Exercise induces lipoprotein lipase and GLUT-4 protein in muscle independent of adrenergic-receptor signaling

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Greiwe, Jeffrey S., John O. Holloszy, and Clay F. Semenkovich. Exercise induces lipoprotein lipase and GLUT-4 protein in muscle independent of adrenergic-receptor signaling. J Appl Physiol 89: 176–181, 2000.—Exercise increases the expression of lipoprotein lipase (LPL) and GLUT-4 in skeletal muscle. Intense exercise increases catecholamines, and catecholamines without exercise can affect the expression of both LPL and GLUT-4. To test the hypothesis that adrenergic-receptor signaling is central to the induction of LPL and GLUT-4 by exercise, six untrained individuals [age 28 ± 4 (SD) yr, peak oxygen uptake 3.6 ± 0.3 l/min] performed two exercise bouts within 12 days. Exercise consisted of cycling at ~65% peak oxygen uptake for 60 min with (block trial) and without (control trial) adrenergic-receptor blockade. Exercise intensity was the same during the block and control trials. Plasma catecholamine concentrations were significantly higher and heart rates were significantly lower during the block trial compared with the control trial, consistent with known effects of adrenergic-receptor blockade. However, blockade did not prevent the induction of either LPL or GLUT-4 proteins assayed in biopsies of skeletal muscle. LPL was significantly increased by 170–240% after exercise compared with before exercise during both the control and block trials. These findings provide evidence that exercise increases muscle LPL and GLUT-4 protein content via signals generated by alterations in cellular homeostasis and not by adrenergic-receptor stimulation.

catecholamines; lipid metabolism; glucose transport; β-blocker; α-blocker

CARBOHYDRATES AND FATTY ACIDS are the main fuel sources utilized during exercise and during recovery from exercise. Lipoprotein lipase (LPL), the rate-limiting factor for the metabolism of triglyceride-rich lipoproteins, and GLUT-4, the major glucose transporter of skeletal muscle, are critical for the acquisition of fatty acids and glucose during and after exercise. The uptake of substrates after exercise replenishes energy stores in preparation for the next bout of exercise and provides fuel for muscle repair and other recovery functions (15).

Exercise depletes high-energy phosphates in muscle and increases the concentration of AMP, causing activation of AMP-activated protein kinase (AMPK). Activation of AMPK probably mediates translocation of GLUT-4 into the sarcolemma with exercise (9, 20). Exercise also increases mRNA and protein levels of both GLUT-4 and LPL in skeletal muscle within 18 h after a bout of exercise (29, 31, 32). The signaling events leading to enhanced expression (probably by activation of transcription) of the GLUT-4 and LPL genes have not yet been identified. Catecholamines increase as a function of exercise intensity (6) and interact with G protein-coupled cell-surface receptors. Both catecholamines and exercise are known to increase intracellular concentrations of cAMP, a signaling molecule implicated in the expression of LPL in skeletal and cardiac muscle (3–5, 27).

Although catecholamines stimulate LPL expression in resting muscle (3–5, 27), it is unclear whether the striking rise in catecholamines seen with exercise contributes to exercise induction of LPL. It is also not known whether catecholamines increase GLUT-4 expression. In this paper, we specifically address the question of whether the increase in skeletal muscle LPL and GLUT-4 protein after exercise is caused by adrenergic-receptor stimulation during exercise. To answer this question, we measured skeletal muscle LPL and GLUT-4 protein content after a single bout of exercise performed in the presence and absence of adrenergic-receptor blockade.

METHODS

Subjects. Subjects were healthy, active, but untrained individuals who regularly performed recreational physical activities. Body composition was estimated from hydrostatic weight. The study was approved by the Washington University Human Studies Committee. Informed consent was obtained from each subject.

Oxygen uptake. A continuous cycle ergometer test was performed by each individual to determine peak oxygen uptake (VO2 peak). The protocol consisted of cycling at 100, 150, and 200 W for 3 min per exercise intensity, followed by 25 W increments every 1 min until exhaustion. An automated on-line system was used to collect and analyze expired air throughout the exercise test (Max-I, Physio-Dyne Instrument, Farmingdale, NY). Each subject met at least two of the following criteria during the VO2 peak protocol: plateau in

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oxygen consumption with increasing work rate, heart rate within 10 beats/min of age-predicted maximal heart rate, and a respiratory exchange ratio exceeding 1.11.

Exercise trials. Two exercise trials, one with and one without adrenergic-receptor blockade, were completed within a 7-to-12-day period. The order of the trials was randomized. Subjects did not exercise the day before each trial.

Adrenergic-receptor blockade was achieved with the nonselective α-blocker phenoxybenzamine (Dibenzyline, Smith-Kline Beecham Pharmaceuticals) and the nonselective β-blocker propranolol (Inderal, Ayerst Laboratories). Phenoxybenzamine is an oral medication. It was not possible to obtain phentolamine, an intravenous α-blocker, for this study. At the time of these experiments, phentolamine use in the United States was limited to medical emergencies because of manufacturing problems. As previously described (36), α-blockade with phenoxybenzamine was started 3 days before the exercise trial at a dose of 10 mg by mouth four times per day (day 1). The dosage was progressively increased to 20 mg four times per day (day 2) and to 30 mg four times per day (day 3), and then subjects ingested 40 mg 1.5 h before the exercise bout on day 4. For β-blockade (24), subjects received a primed (143 μg/kg) continuous intravenous infusion of propranolol, beginning 30 min before the exercise bout. At the start of exercise, the dosage was decreased to 1.4 μg·kg⁻¹·min⁻¹, and infusion of propranolol was continued at this rate until 15 min after the completion of exercise.

On each exercise day, a blood sample was obtained in the morning after a 12-h fast. A vastus lateralis muscle biopsy was performed. Forty-five minutes later, subjects exercised for 1 h on an electrically braked cycle ergometer at ~65% VO₂peak. Oxygen uptake was measured at 15 min-intervals during the exercise bout, and power output was adjusted to maintain ~65% VO₂peak. After the exercise bout, subjects rested in bed for the next 22 h in the Washington University General Clinical Research Center. Blood samples were obtained periodically from an indwelling catheter, and muscle biopsies were performed 8 and 22 h after exercise. Diets during the protocol were standardized and identical for the two trials.

Muscle biopsies. Biopsies were performed as previously described (31). After the initial biopsy, the contralateral leg was used for the second biopsy. The third biopsy was performed 3 cm distal to the site of the initial biopsy. The biopsies for the second trial were performed in the same sequence as the first trial at sites 3 cm distal to the initial biopsies. Muscle samples were immediately cleansed with saline, trimmed of any visible fat, blotted dry, and frozen in liquid nitrogen. Samples were then stored at −80°C for subsequent analysis. Muscle samples from all time points were analyzed for LPL and GLUT-4 protein content by Western blotting as described previously (30, 31). Antibody-bound protein was visualized by using enhanced chemiluminescence (Amersham/Pharmacia Biotech). Protein bands were quantified by densitometry. Data are expressed per protein content. As shown previously (32), acute exercise has no detectable effect on the control protein myosin when this assay is used.

Blood sampling and analysis. A polyethylene catheter was inserted into an antecubital vein in the morning before the initial biopsy and kept patent with saline. Blood samples were obtained from the subjects before the initial biopsy, immediately before exercise, at 30 min of exercise, and just before completion of exercise. In addition, blood samples were obtained every 30 min for the first 2.5 h after exercise and then every 1 h for the next 6 h. Samples were subjected to centrifugation (15 min at 2,000 g), and the supernatant was collected and stored at −80°C until subsequent analyses. Samples were analyzed for insulin (25), catecholamines (33), leptin (23), glucose (Sigma Chemical, St. Louis, MO), and nonesterified fatty acids (Waco Chemicals, Dallas TX). Lipid and lipoprotein levels were measured after a 12-h fast in accordance with the Centers for Disease Control lipid standardization program.

Statistics. Muscle protein content of LPL and GLUT-4 as well as plasma samples were analyzed by using two-way repeated-measures analysis of variance. A Tukey post hoc test was performed when the analysis of variance revealed significant differences.

RESULTS

Subject characteristics are shown in Table 1. Exercise intensities during the two trials were not significantly different (Table 2). Heart rates were significantly lower during exercise for the block trial compared with the control trial (Table 2). Plasma epinephrine (Fig. 1A) and norepinephrine (Fig. 1B) concentrations were significantly higher during exercise for the block trial compared with the control trial, an expected result because receptor blockade interferes with normal catecholamine clearance. Lower heart rates and higher catecholamine levels during the block trial indicate that adrenergic-receptor blockade was successful.

Muscle LPL protein content. Skeletal muscle biopsies were performed before and at 8 and 22 h after the exercise bout for both trials. Muscle was assayed for LPL and GLUT-4 protein content. The absolute amounts of muscle tissue LPL and GLUT-4 were variable between individuals. Therefore, LPL and GLUT-4 protein content were expressed as a percentage of the initial biopsy. LPL and GLUT-4 protein content were lower in the block compared with control trials. The percentage decrease was greater for LPL than for GLUT-4. The percentage decrease in LPL protein content was 10.2% (3.3 ± 0.36%) for controls and 20.9% (6.1 ± 0.35%) for block trials (Table 2).

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177 ± 4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.6 ± 3.8</td>
</tr>
<tr>
<td>VO₂peak, l/min</td>
<td>3.6 ± 0.3</td>
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<tr>
<td>Body fat, %</td>
<td>13.1 ± 2.7</td>
</tr>
<tr>
<td>Serum TC, mmol/l</td>
<td>4.05 ± 0.36 (156.8 ± 14.1)</td>
</tr>
<tr>
<td>Serum LDL, mmol/l</td>
<td>2.44 ± 0.35 (94.3 ± 13.6)</td>
</tr>
<tr>
<td>Serum HDL, mmol/l</td>
<td>1.1 ± 0.1 (42.8 ± 4.1)</td>
</tr>
<tr>
<td>Serum TG, mmol/l</td>
<td>1.1 ± 0.24 (98.7 ± 20.9)</td>
</tr>
</tbody>
</table>

Values are means ± SD; values in parentheses are in mg/dl. VO₂peak, peak oxygen uptake; TC, total cholesterol; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TG, triglycerides. Lipid and lipoprotein levels were assayed in samples obtained after a 12-h fast.

Table 2. Work and heart rate data for each exercise trial

<table>
<thead>
<tr>
<th>Exercise Time, min</th>
<th>Trial</th>
<th>Exercise Intensity, W</th>
<th>Heart Rate, beats/min</th>
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<tbody>
<tr>
<td>15</td>
<td>Control</td>
<td>167 ± 17</td>
<td>149 ± 8*</td>
</tr>
<tr>
<td>30</td>
<td>Block</td>
<td>170 ± 16</td>
<td>125 ± 6</td>
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<tr>
<td>45</td>
<td>Control</td>
<td>153 ± 13</td>
<td>151 ± 9*</td>
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<tr>
<td></td>
<td>Block</td>
<td>160 ± 15</td>
<td>129 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>153 ± 12</td>
<td>153 ± 9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>161 ± 15</td>
<td>127 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference (by using paired t-tests) in heart rate compared with block trial at the same exercise time, P < 0.05.
signals for each individual were compared with signals in the preexercise sample for each trial, and data were analyzed by repeated-measures analysis of variance to focus on differences associated with the intervention rather than differences between individuals. As shown in Fig. 2, left, LPL protein in the control trial was increased 8 h after exercise in the control trial. At 22 h after exercise in the control trial, LPL protein was increased by 170 ± 100% (P < 0.05). The same pattern was seen in the block trial (Fig. 2, right). At 22 h postexercise, LPL protein content was 240 ± 110% greater than baseline (P < 0.05) even though exercise was performed in the setting of both α- and β-blockade.

Muscle GLUT-4 protein content. GLUT-4 protein content was also elevated after exercise bouts in both the control and block trials (Fig. 3). GLUT-4 protein content was already elevated 8 h after exercise in each trial. By 22 h postexercise, GLUT-4 protein content was increased by 51 ± 16% (P < 0.05) in the control trial and by 32 ± 6% (P < 0.05) in the block trial compared with baseline.

For LPL and GLUT-4 in both trials, a significant time main effect was found by two-way repeated-measures analysis of variance. These results suggest that, at least under the conditions of this experiment, the time course of induction of these proteins in human skeletal muscle is similar and unaffected by adrenergic-receptor blockade.

Insulin and glucose affect LPL and GLUT-4 expression. Adrenergic-receptor blockade was associated with lower insulin levels at 0-h (P = 0.04), 0.5-h (P = 0.05), and 1-h (P = 0.02), time points just before and during exercise (Fig. 4A). However, there were no significant differences at other time points and no significant differences at any time points for glucose concentrations (Fig. 4B).

Circulating nonesterified fatty acids tend to increase with exercise because catecholamines promote the activity of hormone-sensitive lipase in adipose tissue. There was a trend for fatty acids to be lower during exercise in the block trial (data not shown), but these differences were not significant. There was no difference in circulating leptin levels between the trials (data not shown).

DISCUSSION

Our results confirm that a single bout of exercise increases skeletal muscle LPL protein content (32). Several previous studies have reported the induction of GLUT-4 protein after 7–10 days of endurance exercise (2, 7). A prolonged, single bout of exercise is known to increase skeletal muscle GLUT-4 expression in rats (29). To the best of our knowledge, the present results represent initial data showing that a single bout of exercise also increases GLUT-4 protein in the skeletal muscle of humans.

The present results also provide insight into the mechanisms underlying the induction of these two proteins that are critical for normal glucose and lipid metabolism. Catecholamines increase as a function of exercise intensity (6), and interact with G protein-coupled adrenergic cell-surface receptors to affect numerous metabolic pathways. Blocking adrenergic receptors by using phenoxybenzamine (a nonspecific α-blocker) and propranolol (a nonspecific β-blocker) during exercise did not prevent the induction of LPL and GLUT-4 protein in human skeletal muscle. These results suggest that, even in the absence of stimulation by catecholamines, skeletal muscle can activate signaling pathways that produce a coordinated response to ensure fuel acquisition.

At least for LPL, several lines of evidence suggest that catecholamines are involved in the response to exercise. Catecholamines increase cAMP, cAMP levels are increased as a function of exercise or in response to elevated

Fig. 1. Plasma epinephrine (A) and norepinephrine (B) concentrations during exercise bouts for control and block (adrenergic-receptor blockade by using phenoxybenzamine and propranolol) trials. A resting sample was obtained (−1 h), and then the subjects exercised for 60 min at ~65% peak oxygen uptake (0–1 h). After exercise, subjects rested in bed for 22 h. Blood samples were obtained at 30-min intervals for 3.5 h (1–4.5 h) after exercise and then every 60 min for an additional 5 h (4.5–9.5 h). A sample was also obtained at 22 h postexercise. Plasma levels were analyzed between control and block trial with a 2-way repeated-measures analysis of variance. A significant interaction was found between control and block trials (P < 0.01).
plasma catecholamine concentrations (17, 21), and a cAMP-response element is present in the human LPL promoter (16). Urinary excretion rates of catecholamines account for >80% of the variability in human skeletal muscle LPL activity (22). Infusion of either epinephrine or isoproterenol increases skeletal muscle LPL expression in humans and rodents (3, 4, 27). Catecholamines also increase LPL activity in rat cardiac muscle (5).

The present results suggest that signaling induced by the interaction between catecholamines and adrenergic receptors is not required for the exercise-induced increase in LPL protein content. A previous study in humans concluded that local contractile activity is responsible for increased LPL activity in muscle (14), but it did not preclude possible local effects of catecholamines released from nerves innervating exercis-
ing muscles. By using systemic adrenergic-receptor blockade, our data also show that catecholamines released locally as a consequence of contractile activity do not play a critical role in LPL induction. Taken together with a study in rats (8), available data indicate that muscle contraction itself induces LPL. The mediator of this effect is unknown, although cAMP is an attractive candidate. cAMP levels are elevated during exercise even with β-blockade (17, 18).

Studies of the effects of catecholamines on GLUT-4 are less consistent. Short-term infusion of epinephrine in rats after surgical removal of the adrenal medulla decreases GLUT-4 transcription (13), suggesting that catecholamines decrease GLUT-4 expression. Rats subjected to β-blockade during 6 wk of exercise training show no increase in muscle GLUT-4, suggesting that catecholamines are necessary for the exercise-induced increase in GLUT-4 (19). The present results do not support this interpretation. One possible explanation for this apparent discrepancy is the fact that the rat study measured GLUT-4 ≥48 h after exercise, a time point at which adaptive changes in GLUT-4 would be reversed (12).

LPL protein content usually reflects LPL enzyme activity in skeletal muscle (31, 35). Under most circumstances, GLUT-4 protein content reflects the capacity of skeletal muscle to carry out glucose transport (10, 29, 30). Protein levels for LPL and GLUT-4 in the present study are probably elevated because of increased message levels. Previous studies by our laboratory using humans show that an increase in skeletal muscle LPL mRNA precedes an increase in LPL protein after exercise (32). Previous studies by our laboratory using rodents show that exercise induces GLUT-4 mRNA and protein (29). Others have demonstrated an increase in GLUT-4 transcription with exercise (26). Therefore, it is likely that exercise activates a transcriptional signaling pathway independent of adrenergic receptors to increase LPL and GLUT-4 protein. A critical issue is the nature of the signaling pathways that modulate activation of transcription by exercise.

AMPK, activated by energy depletion in muscle, is likely to be involved in the induction of exercise-responsive genes. Growing evidence implicates this kinase in GLUT-4 translocation (9, 20), and recent data indicate that AMPK induces GLUT-4 and hexokinase expression (11). However, the downstream mediators of AMPK are unknown. The mitogen-activated protein kinase pathway may be involved. Muscle contraction is a potent activator of this pathway (1). Mitogen-activated protein kinase may be responsible for the increased transcription of immediate-early genes in skeletal muscle after exercise (34).

Insulin-receptor signaling may also be involved in exercise responses. Skeletal muscle contraction has striking metabolic effects in the absence of insulin (28). Consistent with this notion, exercise increases skeletal muscle glucose transport in muscle-specific insulin-receptor knockout mice (37). However, insulin potentiates the exercise effect in these mice without skeletal muscle insulin receptors, suggesting that perhaps insulin receptors in nonmyocytes or insulin interacting with other receptors can affect exercise responses. In the present study, adrenergic-receptor blockade was associated with lower plasma insulin levels during exercise (Fig. 4A). Adrenergic-receptor signaling has complex effects on insulin secretion and action (38). Because lower insulin levels were seen without differences in plasma glucose (Fig. 4B), adrenergic-receptor blockade may modestly enhance insulin signaling during exercise, at least under the conditions of this experiment.

In summary, a single bout of exercise increases human skeletal muscle LPL and GLUT-4 protein content in the setting of both α- and β-adrenergic-receptor blockade. Muscle contraction itself, perhaps acting in response to depletion of high-energy phosphate stores, appears to mediate fuel acquisition by muscle.

We appreciate the commitment of our study subjects.

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