Skeletal muscle GLUT-4 and glucose uptake during exercise in humans

GLENN McCONELL, MICHAEL McCoy, JOSEPH PROIETTO, AND MARK HARGREAVES
Departments of Physiology and Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, Victoria 3052, Australia

McConnell, Glenn, Michael McCoy, Joseph Proietto, and Mark Hargreaves. Skeletal muscle GLUT-4 and glucose uptake during exercise in humans. J. Appl. Physiol. 77(3): 1565-1568, 1994.—The present study examined the relationship between total skeletal muscle GLUT-4 protein level and glucose uptake during exercise. Eight active non-endurance-trained men cycled at 72 ± 1% peak pulmonary oxygen consumption for 40 min, with rates of glucose appearance and disappearance (Rd) determined by utilizing a primed continuous infusion of [3-3H]glucose commencing 2 h before exercise. Muscle glycogen content and utilization, citrate synthase activity, and total GLUT-4 protein were measured on muscle biopsy samples obtained from the vastus lateralis. A direct relationship existed between preexercise muscle glycogen content and glycogen utilization during exercise (r = 0.76, P < 0.05). Citrate synthase activity and glucose Rd at the end of exercise averaged 21.9 ± 3.0 μmol·min⁻¹·g⁻¹ and 27.3 ± 2.5 μmol·kg⁻¹·min⁻¹, respectively. There was a direct correlation between citrate synthase activity and GLUT-4 protein (r = 0.78, P < 0.05); however, at the end of exercise, glucose Rd was inversely related to both GLUT-4 (r = -0.89, P < 0.01) and citrate synthase activity (r = -0.72, P < 0.05). Plasma insulin, which decreased during exercise, was not related to glucose Rd. In conclusion, glucose uptake during 40 min of exercise at 72% peak pulmonary oxygen consumption was inversely related to the total muscle GLUT-4 protein level. This suggests that factors other than the total GLUT-4 protein level are important in the regulation of glucose uptake during exercise.

METHODS

Subjects. Eight active non-endurance-trained subjects volunteered for this study, which was approved by the University of Melbourne Human Research Ethics Committee. Recreationally active non-endurance-trained individuals were chosen because they would be expected to show normal biological variation in aerobic capacity, total GLUT-4 protein levels, muscle glycogen, and citrate synthase activity. Before commencing the study they completed a medical questionnaire and provided written informed consent. The age, height, and weight of these subjects were 24 ± 2 (SE) yr, 175 ± 4 cm, and 67.8 ± 2.1 kg, respectively. At least 1 wk before the trial, peak pulmonary oxygen uptake (Vo2 peak) was determined during incremental cycling (Lode, Groningen, The Netherlands) exercise to volitional fatigue and averaged 3.51 ± 0.14 l/min (range 2.82-3.95 l/min). The subjects were instructed to refrain from strenuous exercise, caffeine, alcohol, and tobacco in the 24 h before the experimental trial. In addition, they were supplied with food for the evening before the trial (3.0 MJ, 80.3% carbohydrate, 6.3% fat, 13.4% protein) to ensure adequate carbohydrate intake and normal muscle glycogen levels for the trial.

Experimental procedures. The subjects reported to the laboratory in the morning after an overnight fast. Catheters were inserted into a vein of one arm for [3-3H]glucose infusion and a vein of the contralateral arm for blood sampling. A resting blood sample was obtained, after which a primed (60 μCi) continuous infusion of [3-3H]glucose in 0.9% saline was commenced. The infusion rate of the glucose tracer (0.48 μCi/min) remained unchanged during 2 h of rest and the 40-min exercise bout. The catheter for blood sampling was kept patent by flushing with 0.9% saline and every 30 min with 0.5 ml of saline containing 10 U/ml heparin. After the catheters were positioned, a muscle sample (50–100 mg) was obtained from the vastus lateralis muscle by using the percutaneous needle biopsy technique with suction. Any visible blood or connective tissue was quickly removed from the sample before it was frozen in liquid nitrogen for later determination of muscle glycogen content, total GLUT-4 protein level, and citrate synthase activity.
After the 2-h rest period, subjects cycled for 40 min at a work load estimated to be equivalent to 70% VO$_2$peak. The laboratory was maintained at 20$\pm$2°C, and a fan circulated air to minimize thermal stress. The subjects drank water ad libitum. Heart rate was monitored (Accurex Polar, Kempele, Finland), and expired air was collected into Douglas bags every 10 min during exercise for the determination of oxygen uptake and respiratory exchange ratio (RER). Blood samples were obtained during the preexercise infusion period (-120, -10, -5, and 0 min) and every 5 min during exercise and were placed on ice in fluoride-heparin tubes. After the trial, these samples were spun and the plasma was frozen and stored at -20°C for later analysis of plasma glucose concentration and [3-3H]glucose. In addition, a portion of the plasma obtained immediately before and after 40 min of exercise was frozen at -20°C for plasma insulin analysis. For plasma lactate determination, 500 $\mu$l of plasma were deproteinized in 8% perchloric acid, spun and then stored at 20°C. Immediately after the trial a second muscle sample was collected for glycogen determination. The pump (Minipuls 2, Gilson, Villiers-le-Bel, France) infusion rate was determined at the end of each trial, and an aliquot of the infusate was frozen for later measurement of [3H]glucose activity.

**Analytic techniques.** Expired air samples were analyzed for oxygen and carbon dioxide contents by using Applied Electrochemistry S-3A/11 and CD-3A electronic analyzers (Ametek, Pittsburgh, PA). These analyzers were calibrated by using commercial gases of known composition. Volume was measured with a Parkinson-Cowan gas meter, calibrated against a Tissot spirometer. Plasma glucose was measured by using an automated glucose oxidase method (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH), whereas plasma insulin concentration was measured by radioimmunoassay (Incstar, Stillwater, MN). Plasma lactate was determined by using an enzymatic technique (15). To determine the specific activity of [3-3H]glucose in each sample, 500 $\mu$l of plasma were deproteinized with 500 $\mu$l of 0.3M Ba(OH)$_2$ and 500 $\mu$l of 0.3M ZnSO$_4$. This tube was then spun at 3,000 rpm for 20 min, and 800 $\mu$l of the supernatant was added to a scintillation vial that was dehydrated to remove tritiated water. The vials were reconstituted with 0.5 ml of distilled water together with 10 ml of scintillant (Beckman Instruments, Fullerton, CA), placed in a refrigerator for 5-6 h, and then counted in the liquid phase using a beta counter (Beckman Instruments, Fullerton, CA). Plasma glucose, glucose Ra and Rd, insulin, lactate, and muscle glycogen before and at the end of exercise. Glucose Rd at the end of exercise (Table 1). Plasma insulin decreased during exercise (Table 1) and was not related to glucose Rd. There was a significant utilization of muscle glycogen in the vastus lateralis and an increase in plasma lactate levels during exercise (Table 1). A direct relationship was observed between the preexercise muscle glycogen content and glycogen utilization during exercise ($r = 0.76, P < 0.05$).

| TABLE 1. Plasma glucose, glucose Ra and Rd, plasma lactate, plasma insulin, and muscle glycogen before and after 40 min of exercise |
|-----------------|-----------------|-----------------|-----------------|
| **Glucose, mmol/l** | Pre | 40 min |
| Glucose Ra, mmol kg$^{-1}$ min$^{-1}$ | 9.4±0.5 | 27.3±2.5* |
| Glucose Rd, mmol kg$^{-1}$ min$^{-1}$ | 9.4±0.5 | 27.3±2.5* |
| Lactate, mmol/l | 0.6±0.1 | 4.3±0.6* |
| Insulin, pmol/l | 51.6±6.0 | 40.5±6.6* |
| Glycogen, mmol/kg wet wt | 115±15 | 55±10* |

Values are means ± SE; $n = 8$ men. Pre, before exercise; 40 min, after 40 min of exercise at 72±1% peak pulmonary O$_2$ uptake; Ra, rate of glucose appearance; Rd, rate of glucose disappearance. Significantly different from Pre: * $P < 0.01$; † $P < 0.05$.
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was a trend for a positive correlation between citrate synthase activity and both muscle glycogen utilization ($r = 0.68, P = 0.10$) and the initial glycogen content ($r = 0.53, P = 0.18$). No significant correlation was found between glucose Rd and either preexercise muscle glycogen and glycogen utilization. In addition, no significant correlations were found between $V_{O_2 \text{peak}}$ and GLUT-4 protein, citrate synthase activity, or glucose Rd or between oxygen consumption during exercise and glucose Rd.

DISCUSSION

The main finding of the present study was that there was an inverse relationship between total GLUT-4 protein and glucose Rd during 40 min of cycling at $72 \pm 1\% V_{O_2 \text{peak}}$ in humans. In addition, because citrate synthase activity and GLUT-4 protein were directly related, there was also an inverse relationship between citrate synthase activity and glucose Rd. Although correlation between variables does not necessarily imply a cause and effect relationship, we used this analysis to investigate the potential associations between a number of intramuscular characteristics and glucose uptake during exercise. Our results suggest that skeletal muscle with an apparent enhanced capacity for glucose transport and oxidation may exhibit lower exercise-stimulated glucose uptake. At first this may seem a surprising result, but it is consistent with findings from endurance-training studies. Endurance training, which increases muscle oxidative capacity and GLUT-4 protein (5, 8, 10), results in lower glucose turnover and oxidation during exercise (3, 18). Taken together, these results suggest that factors other than the total GLUT-4 protein level are involved in the regulation of glucose uptake during exercise. The lower glucose Rd in individuals with higher GLUT-4 protein levels may be due to reduced GLUT-4 translocation and/or inhibition of GLUT-4 intrinsic activity. Alternatively, factors influencing glucose disposal may be involved. For example, early in exercise the rate of muscle glycogen utilization is high and glucose phosphorylation may be reduced as a result of increases in glucose 6-phosphate (12); however, we observed no correlation between glucose Rd and either preexercise muscle glycogen or glycogen utilization during exercise. In the present study, there was also an inverse relationship between glucose uptake during exercise and muscle citrate synthase activity, as has been shown and discussed previously (4). Because GLUT-4 protein levels were directly related to muscle citrate synthase activity, it is likely that the factors that result in an inverse relationship between glucose uptake and muscle oxidative capacity also contribute to the observed relationship between glucose uptake and muscle GLUT-4 levels (Fig. 1).

Insulin-mediated glucose uptake has been shown to be related to GLUT-4 protein levels in both rats (13, 17) and humans (5, 6, 8, 14). In contrast, in the present study we found that glucose uptake during exercise was inversely related to total GLUT-4 protein levels (Fig. 1), suggesting a difference in the relationships between skeletal muscle GLUT-4 protein level and insulin- and contraction-stimulated glucose uptake. This may be due to differential effects of insulin and muscle contractions on GLUT-4 protein recruitment and/or activity. Furthermore, it has been suggested that there are separate insulin- and contraction-recruitable GLUT-4 protein pools in skeletal muscle (7), and, indeed, insulin and contractions have been shown to have additive effects on muscle glucose uptake (9, 20). In addition, alterations in the intramuscular concentrations of metabolites and ions (e.g., adenine nucleotides, creatine phosphate, inorganic phosphate, calcium), with potential effects on glucose transport and/or disposal, that may occur during exercise are unlikely to be present during insulin stimulation. Finally, previous studies using insulin-stimulation have measured near-maximal rates of glucose uptake, whereas in the present study the stimulus for glucose uptake during exercise was likely to be submaximal.

The question arises as to the physiological significance of an elevation in skeletal muscle GLUT-4 protein. It is possible that increased GLUT-4 protein levels enable high rates of glucose uptake during more intense exercise, when substrate demands are greater than in the present study. Furthermore, increased GLUT-4 protein levels may contribute, in part, to high rates of glucose uptake and oxidation late in exercise under conditions of reduced muscle glycogen and maintenance of blood glucose and insulin levels by carbohydrate ingestion (2). Finally, high levels of GLUT-4 protein may facilitate restoration of muscle glycogen during recovery from exercise when carbohydrate is ingested and blood glucose and insulin levels are elevated.

In conclusion, we have observed an inverse relationship between total GLUT-4 protein in skeletal muscle and glucose uptake during exercise. This suggests that factors other than the total GLUT-4 protein level are
important in the regulation of glucose uptake during ex-
ercise.

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Address for correspondence: M. Hargreaves, Dept. of Physiology, 
Univ. of Melbourne, Parkville, Victoria 3052, Australia.

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