Acute exercise and GLUT4 expression in human skeletal muscle: influence of exercise intensity

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Kraniou, Giorgos N., David Cameron-Smith, and Mark Hargreaves. Acute exercise and GLUT4 expression in human skeletal muscle: influence of exercise intensity. J Appl Physiol 101: 934–937, 2006. First published June 8, 2006; doi:10.1152/japplphysiol.01489.2005.—To examine the influence of exercise intensity on the increases in vastus lateralis GLUT4 mRNA and protein after exercise, six untrained men exercised for 60 min at either 39 % peak oxygen consumption (V\textsubscript{O\textsubscript{2 peak}}) (Lo) or 27 ± 2 min at 83 ± 2 % \textit{V}\textsubscript{O2 peak} (Hi) in counterbalanced order. Preexercise muscle glycogen levels were not different between trials (Lo: 408 ± 35 mmol/kg dry mass; Hi: 420 ± 43 mmol/kg dry mass); however, postexercise levels were lower (P < 0.05) in Hi (169 ± 18 mmol/kg dry mass) compared with Lo (262 ± 35 mmol/kg dry mass). Thus calculated muscle glycogen utilization was greater (P < 0.05) in Hi (251 ± 24 mmol/kg) than in Lo (262 ± 34 mmol/kg). Exercise resulted in similar increases in GLUT4 gene expression in both trials. GLUT4 mRNA was increased immediately at the end of exercise (~2-fold; P < 0.05) and remained elevated after 3 h of postexercise recovery. When measured 3 h after exercise, total crude membrane GLUT4 protein levels were 106% higher in Lo (3.3 ± 0.7 vs. 1.6 ± 0.3 arbitrary units) and 61% higher in Hi (2.9 ± 0.5 vs. 1.8 ± 0.5 arbitrary units) relative to preexercise levels. A main effect for exercise was observed, with no significant differences between trials. In conclusion, exercise at ~40 and ~80% \textit{V}\textsubscript{O2 peak} with total work equal increased GLUT4 mRNA and GLUT4 protein in human skeletal muscle to a similar extent, despite differences in exercise intensity and duration.

gene expression; glucose; protein expression

AS THE MAJOR FACILITATIVE glucose transporter in skeletal muscle, GLUT4 is essential for insulin- and contraction-stimulated glucose uptake in this tissue (27). Skeletal muscle GLUT4 levels increase rapidly in response to exercise training (2, 6, 8, 10), decrease with detraining (13) and may contribute to the concomitant changes in insulin action observed with such interventions. In response to a single exercise bout in rats, rates of GLUT4 transcription in skeletal muscle are increased, peaking at 3 h postexercise (16). Consistent with this observation, we have observed increased GLUT4 mRNA expression in human skeletal muscle immediately (11, 14) and 3 h (11) after 60 min of cycle ergometer exercise at ~70–75% peak pulmonary oxygen consumption (\textit{V}\textsubscript{O2 peak}). The mechanisms responsible for the activation of GLUT4 transcription in response to exercise remain to be fully elucidated (3), but increased sarcoplasmic calcium and altered energy status play important roles, with the \textit{Ca}\textsuperscript{2+}/calmodulin-dependent kinase (CaMK) and AMP-activated protein kinase (AMPK) being key links between these stimuli and GLUT4 transcriptional activation (17). Increases in GLUT4 protein have also been observed during recovery from a single exercise bout (5, 12, 19), suggesting activation of posttranscriptional processes.

In the present study, we sought to examine the influence of exercise intensity (with total work equal) on the GLUT4 mRNA and protein responses to acute exercise. During low-intensity exercise (~40% \textit{V}\textsubscript{O2 peak}), activation of calcium-dependent kinases and mitogen-activated protein kinases (MAPK; Ref. 23) would occur to some extent, with little or no activation of AMPK (1, 26). With high-intensity exercise (~80% \textit{V}\textsubscript{O2 peak}), we expected greater activation of CaMK (21), MAPK (23), and AMPK (1, 26), leading to a potentially greater stimulus for GLUT4 transcription. Our hypothesis was that high-intensity exercise would result in higher postexercise GLUT4 mRNA and protein levels than low-intensity exercise, despite increased exercise duration in the latter trial.

METHODS

Subjects. Six healthy, physically active but untrained individuals (22.8 ± 1.6 yr; 84 ± 5 kg; 178 ± 2 cm) gave their written consent to participate in this study. The experimental procedures and possible risks associated with participation were explained to each subject verbally and in writing before commencement of the study. The study was approved by the Deakin University Human Research Ethics Committee and was conducted according to the Declaration of Helsinki.

Experimental protocol. Subjects reported to the laboratory for two trials, conducted at least 1 wk apart. At least 1 wk before their first experimental trial, they performed an incremental, cycle ergometer (Lode Instruments, Groningen, The Netherlands) test to volitional fatigue for the determination of \textit{V}\textsubscript{O2 peak}, which averaged 3.9 ± 0.2 l/min. For 24 h before each experimental trial, subjects consumed a standardized diet consisting of ~75% of total energy intake as carbohydrate, 15% fat, 10% protein, and water ad libitum. They were also asked to refrain from alcohol and caffeine ingestion, tobacco consumption, and vigorous exercise during this period. Subjects reported to the laboratory at 8:00 AM, after an overnight fast, and an initial muscle sample was obtained from vastus lateralis by percutaneous needle biopsy. They then completed an exercise bout comprising either 60 min at ~40% \textit{V}\textsubscript{O2 peak} (Lo) or 27 ± 2 min at ~80% \textit{V}\textsubscript{O2 peak} (Hi). The order of the trials was counterbalanced. An electric fan circulated air to minimize thermal stress. Heart rate was monitored using telemetry (Polar Electro, Kempele, Finland), while expired gases were collected every 10 min during Lo and every 5 min during Hi for measurement of oxygen uptake and estimation of energy expenditure. Total energy expenditure in the two trials was not different (Lo: 478 ± 50 kcal, Hi: 441 ± 44 kcal; P > 0.05).

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Exercise intensity averaged 39 ± 3 and 83 ± 2% \( V_{\text{O}_2 \text{peak}} \) in Lo and Hi, respectively. Plasma lactate increased from resting levels only in Hi (1.1 ± 0.1 vs. 13.5 ± 1.5 mmol/l; \( P < 0.05 \)), whereas there was no significant change in Lo (1.1 ± 0.2 vs. 2.1 ± 0.7 mmol/l). Preexercise muscle glycogen levels were not different between trials (Lo: 408 ± 35 mmol/kg dry mass; Hi: 420 ± 43 mmol/kg dry mass); however, postexercise levels were lower (\( P < 0.05 \)) in Hi (169 ± 18 mmol/kg dry mass) compared with Lo (262 ± 35 mmol/kg dry mass). Thus calculated muscle glycogen utilization was greater (\( P < 0.05 \)) in Hi (251 ± 24 mmol/kg) than in Lo (146 ± 34 mmol/kg).

Exercise resulted in similar increases in GLUT4 gene expression in both trials. GLUT4 mRNA was increased immediately at the end of exercise (~2-fold; \( P < 0.05 \)) and remained elevated after 3 h of postexercise recovery (Fig. 1). When measured 3 h after exercise, total crude membrane GLUT4 protein levels were 106% higher in Lo [3.3 ± 0.7 vs. 1.6 ± 0.3 arbitrary units (AU) units] and 61% higher in Hi (2.9 ± 0.5 vs. 1.8 ± 0.5 AU) relative to preexercise levels. A main effect for exercise was observed, with no significant differences between trials (Fig. 2).

DISCUSSION

The major finding of the present study was that exercise bouts at ~40 and ~80% \( V_{\text{O}_2 \text{peak}} \) increased GLUT4 mRNA and GLUT4 protein in human skeletal muscle to a similar extent, despite differences in exercise intensity and duration. Contrary to our original hypothesis, the high-intensity exercise bout of shorter duration was no more effective than lower intensity exercise of longer duration, with total work output equal, in eliciting these increases in GLUT4 mRNA and protein levels.

Our laboratory has previously reported that a single exercise bout of moderate intensity exercise (1 h at 70–75% \( V_{\text{O}_2 \text{peak}} \)) increases GLUT4 gene expression (11, 14). This is consistent with results in rat skeletal muscle in which there was a transient increase in GLUT4 transcription after acute exercise (16). In our studies, we cannot exclude exercise effects on mRNA stability. The molecular mechanisms responsible for increased GLUT4 transcription after exercise have not been fully elucidated, but increased calcium and altered energy status are thought to play important roles (3, 17). The transcription factor myocyte enhancer factor-2 (MEF-2) is essential for skeletal

![Graph showing GLUT-4 mRNA expression over time](image1)

![Graph showing GLUT-4 protein expression over time](image2)
muscle GLUT4 expression, and our laboratory has observed that exercise reduces MEF-2 association with the transcriptional repressor histone deacetylase 5 (14) and increases MEF-2-DNA binding (15) and MEF2 phosphorylation (14). Collectively, these molecular events contribute to enhanced GLUT4 transcription. Putative upstream kinases involved in these processes include, but are not limited to, CaMK and AMPK. To this end, we hypothesized that high-intensity exercise would activate these kinases to a greater extent, thereby resulting in an enhanced GLUT4 transcriptional response. Rather, it appears that there was sufficient activation of these molecular mechanisms, resulting in enhanced GLUT4 transcription and translation, in both exercise bouts. It is possible that an increase in exercise duration at lower intensity increased the transcriptional response to that exercise, a suggestion consistent with observations in rodent skeletal muscle (7). During relatively low-intensity exercise, there may have been a progressive increase in AMPK activity with increasing exercise duration (22, 25), which, together with ongoing activation of other kinases such as CaMK and MAPK, may have resulted in a GLUT4 transcriptional stimulus equivalent to that provided by the high-intensity exercise bout. Alternatively, low-intensity exercise may have been sufficient to initiate and activate GLUT4 transcription, with no further increases at the higher exercise intensity. Of note, whereas several studies have demonstrated exercise-intensity dependence of several signaling pathways in skeletal muscle (1, 21, 23, 26), other studies have seen that there is no further activation of some kinases as exercise intensity is increased (20). Importantly, in the present study total work output in the two exercise bouts was equal, and this may also explain the similar postexercise GLUT4 responses. Further studies will be needed to resolve the relative importance of exercise intensity, duration, and/or total exercise “impulse” in activation of GLUT4 transcription and translation.

A novel, and somewhat surprising, finding in the present study was the 60–100% increase in skeletal muscle GLUT4 protein expression 3 h after exercise (Fig. 2). However, previous studies in rats have observed rapid (1.5–24 h) upregulation (~50–100%) of GLUT4 protein levels after acute exercise (2, 12, 19). Studies in humans are less clear. Greiwe and colleagues (5) observed increased skeletal muscle GLUT4 levels 8 (~35%) and 22 h (~50%) after exercise, whereas in the study of Wojtaszewski et al. (24) the ~23% increase in GLUT4 protein seen 4 h after exercise was not statistically significant. These studies, and the results of the present study, suggest that the rapid postexercise activation of GLUT4 transcription is followed by enhanced GLUT4 translation. Although we have no measure of translation processes in the present study, an increase in polysome-associated GLUT4 mRNA, which is considered to be translationally active, has been observed in the 5 h after exercise in rats (12). These authors also suggested that the functional importance of enhanced GLUT4 protein expression may relate to the need for postexercise muscle glycogen resynthesis. Previous work in rat skeletal muscle has observed a relationship between muscle glycogen levels and GLUT4 expression during postexercise recovery (4). As expected, in the present study muscle glycogen degradation was greater after high-intensity than low-intensity exercise of longer duration. Despite these differences in postexercise muscle glycogen levels, no differences in postexercise GLUT4 mRNA and protein levels were observed between the two trials, suggesting that muscle glycogen does not have a major influence on postexercise skeletal muscle GLUT4 expression, at least under the conditions of the present study.

Plasma catecholamines were not measured in the present study, but they would have been expected to increase to a greater extent during the higher intensity exercise bout (9). The fact that exercise intensity did not influence GLUT4 gene or protein expression in the present study provides indirect support for the conclusion of Greiwe and colleagues (5) that β-adrenergic activation is not a primary, or necessary, stimulus for increased postexercise GLUT4 expression. In conclusion, exercise at ~40 and ~80% \( V_{\text{O}_2\text{peak}} \) with total work equal, increased GLUT4 mRNA and GLUT4 protein in human skeletal muscle to a similar extent, despite differences in exercise intensity and duration. These adaptive responses in GLUT4 expression may contribute to enhanced insulin action and muscle glycogen storage in the postexercise period.

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GRANTS

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REFERENCES


