

AMP-Activated Protein Kinase (AMPK) Is Activated in Muscle of Subjects With Type 2 Diabetes During Exercise

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Insulin-stimulated GLUT4 translocation is impaired in people with type 2 diabetes. In contrast, exercise results in a normal increase in GLUT4 translocation and glucose uptake in these patients. Several groups have recently hypothesized that exercise increases glucose uptake via an insulin-independent mechanism mediated by the activation of AMP-activated protein kinase (AMPK). If this hypothesis is correct, people with type 2 diabetes should have normal AMPK activation in response to exercise. Seven subjects with type 2 diabetes and eight matched control subjects exercised on a cycle ergometer for 45 min at 70% of maximum workload. Biopsies of vastus lateralis muscle were taken before exercise, after 20 and 45 min of exercise, and at 30 min postexercise. Blood glucose concentrations decreased from 7.6 to 4.77 mmol/l with 45 min of exercise in the diabetic group and did not change in the control group. Exercise significantly increased AMPK $\alpha 2$ activity 2.7-fold over basal at 20 min in both groups and remained elevated throughout the protocol, but there was no effect of exercise on AMPK $\alpha 1$ activity. Subjects with type 2 diabetes had similar protein expression of AMPK $\alpha 1$, $\alpha 2$, and $\beta 1$ in muscle compared with control subjects. AMPK $\alpha 2$ was shown to represent approximately two-thirds of the total α mRNA in the muscle from both groups. In conclusion, people with type 2 diabetes have normal exercise-induced AMPK $\alpha 2$ activity and normal expression of the $\alpha 1$, $\alpha 2$ and $\beta 1$ isoforms. Pharmacological activation of AMPK may be an attractive target for the treatment of type 2 diabetes. *Diabetes* 50:921–927, 2001

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ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; AMPKK, AMPK kinase; DTT, dithiothreitol; FFA, free fatty acid; PI, phosphatidylinositol; PCR, polymerase chain reaction; RT, reverse transcriptase; TBST, Tris-buffered saline with Tween; W_{max} , maximum workload; ZMP, 5-aminoimidazole-4-carboxamide ribonucleotide.

Exercise is an important component of the treatment of type 2 diabetes (1). An acute bout of exercise increases glucose disposal into the contracting muscles, leading to clinically significant decreases in blood glucose concentrations (2–4). Despite the therapeutic effects of exercise on the glycemic control of subjects with type 2 diabetes, the underlying molecular mechanisms responsible for this phenomenon are not fully understood. Both insulin and contraction increase glucose uptake into skeletal muscle through a mechanism that involves translocation of the GLUT4 glucose transporter from an intracellular location to the cell surface (2,3), but there is evidence suggesting that the mechanisms leading to insulin- and exercise-stimulated GLUT4 translocation and glucose uptake are different (3,4).

In type 2 diabetes and severe obesity, there are decreases in insulin receptor tyrosine kinase activity, insulin receptor substrate-1-associated tyrosine phosphorylation, and insulin-stimulated phosphatidylinositol (PI) 3-kinase activation (5–8). Both the obese Zucker rat (9) and subjects with type 2 diabetes (10,11) have defective insulin-stimulated glucose uptake and GLUT4 translocation, despite normal levels of total GLUT4 protein in muscle. In contrast, contraction-stimulated glucose uptake and GLUT4 translocation in the Zucker rat (12–14) and in diabetic subjects (15) are normal, providing evidence that exercise might be able to bypass these defects in insulin signaling.

AMP-activated protein kinase (AMPK) has recently emerged as a potentially key signaling intermediary in the regulation of exercise-induced changes in glucose (16–18) and lipid (16,19) metabolism in skeletal muscle. This enzyme is a member of a metabolite-sensing protein kinase family that is activated in response to alterations in cellular energy levels (20–22). AMPK activation is mediated by increases in the AMP-to-ATP and creatine-to-phosphocreatine ratios through different mechanisms involving allosteric regulation of AMPK subunits, activation by an upstream AMPK kinase (AMPKK), and decreases in the activity of phosphatases (20,21). In the rat, exercise in vivo (23,24), sciatic nerve-stimulated muscle contractions in situ (25,26), and contraction of isolated muscles in vitro (17,22,27) all significantly increase the activity of AMPK. More recently, studies have shown that in nondiabetic

humans, an acute bout of moderate intensity exercise significantly increases AMPK activity (28,29). AMPK is a protein that is fully active as a heterotrimer, consisting of one catalytic subunit (α) and two regulatory subunits (β and γ) (20,21). Two isoforms of the catalytic subunit have been identified ($\alpha 1$ and $\alpha 2$). Whereas AMPK $\alpha 1$ is widely distributed, AMPK $\alpha 2$ is predominantly localized in skeletal muscle, heart, and liver (30).

There is significant evidence in support of a role for AMPK in the regulation of glucose uptake. Most of this evidence comes from studies using the AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), which is phosphorylated within the cell to 5-aminoimidazole-4-carboxamide ribonucleotide (ZMP) (16). ZMP then mimics the effects of AMP, causing activation of AMPK both by allosteric modification and phosphorylation by AMPKK (31). In vitro studies have shown that activation of AMPK with AICAR leads to increases in glucose uptake in skeletal muscle (17,18) and heart (32) through a PI 3-kinase independent pathway, and this increase is associated with GLUT4 translocation to the plasma membrane (32,33). Chronic treatment with AICAR also significantly increases the content of GLUT4 protein in muscle (34,35), an adaptive process similar to that observed with exercise training.

Despite an increasing interest in the potential role of AMPK in the pathogenesis of diabetes (36) and the possible use of AMPK-stimulating agents as treatment for this disease (21,33,35,36), there are no studies evaluating the function of AMPK in this population. Given that subjects with type 2 diabetes have normal exercise-stimulated GLUT4 translocation and glucose uptake in muscle, we hypothesized that if AMPK is the key signaling intermediary mediating this effect, then subjects with type 2 diabetes should have normal activation of AMPK in response to exercise. This is the first study to demonstrate that an acute bout of exercise leads to a significant increase in AMPK $\alpha 2$ activity in the skeletal muscle of people with type 2 diabetes. This finding supports the idea that increasing glucose disposal into skeletal muscle through pharmacological activation of the AMPK could be a novel strategy for the treatment of type 2 diabetes.

RESEARCH DESIGN AND METHODS

A total of 15 male subjects (7 diabetic and 8 healthy volunteers) participated in this study, which was approved by the Ethical Committee at the Karolinska Institute. All subjects were informed of the purpose of the study and written consent was obtained after all procedures were explained. Subjects with evidence of cardiovascular disease or other conditions that would preclude their ability to exercise on a cycle ergometer were excluded. They were also excluded if they had hepatic, renal, or hematologic disorders. All the diabetic subjects were taking sulfonylureas, and to avoid hypoglycemia, the medication was discontinued 24 h before performing the exercise protocol. Subjects were otherwise excluded if they were taking insulin, metformin, thiazolidinediones, cholesterol-lowering medication, or other agents known to affect carbohydrate metabolism. All subjects underwent a 2-h 75-g oral glucose tolerance test to confirm the diagnosis of type 2 diabetes. Subjects in the control group underwent the glucose tolerance test to exclude impaired glucose tolerance, according to defined criteria (37).

Experimental protocol. To determine the appropriate intensity for the acute bout of exercise, subjects performed an incremental workload test on a cycle ergometer several days before participating in the study protocol (15 W/min increase starting at 40 W until exhaustion). The maximum workload the subjects could sustain for 6 min (W_{\max} 6') was calculated (38). All subjects were placed on a weight-maintaining standardized diet for 2 days before the experiment. The diet consisted of 30 $\text{kJ} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ given as 45% carbohydrate, 15% protein, 40% fat, and water ad libitum. All experiments were performed after a period of 6–9 h of fasting.

On arrival at the Clinical Research Center on the day of the exercise protocol, the subjects initially rested for 30 min in the supine position. An intravenous cannula was inserted in an antecubital vein, and a blood sample was taken for determination of pre-exercise insulin (Insulin RIA 100; Pharmacia, Uppsala, Sweden) and free fatty acid (FFA) (Wako Chemicals, Richmond, VA) concentrations, followed by a basal percutaneous needle biopsy obtained from the vastus lateralis muscle. At 5 min after injection of 2–5 ml Citanest for local anesthesia, a needle was inserted through an incision and ~100 mg wet wt muscle tissue was removed (39). The muscle tissue was immediately dissected free from fat and connective tissue and placed in liquid nitrogen. The subjects then exercised on a cycle ergometer at a workload corresponding to 70% of their W_{\max} for a total of 45 min as follows: after 18 min, exercise was stopped, local anesthesia was given as described above, and exercise was continued for an additional 2 min. The time elapsed during this interruption was only 7 ± 2 s. After a total of 20 min of exercise, the subjects moved to a bed and a second muscle biopsy was obtained (20 min sample). Exercise then proceeded, and after 43 min of exercise, the same anesthetic procedure was performed as described above, followed by a third biopsy 2 min later (45 min sample). The time between cessation of exercise and obtaining the biopsies (20 and 45 min samples) was 12 ± 4 s. After the cessation of exercise, the subjects moved to a bed for a 30-min period of rest, followed by a final biopsy. Blood glucose (40) and plasma lactate (41) concentrations were measured before, during, and after exercise using the oxidase method. At the end of the exercise protocol, blood samples were obtained for measurement of insulin and FFA concentrations.

Glycogen concentrations. Muscle samples were homogenized in 1 mol/l HClO_4 at -10°C . An aliquot of muscle homogenate was hydrolyzed in 2N HCl at 100°C for 2 h followed by neutralization with 2N NaOH, and glycogen content was measured by the hexokinase enzymatic method using the glucose HK reagent (Sigma, St. Louis, MO).

Muscle processing. Muscle samples were weighed and then homogenized using a Polytron (Brinkmann Instrument) in ice-cold lysis buffer (1:100, wt/vol) containing 20 mmol/l Tris-HCl (pH 7.4), 1% Triton-X 100, 50 mmol/l NaCl, 250 mmol/l sucrose, 50 mmol/l NaF, 5 mmol/l sodium pyrophosphate, 2 mmol/l dithiothreitol (DTT), 4 mg/l leupeptin, 50 mg/l trypsin inhibitor, 0.1 mmol/l benzamide, and 0.5 mmol/l PMSF, and it was centrifuged at 14,000g for 20 min at 4°C . Supernatants were removed and used for determination of AMPK activity and protein content.

AMPK activity assay. Muscle lysates containing 200 μg protein were immunoprecipitated with specific antibodies to the $\alpha 1$ and $\alpha 2$ catalytic subunits of AMPK and protein A/G beads (Santa Cruz Biotechnology, Santa Cruz, CA). These are anti-peptide antibodies made to the amino acid sequences 339–358 of $\alpha 1$ and 352–366 of $\alpha 2$. Immunoprecipitates were washed in lysis buffer and wash buffer (240 mmol/l HEPES and 480 mmol/l NaCl). Kinase reactions were performed in 40 mmol/l HEPES (pH 7.0), 0.2 mmol/l AMP, 80 mmol/l NaCl, 0.8 mmol/l DTT, 5 mmol/l MgCl_2 , 0.2 mmol/l ATP (containing 2 μCi [^{32}P] γ -ATP), and 0.2 mmol/l synthetic AMPK substrate with the sequence HMRSAMSG-LHLVKRR (42) in a final volume of 40 μl for 20 min at 30°C . At the end of the reaction, a 20- μl aliquot was removed and spotted on Whatman P81 paper. The papers were washed six times in 1% phosphoric acid and one time with acetone. Radioactivity was quantitated with a scintillation counter. Activity was expressed as incorporated ATP (picomoles) per milligram of protein per minute.

Immunoblotting for AMPK $\alpha 1$, $\alpha 2$, and $\beta 1$. Proteins (40 μg) from muscle lysates were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. They were blocked in Tris-buffered saline with 0.05% Tween 20 (TBST) and 5% nonfat milk for 1 h at room temperature. The membranes were incubated overnight at 4°C with the specific antibodies to the $\alpha 1$ and $\alpha 2$ catalytic subunits of AMPK described above and $\beta 1$ antibody (3 $\mu\text{g}/\text{ml}$) (Transduction Laboratories, Lexington, KY) in TBST and 2.5% dry milk. Bound anti-AMPK $\alpha 1$ and $\alpha 2$ antibodies were detected with anti-rabbit immunoglobulin-horseradish-peroxidase-linked whole antibody, and anti- $\beta 1$ antibody was detected using anti-mouse IgG (Transduction Laboratories). The membranes were washed with TBST then incubated with enhanced chemiluminescence reagents (NEN Life Science Products, Boston, MA) and exposed to film. Bands were visualized and quantified using ImageQuant software (Molecular Dynamics).

Determination of expression levels of AMPK $\alpha 1$ and $\alpha 2$ mRNA. Total RNA was isolated by TRI Reagent (Molecular Research Center, Cincinnati, OH), containing guanidinium isothiocyanate and phenol. Total RNA (3.0 μg) was then reverse-transcribed to cDNA in the presence of 10 U/ml cloned Moloney murine leukemia virus reverse transcriptase (RT) (Gibco BRL, Gaithersburg, MD), 125 mmol/l each of dNTPs, 10 mmol/l DTT, 5 ng/ml random hexamer primers, and RT buffer (50 mmol/l Tris-HCl, pH 8.3, 75 mmol/l KCl, 3 mmol/l MgCl_2) to a total volume of 20 μl . A corresponding aliquot of the cDNA mixture synthesized from 100 ng of total RNA was subjected to polymerase chain reaction (PCR). The following PCR oligonu-

TABLE 1
Clinical and metabolic characteristics of the subjects

	Control subjects	Diabetic subjects
Age (years)	49 ± 1.3	53 ± 3.2
BMI	25 ± 1.5	26 ± 1
Glucose (mmol/l)	4.63 ± 0.1	7.6 ± 0.5*
2-h Oral glucose tolerance test (mmol/l)	5.66 ± 0.7	14.8 ± 1.2*
HbA _{1c} (%)	4 ± 0.2	6.4 ± 0.6†
Insulin (pmol/l)	84.4 ± 14	227 ± 49†
FFAs (mmol/l)	0.53 ± 0.05	0.46 ± 0.04
W _{max} (Watts)	120.5 ± 7	142.4 ± 11
Duration of diabetes (years)	—	5.2 ± 1.5

Data are means ± SE; *n* = 8 control (all male) and 7 diabetic subjects (all male). Glucose, insulin, and FFA measurements were performed after an overnight fast. **P* < 0.001 vs. control subjects; †*P* < 0.05 vs. control subjects.

cleotide primers, which hybridize to the conserved sequence of both the human AMPK α1 and α2 cDNAs, were used: sense 5'-CATATGCTGCAGGT-3' and antisense 5'-TIGAGIAGITCAACIGG-3' (I, inosine). The reaction solution for PCR consisted of 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 0.01% (wt/vol) gelatin, 125 μmol/l each of dNTPs, and 0.02 U/μl AmpliTaq Gold (Perkin-Elmer, Foster City, CA). Mixtures were preincubated at 94°C for 9 min to activate the AmpliTaq Gold. The PCR program consisted of the following steps: 94°C for 1 min (denaturation), 40°C for 1 min (annealing), and 72°C for 1.5 min (extension), for a total of 30 cycles. Thereafter, the incubation was continued at 72°C for 8.5 min to complete the polymerization. The amplified cDNAs of AMPK α1 and α2 should yield 840- and 931-bp fragments, respectively (Fig. 5). The products were electrophoretically separated on 4% agarose gels, which were stained with Vista Green fluorescent dye (Amersham International, Buckinghamshire, U.K.), and then analyzed using a Storm imager (Molecular Dynamics). The sequences of the PCR products were confirmed by automated sequencing.

Statistical analysis. Data are presented as means ± SE. For statistical evaluation, a one-way analysis of variance for repeated-measurement was used. Differences between means were determined using the Student-Newman-Keuls test.

RESULTS

Clinical and metabolic characteristics of the subjects. Subject characteristics are summarized in Table 1. There was no significant difference in the mean age, BMI, and W_{max} between the groups. The mean duration of type 2 diabetes was 5.2 years, ranging from 2 to 13 years. Subjects with diabetes had significantly higher fasting plasma glucose, insulin, and HbA_{1c} concentrations compared with the control subjects. The concentration of FFAs was not different between the groups.

Blood glucose, plasma insulin, lactate, FFAs, and muscle glycogen concentrations. The subjects performed cycle ergometry exercise at 70% W_{max}, and blood glucose concentrations were measured during the exercise protocol. As shown in Fig. 1, with 45 min of exercise, blood glucose concentrations significantly decreased from 7.6 ± 0.5 to 4.77 ± 0.6 mmol/l (*P* < 0.05) in the diabetic group. Exercise did not significantly change blood glucose levels in the control group. After 45 min of exercise, baseline plasma insulin concentrations decreased 61% (*P* < 0.05) and 59% (*P* < 0.05) in the diabetic and control groups, respectively. Plasma lactate concentrations increased during exercise, from 0.9 ± 0.13 to 2.58 ± 0.24 mmol/l (*P* < 0.01) and from 0.65 ± 0.06 to 2.21 ± 0.51 mmol/l (*P* < 0.01) in the diabetic and control groups, respectively, and there was no significant difference in plasma lactate concentra-

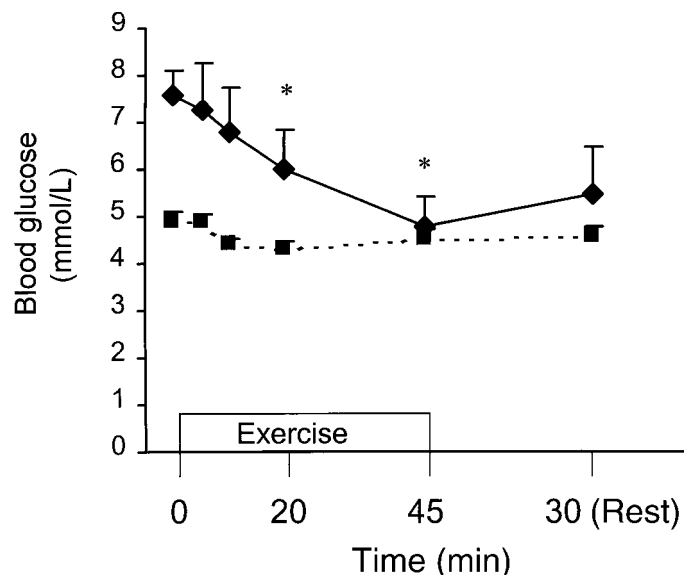


FIG. 1. Exercise decreases blood glucose concentrations in subjects with type 2 diabetes. Seven diabetic subjects (◆) and eight healthy subjects (■) performed cycle ergometry exercise at 70% W_{max} for the period indicated. In the diabetic group, glucose concentrations decreased significantly during the exercise protocol. Data are means ± SE. **P* < 0.05 vs. basal levels.

tions between the groups. The concentration of plasma FFAs did not change after 45 min of exercise in either group.

By the end of the protocol, glycogen concentrations in the vastus lateralis muscle decreased by 30% (*P* < 0.05) in the diabetic group and 33% (*P* < 0.05) in the control subjects (Fig. 2), confirming that this exercise protocol represents a significant metabolic stress that results in significant depletion of glycogen stores in muscle.

Effects of exercise on isoform-specific AMPK activity. The activity of the α1 and α2 AMPK catalytic subunits was measured before exercise, at 20 and 45 min of exercise, and 30 min postexercise. Exercise similarly increased

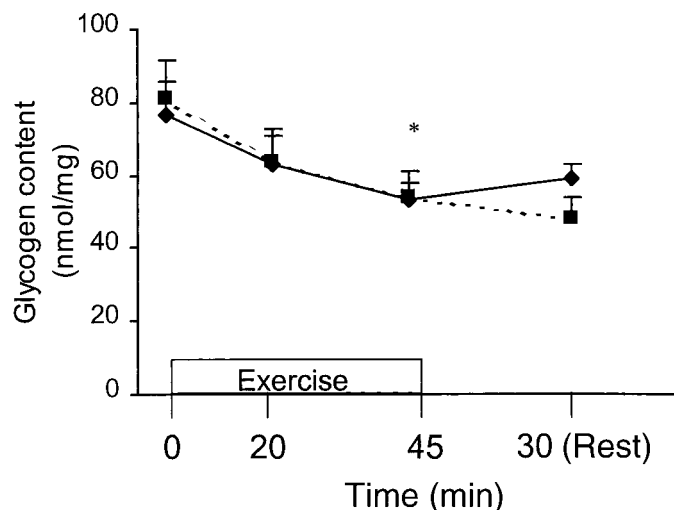


FIG. 2. Glycogen content in vastus lateralis muscle in response to exercise. Diabetic (◆) and control (■) subjects performed cycle ergometry exercise at 70% W_{max} for the period indicated, and muscle biopsies were obtained. Glycogen content was measured as described in RESEARCH DESIGN AND METHODS. There was a significant decrease in glycogen concentrations during exercise in both groups. Data are means ± SE; *n* = 7 and 8 for diabetic and healthy subjects, respectively. **P* < 0.05 vs. basal levels.

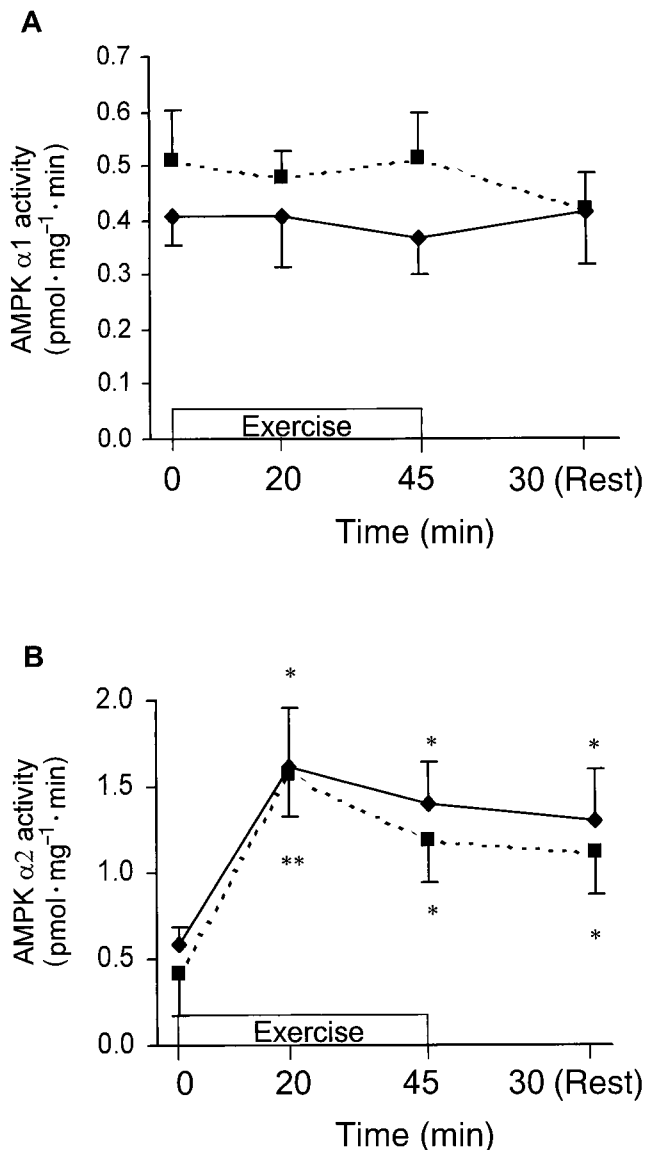


FIG. 3. Exercise increases AMPK $\alpha 2$ activity in subjects with type 2 diabetes. Diabetic (\blacklozenge) and control (\blacksquare) subjects performed cycle ergometry exercise for the period indicated and multiple biopsies of vastus lateralis muscles were obtained. Muscle lysates were immunoprecipitated using AMPK $\alpha 1$ - and AMPK $\alpha 2$ -specific antibodies and subjected to activity assays, as described in RESEARCH DESIGN AND METHODS. Time course of AMPK $\alpha 1$ (A) and $\alpha 2$ (B) activities during exercise at 70% W_{max} . Data are means \pm SE; $n = 7$ and 8 for diabetic and healthy subjects, respectively. * $P < 0.05$ vs. basal of respective group; ** $P < 0.001$ vs. basal of control group.

AMPK $\alpha 2$ activity in both the patients with type 2 diabetes and the control group. In the subjects with type 2 diabetes, AMPK $\alpha 2$ activity was significantly increased by 2.7-fold over basal ($P < 0.05$) at 20 min of exercise (Fig. 3B). In this group, AMPK $\alpha 2$ activity remained elevated at 2.4-fold over basal ($P < 0.05$) at 45 min of exercise and 2.2-fold ($P < 0.05$) over basal 30 min after the cessation of exercise. Interestingly, the two diabetic subjects with the highest BMIs (29 and 31 kg/m²) responded to the same magnitude as the other type 2 diabetic subjects. AMPK $\alpha 1$ activity did not change during exercise in the control or diabetic groups (Fig. 3A). These findings demonstrate that in diabetic subjects, AMPK responds normally to exercise. Thus, if AMPK is a critical mediator of exercise-induced

changes in glucose uptake and fatty acid oxidation in human skeletal muscle, then the AMPK $\alpha 2$ -containing heterotrimer is likely to be the predominant complex responsible for this regulation in both healthy and diabetic subjects.

AMPK $\alpha 1$, $\alpha 2$, and $\beta 1$ protein expression in skeletal muscle. In subjects with type 2 diabetes, processes such as insulin-stimulated glucose uptake (10,11) and fatty acid oxidation (43) in skeletal muscle are impaired. AMPK is thought to have regulatory effects on these metabolic processes, leading to the hypothesis that defects in the AMPK pathway could be involved in the insulin resistance of diabetes (36). To determine whether subjects with type 2 diabetes have abnormal protein content of AMPK $\alpha 1$, $\alpha 2$, and $\beta 1$ in muscle, immunoblotting using isoform-specific antibodies was performed. Figure 4 shows representative immunoblots from two control subjects and two subjects with type 2 diabetes, and graphical results represent the mean content of AMPK $\alpha 1$, $\alpha 2$, and $\beta 1$ at rest from eight control and seven diabetic subjects expressed in arbitrary units. There was no difference in $\alpha 1$, $\alpha 2$, and $\beta 1$ isoform protein content between the control and diabetic groups. **AMPK $\alpha 1$ and $\alpha 2$ mRNA in skeletal muscle.** We also evaluated relative mRNA expression of AMPK $\alpha 1$ and $\alpha 2$ in the resting state by RT-PCR. Total RNA was isolated from muscle in the resting state and then reverse-transcribed to cDNA. Amplification was done by PCR using primers that hybridize to the conserved cDNA sequences of human AMPK $\alpha 1$ and $\alpha 2$, and results are expressed as a percentage of total α mRNA. Relative mRNA expression for $\alpha 1$ and $\alpha 2$, respectively, was 34 and 66% in the control subjects and 36 and 64% in the diabetic group (Fig. 5), and there was no significant difference between groups. The PCR was linear through 30 cycles and saturated after 34 cycles. The accumulation of AMPK cDNA before, during, and after 30 cycles was similar between both groups (data not shown).

DISCUSSION

The major finding of this study was that in subjects with type 2 diabetes, exercise functioned normally to activate the $\alpha 2$ isoform of AMPK. Because the impairment in insulin-stimulated glucose uptake in subjects with diabetes is bypassed by exercise, understanding the signaling pathway by which exercise increases GLUT4 translocation could lead to new approaches for the treatment of insulin resistance (44). Recently, interest has focused on the potential role for AMPK in regulating metabolic responses to exercise in skeletal muscle. Acute activation of AMPK by contraction or AICAR is associated with increases in GLUT4 translocation and glucose uptake in muscle through a PI-3 kinase independent pathway (17,32,33). Manipulation of the AMPK pathway is therefore an attractive approach to increase glucose uptake in muscle and subsequently improve glycemia in subjects with diabetes. Preliminary reports of animal models of diabetes have shown that administration of AICAR causes an acute increase in glucose disposal in muscle (45) and a decrease in blood glucose levels (46), suggesting that AICAR-like compounds capable of activating the AMPK could be a novel group of hypoglycemic agents. The effects of AICAR however, are not limited to AMPK activation in muscle.

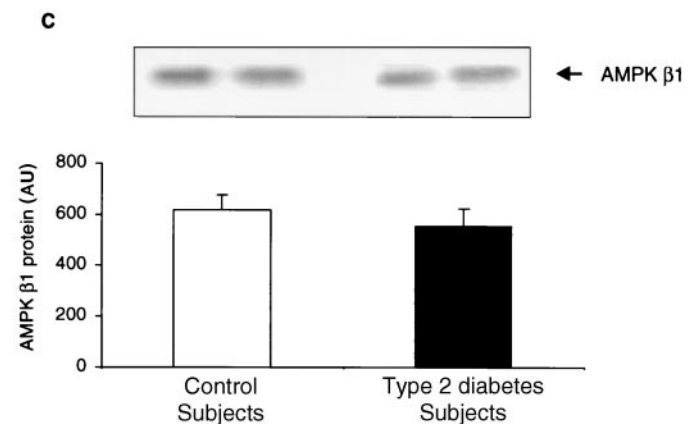
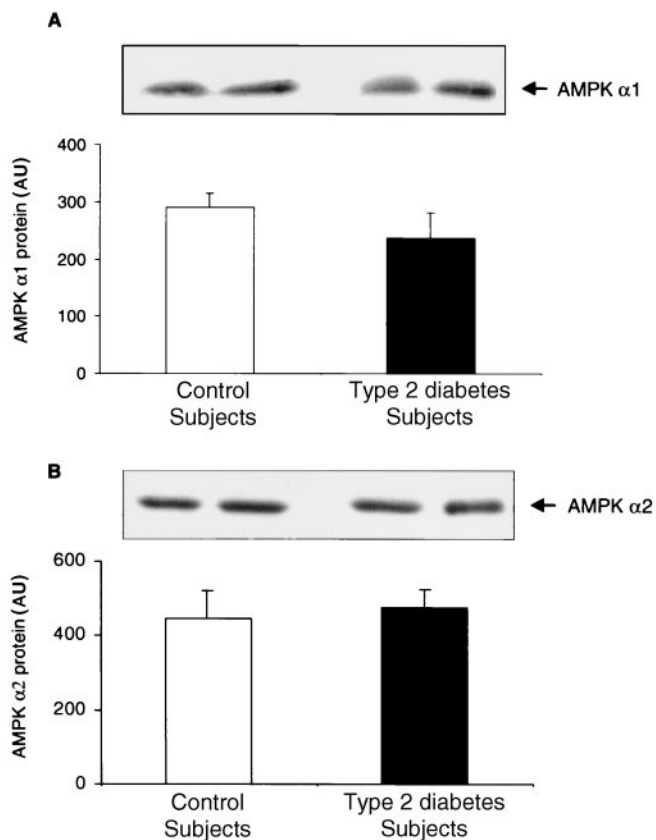


FIG. 4. Immunoblotting of AMPK $\alpha 1$ (A), $\alpha 2$ (B), and $\beta 1$ (C). Protein (40 μ g) from muscle lysates in the resting state were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. Blotting was done with AMPK $\alpha 1$ -, $\alpha 2$ -, and $\beta 1$ -specific antibodies, as described in RESEARCH DESIGN AND METHODS. Data are means \pm SE; $n = 7$ and 8 for diabetic and healthy subjects, respectively.

AICAR has numerous effects on other pathways, including gluconeogenesis (47), glycolysis (48), and cell proliferation (49,50). AICAR is also an adenosine analog, suggesting that some of the effects of the compound on glucose uptake could also be mediated by purine receptors. Therefore, to generate drugs that would function through the AMPK pathway, development of specific activators of AMPK or downstream AMPK substrates will be required.

In the present study, we found an increase in AMPK $\alpha 2$ activity in skeletal muscle of subjects with type 2 diabetes during acute exercise, and this change was associated with a clinically significant decrease in blood glucose concentrations. Although we did not determine muscle glucose disposal during the exercise protocol, previous studies have shown that cycle ergometry exercise performed at similar or lower intensities to our study results in acute increases in leg glucose disposal in subjects with type 2 diabetes, as determined by both radiolabeled glucose (51) and arteriovenous leg balance techniques (52). In the present study, plasma insulin concentrations decreased significantly during exercise, excluding the possibility that the improvement in glycemia was secondary to increased insulin.

AMPK has been proposed to also play an important role in regulating exercise-induced fatty acid oxidation in skeletal muscle (19,21,36). During exercise in the rat, AMPK phosphorylates acetyl-CoA carboxylase (ACC), leading to a decrease in its activity, a fall in malonyl-CoA, de/inhibition of carnitine palmitoyltransferase I, and a subsequent increase in fatty acid oxidation (23,26,53). Activating AMPK with AICAR has similar inhibitory effects on ACC, decreasing malonyl-CoA levels and increasing fatty acid oxidation (16). Recently, it has been shown that similar to the effects in

rodents, acute exercise in humans causes a significant decrease in ACC activity in association with augmented fatty acid oxidation rates (54). Therefore, activating AMPK with exercise or pharmacological agents could potentially result in not only increased glucose disposal in muscle but also decreased malonyl-CoA content; these two mechanisms might ameliorate insulin resistance and improve glycemia.

The isoform-specific changes in AMPK $\alpha 1$ and $\alpha 2$ activity during exercise suggest that these isoforms may play different physiological roles in human skeletal muscle. As mentioned previously, AMPK $\alpha 1$ is widely distributed, and AMPK $\alpha 2$ is primarily expressed in skeletal muscle, heart, and liver (30). In INS-1 cells, AMPK $\alpha 1$ is localized predominantly in the cytosol, whereas AMPK $\alpha 2$ is localized in the cytosol and nucleus (55). The nuclear localization of AMPK $\alpha 2$ raises the possibility that this isoform might regulate gene expression in response to cellular stress. Besides differences in tissue distribution and subcellular localization, substrate specificity for downstream targets between isoforms seems to differ as well (56). An interesting possibility

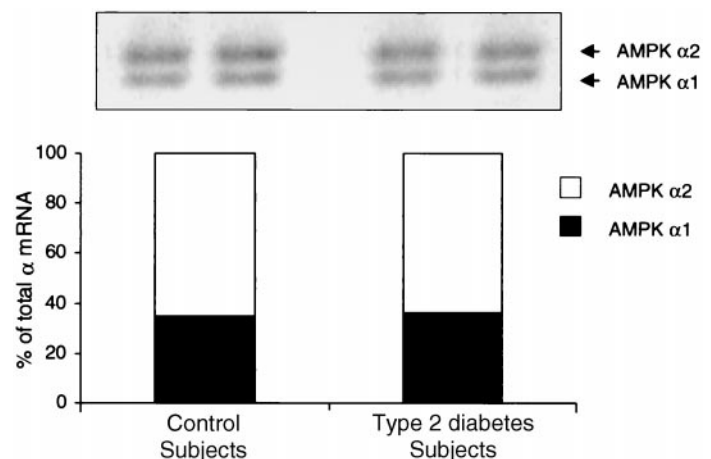


FIG. 5. Determination of relative AMPK $\alpha 1$ (■) and $\alpha 2$ (□) mRNA expression. Total RNA was isolated from muscle, reverse-transcribed to cDNA, and amplified by PCR using the primers described in RESEARCH DESIGN AND METHODS. Data are means; $n = 7$ and 8 for diabetic and healthy subjects, respectively.

is that the differential activation of the AMPK isoforms during exercise could be related to the magnitude of changes in cellular energy levels. Compared with the $\alpha 1$ isoform, liver AMPK $\alpha 2$ activity is more dependent on AMP concentrations (55). In young nondiabetic humans, significant depletion of phosphocreatine and a small decrease in ATP during exercise at 70% $V_{O_{2max}}$ lead to an increase in muscle AMPK $\alpha 2$ activity but no change in $\alpha 1$ (28). A similar finding on differential AMPK activation in humans during moderate-intensity exercise has been confirmed by Wojtaszewski et al. (29). These findings suggest that with this intensity of exercise, $\alpha 2$ -containing rather than $\alpha 1$ isoform-containing AMPK complexes are primarily responsible for activating AMPK-stimulated metabolic changes in skeletal muscle of both healthy and subjects with type 2 diabetes. However, it is also possible that under conditions of extreme exercise intensity, $\alpha 1$ -containing complexes are critical metabolic regulators, because a recent report has shown that 30 s of supramaximal sprint exercise significantly increased the activity of both isoforms (57). Furthermore, the AMPK activity assay used in the present study was performed under saturating AMP concentrations; therefore, some degree of allosteric activation of either α isoform in the intact tissue cannot be completely ruled out.

In type 2 diabetes, several processes within carbohydrate and fat metabolism are dysregulated. AMPK has been proposed to have important regulatory effects on many of these metabolic processes, including fatty acid oxidation and glucose uptake in muscle, cholesterol and triglyceride synthesis in the liver, lipogenesis, and insulin secretion by pancreatic β -cells (36). This has led to the hypothesis that a defect in the AMPK pathway (either primary or secondary to physical inactivity) could be involved in the insulin resistance and dyslipidemia observed in diabetic subjects (36). In the present study, we found that in subjects with type 2 diabetes, not only are the exercise-induced increases in AMPK $\alpha 2$ activity similar in magnitude to those in healthy subjects, but relative mRNA expression of both the α isoforms and protein content of AMPK $\alpha 1$, $\alpha 2$, and $\beta 1$ in skeletal muscle are normal as well. It is thus unlikely that defects in the muscle AMPK of these subjects played a major role in the metabolic alterations of this disease. However, given that these subjects had good exercise tolerance, it is theoretically possible that people with defects in the AMPK pathway were excluded from the study because they had poor exercise tolerance in the first place. In future studies, it will also be important to investigate if AMPK expression and exercise-mediated activation is affected in other subgroups of subjects with different ethnic backgrounds or who are more obese and severely diabetic compared with the subjects included in our study. Although we found normal mRNA expression of the $\alpha 1$ and $\alpha 2$ AMPK isoforms, minor gene mutations would not be detected by RT-PCR; nevertheless, because basal and stimulated kinase activity were normal, even if minor mutations were present, it is unlikely that they affected AMPK activity. The function of AMPK in other tissues (such as islet cells, liver, and fat tissue) of subjects with type 2 diabetes also remains to be determined and will be an important area for future investigations.

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