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Exercise Induces the Translocation of GLUT4 to Transverse Tubules from an Intracellular Pool in Rat Skeletal Muscle

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Exercise stimulates glucose transport in skeletal muscle by increasing the number of GLUT4 glucose transporters at the cell surface. The effect of exercise on GLUT4 content in transverse tubules, which are deep extensions of the plasma membrane involved in myofiber contraction, has never been studied. In the present study, we have investigated whether acute exercise induces translocation of GLUT4 to the transverse tubules. Plasma membranes and transverse tubules were isolated from control and exercised rats by a newly developed membrane fractionation procedure. As expected, acute exercise increased GLUT4 content in plasma membranes (95%; $p < 0.01$). Importantly, exercise also significantly increased GLUT4 content in a transverse tubule-enriched fraction (60%; $p < 0.05$). Furthermore, acute exercise was found to decrease GLUT4 content in an intracellular membrane fraction (−40%; $p < 0.001$). In conclusion, this study demonstrates that GLUT4 is translocated not only to the plasma membranes but also to the transverse tubules in acutely exercized muscles. The results also indicate that GLUT4 is recruited from a novel intracellular membrane fraction.

Skeletal muscle cells depend on extracellular glucose availability to cope with the increased energy demands associated with acute exercise. The rate-limiting step for glucose utilization in skeletal muscle is believed to be its transport accross the cell surface [1]. It is now well documented that insulin stimulates glucose transport by inducing the translocation of GLUT4 from an intracellular compartment to the plasma membrane in rat skeletal muscle [2–5]. Moreover, other studies have shown that acute exercise also increases GLUT4 content in skeletal muscle plasma membranes [4,6–12]. However, more recent studies have shown that another component of the muscle cell surface, the transverse tubules, are involved in insulin-stimulated glucose transport into cardiac and skeletal muscle cells [13–15]. The transverse tubules are membrane folds extending inward from the plasma membrane of muscle cells [16]. These structures are responsible for spreading the action potentials from the nerve terminal up to the sarcoplasmic reticulum leading to fibre contraction [16]. Supporting a role for transverse tubules in myofiber glucose metabolism are the previous observations that these specialized structures also contain insulin receptors as well as high concentrations of glycolytic enzymes [17–19]. As transverse tubules represent at least 60% of the total cell membrane surface in skeletal muscles [20,21], a change in their GLUT4 content is expected to have a significant impact on glucose utilization by myocytes and therefore must be taken into consideration. However, to the best of our knowledge, there are still no studies where the effect of exercise on transverse tubules' GLUT4 protein content has been investigated. Considering that the tubules are responsible for myofiber contraction, it seemed of even greater interest to examine the effects of exercise on GLUT4 content in these structures. On the other hand, the intracellular origin of the GLUT4 appearing in the plasma membrane following stimulation by acute exercise or contraction has been a subject of debate in the last five years. Whereas an exercise-dependent decrease in GLUT4 content in (insulin-responsive) internal membranes has been previously reported [10,12], most studies have failed to detect a significant reduction in

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GLUT4 levels in the insulin-responsive intracellular fraction [4,6–9]. This led to the hypothesis that insulin and exercise may increase glucose transport by recruiting GLUT4 from two distinct intracellular pools.

The aim of the present study was to determine whether acute exercise induces the translocation of GLUT4 to the transverse tubules in addition to the plasma membrane in rat skeletal muscle. We also have attempted to identify the intracellular pool from which GLUT4 is translocated in exercized muscle using a new subcellular fractionation procedure yielding a novel intracellular fraction.

MATERIALS AND METHODS

Animals and exercise protocol. Male Sprague-Dawley rats were either submitted to a bout of exercise on a rodent treadmill for 45 min at 32 m/min at an 8° incline or kept sedentary during that period. Immediately after the bout of exercise, all the rats were killed and hindlimb muscles were rapidly frozen into liquid nitrogen. The tissue samples were then stored at −80°C for later subcellular membrane fractionation. Plasma glucose and lactate levels were measured using a YSI type 2300 STAT Plus automatic glucose analyser (Yellow Springs, OH). Plasma insulin was determined by radioimmunoassay with a rat insulin specific RIA kit from INCSTAR (St. Charles, MO) using rat insulin as standard.

Membrane preparation and immunoblotting. Plasma membranes, transverse tubules and intracellular membranes were isolated using a new procedure recently developed in our laboratory [22] with minor modifications [23]. Our new subcellular fractionation protocol has been extensively characterized with immunologic and enzymatic markers [22] and it allows the simultaneous isolation of both plasma membranes and transverse tubules into separated fractions from the same muscle sample. In this procedure, the use of a strong salt treatment (lithium bromide) also yields an insulin-sensitive intracellular fraction that is greatly enriched in GLUT4 and totally devoid of plasma membranes and transverse tubules markers. Western blot analysis of isolated membrane fractions was performed as previously described [22,23].

RESULTS

The acute bout of exercise did no significantly change plasma glucose levels (C vs E in mM; 6.2 ± 0.5 vs 7.0 ± 0.5) but significantly decreased insulin concentrations as compared to resting animals (C vs E in pM; 744.3 ± 112.1 vs 173.8 ± 39.0; $p < 0.001$). Exercise also significantly increased plasma lactate concentrations (C vs E in mM; 2.3 ± 0.3 vs 3.8 ± 0.4; $p < 0.01$), which probably reflect the anaerobic state induced by this relatively strenuous running protocol.

The subcellular membrane fractions isolated from control and exercized rat muscles were characterized using enzymatic and immunologic markers of plasma membranes and transverse tubules. When compared to the homogenate, 5′-nucleotidase activity in plasma membranes was enriched by ∼17-fold, whereas KpNPPase activity was enriched by ∼50-fold (table 1). Both enzyme activities were barely detectable in transverse tubules (TT1 and TT2) and intracellular membrane fraction

TABLE 1
Protein Recoveries, 5′-Nucleotidase, and KpNPPase Enzymatic Activities in Freshly Isolated Muscle Membrane Fractions

Fraction	State	Protein recoveries	5′-nucleotidase	KpNPPase
PM	C	25.7 ± 2.7	259.7 ± 45.6	1899.4 ± 242.3
	E	25.3 ± 3.2	329.2 ± 57.5	1934.4 ± 243.0
TT1	C	262.4 ± 19.1	57.3 ± 10.8	660.8 ± 177.9
	E	245.2 ± 24.9	69.2 ± 10.8	761.9 ± 103.9
TT2	C	144.3 ± 53.9	22.5 ± 11.2	68.6 ± 34.2
	E	116.0 ± 39.9	34.2 ± 13.8	91.2 ± 54.5
L-IM	C	32.0 ± 6.3	2.2 ± 1.3	22.7 ± 7.4
	E	27.4 ± 6.8	2.3 ± 1.3	26.3 ± 5.5

Values represent mean ± SE of 7 individual membrane preparation. 5′-Nucleotidase activities are in nmol/mg/min; KpNPPase activities are in nmol/mg/hr; and protein yields are in μg of protein/g of muscle weight. C, control; E, exercise; PM, plasma membranes; TT1 and TT2, transverse tubule fractions; L-IM, LiBr-released intracellular membranes. No significant differences were observed between control and exercise-stimulated samples. The muscle weight collected was not different between control and exercised animals (11.7 ± 0.4 g vs 11.4 ± 0.6 g, respectively).

(L-IM). Importantly, no difference in either 5′-nucleotidase and KpNPPase activities or membrane protein recoveries were observed in any fractions indicating that the recovery and distribution of plasma membranes were comparable between control and exercized muscles.

The distribution of immunologic markers of plasma membranes and transverse tubules in the isolated subcellular fractions is shown in figure 1 and table 2. It has been previously reported that both the GLUT1 glucose transporter and the α1 subunit of the Na/K-ATPase are predominantly localized to the plasma membrane in rat skeletal muscle cells [3, 24]. As shown in figure 1 and table 2, the isolated plasma membrane fraction contains high levels of GLUT1 glucose transporter as well as α1-subunit of the Na/K-ATpase, whereas fractions enriched with transverse tubules and intracellular membranes are depleted of these markers. In contrast, the dihydropyridine receptor (DHPr), a transverse tubule marker, was mainly detected in TT1 and TT2 fractions. As for the enzyme markers, exercise neither changed the content nor the distribution of protein markers for plasma membranes (α1-subunit of the Na/K-ATPase) or transverse tubules (DHPr). Furthermore, GLUT1 protein content in the plasma membrane fraction was not changed by exercise.

The effect of exercise on the content and subcellular distribution of GLUT4 in the different fractions is shown in figure 2. A representative Western blot of immunoreactive GLUT4 contents is shown in the upper panel whereas scanning data of relative concentrations of GLUT4 in plasma membranes (PM), transverse tubules (TT1, TT2) and LiBr-released intracellular membranes (L-IM) from control and exercized muscles are presented in the lower panel. In resting skeletal muscle, GLUT4 was mainly located in the intracellular membrane fraction. The intracellular transporter concentration was enriched by ~8-fold as compared to the plasma membrane fraction. Exercise significantly increased GLUT4 contents in both the plasma membrane (PM) (95%; $p < 0.01$) and transverse tubules (TT1) (60%; $p < 0.05$) enriched fractions. However, no significant effect was observed on TT2 GLUT4 content. Furthermore, exercise induced a concomitant reduction in GLUT4 content in the GLUT4-enriched intracellular membrane fraction (40%; $p < 0.001$). When the yields of the different fractions are considered (table 1), the total amount of GLUT4 (yield multiplied by relative GLUT4 content) leaving the L-IM fraction almost fully account (~85%) for the total amount appearing in the PM and TT1 fractions. Moreover, there is about 3.5 times more GLUT4 proteins translocated to the TT1 (10 390 ± 930 relative units/fraction) than to the PM (2 940 ± 430 relative units/fraction) with exercise when considering the total amounts of the transporter in these fractions.

DISCUSSION

To the best of our knowledge, the present study provides the first experimental evidence that

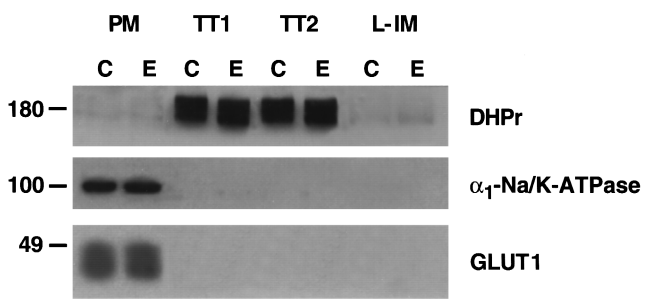

FIG. 1. Membrane distribution and effects of exercise on the content of DHPr, α1-Na/K-ATPase, and GLUT1 in plasma membrane (PM), transverse tubule (TT1 and TT2) and LiBr-released intracellular membrane (L-IM) fractions. 10 μg of subcellular membrane proteins isolated from control (C) and exercised (E) muscles was used for Western blot analysis. PVDF membranes were incubated with either anti-DHPr monoclonal antibody (1:50000)(from Dr. K. Campbell, Univ. of Iowa), anti-αl-Na/K-ATPase (McK-1, from Dr. K. Sweadner, Boston, MA) monoclonal antibody (1:200) or anti-GLUT1 polyclonal antibody (1:2000)(RAGLUTRANS, East Acres, Southbridge, MA). Molecular weight standards (in kDa) are shown on the left.

TABLE 2
Scanning Data of GLUT1, DHPr and α1-Na/K-ATPase Contents in Isolated Membrane Fractions from Control and Exercised Rats

Fraction	State	GLUT1	DHPr	α1-Na/K-ATPase
PM	C	226.9 ± 21.5	N.D.	225.1 ± 24.3
	E	218.9 ± 20.8	N.D.	203.5 ± 45.5
TT1	C	N.D.	495.2 ± 32.0	N.D.
	E	N.D.	520.2 ± 41.4	N.D.
TT2	C	N.D.	539.4 ± 59.6	N.D.
	E	N.D.	545.8 ± 56.4	N.D.
L-IM	C	N.D.	N.D.	N.D.
	E	N.D.	N.D.	N.D.

Values represent mean ± SE of 5 individual membrane preparation. N.D., non detectable; C, control; E, exercise; PM, plasma membranes; TT1 and TT2, transverse tubule fractions; L-IM, LiBr-released intracellular membranes. No significant differences were observed between control and exercise-stimulated samples.

exercise not only induces the translocation of GLUT4 to the plasma membrane but also to transverse tubules. Thus, transverse tubules appear to play a dual role in exercizing muscles: 1) they are necessary for muscle contraction through the transmission of membrane depolarisation to the sarcoplasmic reticulum for Ca^{2+} release, and 2) they provide access of extracellular glucose to the

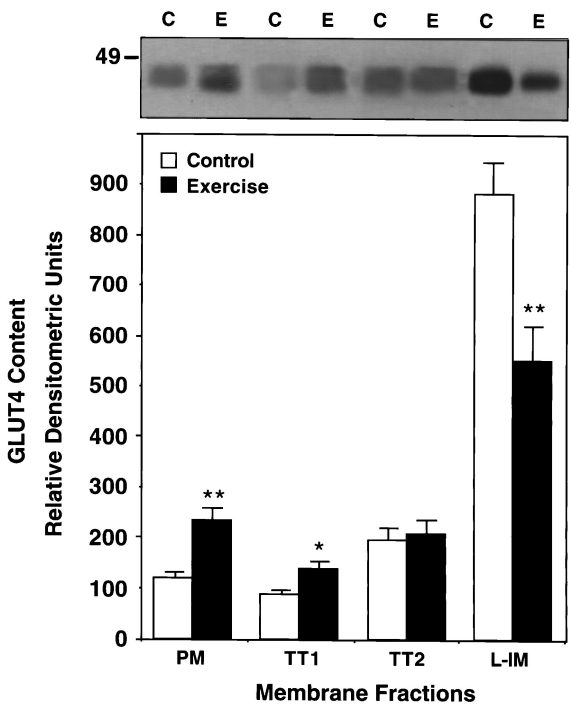

FIG. 2. (Top). Representative Western blot of immunoreactive GLUT4 contents in isolated membrane fractions showing the effects of exercise on the protein contents in plasma membrane (PM), transverse tubule (TT1 and TT2) and intracellular membrane (L-IM) fractions from muscles of control (C) and exercised (E) rats. GLUT4 reactive bands were detected using an anti-GLUT4 polyclonal antibody (1:2000)(IRGT, East Acres, Southbridge, MA). (Bottom) Scanning data of GLUT4 contents (relative densitometric units) in isolated membrane fractions. Bars represent mean ± SE of data obtained from 7 individual membrane preparations, each performed with one control and one exercised rat.

muscle cells by facilitating glucose distribution and transport deep into the myofibers. Supporting the latter role is the previous observations that electrical stimulation of skeletal muscle increase the binding of glycolytic enzymes (glyceraldehyde-3-phosphate dehydrogenase and aldolase) to the transverse tubules [17,19]. Furthermore, these structures represent most (>60%) of the cell surface area in mammalian muscle and therefore, such a quantitatively important structure is likely to contribute to the net entry of glucose and other nutrients in myocytes.

Another interesting observation of the present study is that we could observed a significant exercise-induced decrease in GLUT4 content in a novel intracellular pool that is greatly enriched in GLUT4 transporter proteins but devoid of plasma membrane and transverse tubule markers. We have recently shown, using the same fractionation procedure, that insulin also induces the translocation of GLUT4 to plasma membrane and transverse tubules from the same intracellular pool [22]. Despite the number of studies showing that exercise or muscle contraction increases GLUT4 protein abundance in plasma membranes, the intracellular origin of the recruited GLUT4 transporters still remain a subject of debate. Our study support the hypothesis that insulin and exercise can recruit GLUT4 from the same intracellular pool. It has been recently suggested [4] that changes in insulin levels during exercise may have been responsible for the recruitment of intracellular GLUT4 proteins in some studies. However, in the present study, it is believed that changes in insulin levels are not responsible for the effect of exercise on GLUT4 translocation since plasma insulin, as measured in this study, was in fact significantly decreased by the acute bout of exercise. Nevertheless, the possibility that hypoxia and/or an increase in blood flow (through a greater insulin delivery to the muscle vascular beds) may have contributed to the stimulatory effect of exercise on GLUT4 translocation cannot be completely ruled out in the present study.

It could be argued that exercise induced GLUT4 translocation from another internal pool not isolated by the present fractionation procedure. However this seems unlikely as the total amount of GLUT4 appearing in both PM and TT1 fractions can be almost fully accounted by the amount of GLUT4 leaving the L-IM pool. On the other hand, it is possible that our intracellular fraction is a mixture of “distinct” exercise-sensitive and insulin-sensitive internal pools which would explain its responsiveness to both stimuli. However, the latter hypothesis would then imply that the biochemical nature of these two putative intracellular pools is very similar. Coderre et al. [29] very recently reported the isolation of distinct insulin- and exercise-sensitive GLUT4 intracellular pools. Preliminary analysis of these two pools showed a similar protein composition but a different sedimentation coefficient [34]. It will be interesting to test whether our L-IM intracellular fraction can be separated in two GLUT4-enriched vesicle populations based on possible differences in sedimentation profiles.

In this study, GLUT4 content increased by $\sim$1.6-fold in the TT1 fraction of exercised muscles whereas the transporter content did not change in the TT2 fraction. These data are similar to our previous observations that insulin enhanced GLUT4 contents by $\sim$1.8-fold in TT1 but much less (only $\sim$1.3-fold) in TT2 fractions [22]. The precise nature of the vesicles isolated in those two transverse tubule fractions is still unclear. The TT1 fraction was recovered with mild homogenization whereas TT2 vesicles were released following high-salt treatment for several hours. Moreover, TT2 contains greater amounts ($\sim$8 times) of ryanodine receptor than TT1 [22]. These findings led us to propose that TT1 contains non-junctional tubules whereas TT2 may be enriched in junctional tubules and triadic junctions (where transverse tubules appose the SR) [22,23]. This possibly explains the need for high-salt treatment in releasing TT2 vesicles since triads can form strong complexes with several junctional proteins [25]. The presence of GLUT4 in or near the triads has been previously reported using immunoelectron microscopy in unstimulated cardiac and skeletal muscle [15,26–28]. This is in accordance with the present study showing that TT2 contains a greater amount of GLUT4 in control rat muscle as compared to the plasma membrane and TT1

fractions. Whether the GLUT4 proteins located in the triad membranes are actually in the transverse tubules or the SR, or possibly associated with intracellular organelle(s) will remain speculative until a specific marker of the GLUT4-enriched intracellular pool can be used.

In conclusion, the present study shows that exercise induces the translocation of GLUT4 to transverse tubules in addition to the plasma membrane. Our data also indicate that GLUT4 is translocated from a newly isolated intracellular pool in exercised muscle. Since we have recently shown that insulin also activates the translocation of GLUT4 from that same fraction, these results suggest that this GLUT4-enriched intracellular pool is responsive to both exercise and insulin stimulation. In that regards, it will be of particular interest to examine whether insulin and muscle contraction exert additive effects on GLUT4 translocation from this intracellular pool to the plasma membranes and transverse tubules.

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