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GLUCOSE UPTAKE AND TRANSPORT IN CONTRACTING, PERFUSED RAT MUSCLE WITH DIFFERENT PRE-CONTRACTION GLYCOGEN CONCENTRATIONS

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SUMMARY

1. Glucose uptake and transport, muscle glycogen, free glucose and glucose-6-phosphate concentrations were studied in perfused resting and contracting rat skeletal muscle with different pre-contraction glycogen concentrations. Rats were pre-conditioned by a combination of swimming exercise and diet, resulting in either low (glycogen-depleted rats), normal (control rats) or high (supercompensated rats) muscle glycogen concentrations at the time their hindlimbs were perfused.

2. Compared with control rats, pre-contraction muscle glycogen concentration was approximately 40% lower in glycogen-depleted rats, whereas it was 40% higher in supercompensated rats. Muscle glycogen break-down correlated positively ($r = 0{\cdot}76$; $P < 0{\cdot}001$) with pre-contraction muscle glycogen concentration.

3. Glucose uptake during contractions was approximately 50% higher in glycogen-depleted hindquarters than in control hindquarters; in supercompensated hindquarters it was 30% lower. When rats with similar muscle glycogen concentrations were compared, glucose uptake in hindquarters from rats that had exercised on the preceding day was approximately 20% higher than in hindquarters from rats that had not exercised on the preceding day.

4. Muscle membrane glucose transport, as measured by the rate of accumulation of ^{14}C-3-*O*-methylglucose in the contracting muscles, was 25% lower in super-compensated than in glycogen-depleted muscles at the onset as well as at the end of the 15 min contraction period.

5. Intracellular concentrations of free glucose and glucose-6-phosphate were higher at rest and during the entire 15-min stimulation period in supercompensated muscles than in glycogen-depleted muscles, and glucose uptake during contractions correlated negatively with free glucose ($r = -0{\cdot}52$; $P < 0{\cdot}01$) as well as with glucose-6-phosphate ($r = -0{\cdot}49$; $P < 0{\cdot}01$) concentrations.

6. It is concluded that: (a) The rate of glucose uptake in contracting skeletal muscle is dependent on the pre-contraction muscle glycogen concentration. Regulating mechanisms include limitations of membrane glucose transport as well as of glucose metabolism. (b) Contractions on the preceding day have a stimulating effect on glucose uptake during contractions of the same muscles on the next day.

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INTRODUCTION

Muscle glycogen and blood-borne glucose serve as major fuels for muscular activity. It is reasonable to assume that the rate of combustion of these fuels during muscle contractions to some extent is determined by their availability, and that abundance of one of these fuels might inhibit the utilization of the other fuel. Support for such a contention comes from a study in humans (Gollnick, Pernow, Essen, Jansson & Saltin, 1981) in which glucose extraction was higher across an exercising leg when muscle glycogen concentration was normal than when it was low. Furthermore, in isolated perfused skeletal muscle, it has been demonstrated that during contractions glucose uptake into muscle with high glycogen levels is lower than in muscle with normal glycogen levels (Richter & Galbo, 1986).

Whether the rate of glucose uptake in muscles with high initial glycogen levels is limited by the capacity of glucose disposal within the muscle cell or by decreased membrane transport of glucose, or by both, has not yet been fully elucidated. It has been shown that the glycogenolytic rate is enhanced in contracting muscles with high initial glycogen levels (Gollnick, Piehl, Saubert, Armstrong & Saltin, 1972; Gollnick *et al.* 1981; Richter & Galbo, 1986); this may be accompanied by an increased accumulation of glucose-6-phosphate in the muscle cell (Gollnick *et al.* 1981). Elevated glucose-6-phosphate concentrations inhibit hexokinase, impeding phosphorylation and further metabolism of glucose, transferred from the interstitial space as well as liberated from the branching points of the glycogen molecule by the action of debranching enzyme (Newsholme & Leech, 1983). However, data obtained in adipocyte membrane vesicles suggest that glucose-6-phosphate might also directly decrease membrane transport of glucose (Foley & Huecksteadt, 1984). The present study was, therefore, designed to elucidate the question as to whether differences in the rate of glucose uptake between exercising muscles with high and low initial glycogen levels are solely due to limitations in the capacity of intracellular glucose disposal, or also by limitations of membrane glucose transport.

METHODS

Animals

Male Wistar rats weighing 200–250 g were pre-conditioned in order to obtain three different subgroups with varying muscle glycogen concentrations. Group I (control–rested rats) rested in their cages and had free access to regular rat chow until perfusion. Rats from group II (glycogen-depleted rats) were subjected to 2 h of swimming in water maintained at 32–35 °C, with weights (2·5 % of body weight) attached to their tails. In the 24 h preceding the swim their food intake was restricted to 9 g, and after the swim they were only fed lard until their hindquarters were perfused between 19 and 25 h later. Rats of group III (supercompensated rats) swam following the same protocol as the rats of group II; however, following the swim they received normal rat chow *ad libitum* together with a 10 % glucose drinking solution, until perfusion between 19 and 25 h later. Rats from the three groups were perfused in a randomized order. Rats that had undergone the designed procedures for glycogen depletion ($n = 15$) or supercompensation ($n = 16$), but nevertheless were found to have resting muscle glycogen levels within the normal range observed in control–rested rats (between 20 and 40 μmol (g wet wt)$^{-1}$) at perfusion, were excluded from groups II ($n = 4$) and III ($n = 5$). However, since it was believed that these 'non-responding' exercised rats might constitute an interesting subgroup for making comparisons with control–rested rats, they were assigned to a fourth 'post-hoc' subgroup that will be referred to as the control–exercised group throughout this paper.

Experimental procedures

Following the procedures of pre-conditioning, the rats were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (5 mg (100 g body wt)$^{-1}$) and prepared surgically for hindquarter perfusion in a perfusion cabinet, as previously described (Ruderman, Houghton & Hems, 1971; Richter, Ruderman, Gavras, Belur & Galbo, 1982; Goodman, Ruderman & Aoki, 1978). Before insertion of the perfusion catheters, the rat was heparinized with 500 i.u. of heparin in the inferior vena cava. The perfusate (200 ml) consisted of Krebs–Henseleit solution, 1- to 3-day-old washed bovine erythrocytes at a haematocrit of 30%, 5% bovine serum albumin (Cohn fraction V, Sigma, St Louis, MO, USA), glucose at 6 mM, 0·15 mM-pyruvate and 0·5–1·0 mM-lactate originating from the erythrocytes. No insulin was added. The perfusate was continuously gassed with a mixture of 98% oxygen–2% carbon dioxide, which yielded a pH value of 7·3–7·4 and a partial pressure of carbon dioxide and oxygen in arterial perfusate of typically 35–40 and 400 mmHg, respectively.

The first 25 ml of perfusate that passed through the hindquarter was discarded, whereupon the perfusate was recirculated at a flow of 12·5 ml min^{-1}. After a 20 min equilibration period, the left gastrocnemius–plantaris muscles were freeze-clamped *in situ*, with aluminium clamps cooled in liquid N_2, and resected. Subsequently, the common iliac vessels supplying the biopsied leg were tied off and a clamp was fixed tightly around the proximal part of the leg. Perfusion flow was reduced to 9 ml min^{-1}, which resulted in similar perfusion pressures (50–70 mmHg) as for bilateral perfusion at a flow of 12·5 ml min^{-1}. The hindquarter was allowed to rest for 5 min after which resting perfusate samples were collected. The right leg was then immobilized and a hook electrode was placed around the sciatic nerve and connected to a DISA stimulator (DISA electronic, Herlev, Denmark). The resting length of the gastrocnemius–soleus–plantaris muscle group was adjusted to obtain maximum active tension upon stimulation, whereupon the experimental period was begun. The muscles were made to contract isometrically by stimulating the sciatic nerve electrically with supramaximum (6–10 V) trains of 50 ms and 67 Hz, each impulse in the train lasting for 1 ms. The trains were delivered at a rate of 45 min^{-1} for 15 min. During stimulation, tension developed by the gastrocnemius–soleus–plantaris muscle group was recorded using a locally constructed isometric muscle tension transducer. At the onset of electrical stimulation, perfusate flow was increased to 20 ml min^{-1}, which resulted in a perfusion pressure of 100–130 mmHg. Before and after 1, 5, 10 and 15 min of electrical stimulation, perfusate samples were taken from the venous and arterial tubing for glucose determination. In a subsample of rats, perfusate samples for P_{O_2}, P_{CO_2}, pH and haemoglobin determinations were collected in separate syringes after 5 min of contraction. At the end of the 15 min stimulation period the gastrocnemius–plantaris muscles of the stimulated leg were freeze-clamped *in situ* without interrupting the electrical stimulation or the flow of perfusate. In separate, additional perfusions of glycogen-depleted and supercompensated rats only, the gastrocnemius–plantaris muscles of the stimulated leg were freeze-clamped *in situ* either after 1 or 5 min of electrical stimulation. All muscle samples were powdered under liquid nitrogen and stored at −80 °C until analysed.

In different subsamples of glycogen-depleted and supercompensated rats, muscle membrane glucose transport was measured, either during 5 min of muscle contraction, or during the last 5 min of a 15 min stimulation period. Ten microcuries of 3-*O*-[^{14}C]methyl-D-glucose (specific activity 300–360 mCi mmol^{-1}; New England Nuclear, Boston, MA, USA) were added to the perfusate reservoir. When the isotopes reached the hindquarter, the perfusion was switched to a flow-through perfusion to maintain a constant specific activity. At the end of the exposure period (5 min), gastrocnemius–plantaris muscles were freeze-clamped *in situ*.

In separate perfusions extracellular space before and during electrical stimulation was measured in glycogen-depleted and supercompensated rats. Twelve and a half microcuries of [^{3}H]inulin was added to the perfusate reservoir at the start of the equilibration period (20 min), at the end of which the left gastrocnemius–plantaris muscles were freeze-clamped *in situ* for determination of extracellular space in unstimulated muscles. The gastrocnemius–plantaris muscles of the right leg were then freeze-clamped *in situ* after either 5 or 15 min of electrical stimulation for determination of extracellular space in stimulated muscles.

Analyses, calculations and statistics

Perfusate glucose was measured with a YSI 23 glucose analyser (Yellow Springs Instruments, Yellow Springs, OH, USA). Perfusate haemoglobin was determined with a OSM2 hemoximeter (Radiometer, Copenhagen, Denmark) and the P_{O_2}, P_{CO_2} and pH of the perfusate were measured with an ABL 30 acid–base laboratory (Radiometer, Copenhagen, Denmark). Muscle glycogen was measured by a hexokinase method after acid hydrolysis (Karlsson, Diamant & Saltin, 1971). Muscle concentrations of glucose and glucose-6-phosphate were determined in neutralized perchloric acid extracts with standard enzymatic methods (Lowry & Passoneau, 1972). Concentrations of glucose and glucose-6-phosphate in intracellular muscle water were calculated as previously described, using extracellular spaces measured with [^{3}H]inulin and using a water content of 0·79 ml g^{-1} of perfused muscle (Richter, Ploug & Galbo, 1985). Extracellular space after 1 min of contractions was considered to be the average of the values observed in unstimulated muscles and in muscles after 5 min of contractions.

Uptake of 3-*O*-methylglucose in muscles was determined in perchloric acid extracts and corrected for label in the extracellular space determined by the ^{3}H counts for inulin. Radioactivity was measured in a Packard model 2000 CA liquid scintillation counter (Packard Instruments Company, Downers Grove, IL, USA). From the accumulation of labelled 3-*O*-methylglucose, rates of glucose transport were calculated using a 'specific activity of glucose' determined by the glucose concentration in cell-free perfusate and the 3-*O*-methylglucose counts. The duration of exposure to 3-*O*-methylglucose was short (5 min) to prevent accumulation of 3-*O*-methylglucose in intramuscular water of more than 30% of the extracellular water concentration. Preliminary experiments showed that uptake of 3-*O*-methylglucose is linear with time under these conditions.

Rates of glucose and oxygen uptake were calculated by multiplying arteriovenous differences (ranging between 1 and 7 mg dl^{-1} for glucose) by the flow rate, and were expressed per gram of perfused muscle. With the right common iliac vessels tied off, the perfused muscle mass of the hindquarter of normal rats weighing between 200 and 250 g has been found to be 8·3% of body weight (Richter *et al.* 1982). However, in glycogen-depleted rats, body weight was on average 13% lower than in the two other groups of rats. Experiments set up in order to elucidate the origin of this weight difference showed that the lower body weights in glycogen-depleted rats could be partially explained by differences in liver and intestinal weight, but not by differences in muscle weight. For this reason, perfused muscle mass in glycogen-depleted rats was calculated to be 9·6% of body weight instead of 8·3% as in the two other groups.

Statistical evaluation of the data was done by unpaired *t* test or 2-way analysis of variance, using Scheffe's test for post-hoc multiple comparisons, where appropriate. Data obtained in resting hindquarters were analysed separately from data obtaned in electrically stimulated hindquarters. Data are presented as means ± standard error of the mean (S.E.M.).

RESULTS

Muscle glucose uptake and transport

Glucose uptake at rest was 4·8 ± 0·5 μmol g^{-1} h^{-1} in control–rested hindquarters, higher in glycogen-depleted hindquarters (6·1 ± 0·7 μmol g^{-1} h^{-1}) and lower in supercompensated hindquarters (3·8 ± 0·4 μmol g^{-1} h^{-1}). Glucose uptake in hindquarters from control–exercised rats (5·6 ± 1·3 μmol g^{-1} h^{-1}) was not significantly different from basal glucose uptakes in the other groups of rats. Glucose uptake rate at rest was significantly negatively correlated with muscle glycogen concentration ($r = -0·43$; $Y = 7·59 - 0·08X$; $P < 0·01$). Exclusion of control–rested rats from this regression analysis improved the correlation ($r = -0·50$; $Y = 8·06 - 0·09X$; $P < 0·01$). As shown in Fig. 1, contractions increased glucose uptake in hindquarters from all rats. However, compared with control–rested hindquarters, the contraction-induced increment in glucose uptake rate was larger in hindlimbs from glycogen-depleted and control–exercised rats, whereas it was smaller in supercompensated hindquarters. The final level of glucose uptake during contractions in glycogen-depleted hindquarters was about 2-fold higher than in supercompensated hind-

quarters, and about 50% higher than in control–rested hindquarters. In the latter, glucose uptake during electrical stimulation was about 30% lower than in control–exercised hindquarters. It should be noted that the contraction-induced increase in glucose uptake is not due to the increase in perfusate flow, since it has

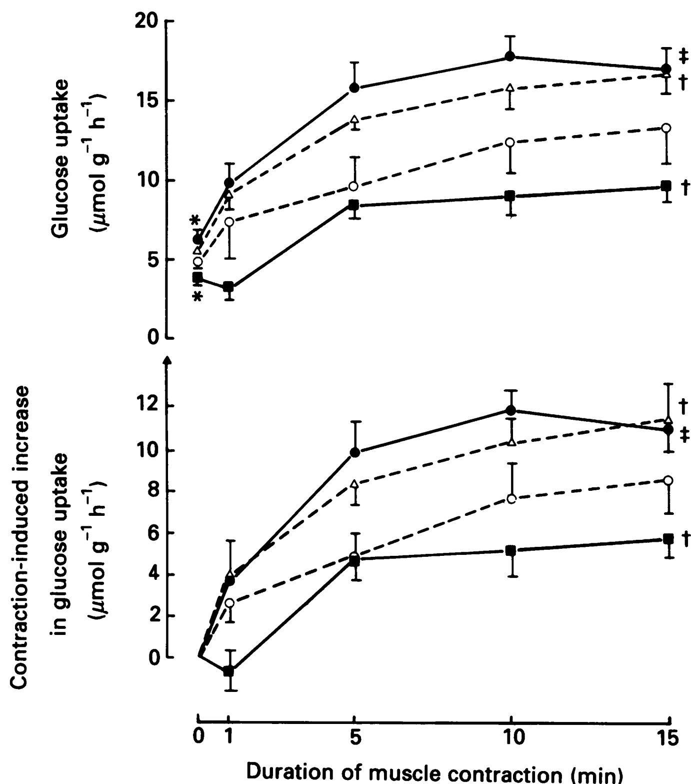

Fig. 1. Glucose uptake before and during contractions in hindquarters with different glycogen levels. Values are means ± S.E.M. of eleven observations, except in control–exercised rats where $n = 9$. Symbols represent control–rested (○), control–exercised (△), glycogen-depleted (●) and supercompensated (■) hindquarters. Contractions were induced by supramaximal (6–10 V) stimulation of the uncut sciatic nerve at a frequency of 45 trains (67 Hz) of 50 ms per minute. Flow was 9 ml/min at rest and 20 ml/min during contractions. Glucose uptakes were calculated from arteriovenous glucose concentration differences times the flow rate. See Methods for further details. * $P < 0.05$ compared with value before contractions in control–rested hindquarters, and † $P < 0.01$, ‡ $P < 0.001$ compared with values during contractions in control–rested hindquarters.

previously been shown that increasing the flow from 12·5 to 25 ml min^{-1} in resting hindquarters does not increase glucose uptake (Ploug, Galbo & Richter, 1984).

Figure 2 shows the relationship between muscle glycogen levels at the onset of contractions and glucose uptake during 15 min of contractions. In the total group of hindquarters perfused ($n = 42$), muscle glucose uptake during electrical stimulation correlated negatively with initial muscle glycogen concentration ($r = -0.67$; $P < 0.001$). However, when control–rested rats, the only group of rats that had not been exercising on the day preceding the perfusion experiments, were excluded from the regression analysis, the correlation improved to $r = -0.77$ ($P < 0.001$).

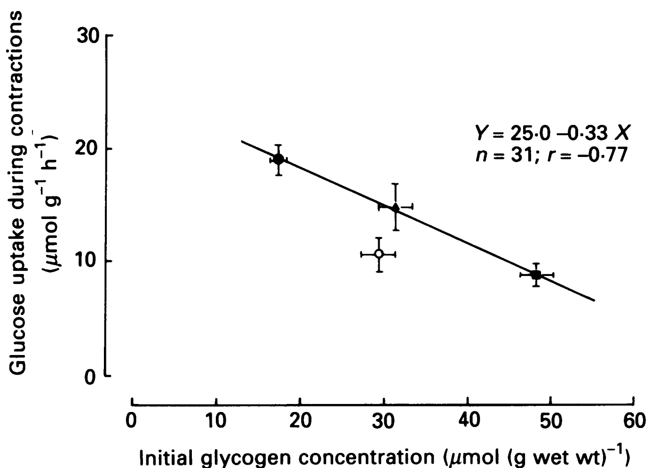

Fig. 2. Relationship between muscle glycogen level and glucose uptake during contractions. Data points represent means ± S.E.M. of eleven observations, except in control–exercised hindquarters where $n = 9$. Symbols represent control–rested (○), control–exercised (▲), glycogen-depleted (●) and supercompensated (■) rats. Regression line was calculated on glycogen-depleted, supercompensated and control–exercised hindquarters only; inclusion of control–rested rats in the regression analysis resulted in a lower correlation coefficient ($r = -0{\cdot}67$; $P < 0{\cdot}001$). See Methods for further details.

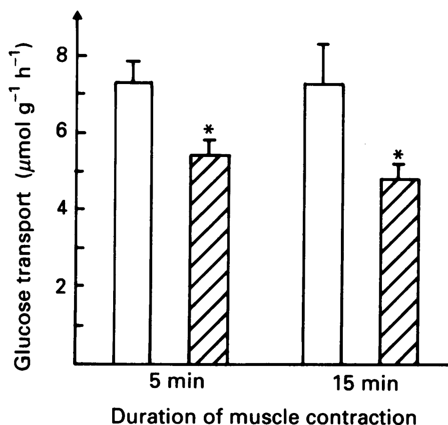

Fig. 3. Glucose transport into contracting muscles with low and high glycogen levels. Values are means ± S.E.M. of eight to nine observations. Open bars represent data from glycogen-depleted hindquarters; filled bars represent data from supercompensated hindquarters. See Methods for further details. * $P < 0{\cdot}05$ compared with corresponding value in glycogen-depleted hindquarters.

Muscle [^{14}C]3-*O*-methylglucose uptake, corrected for 3-*O*-methylglucose present in the extracellular space, was used as a measure of muscle membrane glucose transport in a subsample of glycogen-depleted and supercompensated rats. As shown in Fig. 3, muscle 3-*O*-methylglucose uptake was 25% lower in perfused muscles from supercompensated rats than in muscles from glycogen-depleted rats, both when measured at the onset or at the end of 15 min of electrical stimulation. Extracellular

TABLE 1. Muscle glycogen concentration in perfused hindquarters before and after contractions

Rat group	Before contractions	After contractions	Break-down
Glycogen depleted	17·3 ± 0·9**	6·36 ± 0·8*	10·9 ± 1·0**
Control–rested	29·5 ± 1·8	12·6 ± 1·9	16·8 ± 0·6
Control–exercised	31·2 ± 1·9	16·1 ± 2·5	15·0 ± 2·1
Supercompensated	48·6 ± 2·0**	24·6 ± 2·2**	24·0 ± 1·6**

Values are means ± S.E.M. of eleven observations, except in control–exercised rats where $n = 9$. Values are expressed in μmol (g wet wt)$^{-1}$. Muscle glycogen concentrations were measured in one leg before and in the other leg after 15 min of intermittent short tetanic contractions. See Methods for further details.

* $P < 0.01$ and ** $P < 0.001$ compared with corresponding value in control (either rested or exercised) hindquarters.

space was not different between the two groups of rats. In the total group of rats, extracellular space in resting hindquarters averaged 17·0 ± 2·5 % ($n = 10$), increasing to 23·3 ± 3·5 % ($n = 5$) and 28·4 ± 2·7 % ($n = 5$) after 5 and 15 min of electrical stimulation, respectively.

Muscle glycogen concentration

Compared with control–rested rats, muscle glycogen concentrations before contractions were on average 40 % lower in rats that had undergone procedures for glycogen depletion, whereas they were 65 % higher in supercompensated rats (Table 1). Pre-contraction glycogen levels were similar in control–rested rats and control–exercised rats. During electrical stimulation, muscle glycogen markedly decreased in all groups of rats, the decrease, however, being greater in muscles from rats with high initial glycogen levels (supercompensated) than in muscles from rats with low (glycogen-depleted) or normal glycogen levels. Over the 15 min of muscle contraction, muscle glycogen break-down was more than twice as high in supercompensated rats than in glycogen-depleted rats, the latter in turn breaking down approximately 35 % less glycogen than both control–rested or control–exercised rats. In the total group of rats ($n = 42$), muscle glycogen break-down over 15 min of electrical stimulation correlated significantly with the pre-contraction glycogen concentration ($r = 0.76$; $Y = 4.64 + 0.38X$; $P < 0.001$).

In glycogen-depleted hindquarters perfused during 1 or 5 min of electrical stimulation, mean initial muscle glycogen levels were 20·5 ± 1·3 and 21·6 ± 1·7 μmol (g wet wt)$^{-1}$ respectively, decreasing by 7·3 ± 1·2 and 10·2 ± 1·2 μmol (g wet wt)$^{-1}$ to 13·2 ± 0·7 and 11·4 ± 1·4 μmol (g wet wt)$^{-1}$ after contractions. Corresponding levels in supercompensated hindquarters were 52·1 ± 5·1 and 48·8 ± 2·4 μmol (g wet wt)$^{-1}$ before, decreasing by 10·2 ± 2·8 and 18·9 ± 2·4 μmol (g wet wt)$^{-1}$ to 39·2 ± 2·8 and 29·8 ± 2·9 μmol (g wet wt)$^{-1}$ after contractions (all values $P < 0.001$ compared with corresponding values in glycogen-depleted muscles).

Muscle free glucose and glucose-6-phosphate contractions

Intracellular free glucose and glucose-6-phosphate concentrations in perfused muscles from glycogen-depleted and supercompensated rats before and during 15 min of electrical stimulation are depicted in Fig. 4. Before contractions both

intracellular glucose and glucose-6-phosphate concentrations were slightly, but significantly, higher in muscles from supercompensated rats than in muscles from glycogen-depleted rats. During contractions muscle glucose-6-phosphate concentration was on average between 2- and 3-fold higher in supercompensated muscles

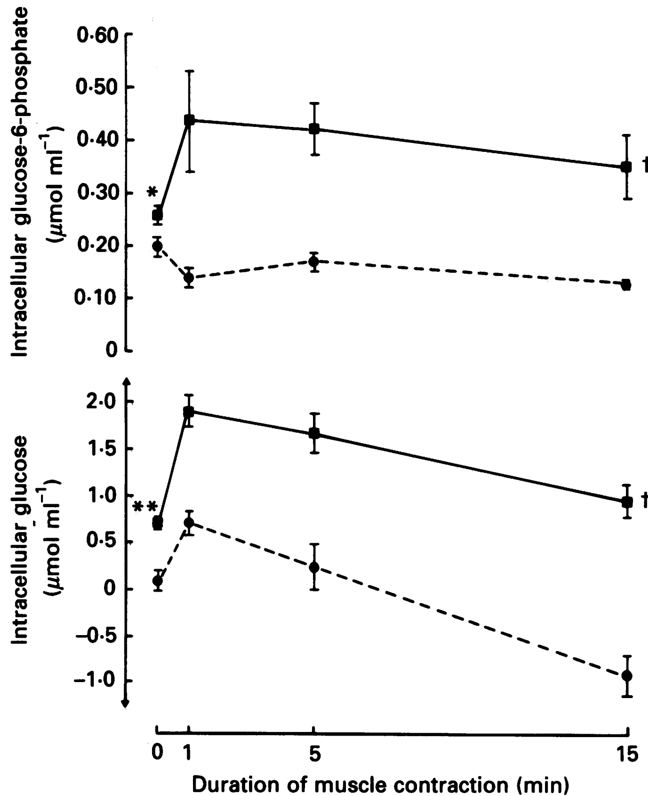

Fig. 4. Muscle glucose-6-phosphate and free glucose concentrations before and during contractions in muscles with high and low glycogen levels. Values are means ± S.E.M. of eleven observations, except for resting values where $n = 33$. Symbols represent glycogen-depleted (●) and supercompensated (■) muscles. Contractions were induced by supramaximal (6–10 V) stimulation of the uncut sciatic nerve at a frequency of 45 trains (67 Hz) of 50 ms per minute, for a period of 1, 5 or 15 min. Values were calculated as intracellular water concentrations using a total water concentration of 79% of wet weight, and extracellular space was determined with [^{3}H]inulin in separate perfusions. See Methods for further details. *$P < 0.05$ and **$P < 0.01$ compared with value before contractions, and †$P < 0.001$ compared with values during contractions in glycogen-depleted hindquarters.

than in glycogen-depleted muscles; this difference was mainly due to the fact that at the onset of electrical stimulation glucose-6-phosphate sharply increased in supercompensated muscles, whereas it remained unchanged in glycogen-depleted muscles. In line with the contraction-induced changes in muscle glucose-6-phosphate in both groups studied, free glucose concentration increased more at the onset of contractions in muscles from supercompensated rats than in muscles from glycogen-depleted rats, and remained higher in supercompensated muscles throughout the

15 min of electrical stimulation. After 15 min of contractions, calculated free intracellular glucose concentration was on average below zero (Fig. 4). This might be due to a slight overestimation of the extracellular space. However, since the size of the extracellular space was found to be similar in supercompensated and glycogen-depleted muscles, the difference in intracellular glucose between the two groups is still valid.

In the total group of rats ($n = 42$), both muscle intracellular glucose ($r = -0.52$; $Y = 14.6 - 3.07X$; $P < 0.01$) and glucose-6-phosphate ($r = -0.49$; $Y = 18.5 - 20.8X$; $P < 0.01$) concentrations, measured after 15 min of contractions, were negatively correlated with glucose uptake.

Muscle performance and oxygen uptake

Muscle tension at the onset and at the end of the 15 min of electrical stimulation were not significantly different between the four groups of rats, expressed either as absolute tension or as a percentage of initial tension. In the total group of rats ($n = 42$) initial muscle tension averaged 1040 ± 20 g, decreasing to 654 ± 19 g or 63 ± 1.4% of initial tension after 15 min of electrical stimulation. Oxygen uptake by the hindquarter was measured after 5 min of contractions in a subsample of glycogen-depleted ($n = 11$) and supercompensated ($n = 11$) rats. Oxygen uptakes averaged 28.8 ± 1.3 and 29.6 ± 1.8 μmol g^{-1} h^{-1} in resting hindquarters, and increased in both groups about 3-fold to 93.2 ± 3.9 and 98.3 ± 3.9 μmol g^{-1} h^{-1} during contractions in glycogen-depleted and supercompensated hindquarters, respectively.

DISCUSSION

The contraction-induced increase in muscle glucose uptake requires both an increase in membrane glucose transport as well as an increase in metabolism of the incoming glucose. Accordingly, the rate-limiting step in glucose utilization during exercise may be either transport or metabolism of glucose. In the present study, identical electrical stimulation of rat muscle perfused with perfusates of identical composition resulted in markedly different rates of glucose uptake depending on the pre-stimulation glycogen concentration (Fig. 1). In addition, the magnitude of glucose uptake was, for a given pre-stimulation glycogen concentration, larger if the rats had exercised the day before perfusion than if they had been rested (Fig. 1). These findings demonstrate that for a given degree of contractile activity the resulting uptake of glucose can vary by at least a factor of two, depending on factors within the contracting muscles. Interestingly, our results further suggest that this modulation of glucose uptake is exerted on the transport rate of glucose across the membrane (Fig. 3) as well as on the metabolism of glucose in the muscle (Fig. 2).

Glucose transport in perfused muscle is increased by contractile activity in the absence of insulin (Ploug *et al.* 1984; Wallberg-Henriksson & Holloszy, 1984), by a decrease in the K_m and an increase in V_{max} of the transport (Ploug, Galbo, Vinten, Jørgensen & Richter, 1987*a*). Studies on membrane vesicles isolated from rat muscle suggest that this increase in glucose transport is at least predominantly (Douen, Ramlal, Klip, Young, Cartee & Holloszy, 1988) if not entirely (Ploug, Vinten, Ohkuwa & Galbo, 1987*b*; Sternlicht, Barnard & Grimditch, 1989) due to an increase

in the intrinsic activity of glucose transporters in the plasma membrane. The rate of glucose transport across the muscle membrane was in the present study measured as the rate of entry of labelled 3-*O*-methylglucose in contracting muscle. Although such a measure does not allow conclusions as to the mechanism behind the different transport rates in muscles with high and low glycogen levels, they for the first time demonstrate that glucose transport in contracting muscle is influenced by the pre-stimulation muscle glycogen concentration. Future studies will have to elucidate whether muscle membrane glucose transport is affected directly by the glycogen concentration or by intracellular metabolites produced during glycogen break-down, and whether the molecular mechanism behind the contraction-induced activation of the glucose transporters is affected by the muscle glycogen concentration. A high concentration of extracellularly added glucose-6-phosphate has been shown to inhibit hexose transport in insulin-stimulated adipocytes and in adipocyte plasma membrane vesicles (Foley & Huecksteadt, 1984). The physiological significance and the mechanism behind this observation is, however, still unclear.

The difference in transport rate (approximately 25%) was, however, not sufficient to account for the difference in rate of glucose uptake (approximately 100%) between muscles with high and low pre-stimulation glycogen concentrations (Figs 1 and 3). Thus, metabolism of glucose must also have been affected. In accordance with this assumption, intracellular free glucose and glucose-6-phosphate concentrations correlated negatively with muscle glucose uptake rate. Free intracellular glucose and glucose-6-phosphate concentrations were, indeed, higher in contracting muscles with high, than in muscles with low, pre-contraction glycogen concentrations (Fig. 4). Similar findings have been reported during dynamic exercise in man (Gollnick *et al.* 1981). In fact, in supercompensated muscle glucose-6-phosphate and free intracellular glucose were increased above resting values during the entire 15 min contraction period, whereas for glycogen-depleted muscle glucose-6-phosphate did not increase and free intracellular glucose was significantly above zero only at 1 min of stimulation (Fig. 4). These findings then indicate that whereas glucose phosphorylation was rate limiting for glucose utilization at all times in supercompensated muscle, only after 1 min of contractions did accumulation of free intracellular glucose indicate phosphorylation as being rate limiting in glycogen-depleted muscle.

The basis for the marked difference in accumulation of glucose-6-phosphate and glucose in contracting muscles with high and low glycogen concentrations is probably the marked difference in rate of glycogen break-down during electrical stimulation. Thus, in accordance with our previous study in perfused rat muscle (Richter & Galbo, 1986) and with several (Bergström, Hermansen, Hultman & Saltin, 1967; Gollnick *et al.* 1972; Galbo, Holst & Christensen, 1979; Jansson, 1980) but not all studies in humans (Klausen & Sjøgaard, 1980; Sahlin, Broberg & Katz, 1989), glycogen break-down was closely positively related to the pre-contraction muscle glycogen level (Fig. 2; Table 1). A high rate of glycogen break-down will, on the one hand, produce glucose-6-phosphate at a high rate which may inhibit hexokinase resulting in intracellular accumulation of glucose, and on the other hand rapid glycogenolysis will also directly produce glucose at a high rate by virtue of the action of the debranching enzyme (Newsholme & Leech, 1983).

Glucose uptake and transport can be increased for many hours after cessation of muscle contractions (Young, Garthwaite, Bryan, Cartier & Holloszy, 1983; Cartee, Young, Sleeper, Zierath, Wallberg-Henriksson & Holloszy, 1989). Studies in perfused rat hindlimbs (Young *et al.* 1983) and in incubated epitrochlearis muscles (Cartee *et al.* 1989) have demonstrated that the rate at which this increase is reversed *in vivo* is to a large extent determined by the diet ingested during the post-exercise period. Thus, carbohydrate deprivation slows, whereas carbohydrate feeding speeds reversal of the increase. Accordingly in the present study, muscle glucose uptake at rest was approximately 30% higher in hindquarters from glycogen-depleted lard-fed rats than in non-exercised control rats, while in supercompensated rats, having free access to carbohydrates in the post-exercise period, it was 20% lower (Fig. 1). No free glucose was detectable intracellularly in muscles from glycogen-depleted rats (Fig. 4), indicating that in these muscles membrane glucose transport was rate limiting for glucose utilization, in line with the earlier observations (Young *et al.* 1983; Cartee *et al.* 1989). In muscles of supercompensated rats, on the other hand, intracellular free glucose and glucose-6-phosphate concentrations were significantly increased (Fig. 4), in turn indicating that in these supercompensated muscles glucose phosphorylation was rate limiting for glucose uptake. In man, kinetics of glucose uptake across the forearm suggest that transport is rate limiting when insulin is low (Yki-Järvinen, Young, Lamkin & Foley, 1987). Similar findings have been described in perfused muscle (Kubo & Foley, 1986). In neither of these studies was the muscle glycogen concentration measured but it was presumably in the normal range. In both studies the rate-limiting step switched from transport to disposal when glucose uptake was increased by insulin. Thus, there seems to be a very delicate balance between transport and disposal of glucose in resting muscle: when glucose supply or glycogen stores are low then transport is limiting while in conditions where glucose supply is more abundant or glycogen stores are plentiful then disposal seems to become rate limiting.

Whether muscle contractions on the preceding day have a sustained effect *per se* on glucose uptake in the same muscles during contractions on the following day is difficult to demonstrate due to the fact that exercise on a preceding day may change the size of pre-contraction muscle glycogen stores on the next day. From the present and previous studies (Gollnick *et al.* 1981; Richter & Galbo, 1986) glycogen is known to be a potent regulator of the rate of glucose uptake in contracting skeletal muscle. In an attempt to overcome this problem, we compared glucose uptake in hindquarters from rats that had normal muscle glycogen concentrations at the time their hindlimbs were perfused, but that had either exercised or not exercised on the day preceding the perfusion experiments. The contraction-induced increase in glucose uptake was greater in hindquarters from exercised rats than in hindquarters from non-exercised rats (Fig. 1). Accordingly, the rate of glucose uptake in hindlimbs from non-exercised rats was approximately 25% lower than one would have predicted based on the relationship between pre-contraction muscle glycogen concentration and glucose uptake during contractions in hindquarters from rats that had been exercised on the preceding day (Fig. 2). These observations thus demonstrate for the first time that contractions on the preceding day have a stimulating effect *per se* on glucose uptake in the same muscles during contractions on the following day. The

mechanism behind this effect remains to be elucidated, but since hindquarters were perfused with media of identical composition, it must be due to mechanisms exerted within the muscle cell, affecting either membrane glucose transport or the rate of glucose phosphorylation.

In conclusion, we have found that: (1) Glucose uptake in resting and contracting skeletal muscle is either directly or indirectly regulated by the magnitude of the pre-contraction muscle glycogen concentration. Regulating mechanisms include modulation of membrane glucose transport as well of glucose metabolism. (2) Exercise on the preceding day has a stimulating effect on glucose uptake during contractions of the same muscles on the following day.

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