Volume 238, number 2, 235–239 FEB 06361 October 1988

Acute exercise increases the number of plasma membrane glucose transporters in rat skeletal muscle

Michael F. Hirshman, Harriet Wallberg-Henriksson*, Lawrence J. Wardzala+, Elizabeth D. Horton and Edward S. Horton

Metabolic Unit, Department of Medicine, University of Vermont, College of Medicine, Burlington, VT 05405, USA

Received 4 July 1988

To determine whether increased glucose transport following exercise is associated with an increased number of glucose transporters in muscle plasma membranes, the D-glucose inhibitable cytochalasin B binding technique was used to measure glucose transporters in red gastrocnemius muscle from exercised (1 h treadmill) or sedentary rats. Immediately following exercise there was a 2-fold increase in cytochalasin B binding sites, measured in purified plasma membranes enriched 30-fold in 5'-nucleotidase activity. This increase in glucose transporters in the plasma membrane may explain in part, the increase in glucose transport rate which persists in skeletal muscle following exercise. Where these transporters originate, remains to be elucidated.

Glucose transporter; Exercise; (Rat skeletal muscle)

1. INTRODUCTION

Contractile activity in skeletal muscle, whether induced by electrical stimulation in vitro or by an acute bout of exercise, has been shown to significantly increase glucose transport activity [1–5]. The mechanism by which contractile activity increases the rate of glucose transport in skeletal muscle is not known, nor is it known whether this mechanism differs from the process by which insulin increases the rate of glucose transport into muscle. It is known, however, that contractile activity increases the rate of glucose transport by increasing its maximum velocity (V_{max}) rather than by a change in the Michaelis constant (K_m) [6,7]. Wardzala and Jeanrenaud [8,9], in studies using incubated rat diaphragm, and Watanabe et al. [10], in studies using perfused rat heart, have shown that insulin stimulates the translocation of glucose transporters from an intracellular pool to the plasma membrane. A similar translocation mechanism has recently been demonstrated in perfused rat skeletal muscle [11]. It has been hypothesized that the increased glucose transport activity in muscle after exercise also may be due to an increase in the number of glucose transporters translocated to the plasma membrane from an intracellular pool. In this case, contractile activity, rather than insulin, would be the stimulus which initiates translocation of these carrier proteins. To test this hypothesis, we have studied the effect of a single acute bout of exercise on the number of plasma membrane glucose transporters in skeletal muscle. This was accomplished by measuring the number of D-glucose-inhibitable cytochalasin B binding sites in purified plasma membranes prepared from red gastrocnemius muscle from sedentary control and acute exercised rats.

Correspondence address: M.F. Hirshman, Metabolic Unit, Department of Medicine, University of Vermont, College of Medicine, Burlington, VT 05405, USA

* *Present address:* Dept of Clinical Physiology, Karolinska Institute, Huddinge Hospital, S-141 86 Stockholm, Sweden

\+ *Present address:* Dept of Medicine, The Washington Hospital Center, 110 Irving St. NW, Washington, DC 20010, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)

Our results indicate that a single bout of exercise increases the number of glucose transporters in the plasma membrane of rat skeletal muscle. These findings suggest that exercise enhances plasma membrane permeability to glucose, at least in part, via an increased number of plasma membrane glucose transporters in muscle.

2. MATERIALS AND METHODS

2.1. *Animals and experimental design*

Male Sprague Dawley rats were received from Charles River Canada (Montreal, Quebec). Animals were housed in a room maintained at 23°C with a 12 h light/dark cycle and allowed free access to food and water for a period of at least 4 days prior to the experiment. Rats, weighing between 180 and 250 g and in the post-absorptive state were either exercised on a Quinton model 42 rodent treadmill at 20 m/min up a 10% grade for 1 h or allowed to remain sedentary. At the end of the one hour period the rats were killed by a blow to the head and cervical dislocation. Portions of red gastrocnemius muscle from both legs were rapidly dissected, trimmed free of fat and connective tissue, and weighed.

2.2. *Plasma membrane preparation*

The preparation of plasma membrane fractions was a modification of the methods described by Philipson et al. [12] and Grimditch et al. [13]. Muscle (1.0–1.4 g) was finely minced, polytroned (Brinkman) twice at setting 8 for 8 s in 5 ml of a buffer containing 20 mM Hepes and 250 mM sucrose (pH 7.4, 37°C, buffer A), homogenized for 10 strokes in a Potter-Elvehjem tissue grinder, and brought up to a volume of 8.3 ml. All subsequent steps were performed at 4°C unless otherwise indicated. At this point, 0.2 ml was removed for marker enzyme and protein determinations. To the remaining volume 0.9 ml of a solution containing 3 M KCl and 250 mM sodium pyrophosphate was added. This mixture was vortex-mixed and centrifuged at 227000 × g_{max} for 50 min. The supernatant was discarded and the pellet resuspended to 4.75 ml with buffer A. Protease inhibitors (0.125 ml of a mixture containing leupeptin, pepstatin and aprotinin each at a concentration of 100 μg/ml) and DNase (0.125 ml, 10000 Kunitz units) were added to the mixture, and it was incubated for 60 min in a 30°C shaking waterbath. The mixture was then diluted with buffer A (1:2), cooled on ice, and centrifuged at 227000 × g_{max} for 50 min. The resulting pellet was resuspended in 34% sucrose and layered on top of 38 and 45% sucrose concentrations. Sucrose buffers 32, 30, 27 and 8% were layered on top to complete the discontinuous gradient. The gradient was centrifuged for 16 h in a Beckman swinging bucket SW 28 rotor at 68000 × g_{max}. Fractions were collected from the gradient at the 8/27% and the 27/30% sucrose interfaces, diluted with buffer A, and centrifuged at 331000 × g_{max} for 60 min. The resulting pellets were resuspended in buffer A to a final volume of 0.6–0.8 ml. An aliquot from each sample was removed for marker enzyme and protein determinations. The remaining sample was used for cytochalasin B binding. All samples were frozen and stored in liquid nitrogen until assayed.

2.3. *Protein and 5'-nucleotidase determinations*

Protein was determined by the Coomassie brilliant blue method (Bio-Rad protein assay, Bio-Rad Laboratories, Richmond, CA) described by Bradford [14] using crystalline bovine serum albumin as the standard. The specific 5'-nucleotidase activity of each homogenate and plasma membrane fraction was also assayed as previously described by Avruch et al. [15].

2.4. *Determination of glucose transporter concentration*

Equilibrium D-glucose-inhibitable [^{3}H]cytochalasin B binding was measured, and the concentration of glucose transporters was calculated as previously described by Wardzala et al. [16,17]. Briefly, Scatchard plots were generated from binding studies in which membranes were incubated with varying concentrations of cytochalasin B in the presence or absence of D-glucose. Cytochalasin E was used to decrease nonspecific binding. The total number of glucose transporters (R_o) and the dissociation constant (K_d) were determined from a linear plot derived by subtraction along the radial axes of binding curves generated in the presence of D-glucose from those in the absence of D-glucose.

2.5. *Statistical analysis*

Data in the text and figures are given as means ± SE. The statistical analyses were performed using Student's t-test. A p value of <0.05 was considered to be statistically significant.

3. RESULTS

3.1. *Body weight, muscle weight and protein recovery*

The mean body weight of the control rats was not significantly different from that of the exercised group (table 1). The total amount of protein recovered from the muscle homogenates (144 vs 142 mg) represented about 12% of the starting wet weight of the muscle in both groups and demonstrates that exercise does not affect the percentage of protein recovery. Fractions 2 and 3 (i.e. the 27% and 30% sucrose fractions), in which most of the plasma membranes are found, were collected and combined together (fraction 2/3). The total amount of plasma membrane protein recovery in fraction 2/3 (499 vs 489 μg) was not affected by exercise (table 1).

3.2. *5'-Nucleotidase recovery, specific activity, and enrichment*

5'-Nucleotidase activity was used as the marker enzyme for plasma membrane recovery. It is important to point out that not only were the protein recoveries very similar between the two groups, but the specific activities of 5'-nucleotidase in the homogenates and fraction 2/3 were also comparable (table 2). The enrichment of

Table 1

Body weights and gastrocnemius muscle weights used in membrane preparations, and protein recovery

	Control	Exercise
Body wt (g)	227 ± 10	234 ± 8
Gastrocnemius wt (g)	1.12 ± 0.07	1.16 ± 0.08
Homogenate protein (mg)	144.1 ± 5.1	141.5 ± 5.8
Plasma membrane protein (μg)	499.6 ± 50.6	488.8 ± 65.1

Results are means ± SE; $n = 8$ for each group

5′-nucleotidase specific activity of fraction 2/3 compared to the homogenate specific activity in both groups is identical (31-fold enriched) and the recovery of 5′-nucleotidase is also the same (9.5%) (table 2).

3.3. *Glucose transporter numbers*

Exercise was associated with an increase in the number of plasma membrane cytochalasin B binding sites per mg plasma membrane protein from 4.14 ± 0.54 pmol/mg for the sedentary group to 8.69 ± 1.36 pmol/mg for the exercise group ($p < 0.01$). This increase in transporter number is not accompanied by a change in K_d (80.5 ± 9.7 nM for the controls vs 89.0 ± 12.9 nM for the exercised, n.s.). In addition the K_d of our binding assays are similar to those seen for diaphragm and adipose cells [8,9,18]. After adjusting for total 5′-nucleotidase recovery in fraction 2/3 we have made an estimate of the number of plasma membrane glucose transporters per gram of muscle. The calculation demonstrates that 19.9 ± 3.0 vs 36.8 ± 3.4 pmol of cytochalasin B binding sites per gram wet weight muscle are located in the plasma membrane of the sedentary and exercised groups, respectively ($p < 0.005$).

Table 2

Marker enzyme specific activities, percent recoveries and fold enrichments from gastrocnemius muscle of exercised and control rats

5′-Nucleotidase	Control	Exercise
Homogenate specific activity (nmol/30 min per mg)	132 ± 14	121 ± 9
Plasma membrane specific activity (nmol/30 min per mg)	3769 ± 638	3494 ± 456
Plasma membrane recovery (%)	9.5 ± 1.1	9.6 ± 1.2
Plasma membrane fold enrichment	31.2 ± 3.3	31.1 ± 3.4

Marker enzyme activities were measured in the original homogenates and plasma membrane fractions using procedures described in section 2. Total 5′-nucleotidase activity of the homogenate and plasma membrane fraction were determined by multiplying their specific activities with their respective protein recoveries from table 1. Percent recovery was the result of dividing total enzyme activity of the plasma membrane by the total enzyme activity of the homogenate multiplied by 100. Plasma membrane fold enrichment is an indication of purity of the plasma membrane marker enzyme specific activity relative to that of the homogenate. Specific activities are the results of triplicate samples from each of 8 separate experiments. Results are means ± SE

4. DISCUSSION

Immediately following one hour of moderate intensity exercise in the rat, a 2-fold increase in the number of glucose transporters measured in purified plasma membranes (5′-nucleotidase enriched) from red gastrocnemius muscle was demonstrated. With an exercise period of only one hour in duration, it is very unlikely that the total number of glucose transporters in the muscle could change, since the time frame for protein synthesis activation is believed to require several hours. Therefore, the increased number of glucose transporters observed in the plasma membrane with exercise is presumed not to represent newly synthesized transporters, but to be derived from either a translocation of glucose transporters from an intracellular pool or through the reorientation and activation of transporters already in the plasma membrane.

Wardzala and Jeanrenaud using rat diaphragm [8,9] and Watanabe and Kono using heart [10] have demonstrated an intracellular pool of glucose transporters that decreases in response to insulin stimulation, concomitant with an increase in glucose transporters seen in the plasma membrane fraction. In skeletal muscle we have been unsuccessful in identifying a fraction specifically representing an intracellular pool of glucose transporters. It is possible that our inability to find an intracellular pool of transporters is associated with the technical problems involved in working

with skeletal muscle. It may also be possible that reserve transporters are in some way associated with the plasma membrane, although masked when in the basal state. Stimulation by exercise or insulin may result in a redistribution or a conformational change, thereby increasing glucose transport activity and cytochalasin B binding. Recent results from Klip and co-workers support the intracellular transporter pool theory [11], while the data of Ploug et al. [19] is consistent with the theory that stimulation increases the redistribution of transporters within the plasma membrane in skeletal muscle [19].

The increase in the number of glucose transporters in the plasma membrane demonstrated in this study may help to explain the increase in the rate of glucose transport observed in muscle following contractile activity [20–26]. However, 4- to 5-fold increases in glucose uptake which have been reported cannot be completely explained by the 2-fold increase in plasma membrane transporters we have found, suggesting an additional activation step. Similar observations have been noted in the adipose cell [27] where insulin stimulation produced approximately a 20-fold increase in glucose transport activity when compared to basal, while only a 5-fold increase in the number of plasma membrane glucose transporters is observed [18]. Since insulin stimulation in muscle [6] and fat [28,29] and contractile activity in muscle are known to stimulate glucose transport by increasing V_{max} without altering K_m [7], this discrepancy may be associated with an increase in the intrinsic activity of plasma membrane transporters. Joost et al. [30] recently demonstrated that the counter-regulation of insulin-stimulated glucose transport by isoproterenol and adenosine deaminase is associated with changes in intrinsic activity of transporters already present in the plasma membrane rather than their translocation.

Since we recover approx. 10% of the 5′-nucleotidase activity, there is still a need for caution when extrapolating results to the intact tissue. In addition, 5′-nucleotidase recovery may not reflect glucose transporter recovery. Similar studies in adipose cells have the advantage of a much larger percent recovery (~50%). It also may be possible that in our membrane preparations we have underestimated the effect of exercise by combining fractions 2 and 3. The 5′-nucleotidase specific activity of fraction 2 is usually 3-fold greater than that of fraction 3, which has been shown to have increased glucose-6-phosphatase activity [13]. This mixture may dilute the plasma membrane by other membrane species.

Despite these potential difficulties, the present study shows that acute exercise of rats increases the number of glucose transporters measured in purified plasma membrane fractions from red gastrocnemius muscle. This increase can explain, in part, the observed increase in skeletal muscle glucose transport activity immediately following exercise. However, changes in the intrinsic activity of the glucose transporter may also play a role. The origin of the increased number of glucose transporters in the plasma membrane following exercise is still unclear.

Acknowledgements: These studies were still supported, in part, by USPHS grant RO1 AM26317-06 (E.S.H.). H.W.-H. was supported by grants from the Swedish Diabetes Association and the Karolinska Institute. The authors would like to acknowledge Laurie J. Goodyear for her help in the preparation of this manuscript.

REFERENCES

[1] Nesher, R., Karl, I.E. and Kipnis, D.M. (1980) Am. J. Physiol. 239, E461–E467.
[2] Nesher, R., Karl, I.E. and Kipnis, D.M. (1985) Am. J. Physiol. 249, C226–C232.
[3] Wallberg-Henriksson, H. and Holloszy, J.O. (1984) J. Appl. Physiol. 47, 1045–1049.
[4] Wallberg-Henriksson, H. and Holloszy, J.O. (1985) Am. J. Physiol. 249, C233–C237.
[5] Wallberg-Henriksson, H. (1986) Acta Physiol. Scand. 127, 39–43.
[6] Holloszy, J.O. and Narahara, H.T. (1965) J. Biol. Chem. 240, 2493–3500.
[7] Narahara, H.T. and Ozand, P. (1963) J. Biol. Chem. 238, 40–49.
[8] Wardzala, L.J. and Jeanrenaud, B. (1981) J. Biol. Chem. 256, 7090–7093.
[9] Wardzala, L.J. and Jeanrenaud, B. (1983) Biochim. Biophys. Acta 730, 49–56.
[10] Watanabe, T., Smith, M.M., Robinson, F.W. and Kono, T. (1984) J. Biol. Chem. 260, 13117–13122.
[11] Klip, A., Ramlal, T., Young, D.A. and Holloszy, J.O. (1987) FEBS Lett. 224, 224–230.
[12] Philipson, K.D., Bers, D.M. and Nishimoto, A.Y. (1980) J. Mol. Cell. Cardiol. 12, 1159–1173.
[13] Grimditch, G.K., Barnard, R.J., Kaplan, S.A. and Sternlicht, E. (1985) Am. J. Physiol. 249, E398–E408.
[14] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.

[15] Avruch, J. and Hoelzl-Sallach, D.F. (1971) Biochim. Biophys. Acta 233, 334–347.
[16] Wardzala, L.J., Cushman, S.W. and Salans, L.B. (1978) J. Biol. Chem. 253, 8002–8005.
[17] Cushman, S.W. and Wardzala, L.J. (1980) J. Biol. Chem. 25, 4758–4762.
[18] Simpson, I.A., Yver, D.R., Hissin, P.J., Wardzala, L.J., Karnieli, E., Salans, L.B. and Cushman, S.W. (1983) Biochim. Biophys. Acta 763, 393–407.
[19] Ploug, T., Galbo, H. and Vinton, J. (1987) Diabetol. 30, 570A–571A.
[20] Richter, E.A., Garetto, L.P., Goodman, M.N. and Ruderman, N.B. (1984) Am. J. Physiol. 246, E476–E482.
[21] James, D.E., Jenkins, A.B. and Kraegen, E.W. (1985) Am. J. Physiol. 248, E567–E574.
[22] James, D.E., Kraegen, E.W. and Chisholm, D.J. (1985) Am. J. Physiol. 248, E474–E480.
[23] Horton, E.S., Horton, E.D., Hirshman, M.F., Pofcher, E. and Ravussin, E. (1982) Diabetes 31, 471a.
[24] Ivy, J.L. and Holloszy, J.O. (1981) Am. J. Physiol. 241, C200–C203.
[25] Ivy, J.L., Young, J.C., McLane, J.A., Fell, R.D. and Holloszy, J.O. (1983) J. Appl. Physiol. 55, 1393–1396.
[26] Ploug, T., Galbo, H. and Richter, E.A. (1984) Am. J. Physiol. 247, E726–E731.
[27] Karnieli, E., Hissin, P.J., Simpson, I.A., Salans, L.B. and Cushman, S.W. (1981) J. Clin. Invest. 68, 811–814.
[28] Crofford, O.B. and Renold, A.E. (1965) J. Biol. Chem. 240, 3237–3244.
[29] Crofford, O.B. (1975) J. Biol. Chem. 250, 7089–7092.
[30] Joost, H.G., Weber, T.M., Cushman, S.W. and Simpson, I.A. (1986) J. Biol. Chem. 261, 10033–10036.