Contraction-stimulated muscle glucose transport and GLUT-4 surface content are dependent on glycogen content

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[1]*Copenhagen Muscle Research Centre, August Krogh Institute, University of Copenhagen, 2100 Copenhagen;* [5]*Steno Diabetes Center and Hagedorn Research Institute, 2820 Gentofte, Copenhagen;* [3]*Medical Research Laboratory, Aarhus Kommunehospital, 8000 Aarhus, Denmark;* [2]*Department of Movement and Sports Sciences, University of Gent, 9000 Gent, Belgium; and* [4]*Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom*

Derave, Wim, Sten Lund, Geoffrey D. Holman, Jørgen Wojtaszewski, Oluf Pedersen, and Erik A. Richter. Contraction-stimulated muscle glucose transport and GLUT-4 surface content are dependent on glycogen content. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E1103–E1110, 1999.—The influence of muscle glycogen content on basal and contraction-induced glucose transport and cell surface GLUT-4 content was studied in rat skeletal muscle. Wistar rats were preconditioned by a combination of swimming exercise and diet, resulting in 40% lower (LG) or threefold higher (HG) muscle glycogen content compared with nonexercised controls (NG). At rest and during contractions, 2-deoxy-D-glucose uptake in perfused fast-twitch muscle, but not slow-twitch muscle, was significantly lower in HG compared with LG. Cell surface GLUT-4 content in the fast-twitch plantaris was 994 ± 180, 1,173 ± 311, and 2,155 ± 243 dpm/g in the basal condition and increased ($P < 0.05$) to 2,285 ± 239, 3,230 ± 464, and 4,847 ± 654 dpm/g during contractions with HG, NG, and LG, respectively, the increase being significantly smaller in HG compared with LG. The contraction-induced increments in glucose transport and in cell surface GLUT-4 content were negatively correlated with the initial glycogen content ($P < 0.01$). In conclusion, glucose transport and cell surface GLUT-4 content in resting and contracting fast-twitch muscle are dependent on the muscle glycogen content.

glucose transporters; exercise; glycogen metabolism; perfused hindlimb; 2-deoxy-D-glucose

THE MOLECULAR MECHANISM leading to contraction-induced glucose uptake in skeletal muscle is still incompletely understood, but it is known to involve translocation of GLUT-4 glucose transporters from the intracellular storage sites to the surface membrane. It has previously also been demonstrated that the muscle glycogen content exerts some influence on contraction-induced glucose utilization (reviewed in Ref. 23). Thus during two-legged ergometer cycling, glucose uptake was higher in the glycogen-depleted leg than in the leg with normal muscle glycogen level (5). In the perfused rat hindlimb, the muscle glucose uptake rate during contractions was negatively correlated with precontraction muscle glycogen content (8). One explanation for this inhibitory effect of glycogen on the contraction-induced muscle glucose uptake may be that high glycogen levels promote contraction-induced glycogen breakdown, which gives rise to high concentrations of glucose 6-phosphate, which in turn inhibit hexokinase. Thus this mechanism suggests that the glucose uptake rate is limited by the inhibition of the glucose phosphorylation step rather than the sarcolemmal glucose transport step (5, 8, 11). However, another possibility exists. As was first shown by Hespel and Richter (8), muscle glucose transport capacity, as measured by the uptake of radiolabeled 3-*O*-methyl-D-glucose, is increased by contractions more in glycogen-depleted than in glycogen-supercompensated muscle (8). Recently this was indirectly confirmed by Kawanaka et al. (12), who found a negative correlation between contraction-induced uptake of 2-deoxy-D-glucose (2DG) and postcontraction muscle glycogen concentrations. These findings suggest that contraction-induced GLUT-4 translocation to the surface membrane may be affected by glycogen levels. Likewise, hypoxia-induced surface labeling of GLUT-4 was shown to be reduced in trained compared with sedentary rat muscle because of a training-induced increase in glycogen level (21). Although hypoxia and contractions do not seem to share the same signaling pathway for increasing muscle glucose transport (3, 29), the latter finding lends credence to the possibility that contraction-induced muscle GLUT-4 translocation may be dependent on muscle glycogen content. Therefore, one aim of the present study was to study directly whether cell surface GLUT-4 content in contracting muscle is dependent on precontraction muscle glycogen content.

After an exercise bout, glucose transport remains elevated for some time, and the reversal is dependent on carbohydrate intake. Thus, if a fat-rich diet is fed after exercise, muscle glucose transport is still somewhat elevated 18 h after exercise, whereas if carbohydrates are fed, baseline levels of transport are reached much sooner (4, 8, 31). The mechanism underlying this effect of carbohydrate feeding and the associated glycogen storage after exercise is not known, and furthermore it is not known whether the increased basal glucose transport is due to persistent GLUT-4 transporters in the surface membrane. Therefore, a second aim of the present study was to elucidate whether the persistent increased glucose transport rate in muscle after exercise, when animals are fed a fat-rich diet, is due to persistent GLUT-4 transporters at the muscle surface membrane.

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METHODS

Animals. All experiments were approved by the Danish Animal Experiments Inspectorate and complied with the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes" (Council of Europe no. 123, Strasbourg, France, 1985). Male Wistar rats (3–4 wk old, 60–100 g) were preconditioned so that we could obtain three different subgroups with varying muscle glycogen concentrations, as described previously (8). A control group with normal muscle glycogen levels (normal glycogen, NG) had free access to regular rat chow and tap water until 3–6 h before perfusion. The rats of the other two groups were subjected to 2 h of swimming in water maintained at 32–35°C, with weights (6% of body weight) attached to their tails. In the 24 h preceding the swim, their food intake was restricted to 4 g (~60% of normal intake). After swimming, they were fed ad libitum with either lard and tap water (low glycogen, LG) or with normal rat chow, tap water, and a 20% glucose drinking solution (high glycogen, HG) until 3–6 h before perfusions. Rats were perfused between 18 and 24 h after the swimming bout.

Surgical procedure. The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight). Surgery was performed as described by Ruderman et al. (26) for isolated hindquarter perfusion.

Perfusion medium. All perfusions were carried out using a cell-free and glucose-free perfusate consisting of Krebs-Ringer bicarbonate buffer solution (KRBB), 4% bovine serum albumin (fraction V, Sigma Chemical) dialyzed (pore size 10–15 kDa) twice for 24 h against 11 volumes of KRBB, 0.15 mM pyruvate, and 4.2 IU/ml heparin, as previously described (28). Media having this composition were used throughout the perfusion in the surface labeling experiments and in the initial stage during the glucose transport experiments. For measurement of glucose transport, 8 mM of 2DG (Sigma) and 1 mM mannitol (Sigma) together with radioactive labeled tracers 2-deoxy-D-[2,6-^{3}H]glucose (specific activity 51 Ci/mmol; Amersham International, UK) and D-[1-^{14}C]mannitol (specific activity 57 mCi/mmol; Amersham International) yielding activities of 0.075 and 0.05 μCi/ml, respectively, were used as previously described (28).

Perfusion procedure. The perfusion medium (100 ml) was gassed with a mixture of 95% oxygen-5% carbon dioxide. The oxygen pressure and pH of the recirculated arterial perfusion medium ranged between 450 and 550 mmHg and 7.3 and 7.4, respectively. The temperature of the perfusate was 35°C, which resulted in a muscle temperature in the calf muscles of ~32°C. With respect to the viability of the presently used muscle preparation, we have previously shown that muscle ATP and creatine phosphate values during 45 min of basal perfusion with a cell-free medium do not change compared with values obtained from rested anesthetized rat muscles (27). The perfusion pressure ranged between 30 and 50 mmHg in resting conditions and between 40 and 60 mmHg during contractions. The initial 10 ml of perfusate were discarded, and thereafter a 15-min equilibration period was carried out, with the hexose-free medium recirculating at a flow of 5 ml/min perfusing both legs (~0.4 $ml \cdot min^{-1} \cdot g\ muscle^{-1}$). After 10 min of equilibration, the left common iliac artery and vein were ligated, and muscle biopsies were taken from the left leg (for determination of precontraction glycogen levels). After the remaining 5 min of the equilibration perfusion, the right leg was made to contract isometrically by electrical stimulation of the sciatic nerve for 10 min while flow was maintained at 5 ml/min. The electrical stimulation was performed with supramaximal trains (25 V) of 100 ms delivered at 2-s intervals and an impulse duration and frequency within the train of 0.1 ms and 100 Hz, respectively. During the last 4 min of contractions, the hindlimb was perfused with the hexose- and tracer-containing perfusate, without recirculation (to ensure a constant concentration of 2DG in the arterial perfusate throughout the exposure time). Immediately after the perfusion, the muscles of the right (stimulated) leg were biopsied. Muscle samples were taken from four different parts of the calf muscles representing a whole range of fiber type distributions. The approximate frequencies of slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), and fast-twitch glycolytic (FG) fibers of rats aged 20–34 days (60–100 g) are taken from Maltin et al. (16) and are given in parentheses (SO:FOG:FG, together with an abbreviation of the muscle name used in this paper). The white, most superficial part of the gastrocnemius (0:20:80; WG), the plantaris (10:50:40; Plant), the red, deep proximal and medial portion of gastrocnemius (10:55:35; RG), and the soleus (55:40:5; Sol) were trimmed of connective tissue, blotted, and freeze-clamped with aluminum clamps cooled in liquid nitrogen. The biopsies were stored at −80°C until analyzed. For measurement of basal glucose transport, both hindlimbs were perfused at a flow of 5 ml/min for 10 min with recirculating hexose-free medium and subsequently for 20 min with the hexose- and tracer-containing medium without recirculation. Immediately after perfusion, the right leg was biopsied. Longer periods of radioactive exposure time were used during basal perfusions (20 min) than during contractions (4 min) to get high enough counts in the muscle extracts during basal perfusions. In a subsample of experiments, arterial and venous perfusate samples were taken at rest and after 5 min of contractions and were analyzed immediately for O_2 pressure (ABL 510 acid-base laboratory, Radiometer). Oxygen uptake was calculated as described previously (3).

Glycogen and glucose transport measurements. Muscle glycogen content was measured as glucose residues by a hexokinase method after acid hydrolysis (13). The 2DG taken up by the different muscles was determined in perchloric acid extracts and corrected for label in the extracellular space determined by the ^{14}C counts for mannitol. Radioactivity was measured in a liquid scintillation counter (model 2000 Tri-Carb, Packard Instruments, Downers Grove, IL). From the intracellular accumulation of 2-deoxy-D-[2,6-^{3}H]glucose, the rate of glucose transport was calculated using a specific activity of hexose determined by the hexose concentration and 2-deoxy-D-[2,6-^{3}H]glucose counts in the perfusate and was expressed as micromoles per gram of muscle per hour.

Photoaffinity labeling of cell surface GLUT-4. Rats were perfused as described previously, with the hexose-free perfusate only. The rested and stimulated legs were perfused simultaneously at a flow of 5 ml/min during an equilibration period (15 min) and 8 ml/min during the 10-min contraction period (100-ms trains at 2-s intervals). A flow of 8 ml/min was chosen in two-legged perfusions because this flow resulted in the same pressure in the arterial tubing as 5 ml/min in one-legged perfusions during contractions. After stimulation, perfusion was stopped, and the plantaris muscles were rapidly but carefully dissected out from rested and stimulated legs and without further stimulation were immediately transferred to a dark room and incubated at 18°C for 8 min in KRBB containing 1 mCi/ml ATB-[2-^{3}H]BMPA, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis(D-mannose-4-yloxy)-2-propylamine (ATB-BMPA; specific activity ≈10 Ci/mmol), prepared as previously described (2). The muscles were then irradiated for 6 min with manual turning over after 3 min in a Rayonet RPR 100 photochemical reactor (RPR 3000 lamps). After irradiation, muscles were blotted and trimmed of visible

tendons and frozen in liquid nitrogen. The frozen muscles were weighed and further processed as described previously (14), except that the immunoprecipitation was carried out overnight at 4°C. Labeled GLUT-4 protein was expressed as disintegrations per minute per gram wet muscle weight.

Total muscle GLUT-4 content. Total crude membranes (TCM) were prepared from 30–40 mg of individual muscles, as described previously (20). TCM samples were separated using a 10% SDS-PAGE gel (Mini Protean II, Bio-Rad Laboratories, Hercules, CA) and then transferred to an Immobilon P membrane (Millipore, Bedford, MA) by semi-dry blotting. Subsequently the Immobilon P membrane was blocked for 2 h in 10 mM Tris and 0.9% NaCl, pH 7.4, or TBS buffer, containing 5% defatted milk powder, followed by incubation with a primary GLUT-4 antibody (goat polyclonal IgG, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Then the membrane was washed and incubated with a secondary alkaline phosphatase conjugated rabbit anti-goat antibody (Pierce, Rockford, IL). A chemifluorescence substrate for alkaline phosphatase (Attophos, Amersham International) was added to the washed membrane, and the antibody-antigen complexes were visualized and quantified with a chemifluorescence scanner (Storm 840, Molecular Dynamics, Sunnyvale, CA). TCM GLUT-4 protein content per microgram protein (measured with Pierce Microtiter Protocol, Rockford, IL) was expressed in arbitrary units relative to a TCM standard.

Statistics. Statistical evaluation of the data was done by *t*-tests or one-way ANOVA by use of the Student-Newman-Keuls method for post hoc multiple comparisons, where appropriate. Correlations were calculated with the Pearson product moment test. Data are presented as means ± SE, and the level of significance was chosen at 0.05.

RESULTS

Muscle glycogen. Muscle glycogen content in glycogen-supercompensated rats (HG) was four- to sixfold higher than in glycogen-depleted (LG) rats (Table 1). The effect of the pretreatment (swimming and diet) on glycogen levels was similar in all of the muscles studied. However, the absolute muscle glycogen content was approximately two- to threefold lower in Sol than in the RG, WG, and Plant. The glycogen breakdown during contractions was two- to threefold higher in HG muscles compared with LG muscles in WG, RG, and Plant ($P <$ 0.05) but not significantly different in Sol. At the end of electrical stimulation, the glycogen content was 4- (Sol), 10- (WG), 12- (Plant), and 16-fold (RG) higher in HG muscles than in LG muscles ($P < 0.05$).

Table 1. *Initial and final muscle glycogen levels and glycogen breakdown in contracting muscles with high and low glycogen content*

	WG	RG	Sol	Plant
Glycogen, μmol/g wet wt				
HG				
Initial	121 ± 8*	160 ± 13*	48 ± 7*	131 ± 12*
Final	76 ± 7*	111 ± 10*	33 ± 5*	86 ± 9*
Breakdown	45 ± 4*	49 ± 4*	15 ± 4	45 ± 3*
LG				
Initial	28 ± 4	25 ± 3	14 ± 1	26 ± 4
Final	8 ± 2	7 ± 2	8 ± 1	7 ± 1
Breakdown	20 ± 2	18 ± 3	6 ± 2	19 ± 3

Data are means ± SE of 6–8 observations. HG and LG, high and low glycogen content, respectively; WG and RG, white and red gastrocnemius, respectively; Sol, soleus; Plant, plantaris. Muscles were contracted by electrical stimulation with 100-ms trains at 2-s intervals. Glycogen breakdown was calculated by initial minus final concentration. *$P < 0.05$ vs. values in LG group.

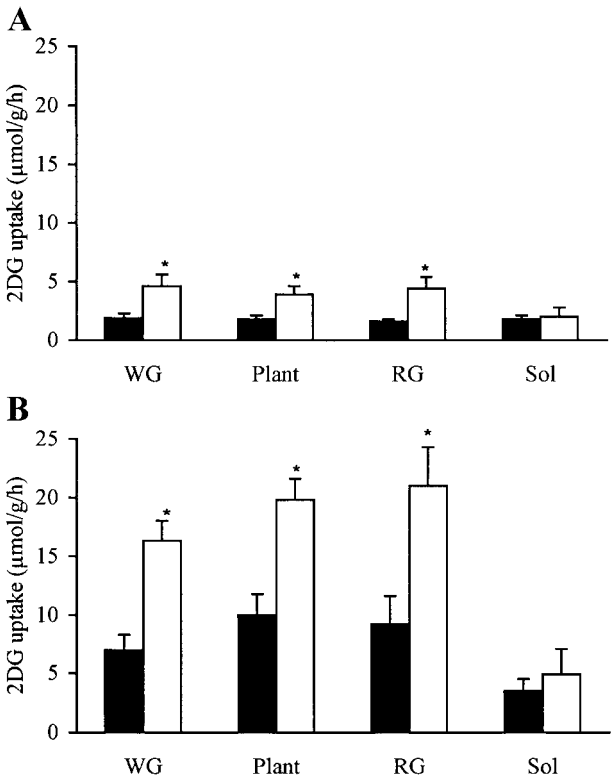

Fig. 1. Basal (*A*) and 10-min contraction-stimulated (*B*) 2-deoxy-D-glucose (2DG) uptake in individual muscles with high (filled bars, HG) and low (open bars, LG) muscle glycogen content. WG and RG, white and red gastrocnemius, respectively; Plant, plantaris; Sol, soleus. Measurements of basal and contraction-stimulated 2DG uptake were done on separate groups of rats. Data are presented as means ± SE (n = 5–11). *Significant difference between HG and LG ($P < 0.05$).

Glucose transport. Basal glucose transport, as measured by uptake of 2DG, was approximately twofold higher in LG than in HG in WG, RG, and Plant, whereas in Sol, basal glucose transport was unaffected by glycogen levels (Fig. 1*A*). Basal glucose transport correlated negatively with the glycogen content in Plant ($r = -0.50$; $P < 0.05$) and RG ($r = -0.40$; $P <$ 0.05) but not in the WG ($r = -0.32$) and Sol ($r = -0.12$). After 10 min of electrical stimulation, glucose transport in WG, RG, and Plant was increased four- to eightfold over basal (Fig. 1*B*). Contraction-induced 2DG uptake in LG was approximately twofold higher than in HG in these muscles ($P < 0.05$). In the Sol, the contraction-induced increase in glucose transport was very limited (~2-fold), and no effect of glycogen content could be observed (Fig. 1*B*). To ensure that the lack of effect of glycogen content in the Sol was not due to this limited increase in glucose transport, we in additional experiments observed that stimulation with a more intense stimulation protocol (200-ms trains at 1-s intervals, instead of 100-ms trains at 2-s intervals) could increase glucose transport in the Sol nearly fivefold over basal,

with no effect of glycogen content (see Table 2). It is our experience that applying this stimulation protocol to muscles of larger rats results in larger increases in glucose transport. Thus, in another set of experiments, we applied the same submaximal and maximal stimulation protocols during perfusion of rats weighing 200–250 g. In these larger rats, the respective increases in Sol 2DG uptake were 6- and 15-fold over basal. Still, there were no differences between HG and LG (Table 2).

The glucose transport rate during contractions in the small rats was significantly negatively correlated with the precontraction glycogen levels in WG ($r = -0.53$; $P < 0.05$), RG ($r = -0.59$; $P < 0.05$), and Plant ($r = -0.74$; $P < 0.05$), but not in the Sol ($r = -0.31$). The glucose transport rate during contractions was also correlated with postcontraction glycogen levels in WG ($r = -0.49$; $P < 0.05$), RG ($r = -0.61$; $P < 0.05$), and Plant ($r = -0.73$; $P < 0.05$), but not in the Sol ($r = -0.18$). Finally, in WG ($r = -0.54$; $P < 0.05$) and in Plant ($r = -0.69$; $P < 0.05$), there was also a significant correlation between glycogen breakdown (calculated as the difference between initial and final glycogen content) and glucose transport.

Cell surface GLUT-4 content. To study whether differences in glucose transport were due to different recruitment of GLUT-4, surface labeling of the Plant was performed. This muscle was chosen because, of the three muscles (WG, RG, and Plant) in which glycogen was found to have an effect on glucose transport, the Plant was the only one that could be dissected out as an intact muscle and incubated. In addition to the HG and LG groups, we also studied a third group of muscles from rested rats with normal glycogen levels (NG). The muscle glycogen content in this NG group (40 ± 3 μmol/g) was intermediate and ~40% higher than in LG ($P < 0.05$) and threefold lower compared with HG ($P < 0.05$; Fig. 2*C*). In the basal state, the glucose transport and the GLUT-4 content at the muscle cell surface were significantly higher in the LG muscles compared with the HG and NG groups (Fig. 2, *A* and *B*). Although there was a significant negative linear correlation between glycogen content on the one hand and basal glucose transport ($r = -0.50$; $P < 0.05$) or basal GLUT-4 surface content ($r = -0.53$; $P < 0.05$) on the other, the relation appears to be curvilinear (Fig. 3, *A* and *B*). Thus the basal glucose transport and cell surface GLUT-4 content are only stimulated in the muscles where glycogen content is below normal, and

Table 2. *2DG uptake in Sol*

	2DG Uptake, μmol·g^{-1}·h^{-1}			Precontraction Glycogen, μmol/g wet wt
	Basal	Submaximal contraction	Maximal contraction	
	60–100 g Rats			
HG	1.8 ± 0.3 (10)	3.5 ± 1.0 (8)	7.8 ± 1.2 (5)	53 ± 5 (23)
LG	2.0 ± 0.8 (11)	4.9 ± 2.2 (6)	10.2 ± 3.8 (5)	13 ± 1 (22)
	200–250 g Rats			
HG	1.5 ± 0.1 (4)	8.6 ± 1.9 (8)	23.7 ± 2.2 (12)	40 ± 4 (24)
LG	1.5 ± 0.3 (5)	10 ± 2.7 (8)	22.5 ± 2.2 (16)	20 ± 1 (29)

Values are means ± SE, with nos. of experiments indicated in parentheses. 2-Deoxy-D-glucose (2DG) uptake was measured in HG and LG Sol muscles during hindlimb perfusions at rest (Basal) and during electrical stimulation (10 min) of submaximal (100-ms trains at 2-s intervals) and maximal (200-ms trains at 1-s intervals) intensities in rats weighing 60–100 g or 200–250 g. Rats weighing 200–250 g were preconditioned and perfused exactly as the 60- to 100-g rats, except that perfusate flow was set at 20 ml/min, resulting in the same perfusion pressure and flow rate/g muscle (0.4 ml·min^{-1}·g muscle^{-1}) as in smaller rats. Precontraction glycogen content was measured in soleus muscle samples obtained from basal perfusions and from nonexercised legs of contraction perfusions.

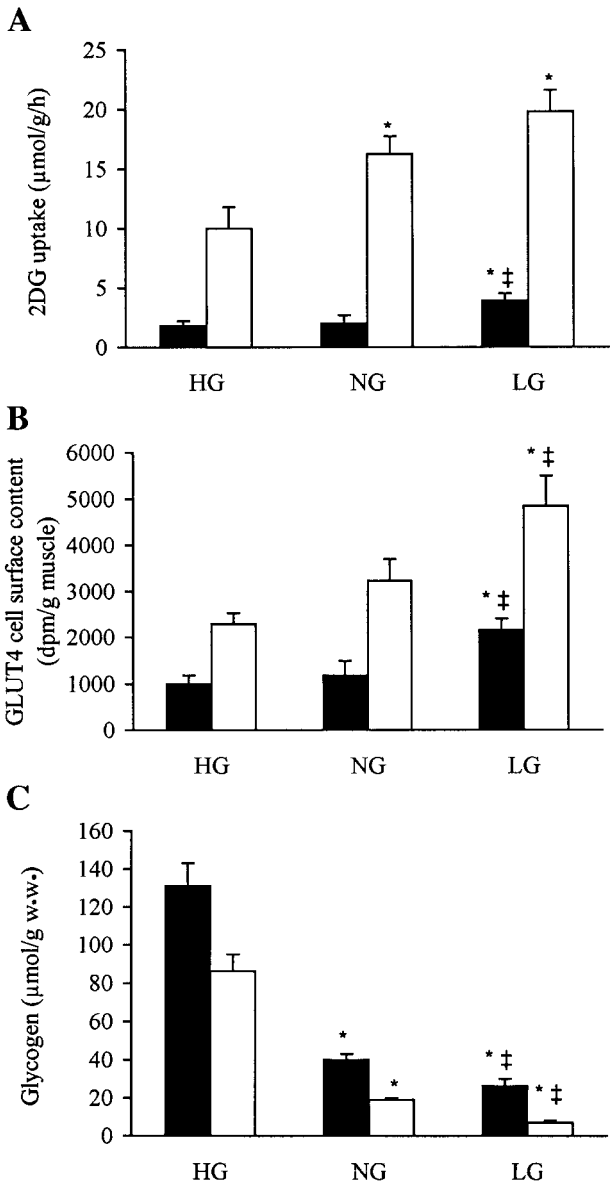

Fig. 2. 2DG uptake (*A*), GLUT-4 cell surface content (*B*), and glycogen content (*C*) in plantaris muscles at rest (filled bars) and after 10 min of contractions (open bars; 100-ms trains with 2-s intervals). w.w., Wet weight. Rats were pretreated to obtain muscles with high (HG), normal (NG), or low (LG) muscle glycogen content. After hindlimb perfusion, plantaris muscles were dissected out of the rested and electrically stimulated legs and were incubated in ATB-BMPA to label cell surface GLUT-4. Glycogen and 2DG uptake were determined in separate experiments, as in Fig. 1. Data are presented as means ± SE (n = 5–8). w.w, Wet wt. * Significantly different from HG ($P < 0.05$); ‡ significantly different from NG ($P < 0.05$).

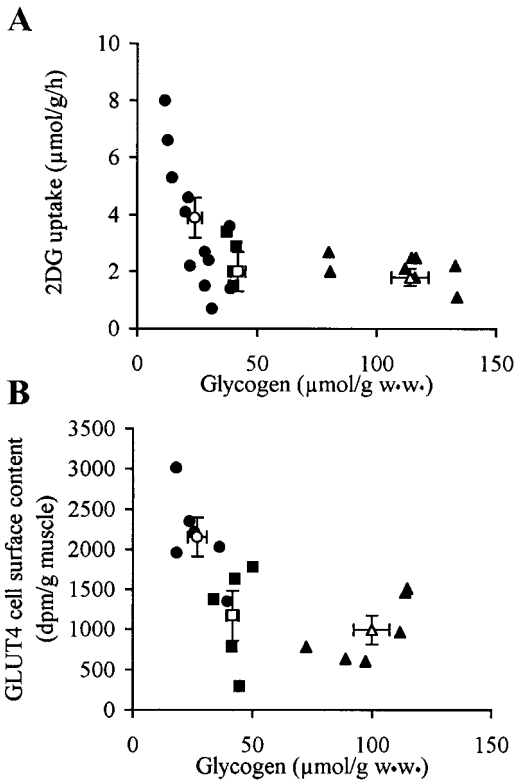

Fig. 3. Basal 2DG uptake (*A*) and basal GLUT-4 cell surface content (*B*) as a function of muscle glycogen content in plantaris muscles with HG (triangles), NG (squares), and LG (circles). Individual data points are given as filled symbols, and group means (± SE) are given as open symbols.

very high glycogen levels do not affect basal glucose transport or surface membrane GLUT-4 content, compared with normal glycogen levels. Contractions increased the amount of GLUT-4 on the muscle cell surface two- to threefold ($P < 0.05$; Fig. 2*B*). The contraction-induced GLUT-4 cell surface content in LG was over twofold higher compared with HG and 50% higher compared with NG ($P < 0.05$). Cell surface GLUT-4 content in NG tended to be higher than in HG ($P = 0.06$; Fig. 2*B*). The contraction-induced increments in both glucose transport rate (as calculated by the contraction-induced glucose transport rate minus the mean glucose transport rate of the basal group) and GLUT-4 cell surface content (as calculated by the difference between cell surface GLUT-4 contents in contracted and rested legs) were significantly negatively correlated with the initial glycogen content (correlation coefficients of −0.70 and −0.59, respectively; $P < 0.05$; Fig. 4, *A* and *B*) and the postcontraction glycogen level (correlation coefficients of −0.69 and −0.53, respectively; $P < 0.05$). Although glucose transport and cell surface GLUT-4 were not measured in the same muscle samples, a positive correlation ($r = 0.95$, $P < 0.01$) was found between the six mean values of both parameters in Plant muscles with HG, NG, and LG at rest and during contractions (Fig. 5).

Total muscle GLUT-4 content. The total GLUT-4 protein content in the muscle of HG, NG, and LG, respectively, was 1.4 ± 0.4, 1.1 ± 0.2, and 1.5 ± 0.4 (arbitrary units) in the Plant ($n = 6$). There were no significant differences between groups.

Body weight, contraction force, and oxygen uptake. Body weight was 86 ± 3, 77 ± 2, and 80 ± 2 g immediately before perfusion in HG, NG, and LG, respectively. The maximal force development during electrical stimulation in the perfused hindlimb was 1.27 ± 0.06, 1.26 ± 0.05, and 1.28 ± 0.04 N/g, and the mean force development was 0.50 ± 0.03, 0.46 ± 0.03, and 0.42 ± 0.02 N/g during 10 min of electrical stimulation in HG, NG, and LG, respectively ($n = 12–14$). There were no significant differences between groups in maximal or mean contraction force. Muscle oxygen uptake was measured immediately before and at the end of the 10-min contraction period in a subsample of HG ($n = 8$) and LG ($n = 7$) rats. Oxygen uptakes were 12 ± 1 and 15 ± 1 $\mu mol \cdot g^{-1} \cdot h^{-1}$ in resting hindquarters and increased to 30 ± 7 and 29 ± 7 $\mu mol \cdot g^{-1} \cdot h^{-1}$ during contractions in HG and LG groups, respectively.

DISCUSSION

During the last two decades, a number of papers have suggested that glycogen plays a role in regulating glucose entry in unstimulated skeletal muscle cells and in muscle cells stimulated with either insulin, hypoxia, or contractions (1, 4, 5, 8–10, 12, 18, 22, 31). The present study is the first to demonstrate that in fast-

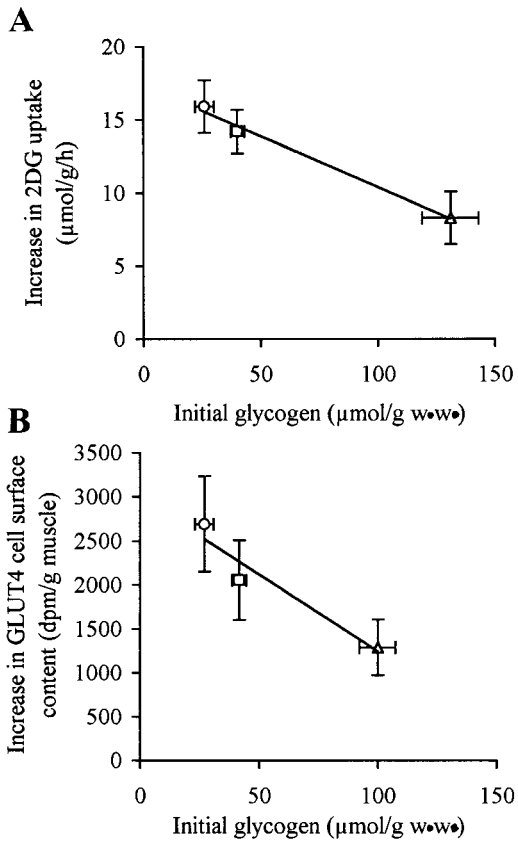

Fig. 4. Effect of initial glycogen content on increments in 2DG uptake (*A*) and cell surface GLUT-4 content (*B*) from rest to contractions, measured in plantaris muscles with HG (△), NG (□), and LG (○). Data are means ± SE ($n = 5–8$).

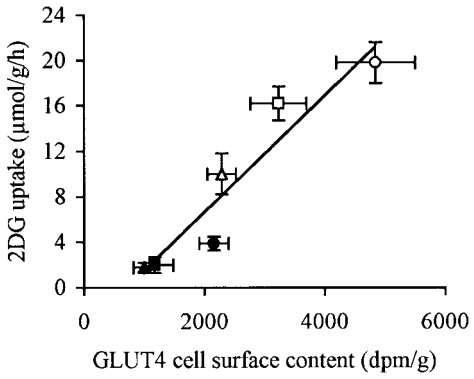

Fig. 5. Correlation ($r = 0.95$, $P < 0.01$) between GLUT-4 cell surface content and 2DG uptake in basal (closed symbols) and contraction-stimulated (open symbols) plantaris muscles with HG (triangles), NG (squares), and LG (circles). Each point is the mean ± SE of 6–8 observations.

twitch muscle, the increment in cell surface GLUT-4 content in response to a standardized contraction stimulus is inversely correlated to precontraction muscle glycogen content. These findings thus suggest that glycogen in muscle modifies GLUT-4 translocation in response to muscle contractions. In addition, we have shown that when muscle glycogen is maintained low by fat feeding after exercise, the surface membrane content of GLUT-4 is increased in the basal state.

The effect of muscle glycogen content on basal and contraction-induced glucose transport was consistently observed in hindlimb muscles with a mixed (RG and Plant) or primarily glycolytic (WG) fiber-type composition. In contrast, glycogen levels did not at all affect glucose transport in the Sol, containing primarily slow-twitch fibers (Table 2 and Fig. 1). This could be interpreted in several ways. First, the Sol is known to have a limited glycogen storage capacity (24, 25), causing threefold lower glycogen levels in Sol compared with the other hindlimb muscles (Table 1). Thus the inhibition of contraction-induced glucose transport during glycogen supercompensation may only be detectable when glycogen levels exceed 100 μmol/g wet weight, a value that can hardly be reached in rat Sol muscles (25). However, this interpretation does not explain why there is no enhanced glucose transport in the highly glycogen-depleted (precontraction glycogen levels below 15 μmol/g in the LG group) Sol muscles. Second, the regulatory role of glycogen on glucose transport may be truly fiber type specific and may therefore be restricted to glycolytic fibers. In this context, it should be realized that the effect of glycogen in fast-twitch muscle may not be due to glycogen itself but rather to another metabolite or reporter of intracellular energy status that changes concomitantly with glycogen. If so, this mechanism would have to be more active in fast-twitch than in slow-twitch fibers, either because of inherent differences between the muscle fiber types or simply because of the different glycogen levels in the different fiber types. Of interest in this regard is the recent work by W. W. Winder and colleagues (see Hayashi et al., Ref. 7, and Merrill et al., Ref. 17), suggesting a role for 5′-AMP-activated protein kinase (AMPK) in contraction-stimulated glucose transport. Differential activation of AMPK, which is dependent on the fuel status of the cell, may help to explain the differential regulation of glucose transport in contracting glycogen-depleted and supercompensated muscles.

It is noteworthy that the results obtained in the nonexercised and non-dietary-manipulated control (NG) group were well within the data obtained in the two groups with extreme muscle glycogen levels (Figs. 2 and 4). This is an important observation, because it signifies that the differences in contraction-induced glucose transport between the groups are not caused by some nonspecific effect of the combination of exercise and diet but rather are genuinely related to differences in muscle glycogen content. Neither were differences in contraction-induced muscle glucose transport and GLUT-4 cell surface content related to differences in total muscle GLUT-4 content, which was similar in the three groups.

It was previously shown in perfused rat hindlimbs that the contraction-induced increase in glucose uptake and transport is dependent on precontraction muscle glycogen concentration (8). In that study, it was concluded that the decrease in contraction-induced glucose uptake in HG compared with LG muscles was due both to decreased glucose phosphorylation and decreased glucose transport capacity in HG. In the present study, we have provided further strong evidence that a major effect of high muscle glycogen levels is a blunted contraction-induced increase in muscle glucose transport capacity. Furthermore, we have for the first time shown that this blunted increase in glucose transport in HG muscle is due to a reduced amount of functional GLUT-4 transporters at the muscle surface membrane.

In the present study, qualitative changes in glucose transport with varying muscle glycogen levels are fully reflected by changes in cell surface GLUT-4, supporting the opinion that the main mechanism for increased glucose transport in contracting muscles is GLUT-4 translocation. However, the absolute increases of contractions over basal are smaller in cell surface GLUT-4 data than in glucose transport data. This is in apparent contrast to a previous study, in which the contraction-mediated increase in GLUT-4 cell surface content fully accounted for the increment in glucose transport after contractions in the soleus (15). This discrepancy may have several explanations. First, in contrast to the in situ measurement of basal glucose transport in the perfused hindlimb, the handling and possibly also stretching of the plantaris (while taking it out for incubation) may have led to increased basal cell surface GLUT-4 content. Second, in the present study, the glucose transport was measured during contractions in the perfused hindlimb, whereas the photolabeling experiments took place a few minutes after cessation of contractions, the time necessary to take out the plantaris and transfer it to the incubation medium. Reversal of the contraction effect may have led to an underestimation of cell surface GLUT-4 content during contractions. Third, the relatively thick plantaris muscle may have represented a diffusion barrier to the photola-

bel, which might contribute to the lower degree of increase in surface labeling compared with glucose transport.

After a bout of glycogen-depleting exercise, carbohydrate feeding speeds and carbohydrate restriction slows the reversal of exercise-induced glucose transport (8, 30, 31). The present results indicate that, in muscles where normal glycogen stores have not yet been completely restored 18–24 h after exercise (as in our LG group), the increased glucose transport is due to a higher number of GLUT-4 molecules at the surface of the muscle cell (Figs. 2 and 3). It seems that, as soon as normal glycogen levels are restored (~40 μmol/g wet weight), the glycogen stores exert a negative feedback signal to stop the GLUT-4 recruitment to the surface membrane. However, muscle glycogen content is not the only regulator of postexercise glucose uptake. Several in vitro studies have shown that the reversal of glucose transport after exercise is not always dependent on glycogen synthesis (6, 19, 30). In these studies, glucose transport returned to baseline or near-baseline levels despite glycogen concentrations being maintained at a low level. Therefore, one may question whether glycogen depletion itself, or another metabolite or signal, is the promoting factor of increased cell surface GLUT-4 content postexercise. Additionally, it is not clear whether GLUT-4 is trapped at the cell surface and unable to return to the intracellular storage sites (decreased endocytosis), or whether GLUT-4 endocytosis is unaffected, but the GLUT-4 translocation to the membrane is continuously stimulated (increased exocytosis).

In conclusion, we have demonstrated that the increased basal glucose transport in glycogen-depleted muscle 18–24 h after exercise is the result of increased appearance of active GLUT-4 molecules at the muscle cell surface. We also provide evidence that contraction-induced glucose transport and GLUT-4 cell surface content are affected by the muscle glycogen content in a concentration-dependent manner. Basal and contraction-induced glucose transport in the soleus muscle is unaffected by glycogen levels, possibly pointing to a fiber type-specific mechanism. These data for the first time show the role of glycogen in regulating GLUT-4 cell surface content in muscle at rest and during contractions.

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