Adrenergic regulation of HSL serine phosphorylation and activity in human skeletal muscle during the onset of exercise

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Talanian, Jason L., Rebecca J. Tunstall, Matthew J. Watt, Mylinh Duong, Christopher G. R. Perry, Gregory R. Steinberg, Bruce E. Kemp, George J. F. Heigenhauser, and Lawrence L. Spriet. Adrenergic regulation of HSL serine phosphorylation and activity in human skeletal muscle during the onset of exercise. Am J Physiol Regul Integr Comp Physiol 291: R1094–R1099, 2006.—Skeletal muscle hormone-sensitive lipase (HSL) activity is increased by contractions and increases in blood epinephrine (EPI) concentrations and cyclic AMP activation of the adrenergic pathway during prolonged exercise. To determine the importance of hormonal stimulation of HSL activity during the onset of moderate- and high-intensity exercise, nine men [age 24.3 ± 1.2 yr, 80.8 ± 5.0 kg, peak oxygen consumption (VO2peak) 43.9 ± 3.6 ml·kg−1·min−1] cycled for 1 min at ~65% VO2peak, rested for 60 min, and cycled at ~90% VO2peak for 1 min. Skeletal muscle biopsies were taken pre- and postexercise, and arterial blood was sampled throughout exercise. Arterial EPI increased (P < 0.05) postexercise at 65% (0.45 ± 0.10 to 0.78 ± 0.27 mM) and 90% VO2peak (0.57 ± 0.34 to 1.09 ± 0.50 mM), HSL activity increased (P < 0.05) following 1 min of exercise at 65% VO2peak [1.05 ± 0.39 to 1.78 ± 0.54 mmol·min−1·kg dry muscle (dm)−1] and 90% VO2peak [1.07 ± 0.24 to 1.91 ± 0.62 mmol·min−1·kg dm−1]. Cyclic AMP content also increased (P < 0.05) at both exercise intensities (65%: 1.52 ± 0.67 to 2.75 ± 1.12, 90%: 1.85 ± 0.65 to 2.64 ± 0.93 μmol/kg dm). HSL Ser660 phosphorylation (~55% increase) and ERK1/2 phosphorylation (~33% increase) were augmented following exercise at both intensities, whereas HSL Ser563 and Ser565 phosphorylation were not different from rest. The results indicate that increases in arterial EPI concentration during the onset of moderate- and high-intensity exercise increase cyclic AMP content, which results in the phosphorylation of HSL Ser660. This adrenergic stimulation contributes to the increase in HSL activity that occurs in human skeletal muscle in the first minute of exercise at 65% and 90% VO2peak.

hormone-sensitive lipase; arterial epinephrine concentration; cyclic adenosine 5’-monophosphate; serine 660 phosphorylation; extracellular regulated kinase 1/2 phosphorylation

CARBOHYDRATE AND LIPID ARE the primary fuels for aerobic energy production in contracting skeletal muscle. Lipids are supplied to muscle from adipose tissue and the degradation of intramuscular triacylglycerol (IMTG) stores (24). IMTGs are hydrolyzed to free fatty acids (FFA) that can be acylated and enter the mitochondria to be oxidized. Adipose tissue and intramuscular lipolysis are thought to be regulated by enzymatic phosphorylation of hormone-sensitive lipase (HSL) (10, 11, 14, 16, 27). HSL can be phosphorylated at four activating sites and one inhibitory site and has a higher affinity to hydrolyze diacylglycerol over triacylglycerol (TG) (1, 19). Much of the early work examined the regulation of HSL activity in adipose tissue, which is predominantly regulated by hormones, leading to the name HSL (16).

During prolonged exercise, both contraction- and hormonal-based mechanisms can activate HSL. Muscle HSL activity, stimulated through contractile-based mechanisms (14, 27), occurs via a calcium-dependent protein kinase C (PKC) (7, 15), which stimulates extracellular regulated kinase 1/2 (ERK1/2) to ultimately phosphorylate HSL on Ser660 (9). As well, epinephrine (EPI)-mediated β-adrenergic stimulation of adenylyl cyclase activity and cAMP has also been shown to activate cAMP-dependent protein kinase A (PKA) to phosphorylate HSL on three sites (Ser563, 659, and 660, rat sequence numbering) (2).

During the onset of exercise, HSL activity and ERK1/2 phosphorylation increased in moderately trained men following 1 min of exercise at 30 and 65% of peak oxygen consumption (VO2peak) (23). Since no changes were observed in venous EPI concentration, it was believed that HSL activity was increased by contraction-based mechanisms only and independent of the β-adrenergic cascade. However, arterial EPI concentrations and muscle β-adrenergic intermediates and HSL phosphorylation sites specific to the cascade have not been measured. At the present time, the importance of the β-adrenergic pathway in activating HSL during exercise onset is unknown.

The aims of this study were to measure arterial EPI concentration, and skeletal muscle cAMP, specific cAMP-mediated HSL phosphorylation sites, and HSL activity during the onset of moderate- and high-intensity cycling exercise. We hypothesized that the β-adrenergic pathway would not be stimulated following 1 min of moderate-intensity exercise and that ERK1/2 would be increased. At this intensity, the mechanism for the increase in HSL activity would, therefore, be contraction based. We also hypothesized that arterial EPI and muscle cAMP and HSL phosphorylation would be increased along with ERK1/2 during the first minute of high-intensity exercise. This would imply that both contraction and hormonal regulation contributed to the increase in HSL activity following the onset of high-intensity exercise.

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ADRENERGIC REGULATION OF HSL DURING ONSET OF EXERCISE

R1095

METHODS

Nine male subjects participated in the study (mean ± SE age 24.3 ± 1.2 yr, body mass 80.8 ± 5.0 kg, V_{O2peak} 43.9 ± 3.6 ml·kg⁻¹·min⁻¹). On average, participants exercised 1–3 days/wk. While most subjects did not limit their exercise to one type, two subjects commonly participated in weight lifting, three subjects commonly participated in basketball, one subject played soccer, one normally cycled, and two normally walked. Before participation, written, informed consent was obtained from each subject following a detailed explanation of the experimental procedures and associated risks of the protocol. The human ethics committees of the University of Guelph and McMaster University approved the study.

Preliminary testing. On each visit, subjects arrived at the laboratory, having abstained from strenuous exercise and caffeine and exercise was averaged to calculate V_{O2peak}. A cadence 20 rpm below their target cycling rate. The final minute of the minute the resistance increased 15–30 W until they could not maintain a cadence 20 rpm below their target cycling rate. The final minute of exercise was averaged to calculate V_{O2peak}.

On the second visit to the laboratory, subjects performed constant-load cycling to verify power outputs corresponding to 65 and 90% V_{O2peak} for the experimental trials. Subjects cycled for 10 min at each power output, separated by ~20 min of rest.

Experimental protocol. Subjects arrived at the laboratory following a 10- to 12-h overnight fast. A Teflon 20-gauge 3.2-cm catheter (Baxter, Irvine, CA) was inserted into the radial artery, and the catheter was kept patent by flushing with 0.9% saline. One leg was prepared for percutaneous needle biopsy sampling of the vastus lateralis muscle. Two incisions were made in the skin and deep fascia under local anesthesia (2% xylocaine without EPI). Immediately before exercise, arterial blood (5 ml) and muscle samples were obtained while the subject remained rested. All muscle samples were immediately frozen in liquid nitrogen for subsequent analysis. Subjects then cycled for 1 min at 65% V_{O2peak} (161 ± 11 W) at a constant cadence (78–88 rpm). Arterial blood samples were obtained between 15–20, 35–40, and 50–55 s of exercise, and a second muscle biopsy was taken immediately following the exercise bout. During the 60 min of recovery, the other leg was prepared for muscle biopsies, and the 1-min exercise procedure was repeated at 90% V_{O2peak} (243 ± 15 W).

Analyses. Arterial whole blood was collected in sodium-heparin tubes. A portion (1.5 ml) was added to 30-μl EGTA and reduced glutathione and centrifuged (10,000 g). Samples were homogenized in ice-cold buffer containing 50 mM HEPES, 150 mM NaCl, 1 mM Na_{2}VO_{4}, 5 mM EDTA, 1.0% Triton X-100, 10% glycerol, and 2 μl protease inhibitor for the determination of ERK phosphorylation. Muscle lysates were solubilized in Laemmli sample buffer with DTT, heated for ~5 min, resolved by SDS-PAGE on 10% polyacrylamide gels, and transferred to a polyvinylidene difluoride membrane. Membranes were blocked with 3% BSA and immunoblotted with a monoclonal mouse phospho-MAPK42/44 antibody (Cell Signaling Technology, Beverly, MA) overnight at 4°C. Membranes were incubated for 1 h at room temperature with the corresponding secondary antimouse antibody, and the immunoreactive proteins were detected with enhanced chemiluminescence and quantified by densitometry.

Rabbit polyclonal antibodies against the peptide and phosphopeptides based on the amino acid sequence (human, Q05469; rat P15304) of human HSL (192–203) EHYYKRNGETGL. Data were expressed as the means ± SE in mmol/kg dry mass; n = 9. Significantly different from rest at the same power output (P < 0.05). *Significantly different than 65% V_{O2peak} at the same time point (P < 0.05).

<table>
<thead>
<tr>
<th>Table 1. Muscle metabolite contents before and after 1 min of exercise at 65% and 90% V_{O2peak}</th>
<th>Rest 1 min</th>
<th>65% V_{O2peak}</th>
<th>90% V_{O2peak}</th>
<th>Rest 1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>81.5 ± 11.8</td>
<td>53.3 ± 16.6*</td>
<td>81.2 ± 11.6</td>
<td>35.6 ± 16.5*†</td>
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<tr>
<td>Cr</td>
<td>38.9 ± 8.0</td>
<td>66.6 ± 12.4*</td>
<td>37.8 ± 6.6</td>
<td>84.1 ± 20.7*†</td>
</tr>
<tr>
<td>ATP</td>
<td>23.9 ± 2.2</td>
<td>23.0 ± 1.4</td>
<td>24.7 ± 1.3</td>
<td>23.4 ± 1.4</td>
</tr>
<tr>
<td>Lactate</td>
<td>7.4 ± 6.9</td>
<td>16.6 ± 8.7*</td>
<td>9.1 ± 4.4</td>
<td>36.6 ± 8.3*†</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry mass; n = 9. V_{O2peak}, peak oxygen consumption; PCr, phosphocreatine; Cr, creatine. *Significantly different from rest at the same power output (P < 0.05). †Significantly different than 65% V_{O2peak} at the same time point (P < 0.05).
RESULTS

Muscle metabolites. PCr decreased following moderate-intensity cycling and decreased to a greater extent following high-intensity cycling (Table 1). Cr changes were reciprocal to PCr. ATP concentrations were unaffected by exercise at both power outputs. Muscle lactate increased following exercise at 65% and to a greater extent at 90% VO₂peak.

Arterial blood measurements. At 65 and 90% VO₂peak, arterial EPI concentrations increased throughout the exercise bout and were significantly higher than rest at 40 and 60 s of exercise at both power outputs (Fig. 1). The increase in arterial EPI with exercise was not different between trials. Arterial lactate concentrations were higher than rest at 40 and 60 s of exercise at both power outputs and were higher at 40 and 60 s at 90 vs. 65% VO₂peak (Table 2). Glucose concentrations were unaffected by exercise at both power outputs.

HSL activity. At rest, HSL activity did not differ between trials but increased from rest following 1 min of exercise at 65 and 90% VO₂peak (Fig. 2). The increase in HSL activity with exercise was not different between trials.

cAMP content and ERK1/2 phosphorylation. Resting cAMP content was similar between trials and increased immediately after each exercise bout (Fig. 3). Increases in cAMP did not differ between trials. One minute of exercise resulted in an increase in ERK1/2 phosphorylation at 65% VO₂peak (1.00 ± 0.31 to 1.35 ± 0.31 arbitrary units) and 90% VO₂peak (1.00 ± 0.52 to 1.31 ± 0.17 arbitrary units).

HSL phosphorylation. HSL contains three PKA-sensitive phosphorylation sites, and two were measured in the present study (Ser659 not measured). Ser660 phosphorylation was increased at 65 and 90% of VO₂peak compared with rest, while Ser563 was unaffected by exercise. In addition, Ser565 phosphorylation (AMPK sensitive) was not altered following exercise (Fig. 4).

DISCUSSION

The results of the present study established that arterial EPI concentrations were elevated and that the β-adrenergic pathway in skeletal muscle was activated (cAMP) following 1 min of exercise at 65% and 90% VO₂peak. Values are means ± SE; n = 9. *Significant increase compared with rest of same power output, P < 0.05.

Table 2. Arterial whole blood lactate, glucose, and epinephrine concentrations during 1 min of cycling at 65% and 90% VO₂peak

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>20 s</th>
<th>40 s</th>
<th>60 s</th>
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<tr>
<td>Lactate, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65%</td>
<td>0.7±0.1</td>
<td>0.8±0.1</td>
<td>1.0±0.1*</td>
<td>1.3±0.1*</td>
</tr>
<tr>
<td>90%</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>1.5±0.1†</td>
<td>2.6±0.2*†</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65%</td>
<td>4.5±0.1</td>
<td>4.3±0.1</td>
<td>4.4±0.1</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>90%</td>
<td>4.4±0.1</td>
<td>4.3±0.1</td>
<td>4.4±0.1</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Epinephrine, nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65%</td>
<td>0.45±0.16</td>
<td>0.74±0.25</td>
<td>0.85±0.19*</td>
<td>0.78±0.15*</td>
</tr>
<tr>
<td>90%</td>
<td>0.57±0.19</td>
<td>0.78±0.16</td>
<td>0.92±0.38*</td>
<td>1.1±0.27*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. *Significantly different from rest at the same power output (P < 0.05). †Significantly higher than 65% VO₂peak at the same time point (P < 0.05).

Fig. 1. Arterial epinephrine concentrations during 1 min of exercise at 65% and 90% peak oxygen consumption (VO₂peak). Values are means ± SE; n = 9. *Significant increase compared with rest of same power output, P < 0.05.

Fig. 2. Hormone-sensitive lipase (HSL) activity before and after 1 min of exercise at 65% and 90% VO₂peak. Values are means ± SE; n = 9. DM, dry mass. *Significant increase compared with rest of same power output, P < 0.05.

Fig. 3. Cyclic AMP content before and after 1 min of exercise at 65% and 90% V˙O₂peak. Values are means ± SE; n = 9. *Significant increase compared with rest of same power output, P < 0.05.
of exercise at both 65 and 90% $\dot{V}O_2\text{peak}$. The increases in cAMP appeared to stimulate PKA activity to increase HSL Ser660 phosphorylation, which ultimately contributed to the increases in HSL activity at the onset of exercise (Fig. 5). A second cAMP-dependent serine phosphorylation site (Ser563) was not affected by exercise. An increase in ERK1/2 phosphorylation following both exercise bouts suggests that both contraction and hormonal regulation contributed to the increase in HSL activity during the onset of moderate- and high-intensity exercise. This conclusion supported our hypothesis for high-intensity exercise but was contrary to our hypothesis for moderate-intensity exercise.

The arterial EPI concentration was significantly increased within 40 s of cycling at 65% $\dot{V}O_2\text{peak}$ in the present study, while previous work reported no increases in venous EPI, even after 3 min of exercise (30). The combined results support previous work demonstrating that venous EPI concentrations do not accurately reflect the arterial EPI concentrations perfusing active muscle during the onset of exercise. These findings also support a role for $\beta$-adrenergic-induced activation of HSL during the transition from rest to moderate- and high-intensity exercise.

cAMP content was increased following 1 min of exercise at both intensities. The increase was likely due to increased adenylate cyclase activity following $\beta$-adrenergic stimulation. To our knowledge, this is the first study to measure an increase in cAMP content in human skeletal muscle within 1 min of exercise. cAMP binds to an inactive cAMP-dependent protein kinase tetrameric enzyme, consisting of two regulatory and two catalytic subunits. Excess cAMP binding to the regulatory subunit results in the release of two activated PKA subunits. PKA has been demonstrated to phosphorylate HSL at the serine sites 563, 569, and 660, leading to increased HSL activity.

The present study measured increased Ser660 phosphorylation and no change in Ser563 phosphorylation. Due to technical limitations, we were not able to measure Ser659. The increase in Ser660 phosphorylation supports the hypothesis that this is an important site for $\beta$-adrenergic activation of HSL (1, 21). However, the role of Ser563 phosphorylation in activating HSL is not well understood, and our results suggest that it is not a primary regulator of HSL activity at the onset of exercise. Roepstorff et al. (19) reported that HSL activity had returned to resting values following 60 min of exercise and that there was no increase in Ser563. Conversely, Watt et al. (25) observed that both Ser563 and 660 increased following 15 and 90 min of moderate-intensity exercise and that both had decreased 120 min after exercise cessation. Therefore, it is uncertain what role Ser563 is playing during prolonged aerobic exercise, but the present results suggest that Ser563 is not a regulatory site at the onset of moderate and intense aerobic exercise.

![Fig. 4. Phosphorylation of HSL Ser660, 563, and 565 before and after 1 min of exercise at 65% and 90% $\dot{V}O_2\text{peak}$. Values are means ± SE, normalized to resting values; n = 6. *Compared with rest, there were significant increases in Ser660 phosphorylation following exercise at 65 and 90%, P < 0.05.](http://ajpregu.physiology.org/)

![Fig. 5. Representative immunoblots for total HSL and HSL phosphorylated at Ser563, 565, and 660.](http://ajpregu.physiology.org/)
This study observed an increase in ERK1/2 phosphorylation following 1 min of exercise. It has been established that ERK1/2 can phosphorylate HSL on Ser600 in adipose tissue (9). However, at the present time, we are unaware of any measurements of Ser600 phosphorylation in rodent or human skeletal muscle. Despite the lack of measurements, there is evidence that contraction-based mechanisms can increase ERK1/2 and HSL activity. It is thought that ERK1/2 can be activated at the onset of contraction through calcium-dependent PKC signaling. Consistent with this hypothesis, a recent study demonstrated that the inhibition of calcium-stimulated PKC in rat skeletal muscle blocked ERK1/2 phosphorylation (6). In addition, using caffeine to stimulate calcium release, they showed that increases in calcium, independent of contractile activity, stimulated hydrolysis by HSL activity. In contrast, high concentrations of calcium have been shown to inhibit IMTG hydrolysis, likely through calcium/calmodulin-dependent kinase II, which phosphorylates HSL on Ser565 and results in the inhibition of HSL activity (29, 32). While it is apparent calcium release increases HSL activity, the role of calcium-stimulated calcium/calmodulin-dependent kinase II appears to contradict its stimulatory role.

Similar to previous observations in our laboratory, HSL activity was increased following 1 min of exercise (23). While it is possible that the observed increase in HSL activity is due to the presence of other neutral TG lipases (15), previous work that used neutralizing HSL antibodies to block HSL activity in tissue lysates demonstrated that the exercise-induced increase in lipase activity was attributable to HSL (19, 28). Interestingly, IMTG hydrolysis can occur in the absence of increases in HSL activity and is likely due to a non-HSL TG lipase (17). While a newly identified TG lipase has been suggested to play a role in hydrolyzing TG to diacylglycerol and FFA (13, 33), the mechanisms and exact role of this TG lipase are presently not well understood.

The rapid increase in muscle HSL activity during the onset of exercise may be due, in part, to the additional need for fuel in active muscle. Basal FFA concentrations are not adequate to supply muscle with the added fuel required during moderate- to high-intensity exercise. While peripheral adipose stores increase plasma FFA concentrations during the onset of exercise, IMTG are likely to be the primary source of FFA for mitochondrial fat oxidation. IMTG pools have been described as an important fuel source during prolonged exercise; however, the role of IMTG as a fuel source during exercise onset is unknown and difficult to measure (22, 24). The rapid increase we observed in HSL activity is similar to exercise-induced increases observed in carbohydrate metabolism, specifically glycogen phosphorylase and pyruvate dehydrogenase activities during the first minute of exercise (12, 26). Skeletal muscle appears to have immediate exercise-induced mechanisms that trigger increases in carbohydrate and lipid metabolism to compensate for the added need for fuel during the onset of exercise.

In summary, this study indicates that β-adrenergic signaling and phosphorylation on Ser660, but not Ser563, are important events in the early exercise-induced increase in HSL activity in human skeletal muscle. We conclude that increases in arterial EPI concentration contribute to the increase in skeletal muscle HSL activity in the first minute of moderate and intense aerobic exercise.

GRANTS

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