Exercise Increases Nuclear AMPK α_2 in Human Skeletal Muscle

Sean L. McGee,[1] Kirsten F. Howlett,[1] Rebecca L. Starkie,[1] David Cameron-Smith,[1] Bruce E. Kemp,[2] and Mark Hargreaves[1]

An acute bout of exercise increases skeletal muscle glucose uptake, improves glucose homeostasis and insulin sensitivity, and enhances muscle oxidative capacity. Recent studies have shown an association between these adaptations and the energy-sensing 5′ AMP-activated protein kinase (AMPK), the activity of which is increased in response to exercise. Activation of AMPK has been associated with enhanced expression of key metabolic proteins such as GLUT-4, hexokinase II (HKII), and mitochondrial enzymes, similar to exercise. It has been hypothesized that AMPK might regulate gene and protein expression through direct interaction with the nucleus. The purpose of this study was to determine if nuclear AMPK α_2 content in human skeletal muscle was increased by exercise. Following 60 min of cycling at $72 \pm 1\%$ of $V_{O_{2peak}}$ in six male volunteers (20.6 ± 2.1 years; 72.9 ± 2.1 kg; $V_{O_{2peak}} = 3.62 \pm 0.18$ l/min), nuclear AMPK α_2 content was increased 1.9 ± 0.4-fold ($P = 0.024$). There was no change in whole-cell AMPK α_2 content or AMPK α_2 mRNA abundance. These results suggest that nuclear translocation of AMPK might mediate the effects of exercise on skeletal muscle gene and protein expression. *Diabetes* 52:926–928, 2003

An acute bout of exercise increases skeletal muscle glucose uptake, improves glucose homeostasis and insulin sensitivity, and enhances muscle oxidative capacity. These positive metabolic adaptations have also been observed in insulin-resistant states and type 2 diabetic subjects and may partly explain the well-recognized benefits of physical activity in the prevention and management of type 2 diabetes (1). Recent studies have shown an association between these adaptations and the energy-sensing 5′ AMP-activated protein kinase (AMPK). AMPK is a heterotrimer, consisting of α, β, and γ subunits, that is activated in response to increases in the AMP-to-ATP ratio (2). There are two α, two β, and three γ isoforms capable of forming AMPK complexes, with skeletal muscle predominantly expressing the α_2, β_2, and γ_1 isoforms (2). AMPK complexes containing the catalytic α_2 subunit are preferentially activated during exercise at intensities $>70\%$ $V_{O_{2peak}}$, in both normal (3) and type 2 diabetic subjects (4). Long-term administration of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), a drug that activates AMPK, to insulin-resistant Zucker rats improves glucose tolerance and other symptoms associated with the disease (5). AICAR administration also increases the expression of several key regulatory proteins involved in glucose metabolism, such as GLUT-4 (6), hexokinase II (HKII [6]), and mitochondrial enzymes (7), similar to exercise training. Consequently, it has been thought that AMPK might mediate the positive effects of exercise on skeletal muscle glucose metabolism through regulation of gene and protein expression. This hypothesis is consistent with the function of the AMPK yeast homologue *Snf*1, which is known to regulate a range of metabolic genes (8). *Snf*1 regulates gene transcription by directly phosphorylating nuclear transcription factors (8). Similarly, AMPK complexes containing the α_2 subunit are preferentially localized to the nucleus (9). Furthermore, inhibition of not only nuclear but also cytosolic AMPK α_2 in islet β-cell cultures is required to relieve inhibition of liver-type pyruvate kinase (L-PK) expression by AMPK (10). This suggests that AMPK translocates to the nucleus where it could then interact with transcriptional regulators, or DNA directly, to control gene expression. Therefore, the purpose of this study was to determine if nuclear AMPK α_2 content was increased in human skeletal muscle following an acute bout of exercise, which we have previously shown to increase GLUT-4 gene expression (11). We hypothesized that exercise would increase nuclear AMPK α_2 abundance.

RESEARCH DESIGN AND METHODS

Subjects. Six active, but untrained, male subjects (20.6 ± 2.1 years; 72.9 ± 2.1 kg; 180 ± 3 cm) were recruited for the study after completing a medical questionnaire and giving their informed written consent. All experimental procedures were approved by the Deakin University Human Research Ethics Committee. At least 7 days before the experimental trial, all subjects performed an incremental cycling (Lode, Groningen, the Netherlands) test to fatigue to determine $V_{O_{2peak}}$, which averaged 3.62 ± 0.18 l/min. This test was also used to select the power output for the experimental trial from the linear relationship between oxygen uptake and power output.

Exercise. Subjects performed 60 min of cycling at $\sim 70\%$ $V_{O_{2peak}}$ after a 12-h overnight fast. Expired air was collected twice, at 15–20 and 40–45 min, to ensure that subjects were exercising at the expected exercise intensity. Subjects ingested tap water ad libitum throughout the exercise bout.

Muscle biopsies. Muscle samples were obtained from the vastus lateralis before exercise, after 10 min of exercise, and immediately following exercise using the percutaneous needle biopsy technique with suction (12). Muscle

samples were immediately frozen in liquid nitrogen and stored for later analysis.

Skeletal muscle fractionation. Nuclear proteins were isolated using a modification of the protocol by Blough et al. (13). Approximately 40 mg of muscle was homogenized in 500 µl of ice-cold buffer A (250 mmol/l sucrose, 10 mmol/l NaCl, 3 mmol/l MgCl$_2$, 1 mmol/l dithiothreitol (DTT), 1 mmol/l PMSF [phenylmethylsulfonyl flouride], and 2 µl/40 mg tissue protease inhibitor cocktail), on ice for ~30 s. The homogenate was then spun in a centrifuge for 5 min at 500g at 4°C. The supernatant, representing a crude fraction, was extracted and stored. The remaining pellet was resuspended in 500 µl of ice-cold buffer B (50 mmol/l Tris, pH 7.5, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l DTT, 50 mmol/l NaF, 5 mmol/l Na pyrophosphate, 50 mmol/l MgCl$_2$, 10% glycerol, 1% Triton X-100, 1 mmol/l PMSF, and 2 µl/40 mg tissue protease inhibitor cocktail) and placed on ice for 10 min, with occasional mixing. The resuspended pellet was spun in a centrifuge for 5 min at 3,000g at 4°C. The supernatant, representing the nuclear fraction, was extracted and stored. Fraction purity was verified by immunoblotting for the nuclear histone 1 (H1), the plasma membrane mitochondrial bound creatine transporter (CreaT), and the plasma membrane and cytosolic GLUT-4. Results are not shown, but the nuclear fraction was dominant for H1 but negative for CreaT and GLUT-4. In contrast, the crude fraction was positive for CreaT and GLUT-4 but negative for H1.

Immunoblotting. Proteins were separated and identified using SDS-PAGE. From each sample, 150 µg of protein was loaded onto 1.5 mm 8% acrylamide gels before undergoing electrophoresis for 70 min at 180 V. Proteins were semi-dry–transferred to a nitrocellulose membrane for 2 h at 50 mA. Membranes were blocked for 1 h in blocking buffer (5% skim milk powder in Tris-buffered saline with 0.25% Tween [TBST]) and exposed overnight at 4°C to AMPK α$_2$ primary antibodies as previously described (14). Membranes were exposed to anti-rabbit horseradish peroxidase–conjugated secondary antibodies at a concentration of 1 in 10,000 in blocking buffer for 60 min. Antibody binding was viewed by enhanced chemiluminescence substrate (Pierce SuperSignal Chemiluminescent; Pierce, Rockford, IL) and a Kodak Image Station 440CF (NEN Life Science Products, Boston, MA). Bands were identified and quantified using Kodak 1D Image Analysis Software (Eastman Kodak, Rochester, NY).

Real time RT-PCR. Total RNA was extracted from ~10 mg of muscle using the acid guanidium thiocyanate-phenol-chloroform extraction technique with modifications (FastRNA Kit-Green; Bio101, Carlsbad, CA). Oligo dT single-stranded cDNA was synthesized using AMV Reverse Transcriptase Kit (Promega A3500, Madison, WI). Forward and reverse primers complimentary to the human AMPK α$_2$ gene (GenBank M20747) were designed using Primer Express software (PE Applied Biosystems, Foster City, CA). The AMPK α$_2$ forward primer sequence (5′ to 3′) was GGG TGA AGA TCG GAC ACT ACG T, while the reverse primer sequence was TTG ATG TTC AAT CTT CAC TTT G. Real-time RT-PCR was performed using the GeneAmp 5700 sequence detector and software (PE Applied Biosystems). Changes in AMPK α$_2$ gene expression were normalized to the housekeeping gene β-actin.

Statistical analyses. All values reported are means ± SEM. One-way ANOVA and least squared difference post hoc tests were used with a significance level of 0.05.

RESULTS

Subjects undertook 60 min of exercise at a workload that elicited a mean absolute oxygen uptake of 2.62 ± 0.11 l/min corresponding to 72 ± 1% of $V_{O_{2peak}}$. Nuclear AMPK α$_2$ content after 10 min of exercise was not different compared with rest. However, nuclear AMPK α$_2$ content was increased 1.9 ± 0.4-fold ($P < 0.05$) after 60 min of exercise compared with rest (Fig. 1A). Whole-cell AMPK α$_2$ protein (Fig. 1B) was similar after 10 and 60 min of exercise when compared with rest. Similarly, AMPK α$_2$ mRNA, expressed as a ratio to mRNA of the housekeeping gene β-actin, was similar after 10 and 60 min of exercise when compared with rest (data not shown).

DISCUSSION

It has previously been shown that GLUT-4 (11), HKII (15), and mitochondrial enzyme (16) mRNA is increased immediately after exercise. Activation of AMPK by AICAR also increases expression of these key metabolic proteins
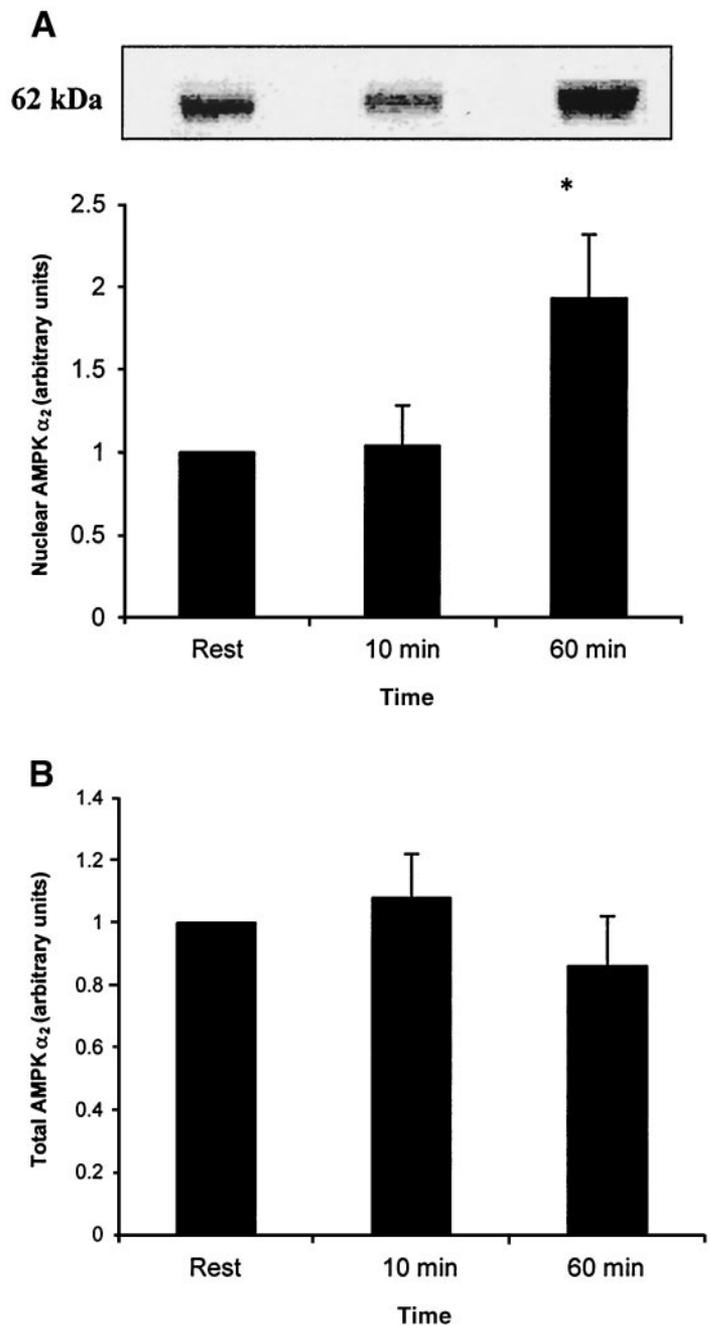

FIG. 1. Nuclear (A) and total (B) AMPK α$_2$ protein in response to exercise. All values are calculated as the fold changes relative to rest and reported as the means ± SEM ($n = 6$). *Significantly different from rest ($P = 0.024$) and 10 min ($P = 0.029$).

(6,7,17,18). The similarity between the effects of exercise and AMPK activation through AICAR on the regulated expression of these proteins has led to the hypothesis that AMPK might mediate the effects of exercise on skeletal muscle gene and protein expression. It has been unclear whether AMPK α$_2$ directly targets nuclear transcription factors, or downstream cytosolic proteins, for this purpose. The results of this study demonstrate for the first time that nuclear AMPK α$_2$ content is increased following an exercise bout that is known to both activate the α$_2$ isoenzyme and induce GLUT-4 gene expression. As whole-cell AMPK α$_2$ content was not changed, it appears that the

increase in nuclear AMPK α_2 content with exercise was the result of nuclear translocation.

The molecular mechanisms underlying the nuclear translocation of AMPK were not examined in the present study. However, in cultured HEK-293 cells it has previously been found that dephosphorylation of the β_1 subunit is associated with the nuclear redistribution of AMPK (19). This has not yet been studied in human skeletal muscle or in AMPK complexes containing the β_2 subunit. Doing so could provide specific pharmacological targets for the treatment and management of insulin resistance and type 2 diabetes, given that AMPK appears to regulate many of the positive chronic adaptations to exercise via altered expression of various metabolic genes. An overview of recent studies suggests that AMPK might regulate gene expression by controlling the activity of various transcriptional regulators. Activation of AMPK has been associated with inactivation of the carbohydrate response element binding protein (20) and p300 transcriptional coactivator (21). Furthermore, AMPK activation increases myocyte enhancer factor 2 (MEF2 [22,23]) and nuclear respiratory factor 1 (NRF-1 [24]) DNA binding. MEF2 is a transcription factor that is required for GLUT-4 gene expression (25), while NRF-1 is thought to be a transcriptional activator of mitochondrial biogenesis (24). It has also been speculated that AMPK itself could participate as part of a transcriptional regulatory complex involved in DNA binding (21); however, there are no data to support this hypothesis at present.

In conclusion, the results from the present study demonstrate that an acute bout of exercise increases skeletal muscle nuclear AMPK α_2 content. The nuclear translocation of AMPK describes a potential mechanism by which AMPK mediates the effects of exercise on the skeletal muscle gene and protein expression. Elucidating the underlying molecular mechanisms mediating AMPK nuclear translocation might be important for the treatment and management of insulin resistance and type 2 diabetes.

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