Rapid Publication

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 $\alpha2$ 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 $\alpha1$ activity. Subjects with type 2 diabetes had similar protein expression of AMPK $\alpha1$, $\alpha2$, and $\beta1$ in muscle compared with control subjects. AMPK $\alpha2$ 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 $\alpha2$ activity and normal expression of the $\alpha1$, $\alpha2$ and $\beta1$ isoforms. Pharmacological activation of AMPK may be an attractive target for the treatment of type 2 diabetes. *Diabetes* **50:921–927, 2001**

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 (α1 and α2). Whereas AMPK α1 is widely distributed, AMPK α2 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 α2 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 kJ · kg^{-1} · 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 (Brinkman 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 tripsin inhibitor, 0.1 mmol/l benzamidine, 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 α1 and α2 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 α1 and 352–366 of α2. 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 HMRSAMSGLHLVKRR (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 α1, α2, and β1. 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 α1 and α2 catalytic subunits of AMPK described above and β1 antibody (3 µg/ml) (Transduction Laboratories, Lexington, KY) in TBST and 2.5% dry milk. Bound anti-AMPK α1 and α2 antibodies were detected with anti-rabbit immunoglobulin–horseradish-peroxidase–linked whole antibody, and anti-β1 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 α1 and α2 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	$7.6 \pm 0.5*$
2-h Oral glucose tolerance test		
(mmol/l)	5.66 ± 0.7	$14.8 \pm 1.2*$
HbA_{1c} (%)	4 ± 0.2	$6.4 \pm 0.6†$
Insulin (pmol/l)	84.4 ± 14	$227 \pm 49†$
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 $\pm$ 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 $\alpha 1$ and $\alpha 2$ cDNAs, were used: sense 5′-CATATGCTGCAGGT-3′ and antisense 5′-TIGAGIIAGITCAACIGG-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 $MgCl_2$, 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 $\alpha 1$ and $\alpha 2$ 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 $\pm$ 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-
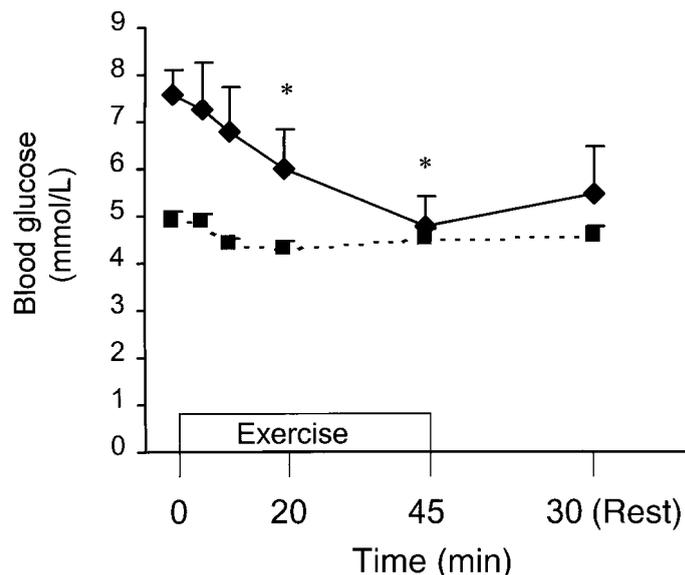

FIG. 1. Exercise decreases blood glucose concentrations in subjects with type 2 diabetes. Seven diabetic subjects (◆) and eight healthy subjects (■) performed cycle ergometry exercise at 70% W_{max} for the period indicated. In the diabetic group, glucose concentrations decreased significantly during the exercise protocol. Data are means $\pm$ 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 $\alpha 1$ and $\alpha 2$ AMPK catalytic subunits was measured before exercise, at 20 and 45 min of exercise, and 30 min postexercise. Exercise similarly increased
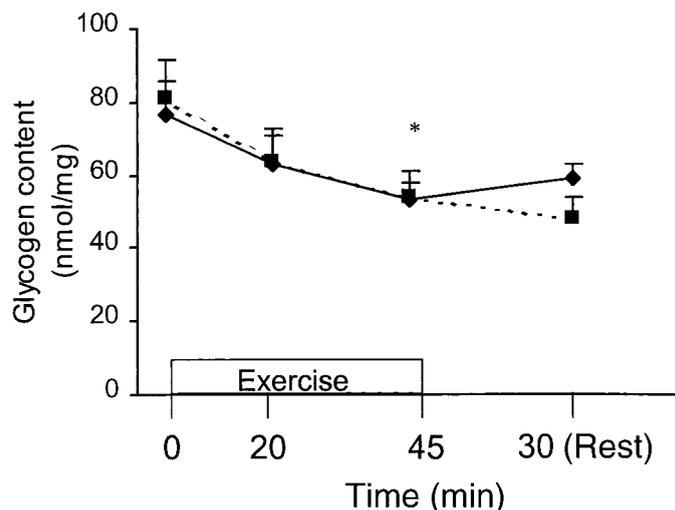

FIG. 2. Glycogen content in vastus lateralis muscle in response to exercise. Diabetic (◆) and control (■) subjects performed cycle ergometry exercise at 70% W_{max} for the period indicated, and muscle biopsies were obtained. Glycogen content was measured as described in RESEARCH DESIGN AND METHODS.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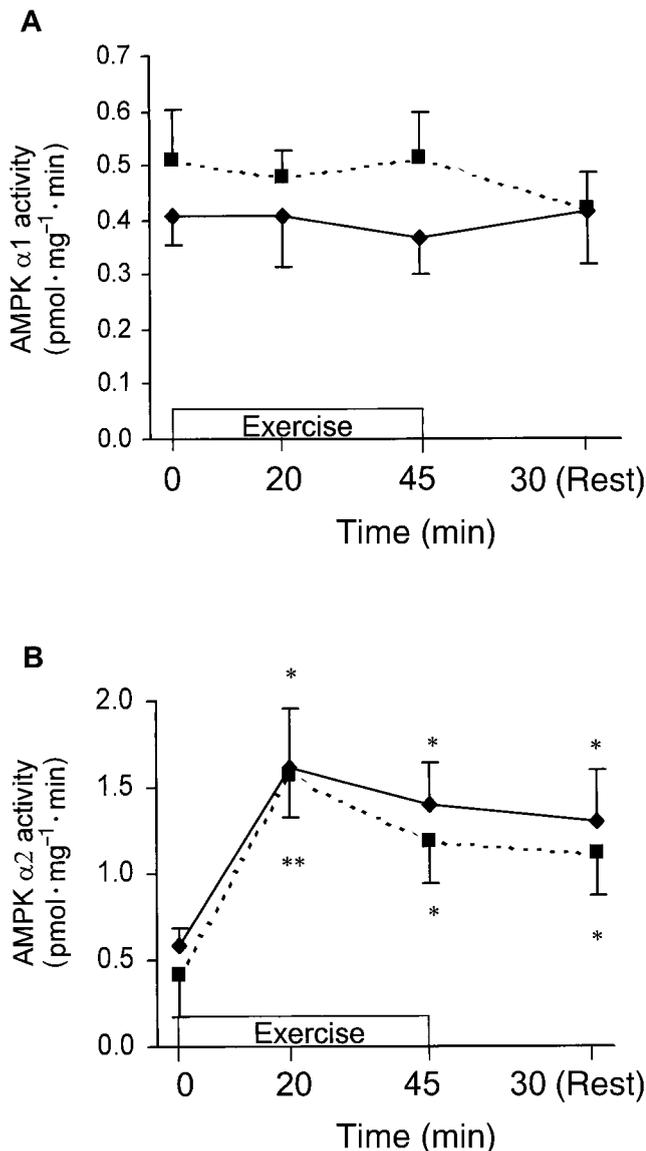
A

B

FIG. 3. Exercise increases AMPK α2 activity in subjects with type 2 diabetes. Diabetic (◆) and control (■) subjects performed cycle ergometry exercise for the period indicated and multiple biopsies of vastus lateralis muscles were obtained. Muscle lysates were immunoprecipitated using AMPK α1– and AMPK α2–specific antibodies and subjected to activity assays, as described in RESEARCH DESIGN AND METHODS. Time course of AMPK α1 (A) and α2 (B) activities during exercise at 70% W_{max}. Data are means ± 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 α2 activity in both the patients with type 2 diabetes and the control group. In the subjects with type 2 diabetes, AMPK α2 activity was significantly increased by 2.7-fold over basal ($P < 0.05$) at 20 min of exercise (Fig. 3B). In this group, AMPK α2 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/m^2) responded to the same magnitude as the other type 2 diabetic subjects. AMPK α1 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 α2–containing heterotrimer is likely to be the predominant complex responsible for this regulation in both healthy and diabetic subjects.

AMPK α1, α2, and β1 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 α1, α2, and β1 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 α1, α2, and β1 at rest from eight control and seven diabetic subjects expressed in arbitrary units. There was no difference in α1, α2, and β1 isoform protein content between the control and diabetic groups.

AMPK α1 and α2 mRNA in skeletal muscle. We also evaluated relative mRNA expression of AMPK α1 and α2 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 α1 and α2, and results are expressed as a percentage of total α mRNA. Relative mRNA expression for α1 and α2, 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 α2 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.
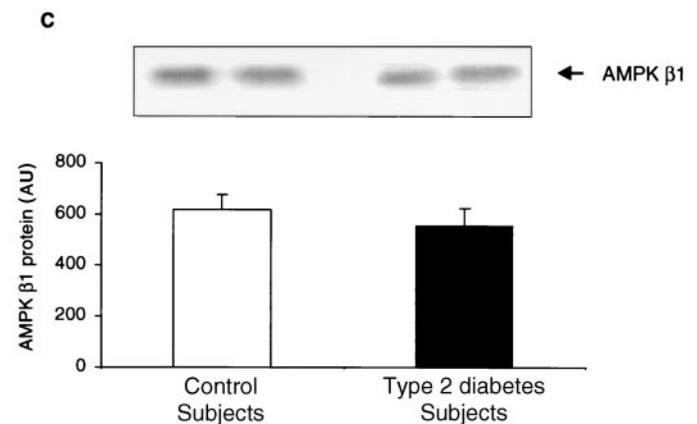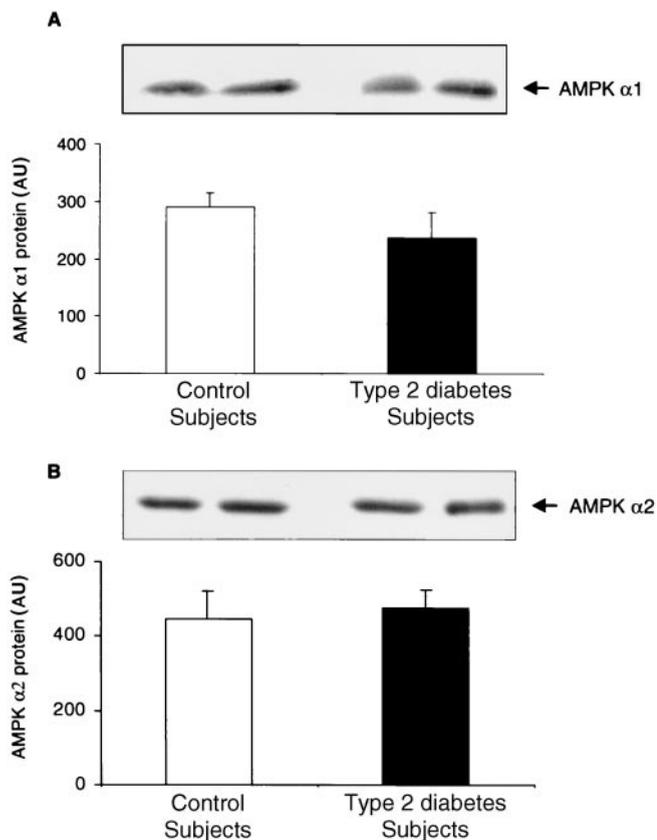
FIG. 4. Immunoblotting of AMPK α1 (*A*), α2 (*B*), and β1 (*C*). Protein (40 μg) from muscle lysates in the resting state were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. Blotting was done with AMPK α1–, α2–, and β1–specific antibodies, as described in RESEARCH DESIGN AND METHODS. Data are means ± SE; $n = 7$ and 8 for diabetic and healthy subjects, respectively.

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 α1 and α2 activity during exercise suggest that these isoforms may play different physiological roles in human skeletal muscle. As mentioned previously, AMPK α1 is widely distributed, and AMPK α2 is primarily expressed in skeletal muscle, heart, and liver (30). In INS-1 cells, AMPK α1 is localized predominantly in the cytosol, whereas AMPK α2 is localized in the cytosol and nucleus (55). The nuclear localization of AMPK α2 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 α1 (■) and α2 (□) 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.

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 α2 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, deinhibition 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

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 Wojtas-zewski et al. (29). These findings suggest that with this intensity of exercise, $\alpha 2$-containing rather that $\alpha 1$ iso-form–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.

REFERENCES

1. American Diabetes Association: Diabetes mellitus and exercise (Position Statement). *Diabetes Care* 21 (Suppl. 1):S40–S44, 1998
2. Holloszy JO, Hansen PA: Regulation of glucose transport into skeletal muscle. *Rev Physiol Biochem Pharmacol* 128:99–193, 1996
3. Hayashi T, Wojtaszewski JF, Goodyear LJ: Exercise regulation of glucose transport in skeletal muscle (Review). *Am J Physiol* 273:E1039–E1051, 1997
4. Goodyear LJ, Kahn BB: Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 49:235–261:235–261, 1998
5. Caro JF, Sinha MK, Raju SM, Ittoop O, Pories WJ, Flickinger EG, Meelheim D, Dohm GL: Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. *J Clin Invest* 79:1330–1337, 1987
6. Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ, Dohm GL: Insulin receptor phosphorylation, IRS-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J Clin Invest* 95:2195–2204, 1995
7. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR: Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. *Diabetes* 46:524–527, 1997
8. Zierath JR, Krook A, Wallberg-Henriksson H: Insulin action in skeletal muscle from patients with NIDDM. *Mol Cell Biochem* 182:153–160, 1998
9. King PA, Horton ED, Hirshman MF, Horton ES: Insulin resistance in obese Zucker rat (fa/fa) skeletal muscle is associated with a failure of glucose transporter translocation. *J Clin Invest* 90:1568–1575, 1992
10. Zierath JR, He L, Guma A, Odegoard Wahlstrom E, Klip A, Wallberg-Henriksson H: Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia* 39:1180–1189, 1996
11. Garvey WT, Maianu L, Zhu JH, Brechtel-Hook G, Wallace P, Baron AD: Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *J Clin Invest* 101:2377–2386, 1998
12. Brozinick JT Jr, Etgen GJ Jr, Yaspelkis BB III, Ivy JL: Contraction-activated glucose uptake is normal in insulin-resistant muscle of the obese Zucker rat. *J Appl Physiol* 73:382–387, 1992
13. King PA, Betts JJ, Horton ED, Horton ES: Exercise, unlike insulin, promotes glucose transporter translocation in obese Zucker rat muscle. *Am J Physiol* 265:R447–R452, 1993
14. Brozinick JT Jr., Etgen GJ Jr, Yaspelkis BB, Ivy JL: Glucose uptake and GLUT-4 protein distribution in skeletal muscle of obese Zucker rat. *Am J Physiol* 267:R236–R243, 1994
15. Kennedy JW, Hirshman MF, Gervino EV, Ocel JV, Forse RA, Hoenig SJ, Aronson D, Goodyear LJ, Horton ES: Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes* 48:1192–1197, 1999
16. Merrill GF, Kurth EJ, Hardie DG, Winder WW: AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol* 273:E1107–E1112, 1997
17. Hayashi T, Hirshman MF, Kurth EJ, Winder WW, Goodyear LJ: Evidence for 5′ AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* 47:1369–1373, 1998
18. Bergeron R, Russell III RR, Young LH, Ren JM, Marcucci M, Lee A, Shulman GI: Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am J Physiol* 276:E938–E944, 1999
19. Ruderman NB, Saha AK, Vavvas D, Witters LA: Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol* 276:E1–E18, 1999
20. Hardie DG, Carling D, Carlson M: The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? *Annu Rev Biochem* 67:821–855, 1998
21. Kemp BE, Mitchelhill KI, Stapleton D, Michell BJ, Chen ZP, Witters LA: Dealing with energy demand: the AMP-activated protein kinase. *Trends Biochem Sci* 24:22–25, 1999
22. Hayashi T, Hirshman MF, Fujii N, Habinowski SA, Witters LA, Goodyear LJ: Metabolic stress and altered glucose transport: activation of AMP-activated protein kinase as a unifying coupling mechanism. *Diabetes* 49:527–531, 2000

23. Rasmussen BB, Winder WW: Effect of exercise intensity on skeletal muscle malonyl-CoA and acetyl-CoA carboxylase. *J Appl Physiol* 83:1104–1109, 1997

24. Rasmussen BB, Hancock CR, Winder WW: Postexercise recovery of skeletal muscle malonyl-CoA, acetyl-CoA carboxylase, and AMP-activated protein kinase. *J Appl Physiol* 85:1629–1634, 1998

25. Hutber CA, Hardie DG, Winder WW: Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am J Physiol* 272:E262–E266, 1997

26. Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB: Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. *J Biol Chem* 272:13255–13261, 1997

27. Ihlemann J, Ploug T, Hellsten Y, Galbo H: Effect of tension on contraction-induced glucose transport in rat skeletal muscle. *Am J Physiol* 277:E208–E214, 1999

28. Fujii N, Hayashi T, Hirshman MF, Smith JT, Habinowski SA, Kaijser L, Mu J, Ljungqvist O, Birnbaum MJ, Witters LA, Thorell A, Goodyear LJ: Exercise induces isoform-specific increases in 5′AMP-activated protein kinase activity in human skeletal muscle. *Biochem Biophys Res Commun* 273:1150–1155, 2000

29. Wojtaszewski JF, Nielsen P, Hansen BF, Richter EA, Kiens B: Isoform-specific and exercise intensity-dependent activation of 5′-AMP-activated protein kinase in human skeletal muscle. *J Physiol* 528 (Pt 1):221–226, 2000

30. Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, House CM, Fernandez CS, Cox T, Witters LA, Kemp BE: Mammalian AMP-activated protein kinase subfamily. *J Biol Chem* 271:611–614, 1996

31. Corton JM, Gillespie JG, Hawley SA, Hardie DG: 5-aminoimidazole-4-carboxamide ribonucleoside: a specific method for activating AMP-activated protein kinase in intact cells? *Eur J Biochem* 229:558–565, 1995

32. Russell RR III, Bergeron R, Shulman GI, Young LH: Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *Am J Physiol* 277:H643–H649, 1999

33. Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW: 5′ AMP–activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* 48:1667–1671, 1999

34. Holmes BE, Kurth-Kraczek EJ, Winder WW: Chronic activation of 5′-AMP-activated protein kinase increases GLUT4, hexokinase, and glycogen in muscle. *J Appl Physiol* 87:1990–1995, 1999

35. Ojuka EO, Nolte LA, Holloszy JO: Increased expression of GLUT-4 and hexokinase in rat epitrochlearis muscles exposed to AICAR in vitro. *J Appl Physiol* 88:1072–1075, 2000

36. Winder WW, Hardie DG: AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol* 277:E1–E10, 1999

37. American Diabetes Association: Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 21 (Suppl. 1):S5–S19, 1998

38. Wallin L, Brundin LH: Physical working capacity determined by different types of bicycle exercise tests. *Clin Physiol* 8:529–537, 1988

39. Bergstrom J: Muscle electrolytes in man. *Scand J Clin Lab Invest* 168:11–13, 1962

40. Hugget AS, Nixon DA: Use of glucose oxidase and O-dianisidin in determinations of blood and urinary glucose. *Lancet* 2:368–370, 1957

41. Clark LC Jr, Noyes LK, Grooms TA, Moore MS: Rapid micromeasurement of lactate in whole blood. *Crit Care Med* 12:461–464, 1984

42. Davies SP, Carling D, Hardie DG: Tissue distribution of the AMP-activated protein kinase, and lack of activation by cyclic-AMP-dependent protein kinase, studied using a specific and sensitive peptide assay. *Eur J Biochem* 186:123–128, 1989

43. Kelley DE, Simoneau JA: Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J Clin Invest* 94:2349–2356, 1994

44. Shepherd PR, Kahn B: Glucose transporters and insulin action: implications for insulin resistance and diabetes mellitus. *N Engl J Med* 341:248–257, 1999

45. Bergeron R, Previs SF, Cline GW, Perret P, Russell III RR, Young LH: The effect of AMP-activated protine kinase (AMPK) stimulation on whole body glucose metabolism in awake lean and zucker diabetic fatty (ZDF) rats (Abstract). *Diabetes* 49 (Suppl. 1):A278, 2000

46. Nakano M, Hayashi T, Yonemitsu S, Masuda I, Masuzaki H, Inque G, Yoshimasa Y, Nakao K: Pharmacological activation of 5′AMP-activated protein kinase (AMPK) in vivo acutely decreases blood glucose and chronically increases muscle GLUT4 in normal and diabetic animals (Abstract). *Diabetes* 49 (Suppl. 1):A12 2000

47. Vincent MF, Erion MD, Gruber HE, Van den Berghe G: Hypoglycaemic effect of AICAriboside in mice. *Diabetologia* 39:1148–1155, 1996

48. Vincent MF, Bontemps F, Van den Berghe G: Inhibition of glycolysis by 5-amino-4-imidazolecarboxamide riboside in isolated rat hepatocytes. *Biochem J* 281:267–272, 1992

49. Samari HR, Seglen PO: Inhibition of hepatocytic autophagy by adenosine, aminoimidazole-4-carboxamide riboside, and N6-mercaptopurine riboside: evidence for involvement of amp-activated protein kinase. *J Biol Chem* 273:23758–23763, 1998

50. Durante P, Gueuning MA, Darville MI, Hue L, Rousseau GG: Apoptosis induced by growth factor withdrawal in fibroblasts overproducing fructose 2,6-bisphosphate. *FEBS Lett* 448:239–243, 1999

51. Wahren J, Hagenfeldt L, Felig P: Splanchnic and leg exchange of glucose, amino acids, and free fatty acids during exercise in diabetes mellitus. *J Clin Invest* 55:1303–1314, 1975

52. Martin IK, Katz A, Wahren J: Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am J Physiol* 269:E583–E590, 1995

53. Winder WW, Hardie DG: Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol* 270:E299–E304, 1996

54. Dean D, Daugaard JR, Young ME, Saha A, Vavvas D, Asp S, Kiens B, Kim KH, Witters L, Richter EA, Ruderman N: Exercise diminishes the activity of acetyl-CoA carboxylase in human muscle. *Diabetes* 49:1295–1300, 2000

55. Salt I, Celler JW, Hawley SA, Prescott A, Woods A, Carling D, Hardie DG: AMP-activated protein kinase: greater AMP dependence, and preferential nuclear localization, of complexes containing the α2 isoform. *Biochem J* 334:177–187, 1998

56. Woods A, Salt I, Scott J, Hardie DG, Carling D: The α1 and α2 isoforms of the AMP-activated protein kinase have similar activities in rat liver but exhibit differences in substrate specificity in vitro. *FEBS Lett* 397:347–351, 1996

57. Chen ZP, McConell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE: AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. *Am J Physiol* 279:E1202–E1206, 2000