Effect of Exercise Intensity on Skeletal Muscle AMPK Signaling in Humans

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The effect of exercise intensity on skeletal muscle AMP-activated protein kinase (AMPK) signaling and substrate metabolism was examined in eight men cycling for 20 min at each of three sequential intensities: low (40 ± 2% VO2 peak), medium (59 ± 1% VO2 peak), and high (79 ± 1% VO2 peak). Muscle free AMP/ATP ratio only increased at the two higher exercise intensities (P < 0.05). AMPK α1 (1.5-fold) and AMPK α2 (5-fold) activities increased from low to medium intensity, with AMPK α2 activity increasing further from medium to high intensity. The upstream AMPK kinase activity was substantial at rest and only increased 50% with exercise, indicating that, initially, signaling through AMPK did not require AMPK kinase posttranslational modification. Acetyl-CoA carboxylase (ACC)-β phosphorylation was sensitive to exercise, increasing threefold from rest to low intensity, whereas neuronal NO synthase (nNOS)α phosphorylation was only observed at the higher exercise intensities. Glucose disappearance (tracer) did not increase from rest to low intensity, but increased sequentially from low to medium to high intensity. Calculated fat oxidation increased from rest to low intensity in parallel with ACCβ phosphorylation, then declined during high intensity. These results indicate that ACCβ phosphorylation is especially sensitive to exercise and tightly coupled to AMPK signaling and that AMPK activation does not depend on AMPK kinase activation during exercise. Diabetes 52:2205–2212, 2003

AMP-activated protein kinase (AMPK) appears to be an important regulator of energy metabolism during skeletal muscle exercise (1,2). The AMPK isoforms α1 and α2 are expressed in skeletal muscle and are activated allosterically by increases in free AMP and by phosphorylation by an upstream kinase, AMP kinase (1,3). Skeletal muscle AMPK kinase phosphorylation of the AMPK at Thr-172 is low at rest and increases during contraction (4–6), but the level of skeletal muscle AMPK kinase activity at rest and during exercise is not known.

Much of the support for AMPK’s role in metabolic control has come from studies using the nucleoside intermediate 5-aminomidazole-4-carboxamide-ribonucleoside (AICAR), which is phosphorylated to form ZMP (AICA-ribotide) and activates AMPK (7). When AICAR is administered to rats in vivo or in vitro in pharmacological doses, it activates AMPK in skeletal muscle and increases glucose uptake and fat oxidation (8–10). However, during exercise, skeletal muscle glucose uptake and fat oxidation do not always change in parallel. For example, during exercise at the same absolute workload, prior exercise training reduces glucose uptake but increases fat oxidation (11). In addition, skeletal muscle glucose uptake increases with increasing exercise intensity (12,13), whereas fat oxidation increases with increasing exercise intensity up to ~65–70% VO2 peak, but then decreases at higher exercise intensities (12,13). A number of human studies now suggest that the regulation of skeletal muscle AMPK α1 and α2 differs depending on the exercise intensity. AMPK α2 is activated during exercise at or above ~60% VO2 peak (14–16), whereas AMPK α1 is only activated during intense sprint type exercise in humans (14–17) or during intense electrical stimulation in vitro in rat muscle (18).

Skeletal muscle fat oxidation depends on fatty acids being transported into the mitochondria via carnitine palmitoyltransferase 1 (CPT1), which is inhibited allosterically by malonyl-CoA (19). AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC)-β (5,20), the enzyme responsible for malonyl-CoA production. Treadmill exercise increases skeletal muscle AMPK activity and ACCβ phosphorylation and reduces ACCβ activity and malonyl-CoA content in rats (5,21). In addition, activation of AMPK, by AICAR treatment, decreases ACCβ activity and malonyl-CoA levels as well as increases fat oxidation.
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in the perfused rat hindlimb (9). ACCβ activity decreases during exercise in humans at and above 60% VO2 peak (22), and decreases in malonyl-CoA are detected at intensities between 85 and 100% VO2 peak but not at 60% VO2 peak (22,23). We found that ACCβ phosphorylation by AMPK at Ser-221 (MRPSSMGLHLVLR) was increased greatly during prolonged exercise at ~60% VO2 peak in human skeletal muscle (16), consistent with the reduction in ACCβ/H9252 activity observed at this intensity (22). Increased AMPK activity is not detected until ~60% VO2 peak (14–16), making it uncertain whether AMPK is responsible for ACCβ phosphorylation and the increased fat oxidation accompanying low-intensity exercise (e.g., 40% VO2 peak) (13). At high exercise intensities (80–100% VO2 peak) (12,13), fat oxidation decreases despite both ACCβ activity (22) and malonyl-CoA levels decreasing (22,23), which otherwise would be expected to drive fat oxidation. The drugs metformin and rosiglitazone, which are widely used to treat type 2 diabetes, have recently been found to activate AMPK in skeletal muscle of rats (24,25), and metformin activates AMPK in people with type 2 diabetes (26). Because diet and exercise can reduce the risk of developing type 2 diabetes, considerable attention has been focused on the possible role of AMPK in these events. For these reasons, it was of interest to systematically examine the effect of low-, moderate-, and high-intensity exercise on AMPK signaling, including AMPK kinase activity and AMPK activity, ACCβ phosphorylation, and fat oxidation.

There is conflicting evidence on the role of nitric oxide (NO) in regulating glucose uptake into skeletal muscle during exercise (27–32). Nevertheless, AMPK is associated with neuronal NO synthase (nNOS) and phosphorylates it during exercise in human skeletal muscle (16,17). Furthermore, AMPK is reported to activate skeletal muscle glucose uptake via a NO synthase-dependent pathway (33). In this study, we examined nNOS, phosphorylation in parallel with glucose uptake at varying exercise intensities.

RESEARCH DESIGN AND METHODS

Subjects. Eight healthy nonsmoking men provided informed written consent to participate in this study, which was approved by the Monash University Standing Committee for Research on Humans. The subjects’ age, weight, and height were 28 ± 2 years, 63.9 ± 3.3 kg, and 179 ± 3 cm, respectively (means ± SE).

Experimental procedures

Preliminary testing. Peak pulmonary oxygen consumption during cycling (VO2 peak) was determined using a graded exercise test to volitional exhaustion on an ergometer (Lode, Groningen, the Netherlands) and averaged 3.08 ± 0.35 l/min (47.9 ± 4.5 ml·kg⁻¹·min⁻¹). On a separate day, subjects completed a familiarization trial in which they cycled for 1 h, spending 20 min at each of three sequential workloads, calculated from the VO2 peak test, to be equivalent to 40% (low intensity), 60% (medium intensity), and 80% (high intensity) of their VO2 peak, respectively. At least 5 days later, the subjects undertook their experimental trial.

Experimental trial. Subjects (overnight fasted) reported to the laboratory in the morning having abstained from exercise, alcohol, and caffeine for 24 h. One catheter was inserted into an antecubital forearm vein for infusion of a glucose, stable isotope tracer ([6,6-2H]glucose; Cambridge Isotope Laboratories, Cambridge, MA) and another into the contralateral forearm for blood sampling. A blood sample was obtained; then a bolus of 41.2 ± 0.5 μmol/kg of the tracer was administered before a 2-h pre-exercise constant infusion (0.58 ± 0.04 μmol·kg⁻¹·min⁻¹), which was continued throughout exercise. The exercise protocol consisted of cycling for 1 h, spending 20 min at each of three sequential workloads: low intensity: 40 ± 2% VO2 peak (75 ± 12 W); medium intensity: 50 ± 1% VO2 peak (132 ± 10 W); high intensity: 70 ± 1% VO2 peak (182 ± 25 W). All subjects completed the protocol.Expired air was sampled into Douglas bags for ~15 min at rest, and then the last 3 min of each 20-min workload. Heart rate was monitored throughout exercise using a heart rate monitor (Polar Favor, Oulu, Finland). Muscle was sampled from the vastus lateralis muscle under local anesthetic using the percutaneous needle biopsy technique, with suction during each 20-min period of exercise at four separate sites (resting and low-intensity samples were obtained from one leg, and medium- and high-intensity samples were obtained from the other leg). The resting muscle sample was frozen in liquid nitrogen within 4 ± 0 s of inserting the needle, with the exercise samples frozen within 13 ± 2 s of the subject stopping exercise. A standard error of the mean was allowed for completion of the biopsy and taping of the area before resuming exercise.

Analytical techniques

Calculation of carbohydrate and fat oxidation. Oxygen and carbon dioxide in the expired air was analyzed using Exercstress OX21 and CO21 electronic analyzers (Clinical Engineering Solutions, Sydney, Australia) calibrated with gases of known composition. Gas volume was measured using a dry gas meter (American Meter Company, Vacummed, Ventura, CA) calibrated against a Tissot spirometer. Expired air analysis was used to calculate oxygen consumption, carbon dioxide production, fat oxidation, and carbohydrate oxidation (34).

Blood analysis. Plasma glucose and lactate were determined using an automated glucose oxidase and lactate oxidase method, respectively (YSI 2300 Stat, Yellow Springs Instruments, Yellow Springs, OH), plasma nonesterified fatty acids (NEFAs) by enzymatic colorimetric methods (NEFA-C test; Wako, Osaka, Japan), and plasma insulin using a human insulin-specific radioimmunoassay kit (Linco Research, St. Charles, MO). Glucose kinetics at rest and during exercise were estimated using a modified one-pool non-steady-state model, as proposed by Steele et al. (35), which has been validated by Radziaz et al. (36). We assumed 0.65 as the rapidly mixing portion of the glucose pool and estimated the apparent glucose space as 25% of body weight. Estimation of plasma glucose disappearance (Rd) and glucose (G) kinetics were determined from the changes in percent enrichment of [6,6-2H]glucose and glucose concentration. The muscles of the legs account for 50–80% of total body glucose uptake during exercise at 55–60% VO2peak (14), and probably a greater proportion during more intense exercise (37). During exercise at 50% of VO2max workload, ~95% of tracer-determined glucose uptake is oxidized (37).

Muscle analysis. A portion (~20 mg) of each muscle sample was freeze-dried and then crushed to a powder with any visible connective tissue removed. Muscle glycogen was extracted by incubating the sample in HCI then NaOH and then analyzed for glucose units using enzymatic methods (38). Muscle metabolites (ATP, creatine phosphate, creatine, and lactate) were extracted using the procedure of Harris et al. (39) and analyzed using enzymatic fluorometric techniques (40). Free ADP and free AMP were calculated as outlined previously (17).

Approximately 70 mg of each frozen muscle biopsy sample (non-freeze-dried) was homogenized in buffer A (50 mmol/l Tris-HCl, pH 7.5, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l dithiothreitol, 50 mmol/l NaF, 5 mmol/l Na3VO4, 10% glycerol, 1% Triton X-100, 10 μg/ml trypsin inhibitor, 2 μg/ml aprotinin, 1 mmol/l benzamidine, and 1 mmol/l phenylmethylsulfonyl fluoride). The homogenates were incubated with the AMPK α1 or α2 antibody–bound protein A beads for 2 h at 4°C. Immunocomplexes were washed with PBS and suspended in 50 mmol/l Tris-HCl buffer (pH 7.5) for AMPK activity assay (41). The AMPK activities in the immune complexes were measured in either the presence or absence of 200 μmol/l AMP. Activities were calculated as picomoles of phosphate incorporated into the SAMS peptide ([ACCα (73–87)Aβ] per minute per milligram total protein subjected to immunoprecipitation. AMPK kinase assays were performed using a two-step reaction with a maltose binding protein (MBP)-AMPK (1–312) fusion construct as substrate (42). The construct consists of the AMPK catalytic core, which is activated after phosphorylation on the activation loop Thr-172. First, the AMPK kinase buffer contained 20 mmol/l Tris-HCl, pH 7.5, 0.1% Tween-20, 10 mmol/l diethiothreitol, 8 mmol/l MgCl2 with 0.4 mmol/l ATP, and 0.12 mmol/l AMP, and MBP-AMPK (1–312) (5 μmol/l) in 10 μl was incubated with 11 μl of the muscle homogenate at 30°C for 30 min. Second, the MBP-AMPK (1–312) activity was determined using a peptide phosphorylation assay, and a 10-μl aliquot of the AMPK kinase reaction was added to the peptide phosphorylation reaction to give a final volume of 40 μl comprising 50 mmol/l HEPES, pH 7.5, 12 mmol/l MgCl2, 0.4 mmol/l ATP, and 0.12 mmol/l AMP, and MBP-AMPK (1–312) (5 μmol/l) in 10 μl was incubated with 11 μl of the muscle homogenate at 30°C for 30 min. The mixture was then heated at 100°C for 5 min and 0.2 ml of sample was loaded onto a 1 cm × 10 cm (diameter × length) polyacrylamide gel. After electrophoresis, the gel was fixed, stained, destained, and dried. The 18 kDa band was excised and described (43) and activities (picomoles of phosphate transferred to the SAMS peptide per minute per milligram protein) were calculated. The AMPK kinase assay was characterized using crude extracts of
TABLE 1
Whole-body fuel oxidation during rest and exercise at low, medium, or high intensity

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>Low intensity</th>
<th>Medium intensity</th>
<th>High intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{O_2} ) (l/min)</td>
<td>0.32 ± 0.06</td>
<td>1.21 ± 0.13*</td>
<td>1.81 ± 0.21†</td>
<td>2.41 ± 0.28‡†</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.84 ± 0.02</td>
<td>0.92 ± 0.01*</td>
<td>0.95 ± 0.01†</td>
<td>0.99 ± 0.00†</td>
</tr>
<tr>
<td>Fat oxidation ( (\mu mol \cdot kg^{-1} \cdot min^{-1}) )</td>
<td>4.9 ± 0.5</td>
<td>10.8 ± 2.1*</td>
<td>9.8 ± 2.6*</td>
<td>3.2 ± 0.8‡‡</td>
</tr>
<tr>
<td>Carbohydrate oxidation ( (\mu mol \cdot kg^{-1} \cdot min^{-1}) )</td>
<td>15.1 ± 3.5</td>
<td>88.9 ± 7.0*</td>
<td>155.4 ± 12.8†</td>
<td>243.1 ± 24.3‡‡</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Different from resting \( (P < 0.05) \), †different from low intensity \( (P < 0.05) \), and ‡different from medium intensity \( (P < 0.05) \), \( n = 8 \).

TABLE 2
Plasma hormones, metabolites, and glucose kinetics during rest and exercise at low, medium, or high intensity

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>Low intensity</th>
<th>Medium intensity</th>
<th>High intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFAs (mmol/l)</td>
<td>0.55 ± 0.06</td>
<td>0.49 ± 0.06</td>
<td>0.37 ± 0.04†</td>
<td>0.34 ± 0.04†</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>567.4 ± 4.2</td>
<td>493.3 ± 3.1*</td>
<td>471.4 ± 4.5*</td>
<td>377.3 ± 3.9‡‡</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.1 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>2.8 ± 0.5*</td>
<td>7.2 ± 0.9*‡</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.9 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.2</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Glucose ( R_a ) ( (\mu mol \cdot kg^{-1} \cdot min^{-1}) )</td>
<td>7.9 ± 0.8</td>
<td>12.1 ± 1.1</td>
<td>19.5 ± 1.7*†</td>
<td>29.8 ± 4.9†‡</td>
</tr>
<tr>
<td>Glucose ( R_d ) ( (\mu mol \cdot kg^{-1} \cdot min^{-1}) )</td>
<td>7.7 ± 0.7</td>
<td>9.9 ± 1.2</td>
<td>18.9 ± 2.2*†</td>
<td>26.5 ± 4.2‡‡</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Different from resting \( (P < 0.05) \), †different from low intensity \( (P < 0.05) \), and ‡different from medium intensity \( (P < 0.05) \), \( n = 8 \).
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TABLE 3
Muscle metabolites during rest and exercise at low, medium, or high intensity

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Resting</th>
<th>Low intensity</th>
<th>Medium intensity</th>
<th>High intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mmol/kg dry wt)</td>
<td>381.1 ± 57.0</td>
<td>339.3 ± 43.5</td>
<td>263.8 ± 52.5†‡</td>
<td>154.6 ± 37.2†‡</td>
</tr>
<tr>
<td>Lactate (mmol/kg dry wt)</td>
<td>2.9 ± 0.4</td>
<td>5.1 ± 0.9</td>
<td>13.4 ± 4.4†</td>
<td>19.7 ± 5.5†</td>
</tr>
<tr>
<td>ATP (mmol/kg dry wt)</td>
<td>22.4 ± 1.3</td>
<td>22.5 ± 1.5</td>
<td>22.4 ± 1.4</td>
<td>20.6 ± 1.3</td>
</tr>
<tr>
<td>Free ADP (μmol/kg dry wt)</td>
<td>108.9 ± 7.3</td>
<td>143.6 ± 16.0</td>
<td>298.3 ± 84.1†</td>
<td>634.5 ± 179.6†‡‡</td>
</tr>
<tr>
<td>Free AMP (μmol/kg dry wt)</td>
<td>0.6 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>5.8 ± 3.2†</td>
<td>27.7 ± 12.8†‡‡</td>
</tr>
<tr>
<td>Free AMP/ATP ratio</td>
<td>0.03 ± 0.00</td>
<td>0.04 ± 0.01</td>
<td>0.22 ± 0.11†‡</td>
<td>1.10 ± 0.47†‡‡</td>
</tr>
<tr>
<td>Creatine (mmol/kg dry wt)</td>
<td>36.9 ± 3.7</td>
<td>43.8 ± 4.9</td>
<td>66.5 ± 10.9†‡</td>
<td>82.0 ± 9.7†‡‡</td>
</tr>
<tr>
<td>Creatine phosphate (mmol/kg dry wt)</td>
<td>77.6 ± 4.9</td>
<td>70.7 ± 4.2</td>
<td>52.4 ± 8.1†‡</td>
<td>35.5 ± 6.4†‡‡</td>
</tr>
</tbody>
</table>

*Different from resting (P < 0.05), †different from low intensity (P < 0.05), and ‡different from medium intensity (P < 0.05). n = 8.

2A). AMPK α2 activity increased (P < 0.05) by approximately fivefold during medium intensity and by approximately eightfold during high intensity compared with resting (Fig. 2B). A large progressive increase (P < 0.05) in phosphorylation of ACCβ at Ser-221 occurred as exercise intensity increased (Fig. 3). The fold increase (P < 0.05) in phosphorylation of ACCβ was ~3-, ~8-, and ~10-fold during exercise at low, medium, and high intensity, respectively, compared with resting. nNOSα phosphorylation tended to increase from resting to low intensity, then increased significantly during medium intensity, and then increased (P < 0.05) further during high intensity. The significant increase in ACCβ phosphorylation from resting to low intensity accompanied the trend for an increase in calculated AMP from 0.6 to 1.0 (μmol/kg dry wt, Table 3), indicating that ACCβ phosphorylation was particularly sensitive to the energy demands of exercise.

DISCUSSION

Activation of AMPK depends on phosphorylation at the activation loop Thr-172 by an upstream AMPK kinase(s), and AMPK activity can be further stimulated allosterically by AMP. AMPK kinase activity has not previously been investigated directly in skeletal muscle, although phosphorylation of AMPK Thr-172 by AMP kinase has been demonstrated in skeletal muscle during exercise (4–6). The results obtained in the present study indicate that there is substantial AMPK kinase activity at rest, in marked contrast to the low resting levels of AMPK activity in skeletal muscle. There was only a modest but significant increase in skeletal muscle AMPK kinase activity during exercise. The high level of AMPK kinase activity indicates that other factors are likely to be important at rest to maintain the AMPK in the inactive dephosphorylated state.

Because AMPK kinase activity was measured in crude extracts, we cannot rule out the possibility that important allosteric regulators may have been diluted out.

It has been reported that AMP binding to AMPK makes it a better substrate for AMPK kinase, and this may explain the low level of AMPK activity in resting muscle despite the presence of high AMPK kinase constitutive activity (44). AMPK α1 activity mirrored the change in AMPK kinase activity at each workload, but, unlike AMPK kinase activity, AMPK α2 activity increased greatly during moderate exercise and then increased further during high-intensity exercise. The apparent increase in AMPK α2 activity from 60 to 80% V02 peak may reflect the fivefold increase in free AMP/ATP ratio from 60 to 80% V02 peak. Further, because there was only a small increase in AMPK kinase activity during exercise compared with AMPK α2 activity, it appears that the major regulation of AMPK α2 activity during exercise is at the level of AMPK α2 rather than AMPK kinase. It is not known whether the apparent increase in AMPK kinase activity during exercise was due to posttranslational modification. Allosteric control of AMPK kinase due to accumulation of AMP in the biopsy extract was considered a possibility based on the report that AMP directly activated partially purified AMPK kinase (45). However, we have tested AMP activation of partially purified AMPK kinase from skeletal muscle, heart muscle, kidney, and liver, and in no case was the AMPK kinase activity stimulated by AMP when MBP-AMP α (1–312) was used as a substrate (S.M. and B.E.K., unpublished data). It has recently been shown that AMPK phosphorylation at Thr-172 increases during in situ stimulations in rat gastrocnemius muscle (5). A correlation between AMPK activity and Thr-172 phosphorylation was also observed (5). Insufficient muscle biopsy material in the present study was
available to correlate Thr-172 phosphorylation with AMPK activity; however, our results suggest that Thr-172 phosphorylation measurements are not a surrogate for direct AMPK kinase activity measurements.

The responsiveness of AMPK activity correlates inversely with the concentration of muscle glycogen (46). Contraction at a low or moderately reduced muscle glycogen content results in a greater level of activation of AMPK during contractions than when starting with a very high muscle glycogen content in both rats (46) and humans (47). The progressive decrease in muscle glycogen content during exercise in the present study paralleled the progressive increase in AMPK α2 activity with increasing workload. The mechanism underlying the inverse relationship between muscle glycogen content and AMPK activation is not known. Addition of glycogen to purified rat liver AMPK does not alter its activity (S.M. and B.E.K., unpublished data) so that suppression of AMPK activation in the presence of high muscle glycogen is not a direct effect of glycogen but rather a consequence of other factors.

Importantly, ACCβ phosphorylation increased during exercise at 40% VO₂ peak (Fig. 3), despite no detectable in vitro increase in AMPK activity at this low intensity of exercise (Fig. 2). Previously we have shown that ACCβ is associated with ACCβ in skeletal muscle (16). This suggests that ACCβ phosphorylation is an especially sensitive measure of in vivo AMPK signaling. Although the trend increase in the free AMP level from rest to 40% VO₂ peak was not statistically significant, the downstream ACCβ phosphorylation was. Similarly, low frequency stimulation in situ and low-intensity running in rats increases skeletal muscle ACCβ phosphorylation and decreases ACCβ activity without a detectable increase in stable AMPK activity (5). We interpret this as indicating that AMPK is very tightly coupled to the metabolic needs of contracting skeletal muscle.

There is evidence that factors other than malonyl-CoA are important in regulating the transport of fatty acids into the mitochondria during exercise in humans (13,22,23,48). The decrease in malonyl-CoA observed by others during exercise at ≥85% VO₂ peak (22) would be expected to increase fat oxidation, but fat oxidation decreases during this intensity of exercise compared with lower intensities (12,13) (Table 1). The reduction in calculated fat oxidation during high-intensity exercise in the present study involving relatively untrained individuals was more exaggerated.
than previous studies using endurance-trained subjects (12,13). Free carnitine decreases with increases in exercise intensity in human skeletal muscle, and it has been suggested that the reduced free carnitine would impair CPT1 activity (13). In addition, there is evidence in humans that the reduction in muscle pH observed during intense exercise may inhibit CPT1 (49). It is also possible that there is a reduction in oxygen in some fibers as the exercise intensity increases resulting in reduced reliance on fat oxidation (50) and that the increased reliance on fast-twitch muscle fibers at high intensities of exercise reduces fat oxidation.

The pattern of response of skeletal muscle AMPK α2 activity and whole-body glucose uptake to increases in exercise intensity were more similar than that of AMPK α1 activity and glucose uptake, suggesting that AMPK α2 may be more directly coupled to glucose uptake than AMPK α1. It should be noted, however, that the data of two recent studies suggest that AMPK activity and glucose uptake during exercise/contraction do not correlate under conditions of high muscle glycogen levels (46,47). Indeed, although whole-body glucose disposal and AMPK α2 activity did not increase significantly during exercise at 40% VO₂ peak in the present study, it is known that contracting skeletal muscle glucose uptake increases at this intensity (51,52).

A small but significant increase in AMPK α1 activity during moderate-intensity exercise was observed in the present study. Previously, we found no significant increase in AMPK α1 activity during 30 min of exercise at 60% VO₂ peak (16), and others (14,15) have found no increase in AMPK α1 activity at 50 and 70% VO₂ max. This variation may depend on the relative contribution of AMPK α1 activation due to allosteric versus Thr-172 phosphorylation control.

We have shown that nNOSµ is a target for phosphorylation by AMPK in human skeletal muscle (17). We found a significant increase in phosphorylation of nNOSµ at Ser-1451 immediately after maximal sprint exercise (17) but only modest effects during lower-intensity exercise at ~60% VO₂ peak (16). In the current study, nNOSµ phosphorylation was also quite variable between subjects and only achieved statistically significant increases at the highest levels of exercise intensity (Fig. 4). The pattern of response of nNOSµ phosphorylation and glucose uptake during exercise of increasing intensity was quite similar.

It is possible that some of the observed effects in the present study may have been due in part to the sequential nature of the protocol. However, the increases in glucose disposal (53) and AMPK α2 activity (54) during exercise were much greater than would be expected from the exercise duration alone, so they must have mainly been an effect of exercise intensity.

In summary, we have found that AMPK kinase is substantially active in resting human skeletal muscle and only modestly increased with exercise. Because AMPK α1 and α2 are essentially inactive at rest, these results are consistent with the idea that the increase in AMP during exercise acts allosterically on AMPK to make it more susceptible to phosphorylation at Thr-172 by AMPK kinase. The modest increase in AMPK kinase activity mirrored that of AMPK α1 activity in that they were both unchanged during exercise at 40% VO₂ peak and then increased to a similar extent during exercise at 60% VO₂ peak, with no further increase at 80% VO₂ peak. AMPK α2 activity exhibited a similar pattern to whole-body glucose use during exercise, with no changes during low-intensity exercise but progressively increases at the higher exercise intensities. Although not measured, it is likely that contracting skeletal muscle glucose uptake increased during low-intensity exercise. ACCβ phosphorylation increased during low-intensity exercise without a measurable increase in AMPK activity and progressively increased with increases in exercise intensity. We interpret these results as indicating that the increase in ACCβ phosphorylation during low-intensity exercise controls.
exercise results from allosteric activation of AMPK by AMP.

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