Sarcolemmal glucose transport and GLUT-4 translocation during exercise are diminished by endurance training

ERIK A. RICHTER, PALLE JENSEN, BENTE KIENS, AND SØREN KRISTIANSEN
Copenhagen Muscle Research Centre, August Krogh Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark

Richter, Erik A., Palle Jensen, Bente Kiens, and Sören Kristiansen. Sarcolemmal glucose transport and GLUT-4 translocation during exercise are diminished by endurance training. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E89–E95, 1998.—Glucose utilization during exercise of a given submaximal power output is decreased after endurance training. The aim of the present study was to elucidate the mechanisms behind this phenomenon by utilizing the sarcolemmal giant vesicle technique. Eight healthy young untrained men endurance trained one thigh for 3 wk. They then exercised both thighs simultaneously at the same work load (77% of peak O2 uptake of the untrained thigh) for 40 min. Training increased muscle GLUT-4 protein by 70% (P < 0.05). Glucose uptake during exercise was 38% lower (P < 0.05) in the trained (T) thigh than in the untrained (UT) thigh because of both a lower (P < 0.05) glucose extraction and blood flow in T. During exercise, sarcolemmal GLUT-4 protein content and glucose transport capacity increased significantly less in T than in UT muscle, and muscle glucose concentration was lower in T compared with UT (P < 0.05) at the end of exercise. It is concluded that, despite a large increase in muscle GLUT-4 with endurance training, exercise of a given submaximal power output increases muscle glucose uptake less in T than in UT muscle. It is suggested that the mechanism behind this phenomenon is blunted exercise-induced translocation of GLUT-4 to the sarcolemma, resulting in a blunted increase in sarcolemmal glucose transport in T muscle.

MATERIALS AND METHODS

Subjects. Eight healthy, young, normal-weight men aged 21–27 yr, with no medical record and especially no history of cardiovascular or endocrine diseases or clotting disorders, served as subjects in the study, which was approved by the Copenhagen Ethics Committee and which conformed with the code of ethics of the World Medical Association (Declaration of Helsinki). None of the subjects was engaged in regular physical activity other than using the bicycle as a means of local transportation. Subjects were included in the study if their maximal pulmonary O2 uptake measured during incremental cycling on a bicycle ergometer was below 52 ml·min−1·kg body wt−1. Average maximal O2 uptake was 47 ± 2 (SE) ml·min−1·kg body wt−1.

Experimental design. After a pretraining muscle biopsy had been obtained from each thigh, subjects trained one thigh for 3 wk using the knee-extensor model (1), which allows dynamic exercise to be performed exclusively with the knee extensors. Forty to 48 h after the last training session, the actual experiment was performed, during which subjects
performed knee extensions with both thighs simultaneously on two completely separate knee-extensor ergometers for 40 min, working at the same absolute power output with each thigh. Arterial and bilateral femoral venous blood was obtained, and bilateral femoral venous blood flow was measured at rest and during exercise. In addition, muscle biopsies were obtained from both thighs at rest and immediately after exercise.

Training. Subjects were accustomed to the one-leg dynamic knee-extensor apparatus (1) with both legs before an incremental knee-extensor test was performed on each leg to determine the peak work capacity of the knee extensors. Pulmonary O₂ uptake was measured, and peak work load for the knee-extensor apparatus (1) with both legs before an incremental knee-extensor test was performed on each leg to determine the peak work capacity of the knee extensors. Pulmonary O₂ uptake was measured, and peak work load for the knee extensors was defined as the workload when the initial linear relationship between workload and pulmonary O₂ uptake changed to an exponential one, indicating the recruitment of accessory muscles to stabilize the body at high workloads. Subjects were included in the study only if the peak workload of the two knee extensors differed by <5%. One week after completing these tests, subjects were instructed to eat a diet containing 8.5 g carbohydrate/kg body weight per day for 2 days to fill the glycogen stores. On the 3rd day the subjects reported to the laboratory in the morning at 0830 after ingesting a light breakfast (2 slices of white bread with a thin layer of butter and jam and one glass of orange juice) at 6AM. A muscle biopsy was obtained from each vastus lateralis muscle under local anesthesia with lidocaine. Subjects then commenced the training program of one thigh. Four subjects trained the dominant thigh and 4 subjects the nondominant thigh. The training consisted of four sessions in the 1st wk, five sessions the 2nd wk, and six sessions the 3rd wk. Training time was gradually increased from 1 to 2 h per session by the end of the 2nd wk. Workload was varied between 70 and 85% of pretraining peak workload. During the training period, subjects also practiced three times for 15 min, during which both thighs worked on two separate knee-extensor ergometers simultaneously at the same power output. The last training session was cut to 1 h, and in addition 15-min two-legged knee extensions were performed at the workload to be used in the final exercise test. This was set as 77% of pretraining peak workload. Subjects were instructed to eat a diet containing 8.5 g carbohydrate/kg body weight for the last 2 days preceding the final exercise experiment. The final experiment took place 40–48 h after the last training session, and subjects were instructed to avoid physical activity during this period.

Experimental protocol. On the morning of the experiment, subjects ate the light breakfast at 0600. They reported to the laboratory at 0830, having used bus, train, or car for transportation. After 30 min of rest in the supine position, Teflon catheters were placed in both femoral veins and one femoral artery under local anesthesia with use of aseptic techniques, and a thermistor probe (Edslab probe 94–030–2.5-F, Baxter Healthcare) for measurement of the vesicle mixture, 4% Nycodenz (Nycomed) in KCl-HEPES buffer (140 mM KCl and 10 mM HEPES, pH 7.4) with 0.37 mg/ml phenylmethylsulfonyl fluoride, collagenase, and aprotinin were added to the suspension of vesicles and collagenase reaction was stopped after addition of 10 mM tri(hydroxymethyl)aminomethane (Tris) preparations (33) and analysis of muscle glycogen, citrate synthase (CS) activity, glucose, and G-6-P. Glycogen was measured as glucose residues by a hexokinase method after acid hydrolysis, as described previously (28). CS, glucose, and G-6-P were analyzed using standard enzymatic methods (28). The remaining part of each biopsy was used for production of total crude membrane (TCM) preparations (33) and analysis of muscle glycogen, citrate synthase (CS) activity, glucose, and G-6-P. Glycogen was measured as glucose residues by a hexokinase method after acid hydrolysis, as described previously (28). CS, glucose, and G-6-P were analyzed using standard enzymatic methods (28). The remaining part of each biopsy was used for production of TCM preparations (33) and analysis of muscle glycogen, citrate synthase (CS) activity, glucose, and G-6-P. Glycogen was measured as glucose residues by a hexokinase method after acid hydrolysis, as described previously (28). CS, glucose, and G-6-P were analyzed using standard enzymatic methods (28). The remaining part of each biopsy was used for production of TCM preparations (33) and analysis of muscle glycogen, citrate synthase (CS) activity, glucose, and G-6-P. 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mM Tris·HCl, and 1 mM EDTA, pH 7.4) and stored at −20°C for subsequent determination of protein concentration and Western blotting (26). The GLUT-4 antibody was a mouse monoclonal antibody produced against a synthetic peptide corresponding to the 13 COOH-terminal amino acids of GLUT-4 (26). Antibody-antigen complexes were visualized within the linear response by the enhanced chemiluminescence detection kit (Amersham, Arlington Heights, IL). To quantify the signal, densiometric scanning was performed (Kern-E n-Tec Software Systems, Copenhagen, Denmark). The GLUT-4 protein content per microgram protein was expressed in arbitrary units relative to a rat heart TCM standard.

On the basis of analysis of different membrane markers, such as p-nitrophenyl phosphatase (plasma membrane), Ca²⁺-ATPase (sarcoplasmic reticulum), succinate dehydrogenase (mitochondrial membrane), and dihydropyridine receptor (T tubule marker), we have previously shown that the vesicles are predominantly of surface membrane origin (26, 33) and that exercise did not change the membrane marker composition of the vesicles (26). It was also verified that the SG vesicle membrane protein yield per milligram wet weight (−1% of the homogenate membrane protein (26, 27)) was not different in vesicles obtained at rest or after exercise (26). Furthermore, exercise did not change the size or appearance of the SG vesicles under a microscope compared with rest (26).

Blood glucose and lactate were measured with a Yellow Springs Instruments analyzer (Yellow Springs, OH). Hemoglobin concentration in blood and O₂ saturation of hemoglobin were measured with an OSM-3 hemoximeter (Radiometer, Copenhagen, Denmark). Expired air was analyzed for O₂ concentration by a Servomex paramagnetic analyzer and for CO₂ by a Beckman infrared CO₂ analyzer. Volume of expiratory air was measured with a Tissot type spirometer.

Statistics. Values measured more than twice in each leg during exercise were compared with a two-way analysis of variance for repeated measures. The Student-Newman-Keuls test was used as a post hoc test. Values measured twice in each leg (before and after training) were compared with the paired t-test. A significance level of 0.05 was chosen.

RESULTS

Muscle adaptations to training. The total muscle GLUT-4 protein content was identical in the two legs before training (Table 1). Training increased the GLUT-4 protein content by 70% in the T leg, whereas no change occurred in the UT leg (Table 1). Similarly, muscle glycogen concentrations were identical in the two legs before training, but after training the resting muscle glycogen concentration was significantly increased by 56% in the T leg, whereas it was unchanged in the UT leg (Table 1). CS activity was measured only after training and averaged 43.8 ± 3.3 µmol/g dry wt in UT muscle and 50.8 ± 2.3 µmol/g in T muscle (P < 0.06). The peak power output during knee-extensor exercise in the T leg increased from 53 ± 4 W before training to 63 ± 4 W after training (P < 0.05), whereas in the UT leg the posttraining values (53 ± 3 W) were similar to the pretraining values (52 ± 4 W).

Glucose uptake and sarcolemmal glucose transport capacity and GLUT-4 content. At rest, blood glucose concentration averaged 4.87 ± 0.17 mmol/l and did not change significantly during exercise (data not shown). At rest, glucose uptake was similar in the two thighs (Fig. 1, Table 2). During exercise, thigh glucose uptake was markedly higher compared with rest; however, the uptake during exercise was on average 38% lower in the T leg than in the UT leg (P < 0.05; Fig. 1, Table 2). This was due primarily to a lower extraction of glucose in the T leg (Table 2), but a lower blood flow (P < 0.05) in the T leg compared with the UT leg contributed to the difference (Table 2). A training-induced decrease in muscle blood flow during exercise of a given power output has been demonstrated previously (24). Sarcolemmal glucose transport capacity was similar in T and UT at rest but increased markedly more with exercise in the UT leg than in the T leg (Fig. 2). Sarcolemmal GLUT-4 content was measured in only seven of the eight subjects because of insufficient vesicle material for Western blotting in one subject. Sarcolemmal GLUT-4 content was similar in UT and T at rest, but the increase with exercise was 60% larger (P < 0.05) in UT than in T (Fig. 3). GLUT-4 intrinsic activity calculated as vesicle glucose transport rate divided by the vesicle GLUT-4 protein content was similar in T and UT at rest (3.2 ± 1.0 and 3.0 ± 0.8 arbitrary units, respectively) and did not increase with exercise (2.7 ± 0.8 vs. 3.4 ± 0.9 in T and UT, respectively).

Muscle concentrations of glucose were similar at rest in UT and T and increased significantly with exercise in

Table 1. GLUT-4 and glycogen in vastus lateralis muscle at rest before and after training

<table>
<thead>
<tr>
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<th>Before Training</th>
<th>After Training</th>
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<tr>
<td></td>
<td>UT</td>
<td>T</td>
</tr>
<tr>
<td>TCM GLUT-4, µg protein</td>
<td>0.32 ± 0.04</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td>Glycogen, µmol/g dry wt</td>
<td>0.29 ± 0.04</td>
<td>0.29 ± 0.04</td>
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</table>

Total crude membrane (TCM) GLUT-4 protein content and glycogen concentration in vastus lateralis muscle at rest in the untrained (UT) and trained (T) legs before and after 3 wk of one-leg endurance training. Values are means ± SE of 8 determinations. *P < 0.05 vs. before training and vs. UT after training. TCM GLUT-4 is given per µg protein in units relative to a rat heart standard.
was markedly higher (\(P < 0.05\)) in both UT and T (Table 3). However, at the end of exercise, the muscle glucose concentration was significantly higher in UT than in T (Table 3). Concentrations of G-6-P were not different between the UT and T muscles either at rest or after exercise (Table 3).

Glycogen breakdown and lactate. At rest, muscle glycogen concentration was 48% higher in the T thigh than in the UT thigh (Table 1). During exercise, muscle glycogen concentrations decreased from 532 ± 23 to 272 ± 33 µmol/g dry weight and from 786 ± 62 to 581 ± 63 µmol/g in UT and T, respectively. Thus the breakdown of glycogen tended to be lower in the T thigh (205 ± 35) compared with the UT thigh (260 ± 40), but the difference was not significant. During exercise, arterial blood lactate concentration increased from an average value of 0.44 ± 0.02 mmol/l at rest to 3.14 ± 0.53 mmol/l after 20 min, whereupon it did not change significantly during the last 20 min of exercise (data not shown). Lactate release was similar in the two thighs at rest, averaging 0.03 ± 0.01 and 0.02 ± 0.01 mmol/min in T and UT, respectively. Release of lactate during exercise was markedly higher (\(P < 0.05\)) in the UT thigh than in the T thigh. Because lactate release in both thighs did not change significantly from 10 to 40 min of exercise, lactate release was averaged over the exercise period and amounted to 0.51 ± 0.36 mmol/min in T and 2.20 ± 0.42 mmol/min in UT (\(P < 0.05\)).

\(O_2\) uptake, heart rate, and perceived exertion. Pulmonary \(O_2\) uptake measured after 20 and 40 min of exercise averaged 1.88 ± 0.12 and 1.93 ± 0.12 l/min, respectively. \(O_2\) uptake was similar in the two thighs at rest and increased similarly with exercise (Table 2). Heart rate averaged 150 ± 5 and 162 ± 4 beats/min after 20 and 40 min of exercise, respectively. Perceived exertion was, after 20 min of knee extensions, 15 ± 1 units in the UT thigh and 10 ± 1 units in the T thigh (\(P < 0.05\)), and after 40 min of exercise the ratings had increased (\(P < 0.05\)) in both thighs to 17 ± 1 and 13 ± 1 units in the UT and T thighs, respectively (\(P < 0.05\)).

**DISCUSSION**

Endurance training is known to decrease utilization of glucose during submaximal exercise at a given absolute power output (5, 30, 31). In the present study we, for the first time, directly demonstrate that the smaller exercise-induced increase in glucose uptake in
Table 3. Concentrations of glucose and G-6-P in muscle

<table>
<thead>
<tr>
<th>Glucose, µmol/g dry wt</th>
<th>G-6-P, µmol/g dry wt</th>
<th>Rest</th>
<th>Exercise</th>
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<tbody>
<tr>
<td>UT</td>
<td>T</td>
<td>UT</td>
<td>T</td>
</tr>
<tr>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>5.1 ± 1.4†</td>
<td>3.0 ± 0.4†</td>
</tr>
<tr>
<td>0.52 ± 0.13</td>
<td>0.38 ± 0.07</td>
<td>0.47 ± 0.12</td>
<td>0.68 ± 0.21</td>
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Values of glucose and glucose 6-phosphate (G-6-P) concentrations were obtained in UT and T legs before and immediately after 40 min of 2-legged knee-extensor exercise. They are means ± SE of 8 observations. P < 0.05 vs. *UT; †rest.

It is well documented that endurance training increases the ability of insulin to stimulate muscle glucose uptake (9, 10, 35), and it is thought that the training-induced increase in muscle GLUT-4 content is at least partly responsible (7, 10, 11). In this light it might seem surprising that exercise-induced muscle glucose transport and uptake are decreased after training despite the increase in muscle GLUT-4 protein. We chose to study the limbs during exercise at the same absolute workload, because our aim was to elucidate the mechanism behind the clearly demonstrated reduction in glucose uptake when muscles work at the same absolute workload after training as compared with before training. It might be argued that the training effect on glucose uptake and transport would disappear if the T and UT leg had worked at the same relative rather than the same absolute exercise intensity. However, because exercise-induced glucose utilization has also been shown to be less in T compared with UT subjects working at the same relative exercise intensity (6, 19), this is not likely, although the difference between T and UT would probably be smaller than when muscles worked at the same absolute intensity. Furthermore, it was recently demonstrated that the rate of disappearance of glucose at the end of 40-min bicycle ergometer exercise at 72% of individual maximal O2 uptake was inversely related to total muscle GLUT-4 content (29). Thus it is clear that the increase in muscle GLUT-4 protein content that occurs with training is not for the benefit of increasing muscle glucose utilization during submaximal exercise. Even during exercise at 100% of maximal O2 uptake, we recently showed a negative correlation between increase in sarcolemmal glucose transport on one hand and total muscle GLUT-4 content on the other (26), suggesting that the inverse relationship between total muscle GLUT-4 content and muscle glucose utilization is also valid during exercise at maximal O2 uptake. The only study in humans that showed increased glucose uptake during exercise in T compared with UT legs is a study in which subjects simultaneously exercised the T and UT legs on a bicycle ergometer at ~75% of maximal O2 uptake during maximal insulin stimulation (8). During maximal insulin stimulation, lipolysis and, in turn, lipid oxidation are greatly suppressed, and therefore the normal increase in fat oxidation during exercise in T vs. UT muscle observed at normoinsulinemia (5, 17, 24) is not possible. It was hypothesized (8) that this inability to increase lipid oxidation brought about the increased glucose utilization in T vs. UT muscle. Taken together with the present study, such a scheme would imply that the rate of lipid oxidation influences GLUT-4 translocation in contracting muscle. This remains to be studied.

From studies in humans it cannot be concluded whether the training adaptation responsible for the blunted exercise-induced increase in glucose transport resides within the muscles or is due to neuromuscular adaptations. To study adaptations within the muscles, in vitro studies of T and UT muscles are necessary. Unfortunately, in vitro studies of T and UT rat muscle do not give a clear answer to the question of whether...
endurance training changes contraction-induced glucose uptake. Some have reported increased contraction-induced glucose transport in trained oxidative fibers but not in trained glycolytic fibers of perfused rat hindlimbs (3, 32). Furthermore, chronic electrical stimulation increased total muscle GLUT-4 and decreased subsequent contraction-induced glucose transport in the extensor digitorum longus and the red part of the tibialis anterior but increased it in the white part of the tibialis anterior muscle (21). However, it has recently been described that the contraction-induced maximal rate of glucose transport is increased in T compared with UT rat epitrochlearis muscle but that it takes more contractions to maximally activate glucose transport in T compared with UT muscle (23). To summarize, in vivo exercise data in humans so far clearly indicate that training decreases exercise-induced glucose uptake at a given power output, but the subjects were, in none of the studies, exercised to the point of glycogen-depleted exhaustion, at which point the highest rate of glucose transport might be expected. In vitro studies of T and UT rat muscle suggest that, at least under some conditions, contraction-stimulated T muscle has a higher maximal rate of glucose transport but that it takes more contractions to reach the maximum than in UT muscle.

It is well known that glucose utilization in contracting muscle is inversely related to the muscle glycogen concentration (13, 18). The prevailing concept is that this is mainly due to higher G-6-P concentrations and ensuing inhibition of hexokinase, leading to accumulation of free glucose in the cell when glycogen levels are high (18). However, contraction-induced glucose transport measured by accumulation of 3-O-methylglucose was also shown to be 25% higher in glycogen-depleted than in glycogen-supercompensated rat muscle (18). In addition, in UT as well as in T rat epitrochlearis muscle, contraction-induced muscle glucose transport correlated negatively with postcontraction muscle glycogen concentrations (23), and insulin-stimulated glucose transport in rat muscle has also recently been found to be negatively associated with glycogen levels (20). Finally, in a preliminary communication it was reported that a part of the GLUT-4 pool is associated with glycogen and that, when glycogen was degraded, GLUT-4 was allowed to translocate to the surface membrane (4). If such a scheme is correct, then part of the decrease in exercise-induced translocation of GLUT-4 in the T muscle in our study (Fig. 3) might be due to the higher glycogen concentration in the T muscle (Table 1). However, no significant correlations between the exercise-induced increase in sarcolemmal glucose transport capacity or GLUT-4 content, on the one hand, and increase in muscle glycogen concentration during exercise or absolute glycogen concentration after exercise, on the other, were observed in the present study. Such correlations might be expected if the GLUT-4–glycogen coupling hypothesis were correct.

The giant vesicle preparation used in the present study was originally described for use in the study of glucose transport in rat muscle by Ploug et al. (33). The membrane components of the vesicles consist almost entirely of sarcolemma and are almost without contamination with intracellular membranes and T tubules (26, 33). It is currently the only sarcolemmal preparation that can be obtained from the limited tissue that is available from needle biopsies of human muscle and that allows the study of both GLUT-4 content and sarcolemmal glucose transport capacity induced by exercise. The preparation responds to exercise in vivo as well as to electrical stimulation in vitro (26, 27, 33). Both maximal and submaximal exercise increases sarcolemmal glucose transport capacity and GLUT-4 content (26, 27) (Figs. 2 and 3), and during submaximal exercise the increases become progressively larger (27) as does glucose uptake (22). Thus, although the magnitude of changes in GLUT-4 content and sarcolemmal transport capacity is only two- to threefold (Figs. 2 and 3), whereas the exercise-induced changes in thigh glucose uptake are much larger (Fig. 1), we believe that the changes in the sarcolemmal vesicles still can be used semiquantitatively to estimate exercise-induced changes in the sarcolemma in situ. In addition, the quantitative importance of the exercise-induced increase in muscle perfusion and possible change in GLUT-4 intrinsic activity in vivo is not known. The two- to threefold increase in GLUT-4 and glucose transport induced by exercise is a feature that this vesicle preparation shares with preparations using subcellular fractionation of rat skeletal muscle, which have been used extensively (12, 14, 15). Furthermore, a recent study using confocal microscopy of human muscle indicates that the multiples of increase in translocation of GLUT-4 during physiological insulin stimulation are barely twofold (38) despite much larger increases in glucose uptake (34). Thus the SG vesicle preparation is suitable for the study of contraction-induced sarcolemmal glucose transport and GLUT-4 content in human skeletal muscle.

In conclusion, in the present study we have shown that, during a given submaximal exercise trial, the increase in glucose uptake was less in endurance-trained than in simultaneously working untrained muscle. We further show for the first time that the mechanism behind this difference in glucose uptake seems to be blunted exercise-induced increase in sarcolemmal glucose transport due to blunted GLUT-4 translocation to the sarcolemma in T compared with UT muscle. Interestingly, this happens despite markedly higher total muscle GLUT-4 stores, which means that endurance training dramatically decreases the fraction of the total muscle GLUT-4 pool that is translocated to the sarcolemma during submaximal exercise.

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Address reprint requests to E. A. Richter.

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REFERENCES