. 1. Linear accumulation of 2DG during a 9-h infusion with skeletal muscle biopsies taken at 3 hourly intervals (3, 6, and 9 h) (n = 4). Values are means ± SD.

ml], and 0.1% [vol/vol] 2-mercaptoethanol). Lysates were obtained from the supernatant fraction after a 10-min centrifugation at 13,000*g*, and precleared for 1 h at 4°C with protein G–Sepharose in PBS (50%, vol/vol) to remove contaminating antibodies present because of the variable amount of blood in the samples. Protein from the muscle lysates (30–40 μg) was separated on Novex SDS/4–12% polyacrylamide gels. After transfer to nitrocellulose, blots were blocked with 5% (wt/vol) nonfat milk in TBST (Tris-buffered saline containing 0.1% [vol/vol] Tween 20) for 1 h and incubated with primary antibodies at 4°C overnight before incubation for 1 h at room temperature with the secondary antibody and development using an enhanced chemiluminescence kit (Amersham Biosciences).

For the muscle samples from the AICAR and 2DG infusion study, antibodies to PKB (Ser^{308}, 1:1,000) and GSK3 (Ser$^{9/21}$, 1:2,000) were used. After phospho-specific analysis, membranes were stripped with 1× Western Re-Probe (Oncogene Research Products, San Diego, CA) and incubated with PKB (1:1,000) and GSK3 (1:2,000). Secondary anti-rabbit antibodies (1:25,000) were from Pierce Biotech (Rockford, IL). Results are expressed relative to the total protein. For the muscle samples from the AMPK time course study, antibodies to ERK1/2 (P-Thr^{202}/Tyr^{204}, 1:1,000), GS (phospho-site 3, 1:1,000), PKB (P-Ser^{473}, 1:1,000), and IRS-1 (total, 1:1,000) were used. Results are expressed relative to β-actin (COOH-terminal fragment). Protein bands were scanned and quantified by densitometry using AIDA Image Analyzer software.

Calculation of glucose uptake. Rates of muscle 2DG uptake were calculated by determining the increase in the concentration of 2DG and 2DG6P between consecutive muscle biopsies over each time period, assuming that the following formula is representative of all hexose uptake:

$$2DGU = ([DG + DG6P])_2 - ([DG + DG6P])_1/t$$

where 2DGU is deoxyglucose uptake, DG is the concentration of 2-deoxyglucose, DG6P is the concentration of 2-deoxyglucose 6-phosphate, t is time, and the subscripted numbers 1 and 2 denote the first and second muscle biopsy samples in which the DG/DG6P concentrations are determined.

Data and statistical analysis. Data are expressed as means ± SE. Results were compared using a paired t test or a repeated-measures ANOVA and a Bonferroni post test (when there were three or more data sets). The null hypothesis was rejected at the 5% level ($P < 0.05$).

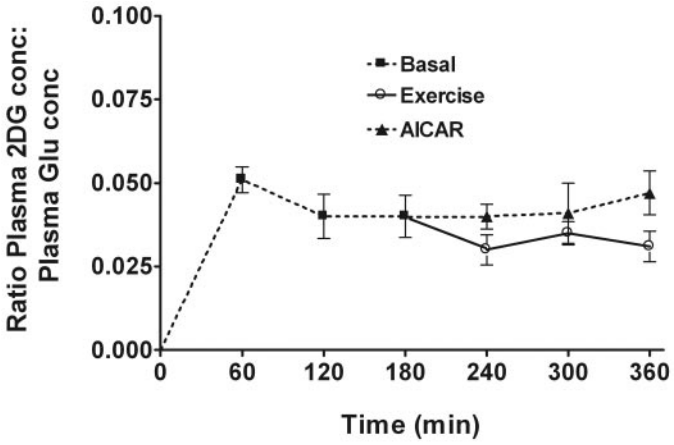


FIG. 2. Ratio of plasma 2DG concentrations to plasma glucose concentration during a 6-h 2DG infusion with a final 3-h AICAR infusion (▲, n = 6) or exercise (○, n = 4). Values are means ± SD.

RESULTS

Accumulation of 2DG under basal conditions. Plasma 2DG concentrations were at steady state throughout the study (data not shown). 2DG muscle uptake increased in a linear fashion in all four individuals (mean R^2 = 0.88 ± 0.09) (Fig. 1).

Glycogen content. There was a nonsignificant fall in muscle glycogen during the study from a 3-h concentration of 19 ± 1.9 to 14 ± 1.5 mmol glucosyl units/kg wet weight of muscle after 9 h.

Plasma lactate and hormone concentrations. Plasma lactate, cortisol, and glucagon remained constant over the course of the study (1.9 ± 0.3 mmol/l, 7.8 ± 1.0 μg/dl, and 28.4 ± 8.4 pg/ml, respectively; grand means).

Stimulation of muscle 2DG uptake during AICAR infusion and exercise

2DG and glucose concentrations. During the AICAR infusion, neither plasma 2DG nor plasma glucose (Table 1) concentrations were significantly different. The ratio of plasma 2DG to glucose was unchanged with AICAR. During exercise, the ratio of plasma 2DG to glucose decreases nonsignificantly immediately after exercise (Fig. 2).

Insulin and lactate concentrations. Plasma insulin (Table 1) and lactate remained constant before and during the AICAR infusion (10.7 ± 2.2 mU/l and 1.7 ± 0.5 mmol/l, respectively). During exercise, the plasma lactate concentration increased from 1.7 ± 0.7 to 3.6 ± 1.5 mmol/l immediately after exercise and remained elevated at 2.7 ± 1.7 mmol/l during recovery.

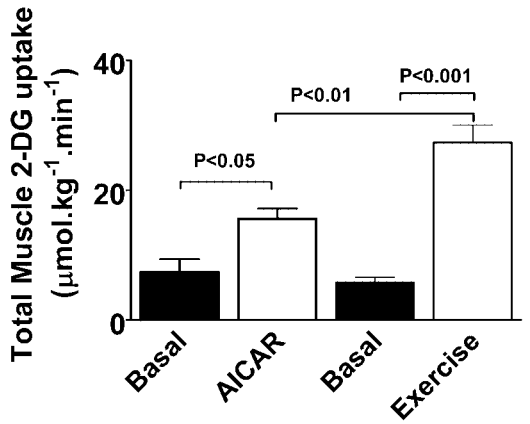
2DG uptake. There was a 2.1-fold increase in 2DG uptake after the 3-h AICAR infusion. In response to exercise, there was a 4.7-fold increase in 2DG uptake (Fig. 3).

Glycogen content. There was a nonsignificant fall in muscle glycogen concentration during the AICAR infusion

TABLE 1
Time course of plasma glucose, insulin, and AICAR concentrations

	2DG infusion				2DG and AICAR infusion		
	0 min	60 min	120 min	180 min	240 min	300 min	360 min
Glucose (mmol/l)	5.0 ± 0.4	5.6 ± 0.4	5.8 ± 1.1	6.7 ± 0.4	6.1 ± 1.0	5.8 ± 1.2	5.7 ± 1.3
Insulin (mU/l)	7.6 ± 1.8	8.3 ± 2.7	10.5 ± 2.1	11.1 ± 2.3	11.2 ± 3.9	13.4 ± 3.5	12.7 ± 3.7
AICAR (mmol/l)					0.18 ± 0.01	0.18 ± 0.04	0.17 ± 0.03

Data are means ± SD.



	2DG	2DG-6P	total 2DG
	concentration (μmol.kg^{-1}.min^{-1})		
Basal	2.6 ± 1.2	4.7 ± 5.0	7.3 ± 4.9
+AICAR	2.7 ± 1.9	12.6 ± 4.5	15.3 ± 4.3
Basal	1.8 ± 0.8	4.0 ± 1.3	5.8 ± 1.6
+Exercise	5.2 ± 2.8	22.1 ± 6.1	27.3 ± 5.4

FIG. 3. Rates of skeletal muscle glucose uptake (micromoles per kilogram per minute) before and after a 3-h AICAR infusion (n = 6). Values are means ± SE.

study: 26 ± 3.7, 21 ± 3.7, and 17 ± 2.4 mmol glucosyl units/kg wet weight for basal, 3 h, and 6 h, respectively.

AMPK activity and phosphorylation of acetyl-CoA carboxylase. There was no significant change in total AMPK α-isoform activity or in the phosphorylation of acetyl-CoA carboxylase (ACC), a downstream target of AMPK, in response to AICAR (Fig. 4*A*). Phosphorylation of AMPK increased immediately after cycling exercise (474%; $P < 0.01$) and remained elevated (162%; $P < 0.05$) for 3 h after exercise (Fig. 4*C*).

Western blot analysis of signaling proteins (data not shown). In response to AICAR, PKB and GSK3 phosphorylation was unchanged.

Time course of AMPK activity

Glucose and hormone concentrations. Glucose, insulin, and glucagon concentrations were stable throughout the AICAR infusion at 4.95 ± 0.58 mmol/l, 6.5 ± 2.2 mU/l, and 22.5 ± 5.7 pg/ml, respectively.

AICAR concentration. The venous plasma AICAR concentration remained constant throughout at 0.18 ± 0.03 mmol/l (Table 1).

AMPK activity and phosphorylation of AMPK and ACC. AMPK α_1 and α_2 activities were unchanged after 20 min or 3 h of AICAR. Phosphorylation of AMPK or ACC also remained unchanged (Fig. 4*B* and *C*).

Western blot analysis of signaling proteins. Phosphorylation of ERK1/2 increased after 20 min and was significant after 3 h of AICAR infusion (Fig. 5). Phosphorylation of GS, PKB, and total IRS-1 levels (Fig. 5) was unchanged.

Metabolites. There was no change in the ratio of AMP, ADP, and ATP during 3 h of AICAR infusion. By 3 h the ZMP concentration was 77% of the AMP concentration, being undetectable at 0 or 20 min (Table 2).

Effect of AICAR on whole-body glucose uptake. The M value increased in all four subjects by a mean of 7% from 9.3 ± 0.6 to 10 ± 0.6 mg · kg^{-1} · min^{-1} ($P < 0.05$). The plasma insulin values rose from a baseline value of 9 ± 1.7 to 159 ± 11.4 mU/l after 180 min of insulin to 171 ± 10.7 mU/l after 180 min of insulin and AICAR ($P < 0.001$, baseline vs. 180 and 360 min). In contrast, plasma cortisol fell from a baseline value of 7.5 ± 0.4 to 5.8 ± 1.1 μg/dl after 180 min of insulin to 4.9 ± 0.9 μg/dl after 180 min of insulin and AICAR ($P < 0.05$, baseline vs. 360 min). There was no change in plasma glucagon concentration (21.2 ± 3.2 pg/ml; grand mean).

DISCUSSION

In healthy young men, an acute AICAR infusion is associated with *1*) a twofold increase in skeletal muscle glucose uptake after 3 h; *2*) no significant change in AMPK α_1 and α_2 activities; *3*) increased ERK1/2 phosphorylation; *4*) no change in phosphorylation of PKB, GS, or total IRS1; and *5*) a small change in whole-body glucose disposal. The in vivo effect of AICAR on skeletal muscle glucose uptake or on AMPK activation has not previously been examined in humans. As a surrogate measure of glucose uptake, we used 2DG, a glucose analog that closely resembles glucose in the characteristics of its transport but is metabolized only to the 6-phosphate derivative. Thus, 2DG6P is effectively trapped within skeletal muscle where its concentration (and that of 2DG) can be determined as a function of glucose uptake, assuming 2DG uptake to be representative of all hexose uptake. 2DG, as [^{14}C]2DG, [^{3}H]2DG (4), or [^{18}F]2DG (for positron emission tomography) (23), is routinely used to determine changes in tissue glucose transport.

We believe that 2DG is a robust surrogate measure of glucose uptake, and the rise in total muscle 2DG during any intervention is not due to methodological reasons (i.e., delayed equilibration of the plasma 2DG with the intracellular space). Postabsorptively, we demonstrated a linear accumulation of total muscle 2DG during a 9-h 2DG infusion (R^2 = 0.88 ± 0.09). Also, exercise, a known stimulus of skeletal muscle glucose uptake, significantly and consistently increased muscle 2DG uptake. 2DG is more likely to inhibit, rather than stimulate, glucose uptake via allosteric inhibition of hexokinase by 2DG6P (34), although 2DG6P is a very poor inhibitor (35). The linear accumulation of 2DG suggests that 2DG is neither stimulating (through a "mass effect") nor inhibiting (through hexokinase inhibition) its own uptake over 9 h.

We observed a 2.1-fold stimulation of glucose uptake with AICAR, consistent with the results of animal studies (13,36). Incubation of human skeletal muscle strips with 1–2 mmol/l AICAR increased glucose transport 2.6-fold (21). The magnitude of the stimulation of 2DG uptake observed with AICAR was less than one-half that seen with acute exercise, a known stimulator of glucose uptake, and may explain the small effect of AICAR on whole-body glucose disposal. Alternatively, the small whole-body effect may be due to the increased muscle glucose uptake being counteracted by increased hepatic glucose output (37).

The stimulation of 2DG uptake after AICAR infusion for 3 h is likely to be insulin independent. Insulin concentrations were at postabsorptive values throughout, and components of the insulin-regulated phosphatidylinositol 3-kinase–dependent signaling pathway (PKB and GSK3) were unaltered 3 h after AICAR. Unlike the results from animal studies or ex vivo human incubations, we saw no significant increase of AMPK activity after 20 min or 3 h of AICAR. This lack of stimulation of muscle AMPK activity with AICAR was surprising, in view of the evidence from

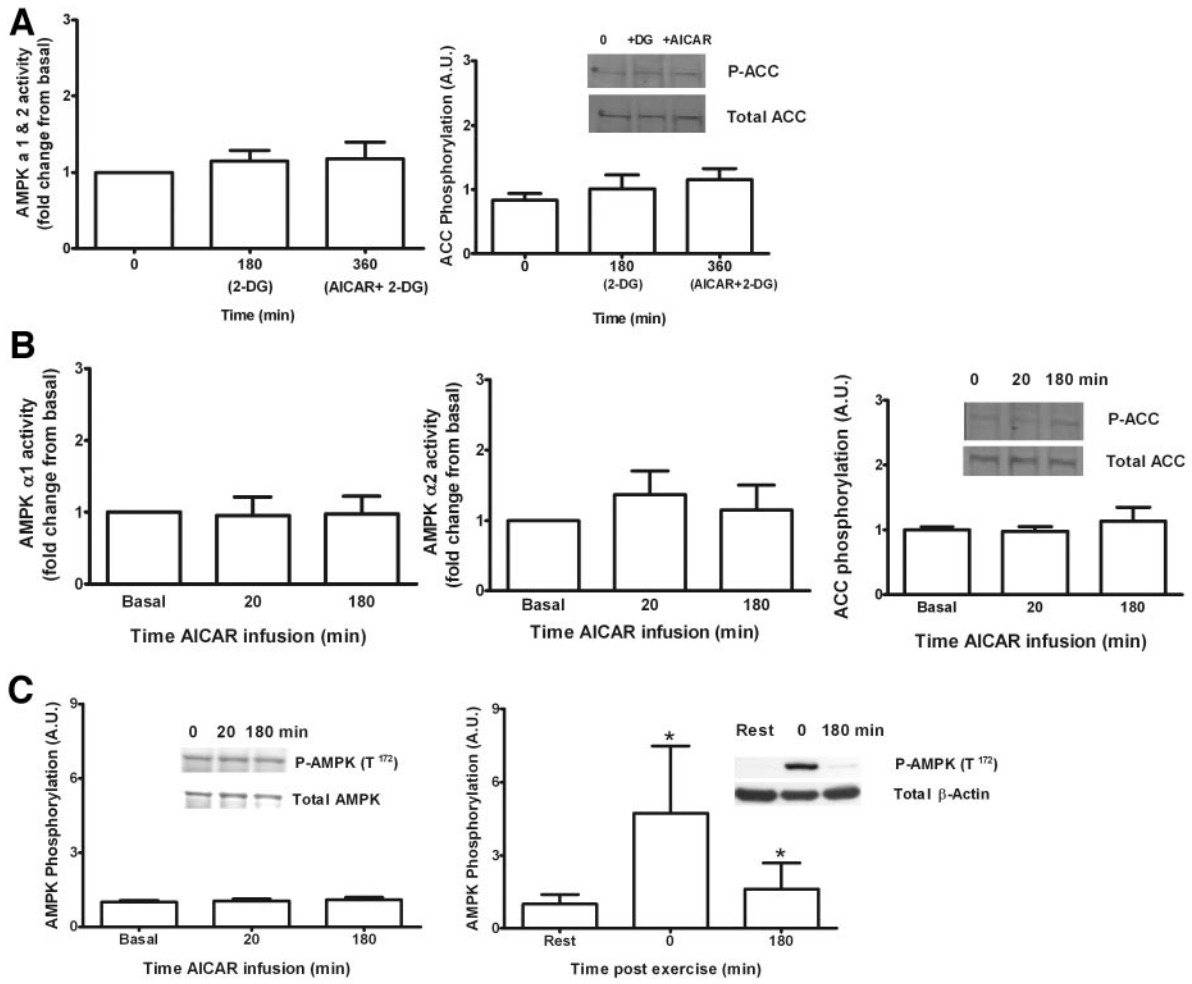


FIG. 4. *A*: AMPK α_1 and α_2 activity and ACC phosphorylation at baseline, after 2DG, and after a 3-h AICAR infusion; NS ($n = 6$). *B*: AMPK α_1 and α_2 activity and ACC phosphorylation at baseline ($n = 4$), after 20-min ($n = 4$) and 180-min ($n = 4$) AICAR infusions. *C*: Representative Western blots of AMPK phosphorylation (Thr^{172}) at baseline ($n = 4$), after 20-min ($n = 4$) and 180-min ($n = 4$) AICAR infusions and at rest, immediately after exercise, and 180 min after exercise ($n = 7$).

studies in transgenic mice demonstrating that AMPK is essential for AICAR-induced glucose uptake (18,19,20). The lack of activation of AMPK at 3 h might reflect a transient activation of AMPK, similar to that observed in rat skeletal muscle in response to muscle contraction, with persistence of glucose uptake despite the rapidly decreasing AMPK activity (38). In rat hepatocytes and adipocytes, AMPK activation by AICAR peaks at 20 min and then rapidly falls (12). However, in additional time course studies, we were unable to detect any change in AMPK activity or phosphorylation after 20 min of AICAR infusion; therefore, transient activation was considered unlikely. Furthermore, phosphorylation of ACC, a downstream target of AMPK, did not change.

This failure to detect any rise in AMPK activity or phosphorylation was not due to our techniques, as immediately after exercise we observed an ~5-fold increase in AMPK phosphorylation, which remained elevated at 3 h. AMPK phosphorylation closely mirrors its activity, suggesting that our methodology would allow us to detect changes in kinase activity in response to AICAR.

There are several possible explanations for the fact that AMPK activation was not observed in parallel with the stimulation of glucose uptake after an AICAR infusion. It may be that in human skeletal muscle a small increase in AMPK activity (relative to exercise-induced AMPK activation) is sufficient to increase subsequent signaling pathways, which stimulate GLUT4 translocation and glucose uptake. However, AMPK activity normally increases 150–200% in response to treatments including AICAR, metformin, or exercise (21,37,39,40). A lesser dose of AICAR ($6\ mg \cdot kg^{-1} \cdot h^{-1}$) is clinically effective in preventing adverse cardiovascular outcomes in patients undergoing coronary artery bypass graft surgery (41). Therefore, this dose of AICAR has clinically and physiologically significant effects in cardiac muscle. Alternatively, AMPK activation in other tissues may regulate skeletal muscle glucose uptake. AMPK $\alpha_2^{-/-}$ mice have reduced insulin-stimulated whole-body glucose utilization and muscle glycogen synthesis rates despite normal glucose transport in isolated muscles (42). This observation suggests an indirect action on muscle, perhaps via adipose tissue or through modulation of the sympathetic nervous system. A final possibility is that AICAR might be stimulating human muscle glucose uptake via an AMPK-independent action (43).

Among a range of signaling proteins, only phosphorylation of ERK1/2 (a member of the mitogen-activated protein kinase family) was significantly increased by AICAR; there were no changes in phosphorylation of PKB or GS or in total IRS-1 expression. In cultured L6 cells, the stimulation of GLUT4 translocation and glucose uptake by AICAR has

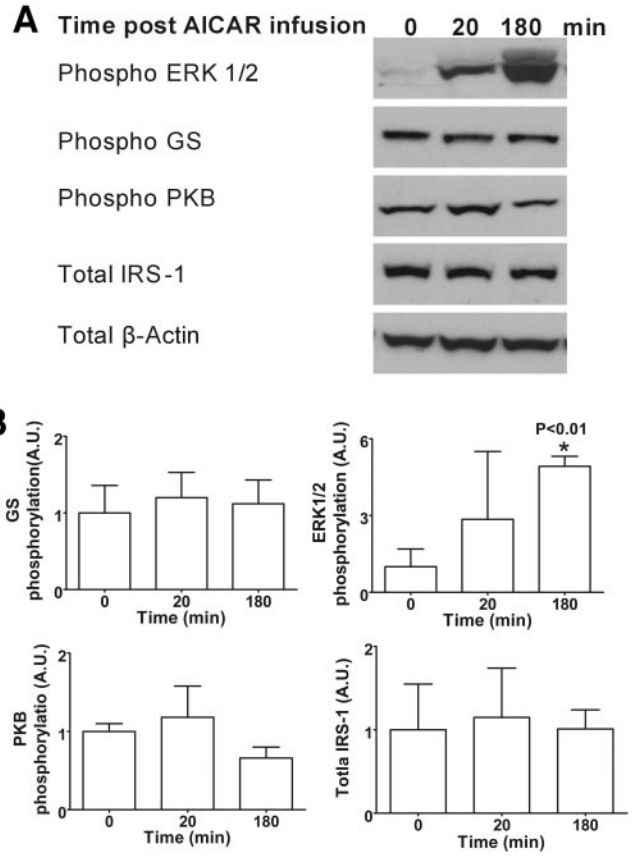


FIG. 5. Representative Western blots of phospho-ERK1/2, GS, S6, PKB, and total IRS-1 at baseline (n = 4) and after 20-min (n = 4) and 180-min (n = 3) AICAR infusions.

been shown to be mediated, at least partly, through sequential activation of mitogen-activated protein kinase kinase 1/2, ERK1/2, phospholipase D, phosphatidic acid, and aPKCs (24). Furthermore, inhibition of any of the components of the ERK pathway (using a mitogen-activated protein kinase kinase-1 inhibitor, a phospholipase D inhibitor, and expression of inactive ERK and protein kinase C-ζ) abolished in parallel the AICAR-induced stimulation of glucose uptake. In our studies, the activation of ERK1/2 does not appear to be downstream of AMPK, as we see no increase in AMPK activity or phosphorylation after 20 min, whereas ERK1/2 phosphorylation has increased. Without the use of specific kinase inhibitors in human in vivo studies, it is not possible to definitively conclude that ERK1/2 mediates the stimulation of human skeletal muscle glucose uptake by AICAR. However, increased ERK1/2 phosphorylation, rather than activity of AMPK, is associated with the stimulation of 2DG uptake.

A decrease in muscle glycogen concentration (~50%) stimulates basal glucose uptake in rat skeletal muscle (44,45). However, we saw a nonsignificant (~20%) fall in glycogen concentration during the AICAR infusion. A similar reduction in glycogen concentration was observed in subjects infused only with 2DG, with no effect on muscle glucose uptake. This finding suggests that increased 2DG uptake after AICAR is mediated by AICAR itself and not falling glycogen stimulating glucose uptake. Finally, the AICAR infusion was well tolerated and was associated with no or minimal side effects in our study and in other studies (41,46).

In summary, we report the first observations that acute in vivo AICAR administration increases human skeletal muscle 2DG uptake and whole-body glucose disposal. In skeletal muscle, this increase was associated with ERK1/2 activation but with no effect on AMPK.

TABLE 2
Skeletal muscle metabolites

Time	ATP	ADP	AMP	ZMP
0 min	21.8 ± 4.5	3.3 ± 0.5	99 ± 43	
20 min	25.1 ± 8.6	3.7 ± 1.3	94 ± 47	
180 min	24.2 ± 3.7	3.7 ± 0.8	91 ± 29	68 ± 13

Data are means ± SD. n = 4 for each time point. ATP and ADP are expressed as millimoles per kilogram of muscle dry weight, and AMP and ZMP are expressed as micromoles per kilogram muscle dry weight.

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