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Glucose formation in human skeletal muscle

Influence of glycogen content

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Eight men exercised at 66% of their maximal isometric force to fatigue after prior decrease in the glycogen store in one leg (low-glycogen, LG). The exercise was repeated with the contralateral leg (control) at the same relative intensity and for the same duration. Muscle (quadriceps femoris) glycogen content decreased in the LG leg from 199 ± 17 (mean $\pm$ S.E.M.) to 163 ± 16 mmol of glucosyl units/kg dry wt. ($P < 0.05$), and in the control leg from 311 ± 23 to 270 ± 18 mmol/kg ($P < 0.05$). The decrease in glycogen corresponded to a similar accumulation of glycolytic intermediates. Muscle glucose increased in the LG leg during the contraction, from 1.8 ± 0.1 to 4.3 ± 0.6 mmol/kg dry wt. ($P < 0.01$), whereas no significant increase occurred in the control leg ($P > 0.05$). It is concluded that during exercise glucose is formed from glycogen through the debranching enzyme when muscle glycogen is decreased to values below about 200 mmol/kg dry wt.

INTRODUCTION

The glycogen molcule has a branched structure, and is composed of glucose units combined by glycoside linkages between C-1 and C-4 and, at the branch points, also between C-1 and C-6. The outermost chains of glycogen are composed of α-1,4-linked glucose, and represent 30–45% of the total number of glucosyl units (Cori, 1957). The degradation of glycogen is catalysed by glycogen phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1), which attacks the α-1,4 linkages and forms glucose 1-phosphate. The remaining core of glycogen, which is resistant to phosphorylase, is called a limit dextrin, and further breakdown of glycogen occurs through the action of the debranching enzyme [1,4-glucan 4-glucosyltransferase (EC 2.4.1.25) and amylo-1,6-glucosidase (EC 3.2.1.3.3)]. The debranching enzyme has been shown to be composed of one polypeptide chain and catalyses two different enzymic reactions (Taylor *et al.*, 1975). Lack of the debranching enzyme results in an accumulation of large amounts of structurally abnormal glycogen in the muscle, and leads to a disease called Cori–Forbes disease, which is characterized by diverse symptoms, including muscle weakness (Edwards & Jones, 1983).

Cleavage of the α-1,6 linkages results in formation of free glucose, which therefore is indicative of the activity of the debranching enzyme. Since about 30–45% of the glycogen molecule exists in the outer branches with α-1,4 linkages (Cori, 1957), it is conceivable that glycogen degradation in the initial stages occurs solely through the action of phosphorylase. Studies *in vitro* with the protein–glycogen particle isolated from rabbit muscle have shown that glycogen degradation at high glycogen concentrations is independent of the debranching enzyme, whereas at lower glycogen concentrations both phosphorylase and the debranching enzyme were active (Nelson *et al.*, 1972).

The aim of the present study was to investigate whether glucose is formed in the muscle during contraction (i.e. reflecting debranching-enzyme activity) and to elucidate the importance of glycogen concentration on this process *in vivo*.

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers participated in the study. Their mean (range) age, height and weight were 29 years (23–40), 183 cm (174–189), 80 kg (70–90). The subjects were informed of the possible risks involved in the experiments before giving their voluntary consent. The study was approved by the Ethical Committee of Huddinge Hospital.

Experimental design

Isometric maximal voluntary contraction (MVC) force of the knee extensor muscles at a knee joint of 90° was assessed before the experiments. The MVC of the leg subsequently used for glycogen depeletion [low-glycogen (LG) leg] and in the control leg was 635 ± 28 N and 605 ± 26 N respectively. The LG leg was chosen arbitrarily and was in four subjects the right leg.

Muscle glycogen content was decreased in one leg by repeated isometric contractions at 66% MVC (during 60 min) with 3 min rest period between each contraction. After 60–90 min rest, local anaesthesia (Citanest, 10 mg/ml) was applied in the skin and the subcutaneous tissue of the lateral aspect of the quadriceps femoris muscle in the LG leg, after which two incisions were made. One muscle sample was then taken by the needle-biopsy technique described by Bergström (1962). Subjects then contracted at 66% of the MVC. The subjects and investigators monitored the force level on a chart recorder. When the subjects demonstrated difficulty in maintaining the predetermined force output, a cuff, placed around the upper part of the thigh, was rapidly

Abbreviations used: MVC, maximal voluntary contraction; LG, low-glycogen.
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inflated (< 1 s) to 240 mmHg and the subject was encouraged to continue the contraction until fatigue, after which the post-exercise biopsy was taken and the cuff was deflated. The cuff was used to ensure that there would be no muscle blood flow between termination of contraction and excision of the post-exercise biopsy. After 30 min rest the procedure was repeated with the control leg. The contraction time with the control leg (35.8 ± 2.2 s) was kept identical with that for the LG leg (36.0 ± 2.3).

Analytical methods

Muscle biopsies were rapidly frozen (~ 2 s) in liquid Freon and maintained at its freezing point by liquid N_2. The muscle samples were freeze-dried, dissected free from any visible blood, fat and connective tissue, and powdered with forceps. The weight of the muscle samples was 5.8–10 mg dry wt. A portion of the muscle sample was extracted in 0.5 M-$HClO_4$ and subsequently neutralized with $KHCO_3$ (Harris *et al.*, 1974). Phosphocreatine, creatine, lactate, glycerol 3-phosphate and glycolytic intermediates were assayed by enzymic techniques as previously described (Harris *et al.*, 1974). Glycerol 3-phosphate was anaysed because previous studies have shown a considerable increase in this metabolite during contraction (Harris *et al.*, 1981). Glucose was analysed by an enzymic technique using hexokinase and glucose-6-phosphate dehydrogenase, as described by Harris *et al.* (1974). The methods were modified for fluorimetric detection of NADH. Muscle metabolites were adjusted to the peak value of total creatine (creatine + phosphocreatine) for each subject in order to correct for the admixture of non-muscle constituents. Total creatine was 114 ± 2, 120 ± 6, 114 ± 4 and 120 ± 6 mmol/kg dry wt. before contraction (LG), after contraction (LG), before contraction (control) and after contraction (control) respectively.

Another portion of the freeze-dried muscle powder (1–3 mg) was used for analysis of glycogen as previously described (Harris *et al.*, 1974). Briefly, the muscle was extracted with KOH (1.0 M), neutralized with HCl and, after enzymic hydrolysis of glycogen with amyloglucosidase and amylase, the released glucose was assayed enzymically.

Statistical methods

Statistical evaluation was performed with Student's paired *t* test, and values are reported as means ± S.E.M.

RESULTS

The initial endurance during contraction at 66 % MVC was 41.3 ± 2.4 s. After the contraction-induced decrease in the glycogen store in the LG leg and the subsequent 60–90 min period of rest, endurance decreased to 36 ± 2.3 s ($P < 0.05$ versus initial value). The contraction time decreased in all subjects except one, where it remained unchanged.

Muscle glycogen content decreased during contraction from 199 ± 17 to 163 ± 16 mmol/kg dry wt. in the LG leg ($P < 0.05$) and from 311 ± 23 to 270 ± 18 mmol/kg in the control leg ($P < 0.05$, Table 1). Both the pre-exercise and the post-exercise glycogen contents were lower in the LG leg than in the control leg ($P < 0.01$). Post-exercise muscle lactate content was higher in the LG leg than in the control leg ($P < 0.05$, Table 1); the reason for this is unclear. The breakdown of glycogen during contraction resulted in an increase in the glycolytic intermediates which was of a similar magnitude to the decrease in glycogen (Table 1). Contraction with the LG leg resulted in a 2–3-fold increase in muscle glucose ($P < 0.01$), whereas no change ($P > 0.05$) occurred in the control leg (Table 1).

Phosphocreatine content decreased during the contraction to about 25 % of the pre-exercise value ($P < 0.001$, Table 1), and there was no difference ($P > 0.05$) in the phosphocreatine content either before or after the contraction between the LG leg and the control leg.

Table 1. Muscle contents (mmol/kg dry wt.) of glycogen, glycolytic intermediates and phosphocreatine

Values are means ± S.E.M. for seven to eight subjects: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ rest versus contraction. Intracellular glucose was estimated from the muscle average glucose, an assumed extracellular glucose concentration of 5 mmol/l and an assumed extracellular water content of 0.33 l/kg dry wt. (Sahlin *et al.*, 1977). GI = glycolytic intermediates (mmol of glucosyl units/kg dry wt.) = glucose + glucose 1-phosphate + glucose 6-phosphate + fructose 6-phosphate + 0.5 (lactate + glycerol 3-phosphate).

	LG leg		Control leg		LG versus control†
	Rest	Contraction	Rest	Contraction	
Glycogen (glycosyl units)	199 ± 17	163 ± 16*	311 ± 23	270 ± 18*	$P < 0.01$
Glucose	1.80 ± 0.14	4.28 ± 0.58**	1.87 ± 0.32	2.13 ± 0.50	$P < 0.05$
Intracellular glucose	0.15	2.63	0.22	0.48	
Glucose 1-phosphate	0.06 ± 0.04	0.40 ± 0.08*	0.04 ± 0.03	0.70 ± 0.09***	n.s.
Glucose 6-phosphate	0.88 ± 0.16	8.52 ± 1.03***	1.36 ± 0.21	10.74 ± 1.87***	n.s.
Fructose 6-phosphate	0.08 ± 0.03	1.77 ± 0.38**	0.20 ± 0.06	2.25 ± 0.50**	n.s.
Glycerol 3-phosphate	0.50 ± 0.06	7.89 ± 0.58***	0.78 ± 0.07	7.44 ± 0.52***	n.s.
Lactate	1.4 ± 0.3	51.1 ± 6.0***	2.6 ± 0.5	45.0 ± 6.3***	$P < 0.05$
GI	3.8 ± 0.3	45.5 ± 4.1***	5.2 ± 0.7	42.7 ± 5.2***	n.s.
Phosphocreatine	87.2 ± 3.5	22.0 ± 4.8***	79.7 ± 2.3	20.5 ± 3.0***	n.s.

† Statistical comparison between post-contraction values; n.s., not significant.

DISCUSSION

Previous studies have demonstrated that there is no major accumulation of glucose in skeletal muscle during isometric contraction (Harris *et al.*, 1981; Katz & Lee, 1988). The unchanged muscle glucose in the contracting control leg, where muscle glycogen was normal, confirms this observation. However, the major finding in the present study was that, in the leg where the initial muscle glycogen was low, a significant accumulation of muscle glucose occurred during the contraction. Previous studies have shown that muscle blood flow is occluded during contraction by the increase in intramuscular pressure when the force exceeds 40% of MVC (Edwards *et al.*, 1972). Since in the present study the contraction force was 66% of MVC, and since any blood flow during the time between termination of contraction and excision of the biopsy (about 10 s) was abolished by the inflated cuff around the thigh (see the Materials and methods section), the muscle could be regarded as a closed compartment. The increase in muscle glucose is therefore not likely to be due to an uptake of glucose from the blood, but is conceivably derived from glycogen through the activity of the debranching enzyme. The present data indicate that a certain extent of the glycogenolysis is catalysed by the debranching enzyme when muscle glycogen decreases below about 200 mmol/kg dry wt. Studies *in vitro* of the glycogen–protein particle have shown that glycogen degradation is dependent on the debranching enzyme when the glycogen concentration is less than 1% (w/w) or 170 mmol/kg dry wt. (Nelson *et al.*, 1972). The present data obtained *in vivo* are thus consistent with these results obtained *in vitro*.

About 7% of the glycosidic linkages in the glycogen molecule are considered to be between C-1 and C-6 (Newsholme & Leech, 1983), and should thus, through the debranching enzyme, give rise to glucose formation. In the present study the ratio between increase in muscle glucose and increase in total glycolytic intermediates (a measure of glycogen decrease) was 0.07 ± 0.03 in the LG leg. The observed fraction of the glycogen breakdown accumulated as free glucose was therefore, in the LG leg, close to the theoretical fraction of α-1,6-glycosidic linkages in the glycogen molecule.

In contrast with the LG leg, muscle glucose did not accumulate in the control leg during contraction, indicating that the debranching enzyme was less active than in the LG leg. The explanation for this might be that a larger absolute amount of glucosyl units are located in the outer branches (i.e. as α-1,4 linkages) in the control leg, owing to the higher glycogen content. Given a certain breakdown of glycogen, it is therefore less likely that an α-1,6 linkage becomes available for the debranching enzyme in the control leg.

It is possible that some of the glucose formed during the initial period of the contraction was phosphorylated by hexokinase, whereas during the latter part of the contraction hexokinase was probably completely inhibited by the increase in glucose 6-phosphate (Colowick, 1973). The increase in glucose 6-phosphate was not significantly different between the legs, but tended to be higher in the control leg (Table 1), and therefore cannot explain the observed difference in glucose accumulation.

We have previously observed large increases in muscle glucose during intensive dynamic exercise when the blood flow is intact and the glycogen content presumably is at a normal value (Katz *et al.*, 1986; Sahlin *et al.*, 1987). The increase in muscle glucose in these studies was considered to be derived from the uptake of glucose from the blood (Katz *et al.*, 1986). The data from the present study, where muscle glucose was unchanged during contraction with normal glycogen contents, support this hypothesis.

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