Journal of Physiology (1993), **460**, *pp.* 443–453
With 3 figures
Printed in Great Britain

ENERGY METABOLISM IN SINGLE HUMAN MUSCLE FIBRES DURING INTERMITTENT CONTRACTION WITH OCCLUDED CIRCULATION

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(*Received 17 January 1992*)

SUMMARY

1. Glycogenolysis in type I and II muscle fibres was investigated in five healthy volunteers during electrical stimulation of the quadriceps muscle group with blood flow occluded.

2. The quadriceps femoris muscles were stimulated intermittently (1·6 s stimulation, 1·6 s rest) at a frequency of 50 Hz for 64 s and isometric contraction force was recorded. Muscle biopsies were obtained at rest prior to and immediately after stimulation. Single muscle fibres were dissected free and were identified as type I and II fibres. ATP, phosphocreatine (PCr) and glycogen contents were measured luminometrically and enzymatically in single fibres and mixed fibre muscle.

3. Electrical stimulation resulted in a marked decline in contraction force and near total depletion of PCr in both fibre types. The ATP turnover rate ($P < 0·05$) and the magnitude of the decline in ATP ($P < 0·05$) were greater in type II fibres. Prior to stimulation the muscle glycogen content was 32% higher in type II fibres compared with type I fibres ($P < 0·01$). During stimulation the rate of glycogenolysis in type II fibres ($4·32 \pm 0·54$ mmol (kg dry matter (DM))$^{-1}$ s^{-1} was twofold greater than the rate in type I fibres ($2·05 \pm 0·70$ mmol (kg DM)$^{-1}$ s^{-1}, $P < 0·05$).

4. The data suggest that the relatively higher rate of glycogenolysis observed in type I fibres during intermittent electrical stimulation with occluded circulation ($2·05 \pm 0·70$ mmol (kg DM)$^{-1}$ s^{-1}), when compared with the corresponding rate recorded during intense contraction with circulation intact ($0·18 \pm 0·14$ mmol (kg DM)$^{-1}$ s^{-1}, $P < 0·05$), may result from an accelerated ATP turnover rate in this fibre type increasing the cellular concentrations of free AMP and inosine 5′-monophosphate (IMP), which are known activators of glycogen phosphorylase.

5. The similarity in the rate of type II fibre glycogenolysis during contraction with circulatory occlusion ($4·32 \pm 0·54$ mmol (kg DM)$^{-1}$ s^{-1}), when compared with the corresponding rate recorded during non-occluded circulation ($3·54 \pm 0·53$ mmol

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(kg DM)$^{-1}$ s^{-1}, $P > 0{\cdot}05$), is in agreement with the suggestion that glycogenolysis in this fibre type is already occurring at a near-maximal rate with circulation intact.

INTRODUCTION

Classically the degree of phosphorylase *b* to *a* transformation, controlled principally by cyclic AMP and calcium activation of phosphorylase kinase, has been considered as the rate determinant of glycogenolysis during skeletal muscle contraction (Cori, 1945; Danforth, Helmreich & Cori, 1962). However, subsequent studies have demonstrated that factors other than the degree of phosphorylase conversion must be of importance to the regulation of glycogenolysis (Cori & Illingworth, 1956; Rennie, Fell, Ivy & Holloszy, 1982; Chasiotis, Sahlin & Hultman, 1983). Inorganic phosphate (P_i), arising from phosphocreatine (PCr) and ATP degradation during contraction, has been suggested to play a central regulatory role by acting as a substrate for glycogen phosphorylation (Chasiotis, 1983). More recently however, Ren & Hultman (1989, 1990) have demonstrated that the rate of skeletal muscle glycogenolysis can vary greatly despite a high intracellular P_i content and extensive *b* to *a* transformation. In agreement with earlier suggestions (Lowry, Schulz & Passoneau, 1964), the authors concluded that a concentration-induced increase in free AMP (fAMP) may be important to the regulation of phosphorylase *a* activity. We have recently demonstrated (Greenhaff, Ren, Söderlund & Hultman, 1991), that the rate of glycogenolysis in human skeletal muscle during short-term maximal contraction with circulation intact, is markedly greater in type II fibres ($3{\cdot}54 \pm 0{\cdot}53$ mmol (kg DM)$^{-1}$ s^{-1}) compared to type I fibres ($0{\cdot}18 \pm 0{\cdot}14$ mmol (kg DM)$^{-1}$ s^{-1}) dissected from the same muscle biopsy. The difference in the rate of glycogenolysis between the two fibre types could have been partly due to the higher phosphorylase activity of type II fibres (Harris, Essen & Hultman, 1976). However, the disparity may also have arisen because of differences in oxidative capacity and/or the rates of anaerobic ATP turnover between the fibre types. Circulatory occlusion is known to reduce skeletal muscle oxygen availability and increase the rate of anaerobic ATP turnover in mixed fibre skeletal muscle (Hultman & Sjöholm, 1983). The aim of the present experiment, therefore, was to investigate the effect of circulatory occlusion on glycogenolysis in type I and II muscle fibres during intense contraction.

METHODS

Subjects. Five healthy volunteers (three males, two females) took part in the present experiment. All subjects participated in some form of regular physical activity, but none was highly trained. Their physical characteristics (mean ± S.E.M.) were: age 31 ± 3 years, height 174 ± 5 cm, weight $77{\cdot}8 \pm 3{\cdot}6$ kg. Prior to commencing the study the experimental procedures were explained to all subjects. All were aware that they were free to withdraw from the study at any point and voluntary consent was obtained in writing from each individual. The study was part of a large project approved by the Ethical Committee of the Karolinska Institute, Sweden.

Experimental protocol. Each subject reported to the laboratory in a 'normal' fed state on the morning of the study. Each was asked to refrain from strenuous exercise for the day prior to reporting to the laboratory. The experiment was performed with the subject lying in a semi-supine position on a bed with both legs flexed over one end at an angle of 90 deg. The particular leg under investigation was chosen at random and was attached via an ankle strap to a strain gauge built into

the frame of the bed. The subject was then asked to perform three maximal voluntary contractions (MVC) to determine the maximal voluntary isometric force of the knee extensors. The isometric force produced was measured with a strain gauge (AB Bofors, Karlskoga, Sweden) and after amplification (direct current amp, Medelec AD6) was displayed on an oscilloscope and recorded on ultraviolet paper (Medelec, Woking, Surrey). The leg was then prepared for electrical stimulation as described previously (Hultman, Sjöholm, Jäderholm-Ek & Krynicki, 1983). Briefly, the muscles of the anterolateral portion of the thigh were stimulated to contract with square wave impulses of 0·5 ms duration, at a frequency of 50 Hz and at a voltage sufficient enough to elicit maximal contraction. Approximately 35 % of the musculature that extends the knee is activated in this way (Hultman *et al.* 1983). Stimulation was intermittent with twenty trains of 1·6 s stimulation being separated by rest periods of 1·6 s. The total contraction time was therefore 32 s. Thirty seconds prior to the initiation of stimulation, a cuff surrounding the proximal portion of the thigh was inflated (250 mmHg) to occlude limb blood flow and remained inflated until a post-stimulation muscle biopsy was obtained. Muscle biopsy samples were obtained (Bergström, 1962) from the vastus lateralis at rest prior to occlusion and immediately after stimulation. Isometric force production was measured throughout the stimulation period. Upon removal from the muscle, biopsy samples were immediately frozen by plunging the biopsy needle into liquid nitrogen. The time delay between the insertion of the biopsy needle and freezing of the sample ranged from 3 to 5 s. The samples were freeze dried and stored at −80 °C until analysed at a later date.

Analytical methods. After fat extraction with petroleum ether, each freeze-dried muscle sample was divided into two approximately equal parts. One part was then dissected free of all visible blood and connective tissue and was pulverized. The powdered muscle was used for the determination of selected muscle metabolites (Harris, Hultman & Nordesjö, 1974). With the use of low power microscopy (× 10 magnification) fragments of individual muscle fibres were dissected free from the second portion of muscle. The ends of each fragment were then cut off, stained for myofibrillar ATPase, and were classified as type I (slow twitch) or type II (fast twitch) fibres (Brooke & Kaiser, 1970; Essen, Jansson, Henriksson, Taylor & Saltin, 1975). Approximately fifty fibre fragments were dissected free from each biopsy sample and after the removal of each end for fibre characterization, the remainder was weighed on a quartz fibre fish-pole balance. The balance was calibrated to an accuracy of 0·01 μg prior to analysis by spectrophotometrically determining the weight of *p*-nitrophenol crystals previously weighed on the balance (Lowry & Passoneau, 1972). Following fibre characterization, eight to twelve fibre fragments of each type were pooled (total weight 16–25 μg). Glycogen was then extracted from type I and type II fibres by adding 20 μl KOH (1 mol l^{-1}), agitating vigorously and warming the samples at 50 °C for a period of 15 min. This procedure resulted in complete digestion of the fibres. Glycogen in a diluted aliquot of the extract was then neutralized and degraded by adding 40 μl HCl (0·25 mol l^{-1}), 40 μl acetate buffer (0·15 mol l^{-1}) and 4 μl amyloglucosidase (50 mg ml^{-1}, Sigma grade II rhizopus mould, 5·95 units mg^{-1}). An aliquot of the supernatant was then assayed for glucose using a fluorimeric modification of the method of Harris *et al.* (1974). Where possible, of the remaining fibre fragments, eight to twelve of each fibre type were used for the determination of ATP and PCr. Individual fibre fragments were extracted in 200 μl trichloroacetic acid (2·5 %) and neutralized with 20 μl $KHCO_3$ (2·2 mol l^{-1}). The determination of single fibre ATP and PCr contents was then performed using the luminometric method of Wibom, Söderlund, Lundin & Hultman (1991).

Calculations. The ATP production per glucosyl unit metabolized (ATP/GU) in mixed fibre muscle was estimated using the following formula:

$$\Delta(\text{mixed muscle lactate}) \times 1{\cdot}5/\Delta(\text{mixed muscle glycogen}),$$

where 1·5 = 1·5 mmol ATP produced from 1 mmol lactate. This value was then used to calculate the single fibre ATP turnover rate (mmol (kg DM)$^{-1}$ s^{-1}) using the following formula:

$$\{\text{ATP/GU}[\Delta(\text{single fibre glycogen})/32]\} + [\Delta(\text{single fibre PCr})/32] + [\Delta(\text{single fibre ATP}) \times 2/32],$$

where, 32 = contraction time (s) and 2 = 2 active phosphates produced per ATP utilized.

Statistical methods. Comparison of glycogen and metabolite contents between type I and type II fibres was achieved using Student's *t* test for paired data. Comparison of percentage force changes and glycogenolytic rates with previously published data from this laboratory (Greenhaff *et al.* 1991) was achieved using Student's *t* test for unpaired data. On all occasions statistical difference was declared at $P < 0{\cdot}05$. Where repeated comparisons were made, e.g. the change in force production

over time, repeated measures ANOVA was employed. When ANOVA resulted in a significant F value ($P < 0.05$), a *post hoc* test was used to locate the differences between means. Values in the text, tables and figures refer to means ± S.E.M.

RESULTS

Muscle contraction force

Muscle contraction force at the onset of electrical stimulation was equal to 177·6 ± 27·6 N. Force production declined progressively with successive contractions and at the end of the stimulation period was equal to 43 ± 7% of the initial contraction force ($P < 0.01$, Fig. 1). The initial decline in force production was similar to that observed previously during electrical stimulation with circulation intact (Fig. 1). However, during the final seven contractions, force production appeared to decline comparatively more rapidly during occlusion. This resulted in a significant difference between treatments nearly being achieved by the end of the stimulation period ($P < 0.15$, two-tailed t test).

Single fibre and mixed muscle ATP and PCr changes

At rest, prior to contraction, there was no difference in the high energy phosphate content of each fibre type (Table 1). Stimulation resulted in a marked decline in the ATP content and almost total depletion of the PCr stores in both fibre types. The magnitude of the decline in ATP was greater ($P < 0.05$) in type II fibres (9·8 ± 1·4 mmol (kg DM)$^{-1}$) when compared with type I fibres (4·7 ± 0·7 mmol (kg DM)$^{-1}$). The magnitude of PCr change was not different when comparing fibre types (83·0 ± 3·1 and 85·8 ± 5·0 mmol (kg DM)$^{-1}$ in type I and II fibres, respectively). There was good agreement between the average single fibre ATP and PCr content when compared with those measured in mixed fibre muscle (whole muscle extract, Table 1).

Mixed muscle metabolite changes

During electrical stimulation, the free glucose content of the mixed muscle sample increased approximately threefold ($P < 0.01$, Table 2). There was a major increase in the muscle hexose monophosphate (HMP) content (Table 2), close to 87% of which was accounted for by the rise in glucose 6-phosphate ($P < 0.001$). Mixed muscle glycogen stores decreased by approximately 90 mmol (kg DM)$^{-1}$ ($P < 0.01$) and muscle lactate content increased by approximately 116 mmol(kg DM)$^{-1}$ ($P < 0.001$).

Single fibre glycogen changes

The muscle glycogen content in type II fibres prior to stimulation (480 ± 24 mmol (kg DM)$^{-1}$) was greater than the corresponding value in type I fibres (364 ± 23 mmol (kg DM)$^{-1}$, $P < 0.01$). The pattern of glycogen degradation during electrical stimulation for each subject is shown in Fig. 2. Stimulation resulted in a high rate of glycogenolysis in type II fibres (4·32 ± 0·54 mmol (kg DM)$^{-1}$ s^{-1}), which was twofold higher ($P < 0.05$) than the rate found in type I fibres (2·05 ± 0·70 mmol (kg DM)$^{-1}$ s^{-1}, Fig. 3).

The mixed muscle glycogen loss of 86 ± 7 mmol (kg DM)$^{-1}$ agreed well with the average glycogen decrease in single fibres of 100 ± 18 mmol (kg DM)$^{-1}$. This assumes each biopsy sample is composed of equal proportions of type I and II fibres. If one

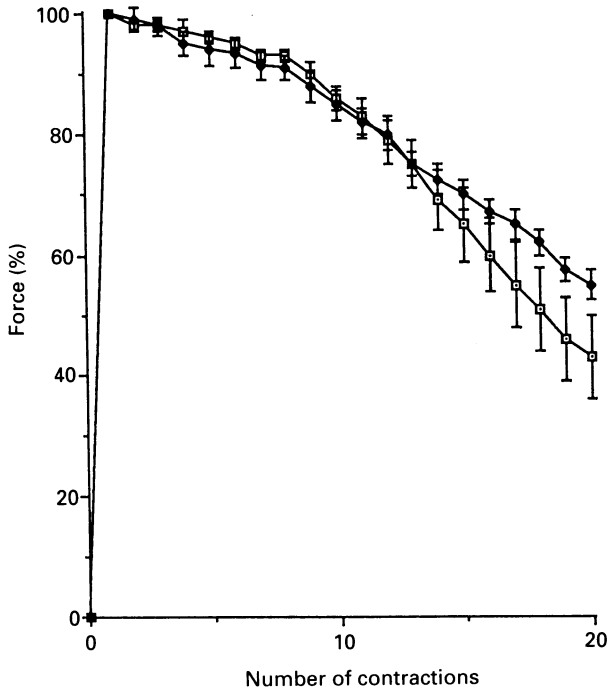

Fig. 1. The percentage change in maximal force production during twenty isometric contractions (1·6 s stimulation at 50 Hz, 1·6 s rest) with leg blood flow open (◆) and with leg blood flow occluded (⊡). Values represent means ± S.E.M. The changes in force production during contraction with blood flow open have been published previously (Greenhaff *et al.* 1991).

TABLE 1. ATP and PCr concentrations in type I and II muscle fibres and in mixed fibre muscle before and after electrical stimulation for twenty contractions (1·6 s stimulation and 1·6 s rest at a frequency of 50 Hz) with blood flow occluded

	Pre-exercise			Post-exercise		
	I	II	Mixed	I	II	Mixed
ATP	25·5 ± 0·8	25·7 ± 0·5	25·8 ± 0·7	20·7 ± 0·6	15·8 ± 1·2*	19·6 ± 1·2
PCr	85·4 ± 2·8	88·5 ± 4·9	83·8 ± 1·9	2·4 ± 0·4	2·7 ± 0·6	6·1 ± 0·4

Values represent means ± S.E.M. and are expressed as mmol (kg DM)$^{-1}$. * indicates type II value significantly different from corresponding type I value ($P < 0·05$).

TABLE 2. Muscle metabolite concentrations in mixed fibre muscle samples obtained before and after electrical stimulation for twenty contractions (1·6 s stimulation and 1·6 s rest at a frequency of 50 Hz) with blood flow occluded

	Pre-exercise	Post-exercise
Glucose	2·27 ± 0·37	6·20 ± 0·93*
Glycogen	401 ± 21	311 ± 32*
Glu 1-P	0·21 ± 0·06	0·91 ± 0·12*
Glu 6-P	1·63 ± 0·28	16·52 ± 1·65**
Fru 6-P	0·28 ± 0·06	3·60 ± 0·51*
Lactate	3·60 ± 0·31	120·00 ± 8·10**

Values represent means ± S.E.M. and are expressed as mmol (kg DM)$^{-1}$. Post-exercise value significantly different from pre-exercise value: * $P < 0·01$; ** $P < 0·001$.

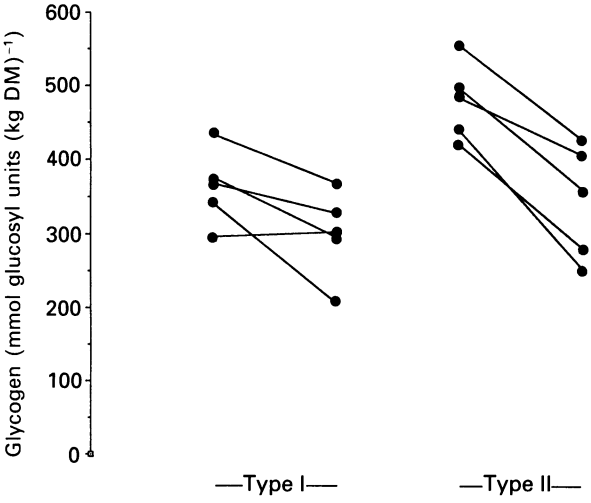

Fig. 2. Individual glycogen contents ($n = 5$) in type I and type II skeletal muscle fibres before and after electrical stimulation (20 × 1·6 s stimulation at 50 Hz interspersed with 1·6 s rest) with leg blood flow occluded.

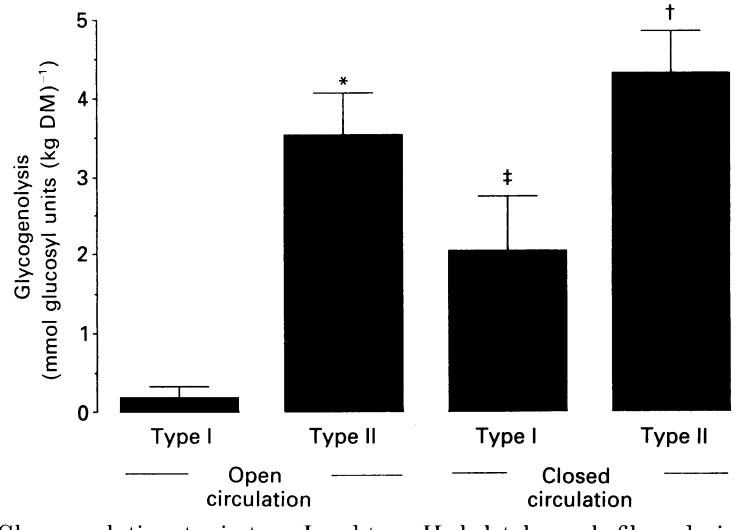

Fig. 3. Glycogenolytic rates in type I and type II skeletal muscle fibres during electrical stimulation (20 × 1·6 s stimulation at 50 Hz interspersed with 1·6 s rest) with leg blood flow open (open circulation) and leg blood flow occluded (closed circulation). Values represent means ± S.E.M. * indicates that the rate of type II fibre glycogenolysis with circulation open was significantly greater than the corresponding rate in type I fibres ($P < 0·01$). † indicates that the rate of type II fibre glycogenolysis with circulation closed was significantly greater than the corresponding rate in type I fibres ($P < 0·05$). ‡ indicates that the rate of glycogenolysis in type I fibres with circulation closed was significantly greater than the corresponding rate with circulation open ($P < 0·05$). Glycogenolytic rates during contraction with leg blood flow open have been published previously (Greenhaff *et al.* 1991).

assumes that each biopsy is composed of 60% type I and 40% type II fibres, the calculated average glycogen decrease would be 93 ± 19 mmol (kg DM)$^{-1}$.

Calculated single fibre ATP turnover rates

The calculated single fibre ATP turnover rates were $6{\cdot}1 \pm 1{\cdot}2$ and $12{\cdot}0 \pm 1{\cdot}0$ mmol (kg DM)$^{-1}$ s^{-1} in type I and II fibres, respectively ($P < 0{\cdot}05$).

DISCUSSION

We have recently demonstrated (Greenhaff *et al.* 1991) that during short-term electrical stimulation (50 Hz, 20 contractions, 1·6 s stimulation 1·6 s rest) with circulation intact, the rate of glycogenolysis in type II fibres ($3{\cdot}54 \pm 0{\cdot}53$ mmol (kg DM)$^{-1}$ s^{-1}) was in excess of the V_{max} (maximal velocity) of phosphorylase measured in whole muscle (1·9–3·4 mmol (kg DM)$^{-1}$ s^{-1}; Chasiotis, 1983), but was in good agreement with the V_{max} of phosphorylase measured in individual type II fibres (Harris *et al.* 1976). Conversely, the glycogenolytic rate in type I fibres, dissected from the same muscle biopsy sample, was close to zero ($0{\cdot}18 \pm 0{\cdot}14$ mmol (kg DM)$^{-1}$ s^{-1}, Fig. 3). The major finding of the present experiment is that when the same stimulation protocol is employed, with circulation occluded, the rate of glycogenolysis is markedly accelerated in type I fibres ($2{\cdot}05 \pm 0{\cdot}70$ mmol (kg DM)$^{-1}$ s^{-1}, $P < 0{\cdot}05$), but does not appreciably change in type II fibres ($4{\cdot}32 \pm 0{\cdot}54$ mmol (kg DM)$^{-1}$ s^{-1}, $P > 0{\cdot}05$; Fig. 3). In this situation, the rate of glycogenolysis is close to the V_{max} of phosphorylase measured in each fibre type (Harris *et al.* 1976), and suggests that both fibre types are maximally activated. This is supported by the finding that PCr was almost totally depleted in both fibre types.

The control of glycogenolysis during skeletal muscle contraction is a highly complex mechanism which can no longer be considered to centre around the degree of transformation of inactive phosphorylase *b* to the active *a* form (Cori, 1945; Danforth *et al.* 1962; Conlee, McLane, Rennie, Winder & Holloszy, 1979; Chasiotis, 1983). The finding that glycogenolysis can occur during short-term contraction (2 s) despite only a negligible rise in P_i (Hultman & Sjöholm, 1983), and, more recently, that glycogenolysis will proceed at a low rate despite a high phosphorylase *a* form and P_i content (Ren & Hultman, 1989), suggests that factors other than the degree of phosphorylase transformation and P_i availability are involved in the regulation of glycogenolysis.

Both AMP and inosine 5′-monophosphate (IMP) have been associated with the regulation of glycogenolysis during exercise, the former being the most potent activator (Cori, Colowick & Cori, 1938; Lowry *et al.* 1964). However, because 90% or more of the total cell content of AMP has been suggested to be bound to cell proteins *in vivo* (Sols & Marco, 1970), it has been questioned whether the increase in fAMP during contraction is of a sufficient magnitude to affect the kinetics of phosphorylase *a*. Recent work (Ren & Hultman, 1990), demonstrates that a small increase in AMP concentration (10 μmol l^{-1}) can markedly increase the *in vitro* activity of phosphorylase *a*. Furthermore, recent *in vivo* evidence (Ren & Hultman, 1990; Sahlin, Gorski & Edstrom, 1990), showing a close relationship between mixed fibre muscle ATP turnover rate and glycogenolysis and glycolysis, suggests an increase in fAMP is a primary stimulus to phosphorylase *a* activation.

It is known from animal studies that predominantly fast twitch muscle has a higher power output and ATP turnover rate in comparison to predominantly slow twitch muscle (Crow & Kushmerick, 1982; Brookes, Faulkner & McCubbrey, 1990). It has also been suggested that the power output (Faulkner, Claflin & McCully, 1986) and ATP turnover rate (Katz, Sahlin & Henriksson, 1986) of human fast twitch muscle fibres are greater than that of slow twitch muscle fibres. The higher ATP turnover rate found in type II fibres ($12{\cdot}0 \pm 1{\cdot}0$ mmol (kg DM)$^{-1}$ s^{-1}), compared with type I fibres ($6{\cdot}1 \pm 1{\cdot}2$ mmol (kg DM)$^{-1}$ s^{-1}, $P < 0{\cdot}05$), in the present study supports this suggestion. As a consequence of a higher ATP turnover rate, fast twitch muscle has a greater capability to generate AMP and IMP (Tullson & Terjung, 1990). Free AMP values close to 30 μmol (kg DM)$^{-1}$ ($\sim$ 10 μmol (l intracellular water)$^{-1}$) have been reported in red fast twitch rat skeletal muscle during ischaemic stimulation (Tullson & Terjung, 1990). While IMP values approaching 3–4 mmol (kg DM)$^{-1}$ (1·0–1·3 mmol (l intracellular water)$^{-1}$) have been reported during stimulation of rat fast twitch skeletal muscle with circulation intact (Aragon, Tornheim & Lowenstein, 1980; Dudley & Terjung, 1985); levels have been shown to increase further with occlusion (Tullson & Terjung, 1990). Based on data from *in vitro* studies (Aragon *et al.* 1980; Ren & Hultman, 1990), this degree of AMP and IMP accumulation would be of a sufficient magnitude to increase the activity of phosphorylase *a* and *b*, respectively. It is plausible to suggest, therefore, that our previously reported finding of a rapid rate of glycogenolysis in type II fibres ($3{\cdot}54 \pm 0{\cdot}53$ mmol (kg DM)$^{-1}$ s^{-1}) during intense contraction with circulation intact, and a negligible rate in type I fibres ($0{\cdot}18 \pm 0{\cdot}14$ mmol (kg DM)$^{-1}$ s^{-1}) dissected from the same muscle biopsy sample (Greenhaff *et al.* 1991; Fig. 3), may be attributable to a selective increase in fAMP and IMP in type II fibres activating phosphorylase. This suggestion is supported by the observed greater decline in the ATP content of rat (Hintz, Chi, Fell, Ivy, Kaiser, Lowry & Lowry, 1982) and human (Söderlund, Greenhaff & Hultman, 1992) type II fibres when compared with type I fibres during electrical stimulation with blood flow intact. The negligible rate of glycogenolysis in type I fibres in this situation (Fig. 3), can be explained by the majority of the ATP demand being met by PCr degradation and the oxidation of glycosyl units, thereby maintaining the fAMP and IMP contents of this fibre type close to zero. This is perhaps not surprising considering the intermittent nature of the stimulation protocol (1·6 s stimulation, 1·6 s rest) and the comparatively high oxidative capacity (Essen *et al.* 1975) and capillary density (Saltin, Henriksson, Nygaard, Jansson & Andersen, 1977) of this fibre type in human tissue. Similar results have been reported during electrical stimulation of slow twitch rat muscle (Meyer, Dudley & Terjung, 1980; Tullson, Whitlock & Terjung, 1990). However, in contrast, IMP accumulation has been observed in type I human muscle fibres during dynamic exercise with muscle blood flow intact (Jansson, Dudley, Norman & Tesch, 1987; Sahlin, Broberg & Ren, 1989), and to approach a level sufficient to activate phosphorylase *b* ($\sim$ 1 mmol (l intracellular water)$^{-1}$). This difference in results may be related to a relatively greater reduction in muscle oxygen availability during dynamic exercise compared with electrical stimulation.

During the present experiment, with circulation occluded, the rate of type I fibre glycogenolysis ($2{\cdot}05 \pm 0{\cdot}7$ mmol (kg DM)$^{-1}$ s^{-1}) was markedly greater than that with circulation intact ($0{\cdot}18 \pm 0{\cdot}14$ mmol (kg DM)$^{-1}$ s^{-1}, $P < 0{\cdot}05$). It is logical to suggest that the increase in glycogenolysis during occlusion was attributable to a reduced

oxygen availability increasing the sarcoplasmic concentration of fAMP in this fibre type. In agreement is the finding that the decline in type I fibre ATP content during the present experiment ($4{\cdot}7 \pm 0{\cdot}7$ mmol (kg DM)$^{-1}$) was greater than the corresponding value with circulation intact ($2{\cdot}6 \pm 0{\cdot}7$ mmol (kg DM)$^{-1}$, $P < 0{\cdot}05$, Greenhaff *et al.* 1991). Furthermore, as it is known there is a near stochiometry between skeletal muscle ATP loss and IMP accumulation (Sahlin & Katz, 1988; Tullson & Terjung 1990), it is plausible to suggest that an IMP activation of phosphorylase *b* (apparent K_m (Michaelis–Menten constant) of phosphorylase *b* for IMP $\sim 1{\cdot}2$ mmol (l intracellular water)$^{-1}$) was partly responsible for the increase in type I fibre glycogenolysis in the present experiment.

The similarity in the rate of glycogenolysis observed in type II fibres in the present experiment ($4{\cdot}32 \pm 0{\cdot}54$ mmol (kg DM)$^{-1}$ s^{-1}) when compared with the corresponding rate with circulation intact ($3{\cdot}54 \pm 0{\cdot}53$ mmol (kg DM)$^{-1}$ s^{-1}, $P > 0{\cdot}05$) is perhaps not surprising as it is known that these fibres are already working close to the V_{max} of phosphorylase when circulation is intact (Greenhaff *et al.* 1991).

We have previously suggested that the decline in whole muscle force production observed during intense electrical stimulation with circulation intact is a result of the disruption of energy metabolism solely in type II fibres, which appear to be close to being maximally activated during this type of exercise (Greenhaff *et al.* 1991; Söderlund *et al.* 1992). The metabolic response of type II fibres during circulatory occlusion appears to be similar to that with circulation intact, and it is therefore plausible to suggest that the apparently greater whole muscle force loss observed towards the end of contraction with circulation occluded (Fig. 1) may be principally the result of fatigue in type I fibres.

It would appear that the sophisticated regulation of glycogenolysis in contracting skeletal muscle is closely related to the energy demand of the muscle, or more specifically, that variation in the rates of glycogenolysis between muscle fibres during intense contraction is a direct function of the degree of single fibre ADP dephosphorylation and rephosphorylation (energy balance). In agreement with previously published data (Ren & Hultman, 1990), the results of the present study suggest that the degree of phosphorylase *b* to *a* conversion and P_i accumulation alone cannot explain the increase in glycolytic rate during exercise. The data point to fAMP and possibly IMP playing a central role in the regulation of single fibre phosphorylase activity during electrical stimulation. The relative contribution made by each will be dependent on the mole fraction of phosphorylase *a* in the contracting muscle. Earlier studies (Chasiotis, 1983) have demonstrated that during the initial 10–20 s of contraction the phosphorylase *a* fraction accounts for 70–80% of the total phosphorylase activity, and that as contraction continues there is a successive decrease in its availability. This suggests therefore, that during the preliminary stages of contraction fAMP will be the primary regulator of glycogen phosphorylation. As contraction continues, however, the parallel accumulation of IMP may be of a sufficient magnitude to activate the increasing fraction of phosphorylase *b*. Further studies involving the estimation of the mole fraction of phosphorylase and fAMP and IMP contents in single muscle fibres are warranted.

This work was supported by grants from the Swedish Medical Research Council. The authors would like to thank Kristina Bodin for her excellent technical assistance.

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