Transient increase in glucose 1,6-bisphosphate in human skeletal muscle during isometric contraction

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Changes in glucose 1,6-bisphosphate and regulators of glucose-1,6-bisphosphate synthase and phosphatase during isometric contraction have been determined. Biopsies were obtained from the quadriceps femoris muscle before and after 20 s of contraction and at fatigue. Glucose 1,6-bisphosphate increased by 35 % after 20 s of contraction ($P < 0.001$) with no further change at fatigue ($P > 0.05$ versus 20 s). P_i , fructose 1,6-bisphosphate and glycerate 3-phosphate, all inhibitors of the synthase, increased significantly during the first 20 s ($P < 0.05$ – 0.001), whereas muscle pH (decrease in which inhibits synthase) decreased continuously. The decrease in the total adenine nucleotide pool, which is stoichiometric with the increase in IMP (an activator of phosphatase), was not significant after 20 s, but was 15 % at fatigue ($P < 0.001$). The rapid increase in glucose 1,6-bisphosphate, despite increases in the inhibitors of synthase, suggests that the synthase was activated, possibly by the substrate glycerate 1,3-bisphosphate and/or a yet unknown activator(s). The lack of any further change in glucose 1,6-bisphosphate during the latter part of contraction may be due to concomitant activation of the synthase and phosphatase.

INTRODUCTION

Glucose 1,6-bisphosphate has been considered to be an important regulator of carbohydrate metabolism (Rose *et al.*, 1977; Guha & Rose, 1982; Beitner, 1985). Although a number of reactions catalysing the production of glucose 1,6-bisphosphate have been suggested (see Eyer *et al.*, 1971), the one catalysed by glucose-1,6-bisphosphate synthase appears to be most specific (Rose *et al.*, 1975) and is likely to be of most significance in contracting muscle *in vivo* (Katz & Lee, 1988). The degradation of glucose 1,6-bisphosphate is apparently catalysed by glucose 1,6-bisphosphatase (Guha & Rose, 1982; Beitner, 1985).

It has been demonstrated that under various physiological and pathological conditions tissue ATP contents are related to glucose 1,6-bisphosphate contents (Beitner, 1985; Yip *et al.*, 1985). For example, anoxia in mouse brain and rat diaphragm, which are mainly dependent on aerobic processes for ATP production, results in rapid depletion of ATP and degradation of glucose 1,6-bisphosphate (Passonneau *et al.*, 1969; Beitner *et al.*, 1979), whereas short-term circulatory occlusion in human skeletal muscle does not affect ATP or glucose 1,6-bisphosphate contents (Katz, 1988). Moreover, when mice are anaesthetized (which decreases the metabolic rate) before decapitation, the rate of glucose 1,6-bisphosphate degradation after decapitation is diminished (Passonneau *et al.*, 1969). On the other hand, we recently observed a 30 % increase in glucose 1,6-bisphosphate while ATP decreased by 17 % in human skeletal muscle during isometric contraction to fatigue (Katz & Lee, 1988). However, since ATP depletion does not become apparent until ~ 25–30 s after initiation of contraction (Hultman & Sjöholm, 1983), it is possible that there is

a biphasic response in glucose 1,6-bisphosphate during contraction.

The purpose of the present study was to determine the glucose 1,6-bisphosphate content in skeletal muscle during the initial and latter phases of contraction. An additional aim was to examine the mechanism(s) for the changes in glucose 1,6-bisphosphate by measuring a number of key regulators of glucose 1,6-bisphosphate synthase and phosphatase.

MATERIALS AND METHODS

Subjects

Eight healthy men whose mean (range) age, weight and height were 27 years (21–35), 81.6 kg (61.4–104.5) and 178 cm (168–188) respectively participated in the study. The subjects were generally in good physical condition, but none was involved in competitive athletics. The subjects were informed of the possible risks of the study before giving their voluntary consents. The experimental protocol was approved by The Arizona State University Human Subjects Research Review Committee.

Experimental design

Isometric maximal voluntary contraction (MVC) force of the left knee extensor muscles was assessed before the experiment (on a separate day) by using a Cybex dynamometer (Lumex, New York, NY, U.S.A.), as previously described (Sahlin & Henriksson, 1984). On the day of the experiment, the subjects reported to the laboratory approx. 2–4 h after a light meal, and assumed the supine position for 10–15 min, after which incisions at biopsy sites (35 % of the distance from the superior

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margin of the patella to the anterior superior iliac spine) over the lateral aspects of both quadriceps femoris muscles were made (one in the right thigh and two in the contralateral thigh). A pre-exercise biopsy (Bergström, 1962) was then obtained from the right thigh.

The subjects were then seated in the Cybex unit and had a tourniquet placed loosely around the left thigh, which was to be the contracting one. The tourniquet was used to ensure that the blood flow to the muscle remained occluded between the end of each contraction and the post-exercise biopsy, to prevent any loss of metabolites into the circulation and aerobic resynthesis of phosphocreatine (Katz & Lee, 1988). Subjects then performed an isometric contraction with the knee extensor muscles at $\frac{2}{3}$ MVC force. The tourniquet was inflated to 250 mmHg after 15 s of contraction, and after 20 s the second biopsy was taken. The subjects then resumed the contraction, at the same force as in the first contraction, to fatigue, with the tourniquet being deflated immediately after initiation of the second contraction. When the subjects demonstrated difficulty in maintaining the predetermined force, the tourniquet was rapidly inflated to 250 mmHg and the subjects were encouraged to continue exercise until fatigue. The third biopsy was taken at fatigue and the tourniquet was deflated. The time between the end of each contraction and freezing of the biopsy sample averaged 12.9 s (range = 9–20). The biopsies were rapidly plunged (< 2 s after excision) into liquid Freon, maintained at its freezing point (-150°C) with liquid N_2 .

The time between the end of the first contraction and the beginning of the second contraction averaged 22 s (range = 15–30). It should be noted that during this period under the present conditions the metabolites remain at their steady-state concentrations (Harris *et al.*, 1981).

Analytical methods and calculations

The biopsies (stored in liquid N_2 until analysis) were freeze-dried, dissected free of solid non-muscle constituents (blood, fat and connective tissue), and powdered. The powder was extracted with 0.5 M- HClO_4 , and the extract was neutralized with KHCO_3 . Metabolites were determined with standard fluorimetric enzymic analyses based on the changes in NAD(P)H (Lowry & Passonneau, 1972; Bergmeyer, 1974). Glucose 1,6-bisphosphate was analysed with the phosphoglucumutase system as previously described (Passonneau *et al.*, 1969; Lowry & Passonneau, 1972). The coefficient of variation for duplicate analyses (on separate occasions) is 3.7% (Katz, 1988).

Muscle metabolites (except for glucose, pyruvate and lactate at rest, and glucose at fatigue, which are all partly of extracellular origin) were adjusted to a total creatine content (sum of phosphocreatine+creatine) of 118.4 mmol/kg dry wt., which is the mean value for the whole material. This adjustment was performed to correct for variability in blood, connective tissue or other solid non-muscle constituents between biopsies.

Statistics

For statistical evaluation, a one-way analysis of variance (ANOVA) with repeated measures was employed. When the ANOVA analysis yielded a significant F value ($P < 0.05$), the location of significance was identified with the Newman-Keuls test. Unless otherwise indicated, values are presented as means $\pm$ S.E.M.

RESULTS

Glucose 1,6-bisphosphate increased in all subjects, from a mean of 100 ± 12 $\mu\text{mol/kg}$ dry wt. at rest to 135 ± 17 at 20 s (Fig. 1). The value at fatigue (150 ± 12 $\mu\text{mol/kg}$ dry wt.) was not significantly different than that after the 20 s contraction. The rate of glucose 1,6-bisphosphate accumulation during the first 20 s of contraction was significantly greater than that during the latter part of contraction (101 ± 22 $\mu\text{mol/min}$ per kg dry wt. versus 18 ± 11 ; $P < 0.05$).

Changes in high-energy phosphates (Table 1) and glycolytic intermediates (except glycerate 3-phosphate, which to our knowledge has not been measured in human skeletal muscle during contraction) (Table 2) are

Fig. 1. Effect of isometric contraction on glucose 1,6-bisphosphate content in muscle

Lines link results for individuals. In one subject insufficient material was obtained in the biopsy after 20 s. The mean values after 20 s and fatigue are significantly higher than at rest ($P < 0.001$).

Table 1. Effect of isometric contraction on high-energy phosphates and creatine in muscle

Values are means ($\pm$ S.E.M.) for seven to eight observations. Contraction time to fatigue averaged 67 ± 4 s. Abbreviations: Cr, creatine; PCr, phosphocreatine; TAN, total adenine nucleotides; P (in footnote), phosphate. *** $P < 0.001$ versus Rest.

	Content (mmol/kg dry wt.)		
	Rest	At 20 s	Fatigue
PCr+Cr	119.1 ± 3.8	118.5 ± 3.7	117.6 ± 5.2
PCr	81.0 ± 1.7	$40.0 \pm 2.1^{***}$	$9.5 \pm 1.3^{***}$
Cr	37.4 ± 1.7	$78.2 \pm 2.2^{***}$	$108.9 \pm 1.3^{***}$
ATP	24.6 ± 0.9	23.6 ± 0.7	$20.2 \pm 0.8^{***}$
ADP	3.07 ± 0.07	3.06 ± 0.12	3.45 ± 0.22
AMP	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.02
TAN	27.8 ± 0.9	26.7 ± 0.8	$23.7 \pm 0.9^{***}$
$\Delta P_i^\dagger$	0	$34.5 \pm 2.5^{***}$	$52.8 \pm 2.6^{***}$

$^\dagger \Delta P_i = -2\Delta\text{ATP} - \Delta\text{ADP} - \Delta\text{PCr} - \Delta\text{hexose monophosphates} - 2\Delta\text{fructose 1,6-}P_2 - \Delta\text{glycerol 3-}P - \Delta\text{glycerate 3-}P$.

Table 2. Effect of isometric contraction on glycogenolytic intermediates in muscle

Values are means ($\pm$ S.E.M.) for seven to eight observations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus Rest.

	Content (mmol/kg dry wt.)		
	Rest	At 20 s	Fatigue
Glucose	1.72 $\pm$ 0.35	2.17 $\pm$ 0.15	3.70 $\pm$ 0.24***
Glucose 1-phosphate	0.13 $\pm$ 0.02	0.29 $\pm$ 0.07**	0.69 $\pm$ 0.07***
Glucose 6-phosphate	0.87 $\pm$ 0.10	5.58 $\pm$ 1.24***	15.00 $\pm$ 1.32***
Fructose 6-phosphate	0.15 $\pm$ 0.03	1.28 $\pm$ 0.36**	3.71 $\pm$ 0.40***
Fructose	0.14 $\pm$ 0.02	0.33 $\pm$ 0.05*	0.38 $\pm$ 0.02*
1,6-bisphosphate			
Glycerol 3-phosphate	0.31 $\pm$ 0.06	3.77 $\pm$ 0.30***	8.90 $\pm$ 0.27***
Glycerate 3-phosphate	0.17 $\pm$ 0.02	0.39 $\pm$ 0.05**	0.36 $\pm$ 0.04**
Pyruvate	0.13 $\pm$ 0.01	0.88 $\pm$ 0.20**	2.07 $\pm$ 0.27***
Lactate	2.1 $\pm$ 0.1	25.9 $\pm$ 2.8***	91.8 $\pm$ 3.5***
Lactate/pyruvate	15.6 $\pm$ 0.8	46.0 $\pm$ 11.1**	53.5 $\pm$ 7.8***

similar to those obtained previously under comparable conditions (Sahlin *et al.*, 1975; Chasiotis *et al.*, 1982; Katz & Lee, 1988).

DISCUSSION

A major finding of the present study is that glucose 1,6-bisphosphate accumulates rapidly in response to intense contraction, but after the initial increase there is no further significant change. These results are consistent with those observed in electrically stimulated rat hindlimb muscles (Bassols *et al.*, 1986).

Theoretically, the rapid increase in glucose 1,6-bisphosphate could be explained by either an activation of glucose 1,6-bisphosphate synthase or an inhibition of the phosphatase, or both processes occurring simultaneously with activation of the synthase predominating. The maximum activity *in vitro* (under optimal conditions and assuming a Q_{10} of 2 and correcting to 37 °C) of glucose 1,6-bisphosphate synthase in mouse skeletal muscle is $\sim 310 \mu\text{mol/min per kg dry wt.}$ (assuming total muscle water = 77%) (Guha & Rose, 1986), whereas that of the phosphatase in mouse skeletal muscle is estimated to be $\sim 20 \mu\text{mol/min per kg}$ (Beitner & Cohen, 1979). It appears that, assuming these enzyme activities are similar to those in human muscle, the rate of glucose 1,6-bisphosphate accumulation during the first 20 s of contraction is primarily attributed to activation of the synthase. This occurs despite the observation that P_i , fructose 1,6-bisphosphate and glycerate 3-phosphate (all inhibitors of the synthase; Rose *et al.*, 1977) increase at this time, whereas pH (low pH inhibits synthase) decreases (see below). Citrate, which is another inhibitor

glycerate 1,3-bisphosphate (Rose *et al.*, 1975). Based on the kinetics of brain synthase, the K_m of the enzyme for glucose 1-phosphate is equivalent to 0.27 mmol/kg dry wt. of muscle (Rose *et al.*, 1977), which is in the range of the present results. Thus increases in glucose 1-phosphate (and possibly glucose 6-phosphate) may at least partly account for the rapid increase in glucose 1,6-bisphosphate. However, we doubt that this is the most important mechanism, for the following reasons. First, glucose 1-phosphate (and glucose 6-phosphate) increase to relatively high concentrations during the latter phase of contraction, whereas glucose 1,6-bisphosphate does not change significantly. Second, we have demonstrated that during euglycaemic hyperinsulinaemia the glucose 1,6-bisphosphate content doubles in human skeletal muscle in the absence of any significant change in glucose 6-phosphate (Katz & Nyomba, 1988), which is in near-equilibrium with glucose 1-phosphate (Newsholme & Leech, 1983). Last, during circulatory occlusion in human muscle, glucose 6-phosphate increases significantly, whereas glucose 1,6-bisphosphate does not change (Katz, 1988).

Alternatively, it is possible that glycerate 1,3-bisphosphate increased in the present study. Accurate measurements of glycerate 1,3-bisphosphate are difficult to make, and we are not aware of any data on this metabolite in muscle during contraction. An estimate of a relative change of glycerate 1,3-bisphosphate can be obtained as follows. One must first assume that the phosphoglycerate kinase reaction is in near-equilibrium, which may or may not be correct (Newsholme & Start, 1973). Nevertheless, the glyceraldehyde-3-phosphate dehydrogenase-phosphoglycerate kinase system is considered to be close to equilibrium *in vivo* (Newsholme & Leech, 1983). Thus:

$$[\Sigma \text{Glycerate 1,3-bisphosphate}] = \frac{[\Sigma \text{glycerate 3-phosphate}] [\Sigma \text{ATP}]}{[\Sigma \text{ADP}] [\text{H}^+]} \times \frac{1}{K_{\text{eq}}} \quad (1)$$

of the synthase, does not change significantly during isometric contraction (Katz & Lee, 1988).

It is possible that the inhibition of the synthase was overcome by increases in the substrates for the enzyme: glucose 1-phosphate (or glucose 6-phosphate) and

where Σ = sum of all ionic species and K_{eq} is the equilibrium constant. The H^+ activity can be estimated from the relationship between lactate and pyruvate and pH in human skeletal muscle derived previously (Sahlin *et al.*, 1975). The pH values are estimated to be 7.05, 6.92 and 6.56 at rest, after 20 s of contraction and at fatigue,

respectively. The ADP content becomes more problematic, since a substantial part of it is apparently not available for enzymic reaction (Veech *et al.*, 1979). Moreover, we do not consider the estimation of free ADP content from the creatine kinase equilibrium and the total tissue contents of the reactants/products to reflect truly the free ADP content at the enzymic site during the contraction (for discussion see Katz *et al.*, 1986). A conservative estimate would be to use the phosphocreatine/creatine ratio in lieu of the free ADP content. Thus it can be calculated that glycerate 1,3-bisphosphate increased ~ 7 -fold during the first 20 s of contraction and ~ 14 -fold at fatigue. If this was indeed the case, then increases in glycerate 1,3-bisphosphate [despite an apparently low K_m , $\sim 0.1 \mu\text{M}$ (Rose *et al.*, 1977)] may at least partly account for the initial increase in glucose 1,6-bisphosphate. This explanation, however, would fail to account for the lack of continued increase in glucose 1,6-bisphosphate during the latter part of contraction, even though activation of the phosphatase may be occurring (see below). Clearly, direct measurements of glycerate 1,3-bisphosphate are needed.

Lastly, although no activator(s) of the synthase is known, the possibility of the existence of such an activator(s), be it allosteric or that it results in covalent modification, cannot be excluded.

During the second part of contraction glucose 1,6-bisphosphate remained constant, suggesting that, if the synthase were at least partly active, then the phosphatase must have been active. It should be noted that, although an IMP-dependent phosphatase has been found in brain (Guha & Rose, 1982), it has, to our knowledge, not yet been found in other tissues (Rose, 1986). Nevertheless, several groups have demonstrated the existence of a glucose-1,6-bisphosphatase in liver and skeletal muscle [see Rose (1986) and Beitner (1985) for references]. This enzyme is apparently activated by Ca^{2+} in a dose-dependent manner in the physiological range (Wakelam & Pette, 1983). Thus contraction itself may activate the phosphatase. Moreover, the decrease in total adenine nucleotides is stoichiometric with the increase in IMP during muscle contraction (Sahlin *et al.*, 1978). Thus, if an IMP-dependent phosphatase exists in skeletal muscle, it was conceivably active during the latter part of contraction, wherein IMP apparently accumulated. Hence a low synthase activity (relative to the activity during the initial phase of contraction) together with a near-maximal activation of the phosphatase may explain the lack of any measurable change of glucose 1,6-bisphosphate during the latter part of contraction. At any rate the present findings are not inconsistent with the relationship between ATP and glucose 1,6-bisphosphate (see the Introduction).

In conclusion, intense contraction results in a biphasic response in glucose 1,6-bisphosphate and changes in a number of regulators of glucose 1,6-bisphosphate synthase and phosphatase. Thus muscle contraction may be a useful model with which to study the regulation of glucose 1,6-bisphosphate.

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