Effects of exercise training on muscle GLUT-4 protein content and translocation in obese Zucker rats

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Brozinick, J. T., Jr., G. J. Etgen, Jr., B. B. Yaspelkis III, H. Y. Kang, and J. L. Ivy. Effects of exercise training on muscle GLUT-4 protein content and translocation in obese Zucker rats. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E419–E427, 1993.—The rates of muscle glucose uptake of trained (TR) and untrained (UT) obese Zucker rats were assessed by hindlimb perfusion under basal conditions (no insulin) in the presence of a maximally stimulating concentration of insulin (10 mU/ml) and after muscle contraction elicited by electrical stimulation of the sciatic nerve. Perfusate contained 28 mM glucose and 7.5 μCi/mmol of 2-deoxy-D-[^{3}H]glucose. Muscle GLUT-4 concentration was determined by Western blot analysis and expressed as a percentage of a heart standard. The rates of insulin-stimulated glucose uptake were significantly higher in the plantaris, red gastrocnemius (RG), and white gastrocnemius (WG), but not the soleus or extensor digatorum longus (EDL) of TR compared with UT rats. After muscle contraction the rates of glucose uptake in the TR rats were significantly higher in the soleus, plantaris, and RG. TR rats had significantly higher GLUT-4 protein concentration and citrate synthase activity than the UT rats in the soleus, plantaris, RG, and WG. Basal plasma membrane GLUT-4 protein concentration of TR rats was 144% above UT rats ($P < 0.01$). Stimulation by insulin and contraction resulted in a significant increase in plasma membrane GLUT-4 protein concentration in UT rats only. However, plasma membrane GLUT-4 protein concentration in insulin- and contraction-stimulated TR rats remained 53% and 30% greater than that of UT rats, respectively ($P < 0.05$). Exercise training did not alter basal, insulin-, or contraction-stimulated GLUT-4 functional activity. These results indicate that the increases in insulin- and contraction-stimulated muscle glucose uptake after aerobic exercise training were due in part to an increased plasma membrane GLUT-4 protein concentration and not to an increased GLUT-4 protein translocation or enhanced GLUT-4 functional activity.

glucose transporter; 2-deoxyglucose; insulin; insulin resistance; muscle contraction

THE OBESE Zucker rat exhibits severe skeletal muscle insulin resistance, which is characterized by a pronounced decrease in insulin-stimulated glucose uptake (6–8, 16, 17, 28). The locus of this insulin resistance is thought to reside in the glucose transport process (6, 16, 28). Exercise training has been shown to improve the muscle insulin resistance of the obese Zucker rat (2, 6, 16, 17). The improvements with exercise training are both exercise intensity and fiber-type specific and appear to occur only in muscles that are substantially recruited during exercise (2, 6, 16).

It has recently been demonstrated that insulin-sensitive tissues express a unique glucose transporter protein (GLUT-4), which regulates facilitated glucose transport in response to both insulin and muscle contraction (9, 14, 15). Insulin- or contraction-activated glucose transport in skeletal muscle is thought to involve the rapid reversible translocation of GLUT-4 protein from an intracellular pool to the plasma membrane (9, 14, 15).

Recent evidence from our laboratory (2) has shown that the increase in insulin-stimulated glucose uptake in muscle of the obese Zucker rat after exercise training is directly related to an increase in the total muscle GLUT-4 protein concentration. However, the total muscle GLUT-4 protein concentration may not be as important as its distribution and possibly its ability to be activated. The mechanism through which exercise training improves the skeletal muscle insulin resistance of the obese Zucker rat is unknown. Therefore, the first purpose of this study was to determine if exercise training alters the distribution, functional activity, or insulin-stimulated translocation of GLUT-4 protein in the skeletal muscle of the obese Zucker rat.

Muscle contraction in the absence of insulin has been shown to stimulate glucose uptake (5, 22). Despite the well-documented insulin resistance of the obese rat, recent evidence from our laboratory has shown contraction-stimulated glucose uptake is not defective in this model (5). This suggests that contraction and insulin stimulate glucose transport via separate pathways (9, 10, 17). As previously noted, insulin-stimulated muscle glucose uptake is increased in the muscle of the obese Zucker rat by exercise training (6, 16, 17). However, it is unknown if contraction-stimulated muscle glucose uptake is increased in the obese Zucker rat with exercise training. Therefore, the second purpose of this study was to determine if exercise training increases the ability of contractile activity to stimulate muscle glucose uptake in the obese Zucker rat and if such an adaptation could be explained by an increased GLUT-4 protein concentration, distribution, or functional activity.

METHODS

Experimental animals. Thirty-eight obese Zucker rats 4–5 wk of age were divided into two groups: exercise trained ($n = 21$) and sedentary control ($n = 17$). The exercise-trained and sedentary groups were further subdivided into basal, contraction-stimulated, or insulin-stimulated groups. All rats were housed three per cage and were provided water and laboratory chow ad libitum. The temperature of the animal room was maintained at 21°C, and an artificial 12:12-h light-dark cycle was set.

The training protocol consisted of running on a motor-driven treadmill (Quinton Instruments, Seattle, WA) up an 8% grade, 5 days/wk for 6–7 wk. Both treadmill speed and duration were increased rapidly such that by the 3rd wk of training the rats were capable of running five 18-min bouts separated by 5-min rest periods, at 24 m/min. This exercise intensity was selected because it has been found to improve the insulin resistance of both fast-twitch red and white muscle fibers of the obese Zucker rat (2, 6). The temperature of the exercise room was maintained at 21°C.

Surgical preparation and hindlimb perfusion. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body wt) after a 12-h fast and 48–56 h after

the last exercise bout. The surgical procedure for hindlimb perfusion and the perfusion apparatus were similar to those previously described by Ruderman et al. (25). Additionally, both sciatic nerves of the muscle-contraction rats were surgically exposed, and miniature electrodes were attached. After completion of the surgical preparation, cannulas were inserted into the abdominal aorta and vena cava of the rats, and their hindlimbs were washed out with 50 ml of Krebs-Henseleit buffer (pH 7.4). Immediately thereafter, the cannulas were placed in line with the perfusion system, and the hindlimbs were allowed to stabilize during a 15-min nonrecirculating washout period. The perfusion medium consisted of Krebs-Henseleit buffer (pH 7.4) containing 4% dialyzed bovine serum albumin (Cohn fraction V, US Biochemical), 30% washed time-expired human red blood cells, 0.3 mM pyruvate, and 0.5 mM glucose. Perfusate flow rate during the washout period was 15 ml/min. After the washout, perfusate samples were taken from the venous line for determination of insulin, and the rat was killed with an intracardiac injection of pentobarbital sodium. Insulin analysis indicated that the washout procedure effectively lowered the insulin concentration of the hindlimbs below the level of detection (<2.5 μU/ml).

For determination of basal and insulin-stimulated glucose uptake, the perfusate was changed to one containing 28 mM glucose, 2-deoxy-D-[^{3}H]glucose (2-[^{3}H]DG, 7.5 μCi/mmol glucose), 2 mM mannitol (60 μCi D-[1-^{14}C]mannitol/mmol), and 0.3 mM pyruvate. This concentration of glucose was selected because it is approximately fourfold higher than the average glucose concentration that results in a half-maximal rate of glucose uptake into muscle (19), thereby ensuring that the maximum velocity of uptake was measured. When insulin-stimulated glucose uptake was determined, the perfusate also contained 10 mU/ml insulin. Perfusion times were 10 min for basal and 7 min for insulin- and contraction-stimulated rats, and the perfusate flow rate was maintained at 15 ml/min.

For contraction-stimulated glucose uptake, the skin was reflected from both hindlimbs of the rat during the initial 15-min perfusion period. A section of the calcaneus, with the Achilles tendon still attached, was then clipped from each foot. The triceps surae of both hindlimbs were then reflected away from the tibia, and jewelers' chains were attached to both calcaneus-Achilles tendon interfaces. Both hindlimbs of the rat were immobilized in a specially designed acrylic cradle that allowed adjustment of muscle lengths to achieve maximal twitch tension. One hindlimb was connected to a force transducer/chart recorder to monitor the force of contraction.

After the 15-min washout period, muscle contraction was initiated by stimulating the sciatic nerves with 200-ms trains of 100 Hz, with each impulse in a train being 0.1 ms. The trains were delivered at a rate of 1/s at a supramaximum voltage (8–12 V) for 2 × 5 min separated by a 1-min rest period. Immediately before the initiation of contraction, the flow rate was increased to 25 ml/min to ensure proper oxygen and substrate delivery to the hindlimb muscles. On completion of the second stimulation period, the perfusate was changed to that used for the measurement of basal glucose uptake, and the flow rate was reduced to 15 ml/min. All perfusions were performed at 37°C.

Immediately after each perfusion, the hindlimbs of the rats were washed out with 30 ml of Krebs-Henseleit buffer to remove intravascular erythrocytes. The soleus, plantaris, red (RG) and white (WG) portions of the gastrocnemius, and the extensor digatorum longus (EDL) were then removed from the left leg and blotted on gauze dampened with Krebs-Henseleit buffer. The muscles were clamped frozen in tongs cooled in liquid N_2 and stored at −80°C until analyzed for 2-deoxy-D-glucose (2-DG) uptake, citrate synthase activity, and GLUT-4 protein content. These muscles were chosen because they are composed of mixed and homogeneous sections of the three basic skeletal muscle fiber types of the rat (1). Additionally, because the EDL is not extensively recruited during treadmill exercise (6, 16), it was chosen to differentiate between the local and systemic effects of exercise training on muscle glucose uptake.

Determination of 2-DG uptake. Muscle samples were weighed, homogenized in 1 ml of 10% trichloroacetic acid (TCA) at 4°C and centrifuged in a microcentrifuge (Fisher Scientific, Houston, TX) for 10 min. Duplicate 0.3-ml samples of the supernatant were transferred to 20-ml scintillation vials containing 10 ml of Scintiverse E (Fisher Scientific, Pittsburgh, PA) and vortexed. For determination of perfusate specific activity, well-mixed samples of the arterial perfusate were obtained during perfusion. The samples were deproteinized in 10% TCA and treated the same as the muscle homogenates. The samples were counted for radioactivity in a LS-350 liquid scintillation spectrophotometer (Beckman, Fullerton, CA). Efficiency and channel crossover were determined by counting ^{3}H and ^{14}C standards of known activities. The accumulation of intracellular 2-[^{3}H]DG, which is indicative of muscle glucose uptake, was calculated by subtracting the concentration of 2-[^{3}H]DG in the extracellular space from the total muscle 2-[^{3}H]DG concentration. The 2-[^{3}H]DG in the extracellular space was quantified by measuring the concentration of [^{14}C]mannitol in the muscle homogenate.

Tissue preparation for determination of citrate synthase activity and total GLUT-4 protein concentration. Citrate synthase activity and GLUT-4 protein concentration were determined from the same muscle homogenates. Muscle samples were weighed and then homogenized in an HES buffer [20 mM *N*-2-hydroxyethylpiperzine-*N*′-2-ethanesulfonic acid (HEPES), 1 mM EDTA, 250 mM sucrose, pH 7.4, 1:20 wt/vol] on ice with three consecutive 15-s bursts of a Virtishear homogenizer (Virtishear, Gardiner, NY) set at its highest speed. For citrate synthase activity a 300-μl aliquot was further diluted 1:2 in HES buffer and 1:10 in 0.1 M tris(hydroxymethyl)aminomethane (Tris) and 0.4% Triton X-100, pH 8.1. Citrate synthase activity was determined spectrophotometrically according to Srere (29). Total GLUT-4 protein concentration was determined by Western blot analysis.

Membrane preparation. Plasma and intracellular membranes were prepared from muscle innervated by the sciatic nerve. Approximately 6 g of mixed rat skeletal muscle consisting of the biceps femoris, flexor halicus longus, semimembranosus, semitendinosus, and tibialis anterior from both legs, and the plantaris, and RG and WG from the right leg were removed at the end of perfusion, cleaned of fat and connective tissue, and weighed. This muscle was minced in a buffer consisting of 255 mM sucrose, 100 mM Tris, and 0.2 mM EDTA, pH 7.6. The minced muscle was next homogenized (Brinkman PT-10 Polytron) at a slow speed and then homogenized again with a Potter-Elvehjem Teflon-glass tissue grinder and brought up to a volume of 25 ml with homogenization buffer. A 0.5-ml aliquot was removed for measurement of marker enzymes and protein, and the homogenate was then centrifuged at 34,000 *g* for 20 min. The resulting pellet was used for purification of plasma membranes while the supernatant was used for the purification of microsomal membranes according to Hirshman et al. (15).

Each plasma and microsomal membrane suspension was weighed to determine the exact volume. An aliquot of each suspension was removed for marker enzymes and protein determinations, and the remainder was used for Western blotting. All samples were stored at −80°C until assayed.

Protein and marker enzyme assays. Homogenate and membrane protein concentrations were determined for each preparation by the Coomassie Brilliant Blue method (Bio-Rad protein assay, Richmond, CA) as described by Bradford (4), using crystalline bovine serum albumin as the standard. K^+-stimu-

lated-*p*-nitrophenol phosphatase (K^+-pNPPase) specific activity was measured as a marker for plasma membranes by the method of Bers et al. (3). UDP galactose-*N*-galactosyltransferase, an enzyme marker associated with the Golgi apparatus, was also measured in each fraction by the method of Fleisher (11). To determine if there was sarcoplasmic reticulum contamination of the plasma or microsomal membrane fractions, both homogenate and membrane fractions were assayed for Ca^{2+}-stimulated ATPase (Ca^{2+}-ATPase) activity by the method of Seiler and Fleischer (27).

Western blotting. A 100-μl sample of the tissue homogenate or a 50-μl sample of the membrane suspension was diluted 1:1 with Laemmli sample buffer (21). Aliquots of the diluted samples containing 75 μg of homogenate, 15 μg of plasma membrane, or 30 μg of microsomal membrane protein, along with molecular weight markers (Bio-Rad, Richmond, CA) were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) run under reducing conditions on a 12% resolving gel. Samples from lean and obese rats were run on the same gels. All membrane samples were run on duplicate gels. Resolved proteins were transferred to polyvinylidene difluoride (PVDF) sheets (Bio-Rad) by the method of Towbin et al. (31) utilizing the buffer system of Kyhse-Andersen (19), and a Bio-Rad SD semidry transfer unit. All subsequent incubations were carried out in Tween-Tris buffered saline (TTBS, 0.05% Tween-20) composed of 20 mM Tris, 500 mM NaCl (pH 7.5, at 25°C). After transfer, the PVDF sheets were blocked in TTBS and 5% nonfat dry milk, pH 7.5. Next, the sheets were washed in TTBS for 20 min and then incubated for 1 h with the polyclonal GLUT-4 antibody F349 (donated by Dr. Mike Mueckler, Washington University, St. Louis, MO) at a titer of 1:500 in TTBS, 1% milk, and 0.02% sodium azide. The PVDF sheets were then washed in TTBS for 20 min followed by incubation with ^{125}I-labeled goat anti-rabbit immunoglobulin G (New England Nuclear, Boston, MA) at a concentration of 0.3 μCi/ml for 1 h at 37°C. The sheets were then washed and air dried. Antibody binding was visualized by autoradiography that was performed at −70°C with Kodak X-Omat AR film and Du Pont Cronex Lightning Plus intensifying screens. Labeled bands were traced, cut out, and counted in a Beckman model 5500 gamma counter. Each band was corrected for background radioactivity, and the counts were expressed as a percentage of a standard (100 μg of heart homogenate protein) run on each gel.

Statistical analysis. The data were analyzed by multivariate analysis of variance using the MANOVA procedure (SPSS/PC+ 4.0) with a priori comparisons using the appropriate contrasts to test the effects of training status and treatment and interactions between these main effects. A level of $P < 0.05$ was considered to be statistically significant. All values were expressed as means ± SE.

RESULTS

There were no significant differences in body weights, muscle weights, or muscle protein between trained and untrained rats in each of the experimental conditions (Table 1). Basal and insulin- and contraction-stimulated glucose uptakes of individual muscles or sections of muscle are shown in Fig. 1. Under basal conditions, glucose uptake was not significantly different between trained and untrained rats. Exercise training increased insulin- and contraction-stimulated glucose uptake in the muscles of the obese Zucker rat, but the response was fiber-type specific. Insulin-stimulated glucose uptake was increased in the plantaris, RG and WG, but not in the soleus. In contrast, contraction-stimulated glucose uptake was increased in the plantaris, soleus, and RG, but not in the WG. Neither insulin- nor contraction-stimulated glucose uptake was increased in the EDL by exercise training.

Table 1. *Body and muscle weights used in membrane preparations and protein recovery*

	Basal	Contraction	Insulin
	Untrained		
n	5	6	6
Body weight, g	315.2±21.3	301.8±9.8	293.0±8.2
Muscle weight, g	5.32±0.43	5.81±0.30	5.11±0.21
Total protein			
Homogenate, mg	750.0±31.7	770.1±36.5	686.2±32.5
Plasma membrane, mg	1.79±0.15	1.64±0.17	1.53±0.22
Microsomal membrane, mg	0.80±0.13	0.62±0.11	0.80±0.17
	Trained		
n	6	9	6
Body weight, g	290.2±9.9	290.5±5.1	281.5±7.1
Muscle weight, g	5.47±0.30	5.71±0.61	5.25±0.20
Total protein			
Homogenate, mg	769.8±29.6	787.0±7.56	725.4±50.9
Plasma membrane, mg	1.56±0.09	1.64±0.60	1.53±0.07
Microsomal membrane, mg	0.83±0.17	0.75±0.69	0.62±0.06

Values are means ± SE; *n*, no. of rats.

Exercise training significantly increased citrate synthase activity and total GLUT-4 protein concentration of the soleus, plantaris, RG, and WG, but not the EDL (Fig. 2). The magnitude of change in GLUT-4 protein concentration in the different fiber types was very similar to that which occurred for citrate synthase activity.

In both untrained and trained rats, a significant positive correlation ($r = 0.79$) was found for insulin-stimulated glucose uptake and total GLUT-4 protein concentration in muscle composed of predominately fast-twitch fibers (plantaris, RG, and WG) (Fig. 3). For contraction-stimulated glucose uptake and GLUT-4 protein concentration, significant correlations were found for the fast-twitch muscles ($r = 0.58$) and soleus ($r = 0.48$) (Fig. 4).

Specific activities, percent recoveries, and fold enrichments of the enzyme K^+-pNPPase, a plasma membrane marker enzyme, are given in Table 2. K^+-pNPPase specific activity in the plasma membrane was not altered by exercise training. K^+-pNPPase activity was enriched ~34-fold in the plasma membrane fraction and ~10-fold in the microsomal membranes compared with the crude homogenate. Degree of enrichment did not differ between trained and untrained rats. Furthermore, neither muscle contraction nor insulin stimulation had an effect on percent recovery in trained or untrained rats.

Provided in Table 3 are the specific activities, percent recoveries, and degrees of enrichment (expressed as multiples) of galactosyltransferase. Galactosyltransferase activity was enriched ~25- to 30-fold in the plasma membrane and ~60- to 70-fold in the microsomal membranes compared with the crude homogenate. The degree of enrichment did not differ between untrained and trained rats. Furthermore, neither muscle contraction nor insulin stimulation had an effect on degree of enrichment in untrained or trained rats. Percent recoveries of galactosyltransferase in the plasma and microsomal membranes were approximately equal (5–7%) and were not altered by training, muscle contraction, or insulin stimulation.

Specific activities, percent recoveries, and degree of

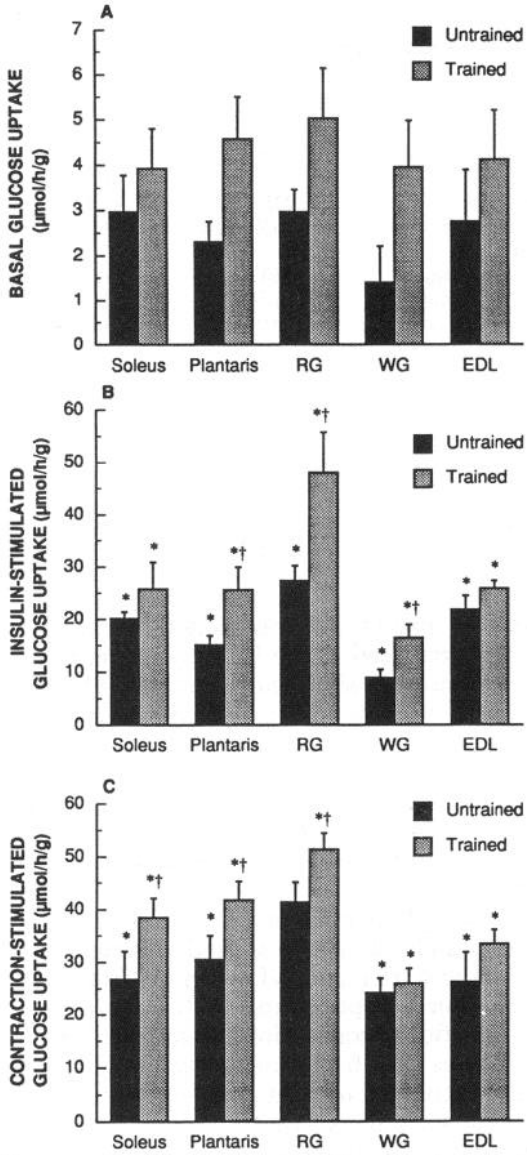

Fig. 1. Glucose uptakes for soleus, plantaris, red gastrocnemius (RG), white gastrocnemius (WG), and extensor digatorum longus (EDL) for trained and untrained obese Zucker rats during basal conditions (*A*), insulin stimulation (*B*), and after muscle contraction (*C*). Values are means ± SE. * Significantly different from basal ($P < 0.05$). † Significantly different from untrained ($P < 0.05$).

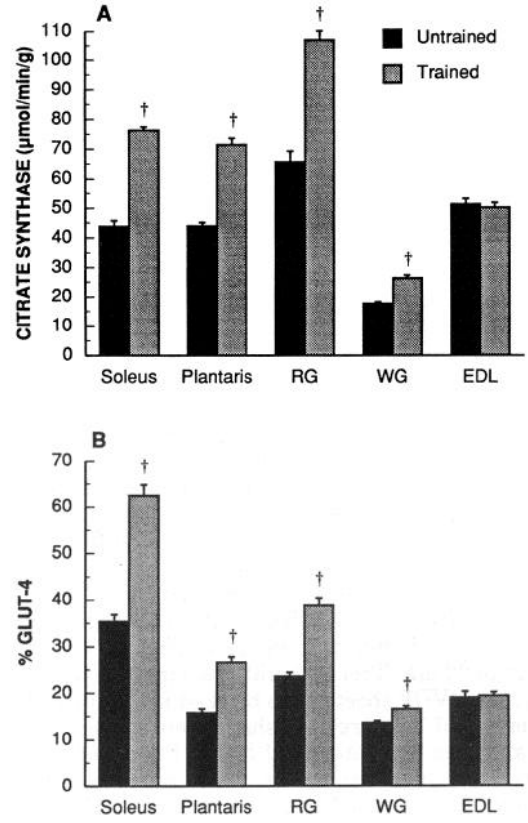

Fig. 2. Citrate synthase acivity (*A*) and GLUT-4 protein content expressed as percentage of heart standard (*B*) of soleus, plantaris, RG, WG, and EDL of trained and untrained obese Zucker rats. Values are means ± SE. † Significantly different from untrained ($P < 0.05$).

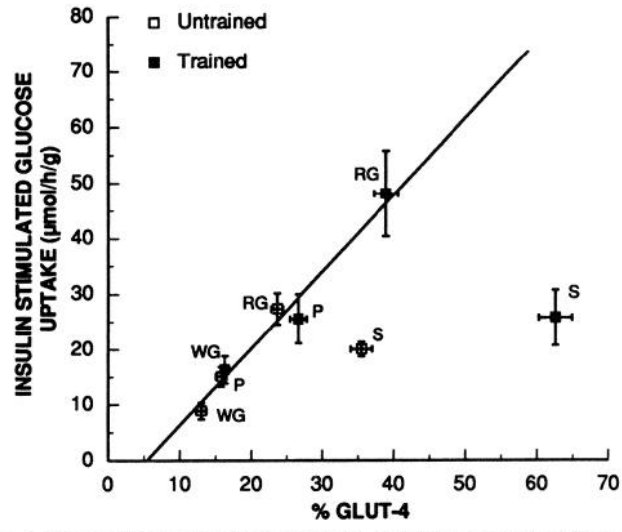

Fig. 3. Correlation between total GLUT-4 protein content and insulin-stimulated glucose uptake in plantaris (P), RG, and WG. Data shown in Figs. 1 and 2 were analyzed by linear regression to correlate values for glucose uptake and GLUT-4 protein content. Values are means ± SE. Correlation coefficient for both trained and untrained, $r = 0.79$. S, soleus.

enrichment (expressed as multiples) of the enzyme Ca^{2+}-ATPase, a marker of sarcoplasmic reticulum, are given in Table 4. The Ca^{2+}-ATPase activities indicated that there was virtually no contamination of the plasma membrane preparation with sarcoplasmic reticulum, as the degree of enrichments of Ca^{2+}-ATPase were less than one for both untrained and trained rats. Ca^{2+}-ATPase was not detected in microsomal membranes.

Under basal conditions, plasma membranes from the trained rats had a 2.5-fold greater GLUT-4 protein concentration than plasma membranes from untrained rats (Fig. 5*A*). Insulin and contraction stimulation significantly increased plasma membrane GLUT-4 protein concentration (~twofold increase) only in the untrained rats. GLUT-4 protein concentration in insulin- and contraction-stimulated plasma membranes from trained rats, however, were 53 and 30% greater, respectively, than the GLUT-4 protein concentration of contraction- and insulin-stimulated plasma membranes from untrained rats.

Under basal conditions there were no significant differences in microsomal GLUT-4 protein concentration

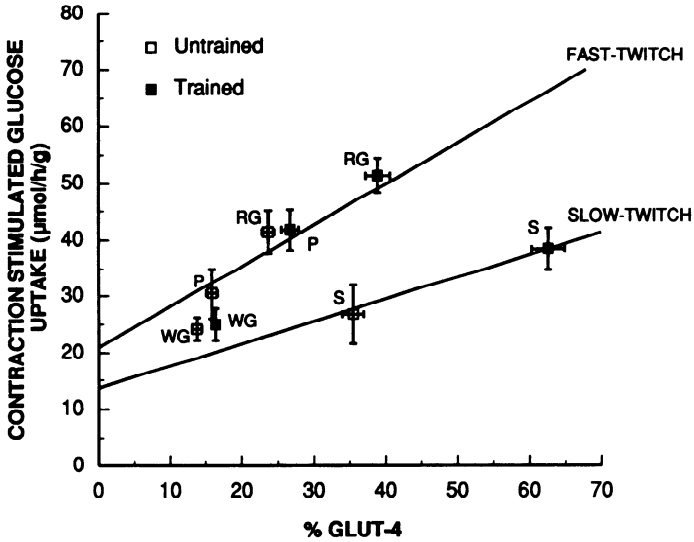

Fig. 4. Correlation between total GLUT-4 protein content and contraction-stimulated glucose uptake. Results shown in Figs. 1 and 2 were analyzed by linear regression to correlate values for glucose uptake and GLUT-4 protein content. Values are means ± SE. Correlation coeffecients for fast-twitch fibers, $r = 0.59$, and slow-twitch fibers, $r = 0.48$.

Table 2. *K^+-stimulated p-nitrophenol phosphatase activities, percent recoveries, and degree of enrichment of membrane fractions from basal, contracted, and insulinized muscle from untrained and trained rats*

K^+-pNPPase	Basal	Contraction	Insulin
	Untrained		
n	5	6	6
Homogenate			
Specific activity, nmol·mg^{-1}·min^{-1}	3.43±0.24	3.65±0.20	3.53±0.29
Plasma membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	109.2±12.5	126.1±14.9	102.5±9.2
Recovery, %	7.80±1.14	6.27±0.62	6.27±0.42
Multiples of enrichment	32.4±3.9	34.8±8.4	30.3±2.1
Microsomal membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	30.7±8.2	42.8±7.1	37.6±7.0
Recovery, %	0.97±0.09	0.94±0.2	1.10±0.13
Multiples of enrichment	9.0±1.9	11.8±1.2	11.2±1.6
	Trained		
n	6	9	6
Homogenate			
Specific activity, nmol·mg^{-1}·min^{-1}	3.53±0.12	3.43±0.69	3.88±0.40
Plasma membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	123.2±5.7	115.4±4.6	120.1±4.8
Recovery, %	7.78±0.39	6.80±1.28	6.88±0.49
Multiples of enrichment	34.2±2.0	32.2±2.8	31.5±2.6
Microsomal membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	40.6±3.9	41.1±5.2	50.6±3.4
Recovery, %	1.23±0.41	1.12±0.15	1.09±0.19
Multiples of enrichment	11.0±1.3	11.0±1.4	12.1±1.1

Values are means ± SE; n, no. of rats. K^+-pNPPase, K^+-stimulated p-nitrophenol phosphatase.

between trained and untrained rats (Fig. 5*B*). Similarly, muscle contraction and insulin stimulation did not cause a significant change in microsomal GLUT-4 protein concentration in either the trained or untrained rats.

Table 3. *UDP galactose-N-acetylglucosamine galactosyltransferase activities, percent recoveries, and degree of enrichment of membrane fractions from basal, contracted, and insulinized muscle from trained and untrained rats*

Galactosyltransferase	Basal	Contraction	Insulin
	Untrained		
n	7	6	6
Homogenate			
Specific activity, nmol·mg^{-1}·2 h^{-1}	1.14±0.08	1.05±0.09	1.12±0.20
Plasma membrane			
Specific activity, nmol·mg^{-1}·2 h^{-1}	27.5±3.2	27.4±3.1	29.5±5.8
Recovery, %	5.01±0.71	5.22±0.56	6.12±1.21
Multiples of enrichment	24.6±3.7	27.4±4.3	29.5±6.8
Microsomal membrane			
Specific activity, nmol·mg^{-1}·2 h^{-1}	68.4±7.6	69.9±9.5	67.75±4.0
Recovery, %	5.95±1.31	5.21±0.58	6.86±1.98
Multiples of enrichment	61.0±7.9	66.7±9.1	67.4±2.4
	Trained		
n	6	9	6
Homogenate			
Specific activity, nmol·mg^{-1}·2 h^{-1}	1.12±0.21	1.01±0.65	1.26±0.16
Plasma membrane			
Specific activity, nmol·mg^{-1}·2 h^{-1}	30.3±2.7	22.3±2.3	28.6±2.3
Recovery, %	6.26±1.04	5.49±1.82	5.37±0.76
Multiples of enrichment	29.5±5.9	26.5±3.9	24.9±4.3
Microsomal membrane			
Specific activity, nmol·mg^{-1}·2 h^{-1}	64.6±4.5	60.8±5.2	63.5±5.5
Recovery, %	6.81±1.20	6.81±1.90	5.60±1.17
Multiples of enrichment	65.0±11.7	70.8±5.9	61.49±9.3

Values are means ± SE; n, no. of rats. There were no significant differences between groups.

The ratio of hindlimb glucose uptake to plasma membrane GLUT-4 protein concentration was determined to provide an estimate of GLUT-4 functional activity (see Table 5 and DISCUSSION for details). Hindlimb glucose uptake, as determined by averaging the glucose uptakes of the plantaris, RG, and WG, was not significantly different between trained and untrained obese rats under basal conditions. However, both insulin- and contraction-stimulated glucose uptakes were significantly increased by training (Fig. 6). GLUT-4 functional activity was the same for untrained and trained rats under basal, insulin-, and contraction-stimulated conditions. However, insulin increased GLUT-4 activity above basal by ~5-fold, whereas contraction increased GLUT-4 activity above basal by ~10-fold.

DISCUSSION

In the present study, exercise training resulted in an increased insulin-stimulated glucose uptake and total GLUT-4 protein concentration in the plantaris, RG, and WG of the obese Zucker rat. In agreement with previous research from our laboratory, the increases in insulin-stimulated glucose uptake and total GLUT-4 protein concentration occurred only in muscles that displayed an increase in citrate synthase activity (2, 6, 16). In addition,

Table 4. *Ca^{2+}-stimulated ATPase specific activities, percent recoveries and degree of enrichment of membrane fractions from basal, contracted, and insulinized muscle from untrained and trained obese rats*

Ca^{2+}-Stimulated ATPase	Basal	Contraction	Insulin
	Untrained		
n	5	6	6
Homogenate			
Specific activity, nmol·mg^{-1}·min^{-1}	105.6±17.6	96.8±4.5	104.7±15.0
Plasma membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	63.0±34.5	60.7±20.9	24.0±14.6
Recovery, %	0.15±0.11	0.14±0.06	0.18±0.14
Multiples of enrichment	0.58±0.37	0.57±0.21	0.40±0.26
Microsomal membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	ND	ND	ND
	Trained		
n	6	9	6
Homogenate			
Specific activity, nmol·mg^{-1}·min^{-1}	96.5±9.4	96.8±4.5	105.0±9.0
Plasma membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	31.1±19.2	3.58±2.18	24.0±11.2
Recovery, %	0.09±0.05	0.01±0.09	0.06±0.03
Multiples of enrichment	0.40±0.23	0.04±0.22	0.28±0.14
Microsomal membrane			
Specific activity, nmol·min^{-1}·mg^{-1}	ND	ND	ND

Values are means ± SE; *n*, no. of rats. ND, not detected.

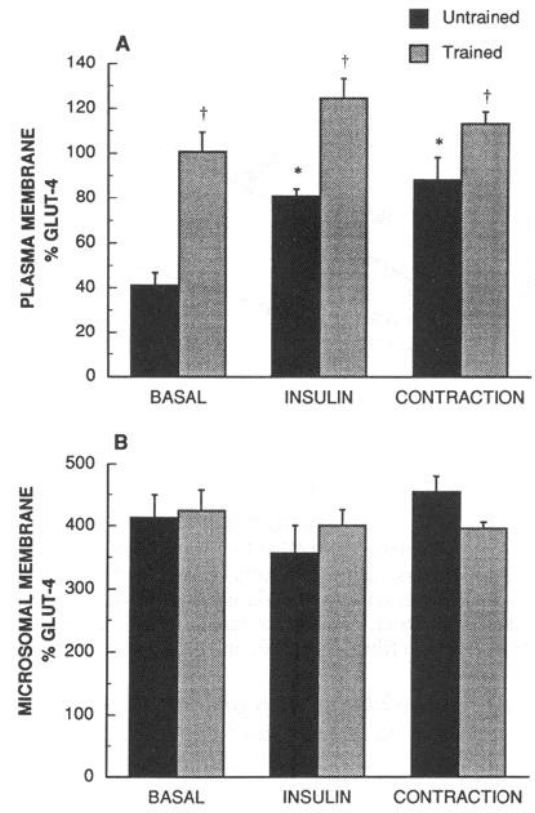

Fig. 5. Plasma membrane (*A*) and microsomal (*B*) GLUT-4 protein content of trained and untrained obese Zucker rats expressed as a percentage of heart standard. Values are means ± SE. * Significantly different from basal. †Significantly different from untrained ($P < 0.05$).

Table 5. *Functional activity of plasma membrane GLUT-4 protein from untrained and trained obese rats*

	Basal	Insulin Stimulated	Contraction Stimulated
Trained	2.22±0.34	12.48±1.98*	21.21±2.28*†
Untrained	2.62±0.81	11.74±1.95*	21.30±8.55*†

Values are mean activities (nmol glucose uptake·%GLUT-4^{-1}·h^{-1}) ± SE. Functional activity was calculated by dividing hindlimb glucose uptake (μmol·g wet wt^{-1}·h^{-1}) by %GLUT-4 protein per gram muscle. %GLUT-4 protein per gram muscle was determined by multiplying %GLUT-4 protein per milligram plasma membrane protein by milligram plasma membrane protein per gram muscle. * Significantly different from basal. † Significantly different from insulin-stimulated rats.

the increases in glucose uptake, GLUT-4 protein concentration and citrate synthase activity were proportional and occurred in parallel (Figs. 1, 2, and 3). These results suggest that the improvement in muscle insulin resistance with exercise training is related to the degree to which the muscle is trained. Previous findings from our laboratory have also shown that the muscle of trained obese Zucker rats exhibit coordinated increases in the activity of citrate synthase and that of enzymes involved in intracellular glucose metabolism (6, 16, 30). Taken together, these findings suggest that the training-induced increases in insulin-stimulated glucose uptake and total GLUT-4 protein concentration are coregulated with an increased expression of enzymes that control the intracellular disposal of glucose. Therefore, as the capacity of the muscle to transport glucose is increased, its capacity to metabolize glucose is increased proportionally.

It is noteworthy that the soleus, despite displaying a 77% increase in GLUT-4 protein concentration and 74% increase in citrate synthase activity, did not show an improvement in insulin-stimulated glucose uptake. This lack of an increase in insulin-stimulated glucose uptake after exercise training agrees with previous findings from our laboratory (6, 15) and those of Crettaz et al. (7). The present study extends our previous findings in that the absence of an improvement in the soleus could not be attributed to a lack of an increase in total GLUT-4 protein concentration. It was found, however, that the soleus had significantly increased contraction-stimulated glucose uptake following exercise training. A possible explanation for these observations is that there are two pools of GLUT-4 protein in muscle and that exercise training only increases the contraction regulated pool in the soleus of the obese Zucker rat.

A significant correlation ($r = 0.79$) was found between insulin-stimulated glucose uptake and total GLUT-4 protein concentration for individual fast-twitch muscles of both trained and untrained rats. This relationship is similar to that previously found by Banks et al. (2). It was observed that the coordinates for the individual muscles of both trained and untrained rats were on the same regression line (Fig. 4). This indicates that the increase in glucose uptake was directly proportional to the increase in total GLUT-4 protein concentration. Therefore, the

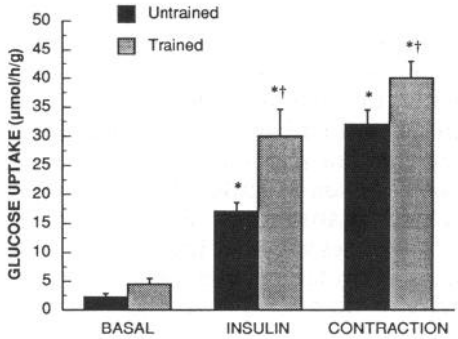

Fig. 6. Hindlimb glucose uptake determined by averaging glucose uptakes of plantaris, RG, and WG for trained and untrained obese Zucker rats during basal conditions, insulin stimulation, and after muscle contraction. Values are means ± SE. * Significantly different from basal. †Significantly different from untrained ($P < 0.05$).

results suggest that the increase in insulin-stimulated glucose uptake with exercise training was mainly due to the increase in total muscle GLUT-4 protein. This hypothesis is supported by the Western blot analysis of the isolated membrane fractions from hindlimb muscles.

Western blotting of basal plasma membranes from trained and untrained rats showed that the basal plasma membranes from trained rats had a 144% greater GLUT-4 protein concentration than that of untrained rats. Insulin stimulation resulted in a significant increase in plasma membrane GLUT-4 protein concentration in untrained rats but not trained rats. Nevertheless, because of the elevated basal plasma membrane GLUT-4 protein concentration after training, the plasma membrane GLUT-4 protein concentration of trained insulin-stimulated rats was 53% greater than that of untrained rats under the same condition. No difference in the microsomal GLUT-4 protein concentration of the trained and untrained rats was observed. Furthermore, insulin did not cause a significant decline in microsomal membrane GLUT-4 protein in trained or untrained rats.

To obtain a relative estimate of GLUT-4 functional activity, the ratio of glucose uptake to total plasma membrane GLUT-4 protein content was determined (Table 5). Glucose uptake was determined by averaging the glucose uptakes of the plantaris and RG and WG (Fig. 6). Plasma membrane GLUT-4 content was determined as the percent GLUT-4 protein per gram muscle. This was based on the protein concentration used in the Western blot analysis and the percentage of plasma membrane recovered. Insulin stimulation caused a significant increase in GLUT-4 protein functional activity; however, this was not different between trained and untrained rats. Therefore, the present results indicate that the exercise training-induced increase in insulin-stimulated glucose uptake was not due to an increase in the translocation of GLUT-4 protein to the plasma membrane or the activation of GLUT-4 protein associated with the plasma membrane.

Recent research suggests that the muscle insulin resistance of the obese Zucker rat is due to a defect in insulin-stimulated GLUT-4 protein translocation from its intracellular pool to the plasma membrane (18) and possibly a reduced functional activity (Brozinick, Etgen, Yaspelkis, and Ivy, unpublished observations). Because the increase in insulin-stimulated glucose uptake after exercise training was not associated with either insulin-stimulating event, it can be concluded that the increase in GLUT-4 protein concentration was the main reason for the improvement in muscle insulin resistance in the obese Zucker rat. This suggests that exercise training does not correct the actual cellular defects associated with the muscle insulin resistance in the obese Zucker rat but causes an increase in plasma membrane GLUT-4 protein which compensates for these defects.

The increase in plasma membrane GLUT-4 protein concentration in response to exercise training does not appear to be specific to the obese Zucker rat, but rather a normal adaptation to aerobic exercise training. Recently, Goodyear et al. (13) reported that exercise training increased the plasma membrane but not the microsomal membrane GLUT-4 protein concentration of non-insulin-resistant rats. In addition, this group also reported that neither the insulin-stimulated GLUT-4 protein translocation process nor the functional activity of the GLUT-4 protein, as assessed by plasma membrane vesicle transport, was found to be altered by the training protocol.

It is possible, however, that the inability of Goodyear et al. (13) as well as the present study to demonstrate an increase in the intracellular concentration of GLUT-4 protein as a result of exercise training was due to the procedure used to isolate the intracellular GLUT-4 protein pool. That is, exercise training could have increased the intracellular pool of GLUT-4 protein, but the membrane isolation procedure used was unable to detect this change. This may also explain the lack of response of the microsomal GLUT-4 protein to insulin stimulation. However, this is unlikely as we have found (G. J. Etgen, A. R. Memon, G. A. Thompson, Jr., and J. L. Ivy, unpublished observations), as have others using the same isolation procedures (12, 13, 15), that the GLUT-4 protein concentration of the microsomes declines significantly with insulin stimulation in non-insulin-resistant rats.

Like insulin, muscle contraction is believed to stimulate skeletal muscle glucose uptake by causing the translocation of GLUT-4 protein to the plasma membrane, although muscle contraction is thought to stimulate translocation through a mechanism distinct from that of insulin (5, 9, 10, 12, 14). Recently, Ploug et al. (23) reported that exercise training increased contraction-stimulated glucose transport of the soleus (slow-twitch, oxidative fibers), but not that of the RG (fast-twitch oxidative fibers) or WG (fast-twitch glycolytic fibers) of non-insulin-resistant (Wistar) rats. In the present study, exercise training resulted in an increased contraction-stimulated glucose uptake and total GLUT-4 protein concentration in the soleus, plantaris, and RG. Although the WG responded to exercise training with an increased GLUT-4 protein concentration, it did not demonstrate an increased contraction-stimulated glucose uptake. The reason for the difference in results is not known but could be related to the strain of rat investigated or more likely the means by which glucose uptake was determined. Both

studies do demonstrate, however, that exercise training-induced alterations in insulin- and contraction-stimulated glucose uptakes are fiber-type specific. The results of the present study suggest that the contraction-regulated glucose transport system of the oxidative fibers and the insulin-regulated glucose transport system of the fast-twitch fibers are improved with exercise training in the obese Zucker rat.

Western blotting of plasma membranes from trained and untrained rats showed that muscle contraction resulted in a significant increase in plasma membrane GLUT-4 protein concentration in untrained, but not trained rats. Nevertheless, because of the elevated basal plasma membrane GLUT-4 protein concentration after training, the plasma membrane GLUT-4 protein concentration of trained contraction-stimulated rats was 30% greater than that of untrained rats. As with insulin stimulation, contraction caused a significant increase in the functional activity of the GLUT-4 protein in the trained and untrained rats. There was also no significant difference in contraction-stimulated GLUT-4 functional activity between the trained and untrained rats. Furthermore, muscle contraction did not result in a significant decline in GLUT-4 protein concentration in either trained or untrained microsomal membranes. These results provide evidence that exercise training did not increase contraction-stimulated glucose uptake in the obese rats by improving the GLUT-4 protein translocation process or the functional activity of the GLUT-4 protein.

Whether the increase in contraction-stimulated glucose uptake after training can be attributed to the increase in total GLUT-4 protein concentration is questionable. Unlike the results for insulin-stimulated glucose uptake, a high correlation between total GLUT-4 protein and glucose uptake for the different muscle fiber types was not found. When the muscles composed of predominantly fast-twitch fibers (plantaris, RG and WG) and the soleus (slow-twitch fiber) were analyzed separately, the correlations were $r = 0.59$ and 0.48, respectively. Although these are significant correlations they are not very robust. Therefore, it is possible that the improvement in contraction-stimulated glucose uptake in the obese rat was only partially due to the increased total GLUT-4 protein concentration.

It is interesting that the plasma membrane GLUT-4 protein concentration of trained and untrained rats was significantly different, whereas basal glucose uptake was not. Also, that insulin- and contraction-stimulated glucose uptakes of the trained rats increased without a significant increase in plasma membrane GLUT-4 protein concentration. This suggests that in the basal state there are large numbers of immunologicaly detectable GLUT-4 transporters associated with the plasma membrane that are inactive. It further suggests that GLUT-4 protein has a second level of regulation besides translocation, in which the GLUT-4 transporters are in close association with the plasma membrane but are unable to transport glucose. This hypothesis is supported by recent findings that insulin, in addition to causing the translocation of GLUT-4 to the plasma membranes of adipocytes, also "activates" or "unoccludes" GLUT-4 transporters that are already present in the plasma membrane (26, 32).

In summary, it was found that aerobic exercise training increased the insulin- and contraction-stimulated skeletal muscle glucose uptakes of the obese Zucker rat. The improvements in insulin- and contraction-stimulated glucose uptake were fiber-type specific. Insulin-stimulated glucose uptake was increased only in fast-twitch fibers and contraction-stimulated glucose uptake was increased only in oxidative fibers. Furthermore, these training-induced increases were not the result of an adaptation in the GLUT-4 protein translocation process or functional activity of the GLUT-4 transporter.

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