Effects of AICAR and exercise on insulin-stimulated glucose uptake, signaling, and GLUT-4 content in rat muscles

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The ameliorated insulin sensitivity after exercise is mainly due to enhanced insulin action on skeletal muscle, the predominant tissue for insulin-stimulated glucose disposal (10). In skeletal muscle, insulin stimulation leads to activation of a specific intracellular signaling cascade involving the insulin receptor substrate (IRS) family, the lipid kinase phosphatidylinositol (PI) 3-kinase, and also the serine/threonine kinase PKB/Akt (1). Activation of the insulin-signaling cascade leads to a recruitment of the insulin-sensitive glucose transporter (GLUT-4) from an intracellular pool to the surface membrane, thus allowing glucose to enter the cell (24). This transport of glucose across the sarcolemma through GLUT-4 is thought to be the rate-limiting step for glucose utilization (8).

The molecular mechanism of the increased insulin action after long-term exercise in skeletal muscles might partly be explained by an increased GLUT-4 expression (9) and to some extent also by enhanced activity in the insulin-signaling cascade (5, 17). Although several studies have shown that GLUT-4 expression is enhanced only in muscles activated by the chosen exercise program (9), little is known about the muscle fiber specificity of the increased insulin-signaling activity.

The 5′-AMP-activated protein kinase (AMPK) has recently been suggested as a potential candidate in the signaling process in response to exercise (19, 28). Interestingly, both long-term treatment of rats with 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), which is an AMPK activator (3, 13, 29), and exposure for 18 h of in vitro incubated rat epitrochlearis muscle to AICAR (23) result in enhanced expression of GLUT-4 in skeletal muscles. Furthermore, the increased GLUT-4 expression is associated with a concomitant fiber type-related increase in the level of maximally insulin-stimulated glucose uptake (3), suggesting that at least parts of this beneficial adaptation to long-term exercise can be mimicked through chronic AMPK activation. The possible effects of repetitive AMPK stimulations with AICAR on activity in the insulin-signaling pathway are, however, still to be defined.

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Consequently, the aim of the present study was to determine whether the observed increase in insulin-stimulated glucose uptake in muscles from long-term exercised rats and rats exposed to repetitive pharmacological AMPK activations by chronic AICAR administration are followed by fiber-type-specific changes in insulin signaling and/or fiber-type-specific changes in GLUT-4 expression.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats (weight ~70 g) were housed under controlled temperature (22–23°C) and lighting (12:12-h light/dark cycle) and were given free access to water and a standard rat diet. Animals were supplied from M&B A/S (Ry, Denmark) and randomly divided into either an AICAR, exercise, or control group. All experimental procedures were approved by the Danish Animal Experiments Inspectorate and complied with the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes.

**Animal protocol for AMPK-studies.** To determine the level of isoform-specific activation of the AMPK system, rats were killed immediately either after a 60-min treadmill run or 60 min after a single AICAR injection (1 mg/g body wt). Soleus [muscle fiber composition (2): ~84% type I, 16% type IIa, and 0% type IIb], extensor digitorum longus (EDL) [muscle fiber composition: ~3% type I, 59% type IIa, and 38% type IIb], and epitrochlearis [muscle fiber composition (22): ~15% type I, 20% type IIa, and 65% type IIb] were rapidly dissected out, snap-frozen in liquid nitrogen, and stored at ~80°C until analyzed. Individual muscles were homogenized as described by Musi et al. (21) with minor modifications. Muscles were homogenized in ice-cold lysis buffer (1:25 wt/vol) [20 mM Tris, 50 mM NaCl, 5 mM Na2HPO4, 50 mM NaF, 250 mM sucrose, 2 mM DTT, 1% Triton X-100, 2 μg/ml aprotinin, 5 μg/ml leupeptin, 0.5 μg/ml pepstatin, 10 μg/ml antipain, 1.5 mg/ml benzamidine, and 100 μmol/l 4-[(2-aminoethyl)-benzenesulfonyl fluoride, hydrochloride (AESAHF); pH 7.4] with a homogenizer operating at maximum speed twice for 20 s. Insoluble materials were removed by centrifugation at 14,000 g for 20 min at 4°C, and protein content on the supernatant was determined with a bichinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL).

**Isoform-specific AMPK activity assay.** Isoform-specific AMPK activity was assessed as previously described (21) with minor modifications. Antibodies against the catalytic α1 and α2 subunits of AMPK (Upstate Biotechnology, Lake Placid, NY) coupled to protein A-agarose (Sigma Chemical, St. Louis, MO) were used for immunoprecipitating from aliquots of protein (200 μg). The immune complexes were washed twice in ice-cold lysis buffer and twice in wash buffer (240 mM HEPES and 480 mM NaCl; pH 7.0). AMPK activity was assessed in a buffer containing 40 mM HEPES, 80 mM NaCl, 0.2 mM AMP, 0.2 mM MgATP, 6 μCi [γ-32P]ATP 5 mM MgCl2, and 0.2 SAMS peptide (Upstate Biotechnology) for 20 min at 30°C. The reaction was stopped by spotting 20 μl of reaction aliquot on Whatman P81 paper and dropping it into 1% phosphoric acid. The papers were washed six times in 1% phosphoric acid and once with acetone, and radioactivity was quantified by scintillation counting (Wallac 1409, Wallac, Turku, Finland).

**Immunoblotting for phospho-AMPK (Thr172).** Aliquots of protein were resolved by SDS-PAGE by use of the Bio-Rad Mini Protein II system (10% polyacrylamide gels), transferred to nitrocellulose, blocked with 5% nonfat milk in TBST (10 mM Tris, 150 mM NaCl, pH 8.0, and 0.1% Tween 20), and incubated with anti-phospho-AMPK (Thr172) (Cell Signaling, Beverly, MA) for protein expression of both phosphorylated AMPK-α1 and α2. The membranes were then washed and incubated with secondary horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Pierce Chemical) as secondary antibody, and proteins were visualized by BioWest enhanced chemiluminescence (UVP, Upland, CA) and quantified by UVP BioImaging System.

**Protocol for long-term exercise and chronic AICAR administration.** Animals in the long-term exercise group were accustomed to running for 5 min/day for 2 days on an Exer-3/6 rodent treadmill (Columbus Instruments, Columbus, OH). Subsequently the workload and duration were increased to 20 min/min, at a 10% incline, 60 min/day for 5 successive days. AICAR-exposed animals received daily subcutaneous injections of AICAR (Toronto Research Chemicals, North York, ON, Canada) (1 mg/g body wt) for 5 days as described previously (29). All treadmill running and AICAR injections were carried out in the morning after the rats had free access to food during the night. Sedentary rats served as control animals.

**Muscle preparations for long-term study.** Rats were killed by cervical dislocation after an overnight fast and 24 h after the last training session or AICAR injection. Therefore, the results obtained in the long-term study reflect the chronic adaptations induced by exercise or AICAR administration. Soleus, EDL, and epitrochlearis were rapidly but carefully dissected out and used for measurements of glucose uptake, insulin signaling, and total GLUT-4 content. Muscles used for determination of total GLUT-4 content were snap-frozen in liquid nitrogen directly after removal and stored at ~80°C until assayed.

**Muscle incubation.** All muscles (epitrochlearis, EDL, and soleus) used for measurement of glucose uptake or insulin signaling were incubated for 20 min at 30°C in 5 ml of oxygenated Krebs-Henseleit buffer (117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 24.6 mM NaHCO3; pH 7.4) containing 5 mM HEPES, 20 mM mannitol, and 0.1% BSA in the presence or absence of insulin (60 nmol/l) by using a shaking water bath allowing continuously gassing of the buffer (95% O2-5% CO2). Muscles used for insulin-signaling measurements were trimmed and frozen in liquid nitrogen and stored at ~80°C until analyzed, whereas muscles used for measurement of glucose uptake were further incubated for 10 min in Krebs-Henseleit buffer containing 5 mM HEPES, 12 mM [14C]mannitol (8 μCi/ml), and 8 mM 3-O-[14H]methylglucose (3-OMG; 437 μCi/ml) (NEN, Boston, MA) with or without insulin. Glucose transport activity was assessed as previously described (20) and presented as micromoles glucose analog accumulated per milliliter of intracellular water per hour.

**Muscle preparations for insulin-signaling assays.** Incubated muscles were homogenized as described by Wojtaszewski et al. (30). In short, muscles were homogenized in ice-cold solubilization buffer (50 mM HEPES, 137 mM NaCl, 10 mM Na2PO4, 10 mM NaF, 1 mM MgCl2, 1 mM CaCl2, 1% NP-40, 10% glycerol, 2 mM Na3VO4, 2 μg/ml aprotinin, 5 μg/ml leupeptin, 0.5 μg/ml pepstatin, 10 μg/ml antipain, 1.5 mg/ml benzamidine, and 100 μmol/l AEBSF; pH 7.4) and rotated for 1 h at 4°C. Insoluble materials were removed by centrifugation at 16,000 g for 60 min at 4°C, and protein content on the supernatant was determined with a bichinchoninic acid protein assay reagent (Pierce Chemical).

**PI3-kinase assay.** PI3-kinase activity was assessed as previously described (30) with minor modifications. Briefly, aliquots of protein were immunoprecipitated overnight with protein A-agarose-coupled anti-IRS-1 or anti-IRS-2 antibody
(Upstate Biotechnology). The immune complexes were washed, and PI3-kinase activity was assessed directly on the protein A-agarose complex in a buffer containing 10 mM Tris-HCl, 1 mM EDTA, 1 mM MgCl₂, 75 μM ATP, 50 mM NaCl, and 6 μCi [γ-³²P]ATP (NEN). Reaction products were resolved by thin-layer chromatography and were quantified by using a phosphoimager (Packard BioScience, Meriden, CT).

Protein expression and phosphorylation of PKB/Akt. Aliquots of protein were resolved by SDS-PAGE as described in Immunoblotting for phospho-AMPK (Thr172). Anti-PKB/Akt antibody (New England BioLabs, Beverly, MA) was used for protein expression of PKB/Akt and antiphospho-PKB/Akt (Ser473) antibody (New England BioLabs) for expression of phosphorylated PKB/Akt. Proteins were visualized and quantified as described in Immunoblotting for phospho-AMPK (Thr172).

PKB/Akt activity assay. Aliquots of protein were immunoprecipitated overnight with protein G-agarose coupled anti-PKB/Akt antibody (Upstate Biotechnology) or protein A-agarose coupled PKB/Akt2 antibody (Aviva Antibody, San Diego, CA). The complex was washed twice in solubilization buffer containing additional NaCl (500 mM in total), twice in buffer containing 50 mM Tris, 0.1 mM EGTA, 5 mM DTT, 5 mM -glycerol phosphate, 5 mM EDTA, 1 mM Na₂VO₄, and 1 mM DTT; pH 7.2). PKB activity was assessed in kinase buffer containing 0.1 mM ATP, 1.88 mM MgCl₂, 4 μCi [γ-³²P]ATP, 0.01 mM PKA-inhibitor peptide, 0.1 mM PKB-specific peptide (Upstate Biotechnology) at a final volume of 40 μl at 30°C for 10 min. At the end of the reaction, a 20-ml aliquot was removed and spotted on Whatman P81 paper. The papers were washed six times for 20 min in 1% phosphoric acid and once with acetone, and radioactivity was quantified by scintillation counting (Wallac 1409, Wallac).

Total muscle GLUT-4 content. Total crude membranes were prepared from ~20 mg of epitrochlearis, EDL, or soleus muscles as previously described (3). Proteins were visualized and quantified as described in Immunoblotting for phospho-AMPK (Thr172).

Statistical analysis. All data are presented as means ± SE. Statistical evaluation of the data was done by one-way ANOVA. When analysis revealed significant differences, a post hoc test was used to correct for multiple comparisons (Student-Newman-Keuls test). Differences between groups were considered statistically significant if P < 0.05.

RESULTS

Immunoblotting for phospho-AMPK (Thr172) and isoform-specific acute AMPK activity. A single injection of AICAR or 60 min of treadmill run resulted in a rise in AMPK-α2 activity (Fig. 1) and phospho-AMPK (Thr172) expression (Table 1) in all three muscles investigated. The greatest increase was observed in epitrochlearis muscles, in which AMPK-activity rose 5.6-fold in AICAR-injected animals and 3.7-fold in exercised animals (P < 0.01, n = 5–6). Protein amount of phosphorylated AMPK rose 2.2-fold after an AICAR injection and 1.6-fold after exercise (P < 0.01, n = 5–6). In EDL and soleus muscles, AICAR injection and exercise both resulted in ~3-fold rise in AMPK activity (P < 0.01, n = 5–6); likewise, AICAR injection and exercise resulted in a significant ~40% rise in phosphorylation in EDL (P < 0.01, n = 5–6) whereas only a nonsignificant tendency to an increase in AMPK phosphorylation was noted in soleus muscles during both regimes. No significant changes in AMPK-α1 activity were observed (Table 1).

3-OMG transport. Maximal insulin-stimulated glucose transport activity was markedly increased in the predominant type IIb fiber-containing epitrochlearis muscles from both exercised (9.40 ± 0.49 μmol·ml⁻¹·h⁻¹) and AICAR-injected (12.07 ± 0.87 μmol·ml⁻¹·h⁻¹) animals compared with the control group (7.15 ± 0.45
Table 1. Phospho-AMPK (Thr172) expression and AMPK-α1 activity

<table>
<thead>
<tr>
<th></th>
<th>Epitrochlearis</th>
<th>EDL</th>
<th>Soleus</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>100 ± 5.0</td>
<td>100 ± 4.4</td>
<td>100 ± 3.7</td>
</tr>
<tr>
<td>AMPK-α1 activity</td>
<td>100 ± 20.1</td>
<td>100 ± 8.9</td>
<td>100 ± 7.4</td>
</tr>
<tr>
<td>Exercise</td>
<td>155 ± 16.5*</td>
<td>142 ± 5.4*</td>
<td>128 ± 14.7</td>
</tr>
<tr>
<td>AMPK-α1 activity</td>
<td>101 ± 31.9</td>
<td>125 ± 19.1</td>
<td>78.8 ± 6.8</td>
</tr>
<tr>
<td>AICAR</td>
<td>223 ± 24.6*</td>
<td>133 ± 11.1*</td>
<td>109 ± 7.5</td>
</tr>
<tr>
<td>AMPK-α1 activity</td>
<td>77.2 ± 10.5</td>
<td>118.9 ± 14.7</td>
<td>85.1 ± 13.7</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as percentage of the same muscle from control animals; n = 5–6 rats. EDL, extensor digitorum longus; AMPK, 5′-AMP-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside. *P < 0.01 vs. control.

μmol·ml⁻¹·h⁻¹), resulting in a 31 and 69% (P < 0.01, n = 12–18) increase in exercised and AICAR-injected animals, respectively (Fig. 2A). Furthermore, a significant increase in maximally insulin-stimulated glucose uptake was found in the mixed type II EDL muscle in both the exercised and AICAR-injected animals compared with controls [on average 24.5 and 27.2% (P < 0.01, n = 12–18) for exercise and AICAR, respectively] (Fig. 2B). In contrast, insulin-stimulated 3-OMG-transport did not differ between the groups in the red type I soleus muscle. Basal noninsulin-stimulated glucose transport was significantly (P < 0.05) lower in AICAR-exposed animals in all muscle types compared with controls and exercised animals (76.2% in epitrochlearis, 54.3% in EDL, and 82.8% in soleus compared with control animals).

**IRS-1- and IRS-2-associated PI3-kinase activity.** As shown in Table 2, maximal insulin stimulation (60 nmol/l) led to a 9.0-, 8.5-, and 8.0-fold increase in IRS-1-associated PI3-kinase activity over basal activity in epitrochlearis, EDL, and soleus, respectively. In response to AICAR exposure, insulin-stimulated IRS-1-associated PI3-kinase activity was further enhanced in all three muscles characterized by different fiber-type composition (60.6% in epitrochlearis, 36.1% in EDL, and 80.1% in soleus compared with control animals; P < 0.05, n = 12–14). In contrast, muscles from exercised rats did not differ in insulin-stimulated IRS-1-associated PI3-kinase activity from control rats, even in the epitrochlearis muscles, in which exercise resulted in a 31% increase in insulin-stimulated glucose transport compared with control rats. In all three muscles, basal IRS-1- and IRS-2-associated PI3-kinase activity as well as insulin-stimulated IRS-2-associated PI3-kinase (Table 2) showed no significant difference between the three groups.

**Protein expression, phosphorylation, and activity of PKB.** Neither exercise nor AICAR administration altered PKB protein expression (data not shown). Insulin-stimulated PKBα and β activity and phosphorylated PKB were increased by ~50% in all of the three muscles in the AICAR-injected group compared with controls as shown in Table 3. However, in the exercised animals, insulin did not increase PKB activity further compared with the sedentary controls in either epitrochlearis, EDL, or soleus muscles, consistent with our results for IRS-1-associated PI3-kinase activity.

**Muscle GLUT-4 protein content.** Measurements of total crude membrane content of GLUT-4 protein in the three investigated muscles are displayed in Fig. 3.
Muscles from AICAR-exposed and exercised animals showed an increase in GLUT-4 content that was most prominent in epimysial with a 40.8% (P < 0.05) increase in the exercised-trained and a 97.8% (P < 0.01) increase in the AICAR-injected group compared with the sedentary controls (n = 8–12). In the EDL muscle, AICAR administration and long-term exercise resulted in an almost equal ~45% increase in GLUT-4 content (P < 0.01, n = 6–12). In contrast, GLUT-4 content in the soleus muscles did not differ significantly from control animals (n = 6–12).

**DISCUSSION**

The present study demonstrates that 5 days of AICAR exposure or long-term treadmill exercise increased insulin-stimulated 3-OMG transport in rat skeletal muscles in a fiber-type-specific manner and that this was almost paralleled by an enhanced GLUT-4 expression in the same muscles investigated. Our data from the isoform-specific AMPK activity in these muscles indicate that the adaptations could be mediated through this system, as the acute effect of either one injection of AICAR or a single bout of exercise increases AMPK-α2 activity with the same fiber-type specificity as the increased GLUT-4 expression. AMPK-α1 activity does not seem to be involved in the adaptations because neither AICAR injections nor treadmill exercise increased AMPK-α1 activity.

The effect of long-term AMPK-activation on the activity in the insulin-signaling cascade is another potential cause for increased insulin-stimulated glucose uptake. The molecular mechanisms by which AMPK activity augments insulin signaling have not yet been revealed, but a recent study has shown that AMPK can phosphorylate IRS-1 on Ser-789 in mouse C2C12 myotubes when these are exposed to AICAR, thereby establishing a link to the insulin-signaling cascade (14). This phosphorylation does not alone affect the activity of the IRS-1-associated PI3-kinase, but simultaneous AICAR exposure enhances the activity induced by insulin.

Interestingly, our study showed that long-term AICAR administration increased the activity in the proximal insulin-signaling cascade 24 h after the last injection of AICAR. The increase was noticed on the isolated IRS-1 through association with the insulin receptor substrate; IRS, insulin receptor substrate; PI3, phosphatidylinositol 3-kinase. *P < 0.05 vs. control.

**Table 2. PI3-kinase activity**

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Epimysial</th>
<th>EDL</th>
<th>Soleus</th>
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<tbody>
<tr>
<td></td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Control</td>
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</tr>
<tr>
<td>IRR-1 PI3 activity</td>
<td>11.2 ± 2.8</td>
<td>11.8 ± 1.1</td>
<td>12.6 ± 1.4</td>
</tr>
<tr>
<td>IRR-2 PI3 activity</td>
<td>17.2 ± 3.4</td>
<td>23.2 ± 7.1</td>
<td>24.1 ± 9.1</td>
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<tr>
<td>Exercise</td>
<td></td>
<td></td>
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<tr>
<td>IRR-1 PI3 activity</td>
<td>11.6 ± 2.8</td>
<td>14.5 ± 1.3</td>
<td>11.6 ± 1.2</td>
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<tr>
<td>IRR-2 PI3 activity</td>
<td>12.1 ± 3.4</td>
<td>28.7 ± 5.0</td>
<td>28.9 ± 8.5</td>
</tr>
<tr>
<td>AICAR</td>
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<tr>
<td>IRR-1 PI3 activity</td>
<td>13.4 ± 2.7</td>
<td>16.3 ± 3.0</td>
<td>10.1 ± 1.8</td>
</tr>
<tr>
<td>IRR-2 PI3 activity</td>
<td>11.5 ± 1.6</td>
<td>15.9 ± 5.1</td>
<td>32.1 ± 9.0</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as percentage of the same muscle from control animals; n = 12–14 rats. IRS, insulin receptor substrate; PI3, phosphatidylinositol 3-kinase. *P < 0.05 vs. control.

**Table 3. PKB phosphorylation and activity**

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Epimysial</th>
<th>EDL</th>
<th>Soleus</th>
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<tbody>
<tr>
<td></td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospho-PKB</td>
<td>7.1 ± 1.7</td>
<td>12.9 ± 1.1</td>
<td>9.5 ± 3.7</td>
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<tr>
<td>PKBα activity</td>
<td>41.5 ± 7.9</td>
<td>28.7 ± 10.1</td>
<td>20.8 ± 3.7</td>
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<tr>
<td>PKBβ activity</td>
<td>44.8 ± 9.2</td>
<td>39.3 ± 5.1</td>
<td>22.9 ± 11.0</td>
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<tr>
<td>Exercise</td>
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<tr>
<td>Phospho-PKB</td>
<td>7.7 ± 3.4</td>
<td>11.3 ± 2.1</td>
<td>12.5 ± 3.6</td>
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<tr>
<td>PKBα activity</td>
<td>28.6 ± 9.0</td>
<td>18.4 ± 2.9</td>
<td>19.0 ± 3.3</td>
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<td>PKBβ activity</td>
<td>31.7 ± 12.5</td>
<td>19.7 ± 15.3</td>
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<td>AICAR</td>
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<tr>
<td>Phospho-PKB</td>
<td>13.4 ± 5.6</td>
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<td>PKBα activity</td>
<td>40.6 ± 6.6</td>
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<td>17.8 ± 8.7</td>
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<tr>
<td>PKBβ activity</td>
<td>49.4 ± 18.0</td>
<td>38.3 ± 11.1</td>
<td>35.3 ± 22.6</td>
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Values are means ± SE and are expressed as percentage of the same muscle from control animals; n = 6–10 rats. PKB, protein kinase B. *P < 0.05 and †P < 0.01 vs. control.
no increase in the activity of either insulin-stimulated IRS-1 or IRS-2-associated PI3-kinase compared with sedentary control animals.

To further elucidate the effects on the insulin signaling, we looked at two isoforms of the serine/threonine kinase PKB/Akt, a target for PI3-kinase activity that has been suggested as an intermediate in the insulin-signaling cascade downstream of the PI3-kinase level (4). PKBα/Akt1 is widely expressed and is the predominant isoform in most tissues, whereas PKBβ/Akt2 is mostly expressed in insulin-sensitive tissues (16), and recent studies of mice lacking PKBβ/Akt2 have shown insulin resistance and a diabetes mellitus-like syndrome, indicating an important role for this isoform in insulin signaling (6). Long-term AICAR administration also increased the activity of both the α and β isoforms of PKB in all muscles examined; in contrast, no enhanced activity was noticed in the exercised animals compared with controls.

The increase in insulin signaling in the AICAR-treated rats was roughly equal in the three muscles investigated and did not follow the fiber-type specificity of the increased insulin-stimulated glucose transport. This finding shows that it is possible to increase the activity in the proximal part of the insulin-signaling pathway without increasing glucose uptake. Interestingly, in rats undergoing surgical stress, PI3-kinase and PKB activity have also been found to be increased even though glucose uptake in skeletal muscles was decreased (25).

The fact that the insulin-signaling activity was found to be enhanced after chronic AICAR treatment but not after exercise may implicate differences in the response of muscles to chronic AICAR exposure and long-term exercise. However, Chibalin et al. (5) found that long-term exercised rats exhibited elevated insulin-signaling activity in epitrochlearis muscles 24 h after the last bout of exercise. This is in agreement with our data from the AICAR-injected group. The discrepancy between the unchanged insulin-signaling activity in our exercised animals and the study by Chibalin et al. may be due to the training protocol used. In the latter, rats were exercised by 6 h of swimming every day in contrast to the 1 h of treadmill running used in our study. The long 6-h training program might have increased the AMPK activity for a much longer period than the 1-h treadmill running we were using, and this might have resulted in the increase in insulin-signaling activity. In this context, it would be important in future studies to clarify the duration of the elevated AMPK activity in skeletal muscles after in vivo AICAR administration and to define the muscle fiber-type specificity of very long daily exercise like 6 h of swimming.

The marked effect on insulin-stimulated glucose uptake in our exercise group without any changes in insulin-signaling activity demonstrates that increased signaling is not mandatory for increased glucose uptake. This finding is in agreement with two very recent studies, one on healthy middle-aged men completing a 7-day exercise program that resulted in an increased insulin-stimulated glucose transport but no observed changes on the PI3-kinase or PKB activity (26), and another study on the obese Zucker rat in which a 7-wk exercise program also enhanced insulin-stimulated glucose transport without any effect on insulin signaling (7).

In summary, our results show that increased insulin-stimulated glucose transport after exercise and AICAR exposure correlates with the increase in GLUT-4 protein expression. Enhancement of the insulin signal transduction after chronic AICAR adminis-
tration as shown in this study or by different forms of long-term exercise might also be involved, although we found the activity of the insulin-signaling pathway to be increased in type I muscle fibers after AICAR exposure without a concomitant increase in glucose uptake. This finding could be important for the assessment of future initiatives in the treatment of Type 2 diabetes mellitus because measures focusing solely on increasing the activity in the insulin-signaling cascade may not always result in a beneficial effect on the deranged glucose transport.

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REFERENCES