Muscle metabolism during fatiguing exercise

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Abstract In humans after short-term maximal exercise muscle glycogen levels are reduced to 75-80% of the initial muscle content. However, data obtained from maximally activated rat muscles show that after only a few seconds of exercise total glycogen depletion had occurred in many fast fibres. It is argued that during short maximal exercise, effects of glycogen depletion in some fibres may lead to the inability to sustain the required high level of exercise. During short maximal exercise and at the end of prolonged heavy exercise large amounts of IMP and ammonia are produced. In experiments with rat muscles it was found that the extent of loss of force (and power) at the end of all kinds of exercise, was related to the amount of IMP produced. It is suggested that the IMP produced may help to control the energy flux through the muscle cells by inhibiting contractile function and thus protect the muscle against an energy crisis. The high rate of production of IMP by muscles which are glycogen depleted indicate that IMP might be a linkage between glycogen depletion and fatigue.

Glycogen and endurance

In the late 1960's several reports by Scandinavian investigators showed that glycogen stored in skeletal muscles was a major determinant for endurance of long-term heavy exercise. First it was shown that performance time was linearly related to the amount of stored glycogen in the muscle before exercise (Ahlborg et al. 1967). In a subsequent paper it was demonstrated that work time was affected by manipulation of the amount of glycogen storage by different diets (Bergström et al. 1967). The conclusion from these papers, i.e. that glycogen was a limiting factor for performance of long-term heavy exercise, was universally accepted and used by endurance athletes to improve performance by different "carbohydrate loading" regimes.

In contrast to long-term exercise, relatively high muscle glycogen levels were found after fatiguing short-term high-intensity exercise (e.g. Boobis et al. 1983, Sahlin et al. 1975, 1976, 1989). It has further been demonstrated that human high-intensity exercise performance was not impaired by low muscle glycogen concentrations (varying from 153 to 426 μmol glucose units/g^{dw}; Symons & Jacobs, 1989). Also the rate of glycogenolysis during short-term tetanic stimulation of rat muscles was not affected by glycogen levels between 80 and 165 μmol/g^{dw}; Spriet et al. 1990). Based on these data glycogen depletion seems to play a non-significant role in fatigue during short-term high-intensity exercise. However, all these data were obtained from whole muscle samples. During high-intensity exercise it is necessary to recruit almost all the muscle fibres (e.g. Vøllestad et al., 1985). Reduction in muscle performance may occur by fatigue of all fibres, but it is more rational to expect a selective fatigue of some muscle fibres which have a low resistance against fatigue. Since almost all fibres are active, fatigue of only a few fibres will already result in a decrease of performance, with only little effect on whole muscle metabolism. Therefore it is necessary to investigate metabolic changes in different fibre populations of muscles.

In order to investigate changes in glycogen levels in different fibre types, histochemical techniques for fibre typing and substrate staining were used. The periodic acid-Shiff (PAS) stain is specific for glycogen (see e.g. Vøllestad et al. 1984) and is used for detection of glycogen depletion in fibre types and in individual muscle fibres. Using these techniques, it was shown that during long-term exercise the slow-oxidative fibres were depleted first, followed by the fast-oxidative and finally the fast-glycolytic fibres in man (Gollnick et al. 1973) and rat (Armstrong et al. 1974). In contrast, these reports showed that during high intensity exercise, fast-glycolytic fibres started to use glycogen immediately at the start of exercise.

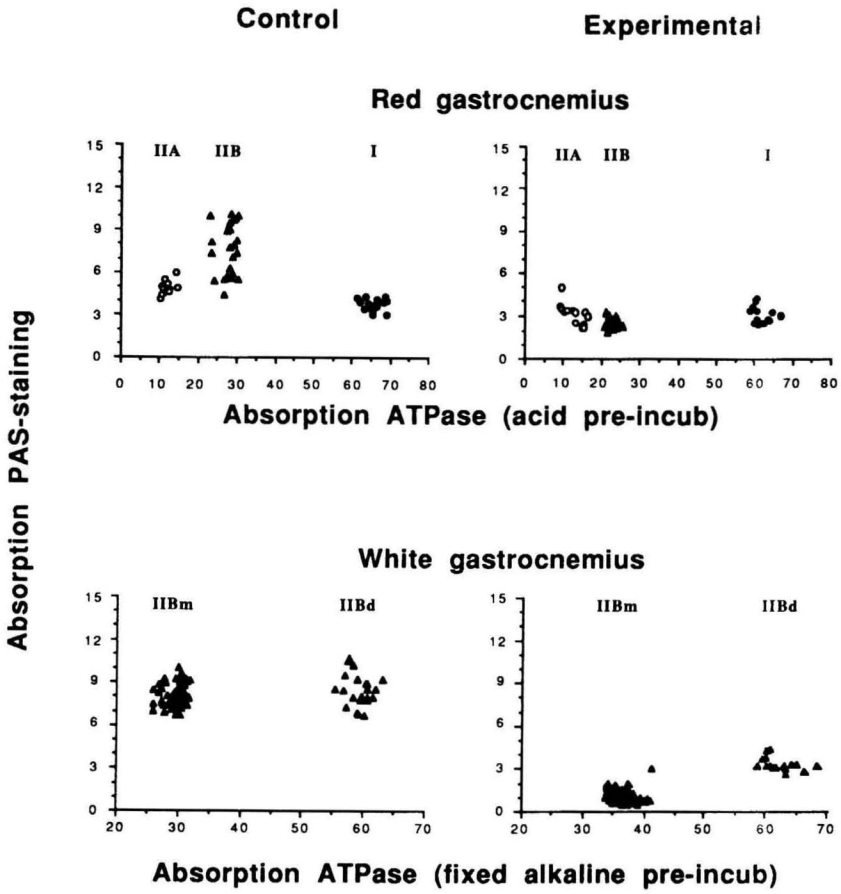

Figure 1. Example of changes in PAS-absorption in different fibre types in red and white rat medial gastrocnemius muscles.
The experimental muscle was maximally stimulated to perform 40 successive dynamic contractions within 10s (see group C in Fig. 3). The controls were resting contralateral muscles. The fibres were classified according to Lind & Kernell (1991). The absorption data are given in arbitrary units. These experiments were performed in collaboration with A.Lind and D.Kernell of the Department of Neurophysiology, University of Amsterdam, The Netherlands.

In a recent study we investigated glycogen degradation in fibres of in situ medial gastrocnemius muscles. These muscles of anaesthetized rats were maximally stimulated (1mA; 120Hz) at a temperature of 36°C using an isovelocity measuring device (de Haan et al. 1989). The muscles performed 40 dynamic contractions (duration 84ms) within 10s. During the 10s exercise (total active duration: 3.4s) work output per contraction decreased to <10% of the output in the first contraction (see group C in Fig. 3). At the end of the exercise the muscles were frozen in isopentane pre-cooled in liquid nitrogen. 10µm sections were cut from the midsection of the muscles and stained for fibre typing and PAS. Identification of fibre types I, IIA, IIBd and IIBm occurred as described by Lind & Kernell (1991) by a combination of ATPase stainings after an acid pre-incubation (Brooke & Kaiser, 1970) and after a fixed alkaline pre-incubation (Guth & Samaha, 1970).

Figure 1 presents examples of PAS absorption measurements of fibres in resting and exercised muscles. In the red portion of the gastrocnemius muscle all 4 fibre types are present, while in the white portion of the muscle only types IIBd and IIBm are seen (Fig. 1). Although all fibres were maximally stimulated, glycogen utilization (as judged by the change in PAS absorption) was most pronounced in the type IIBm fibres and hardly any changes in PAS absorption occurred in the type I fibres. These data show that in rats severe glycogen depletion of fast fibres can occur in short duration maximal exercise of only a few seconds. Differences exist between rats and humans with respect to total content of glycogen and possibly in maximal rates of glycogen degradation. Nevertheless, effects resulting from glycogen depletion may play also a role in fatigue during short-term high-intensity exercise in humans. This is supported by the observations of Gollnick et al. (1974) who showed that after the first exercise bout (3min at 120% VO_{2max}) some FT fibres were already glycogen depleted.

Glycogen depletion patterns and motor unit recruitment

The data in Fig. 1 show that the absence of a change in PAS-absorption in slow fibres (Type I) does not necessarily mean that these fibres were not active during exercise, because in this preparation all fibres were maximally activated. Thus although during high-intensity exercise in humans only little evidence of glycogen oxidation is seen in the slow-twitch fibres (Gollnick et al. 1973), one cannot conclude that those fibres had not been active during this type of exercise. Clearly care is needed in interpreting glycogen depletion patterns in terms of fibre activity (and fibre recruitment). The relation between glycogen depletion patterns and fibre activity may also be disturbed by the existence of differences between fibres with respect to the possibilities of utilizing other substrates (like free-fatty acids and amino acids) as well as glycogen for ATP generation. Moreover, some reports suggest that also non-exercising muscles in the rat may utilize glycogen at a similar rate as the exercising muscles (McDermott et al. 1987, Bonen 1989). In these studies comparison was made between the changes in glycogen levels of rat hindlimb muscles after treadmill exercise with and without hindlimb suspension. All types of muscle (white gastrocnemius as well as soleus muscle) showed similar glycogen degradations irrespective of their activity. They further showed that the loss of glycogen in the non-exercising muscles was dependent on the increase in the plasma epinephrine concentration (McDermott et al. 1987). This dependency on epinephrine is in agreement with earlier reports from Gorski et al. (1978) and Richter et al. (1981, 1982). They suggested that the first enhancement of glycogen utilization during exercise is induced by the onset of contractile activity, but that later in the exercise epinephrine is necessary to maintain an enhanced rate of degradation. The findings that muscles which had not been activated also showed glycogen degradation further demonstrates, that one should be careful with using glycogen depletion patterns to study motor-unit recruitment patterns during exercise.

Glycogen depletion and fatigue: why?

In spite of the enormous attention given to glycogen oxidation and fatigue, it remains unclear why low glycogen levels results in fatigue. The most popular theory on why glycogen is necessary for ATP production is the anaplerotic theory (Conlee 1987). According to this theory glycogen oxidation provides pyruvate, which can be used for reactions to supply intermediates of the tricarboxylic acid (TCA) cycle. Oxaloacetate can be formed by carboxylation of pyruvate (by pyruvate carboxykinase) and of phosphoenolpyruvate (PEP; by PEP carboxykinase). α-Ketoglutarate can be formed by the aminotransferase reaction, where pyruvate is converted to alanine at the expense of glutamate (by Glutamate-Pyruvate Transaminase). During short-term high-intensity exercise (Essén & Kayser 1978) and during the first minutes of prolonged exercise there is an increase in the amount of TCA cycle intermediates (TCAI; Sahlin et al. 1990). An increase in TCAI is needed to obtain a high flux through the cycle, which is necessary to feed the mitochondria with reduction equivalents (NADH) for the respiratory chain. During prolonged exercise a decrease of TCAI is observed (Sahlin et al. 1990).

It has been suggested that degradation of branched-chain amino acids (BCAA) is one of the major reactions which leads to draining of intermediates from the TCA cycle (Wagenmakers et al. 1991). Through activation of the branched-chain 2-oxo acid dehydrogenase complex, BCAA are converted to 2-oxo acids, while α-ketogluterate is aminated to glutamate. Glutamate

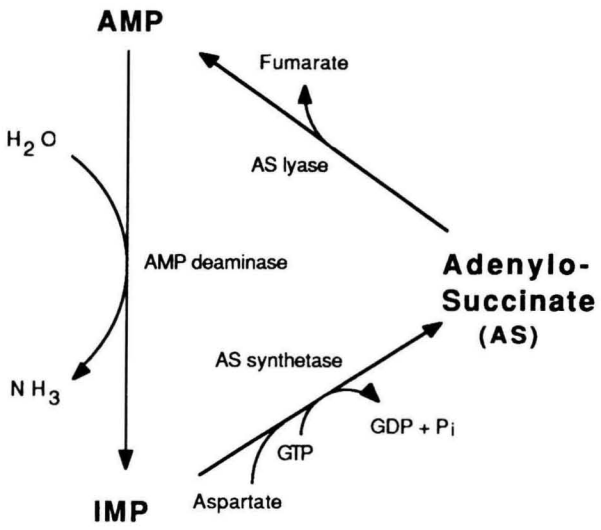

Figure 2. The Purine Nucleotide Cycle (Lowenstein 1972).

can then be used to form glutamine or alanine (by the Glutamate Pyruvate Transaminase reaction). The release of glutamine and alanine from the muscles during prolonged exercise (Sahlin et al. 1990) indicate that draining of TCAI occurs. Thus, in order to maintain a high flux through the TCA cycle it is necessary to remain supplying the TCA cycle with intermediates. It has been suggested that when muscle glycogen is depleted, the supply of pyruvate will be reduced leading to a limitation of anaplerotic reactions. Because of the resulting decrease in maximal rate of ATP production transient increases in ADP and AMP will occur, which will then lead to contractile failure (Sahlin et al. 1990).

Purine Nucleotide Cycle and fatigue

It was further argued that the occurrence of transient increases in ADP and AMP was supported by the observed increases in inosine-5'-monophosphate (IMP) and ammonia, because ADP and AMP are both activators of the enzyme AMP-deaminase (Sahlin et al. 1990). This reaction is part of the purine nucleotide cycle (PNC; Fig. 2; Lowenstein 1972), which cycle is active during exercise (Aragon & Lowenstein 1980). There are several functions suggested for the PNC (Tullson & Terjung 1989; Lowenstein 1972, 1990):

1. During one complete cycle one aspartate is deaminated to fumarate. Thus the cycle can provide TCAI from amino acid sources and thus support the replenishment of TCA cycle intermediates. Because of the relatively low activities of the enzymes for reamination of IMP to AMP this replenishment probably does not occur during short maximal exercise.

In the other suggested functions only the AMP deaminase reaction is involved.

2. One of the main functions is thought to be the control of relative concentrations of ATP, ADP and AMP. The maintenance of a relatively high ATP/ADP ratio (and phosphorylation potential) is important for many cellular reactions.
3. Produced NH_3 may take up H^+-ions and thus serve as a pH-buffer. NH_3 is also an activator of phosphofructokinase and hence glycolysis.
4. Produced IMP may stimulate glycogen degradation by activation of the enzyme phosphorylase b.

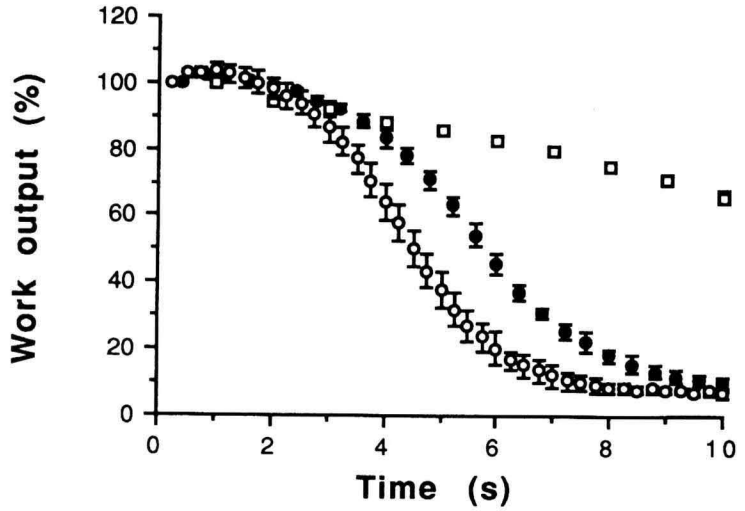

Figure 3. Changes in work output (mean ± SD) during exercise.
Rat medial gastrocnemius muscles were maximally stimulated to perform a series of contractions within 10s.
Group A (..): 10 contractions (duration 342ms shortening velocity (v) 80mm/s).
Group B (o): 25 contractions (duration 134ms: v=50mm/s).
Group C (o): 40 contractions (duration 84ms: v=80mm/s).
Total stimulation time was ~3.4s in all groups. Shortening distance was 6mm (~17% of the muscle belly length). (Data from de Haan, 1990).

All these suggested functions will help to preserve a high rate of ATP production in order to sustain the exercise. For living cells it is important to control the energy flux and thus to maintain a balance between ATP producing and ATP utilizing processes. Occurrence of an imbalance may result in loss of main cell functions and necrosis. Therefore muscle cells should not only control ATP producing reactions but should also control ATP utilizing processes (as contractile activity).

It has been suggested that the production of IMP may play a role in the control of contractile activity and thus serve as a protecting mechanism (Berden et al. 1986; Westra et al. 1986). It has been shown that the loss of force during one continuous or during series of repeated isometric or dynamic contractions co-incided with an increase in muscle IMP content (Westra et al. 1986; de Haan et al. 1988, 1991). In recent experiments different protocols of short-term high-intensity exercise of rat medial gastrocnemius muscles lead to different extents of loss of performance (Fig. 3). In groups A, B and C the muscles performed 10, 25 and 40 contractions within 10s with shortening velocities of 20, 50 and 80mm/s, respectively.

Total stimulation time during the 10s exercise was 3.4s in all protocols. Whereas ~90% of the initial work output was lost at the end of the exercise in groups B and C, the loss of work output was only ~35% in group A (Fig. 3). Muscle phosphocreatine and lactate concentrations at the end of exercise were similar for the 3 groups (Table 1). However, reductions in ATP and concommitant productions of IMP were higher for the 2 groups with the greatest loss of performance. The very high IMP concentrations after exercise in group C correspond with the large depletion of glycogen found at the end of this exercise (Fig. 1). Formation of IMP and NH_3 in glycogen depleted muscles and fibres have also been reported for human muscles (Norman et al. 1988; Spencer et al. 1991; Broberg & Sahlin 1989).

Table 2. Metabolite concentrations of resting muscles (Control) and of muscles sampled after the 10s exercise period of the 3 different groups. (Data from de Haan, 1990)

	Control (n=18)	Group A (n=6)	Group B (n=6)	Group C (n=6)
PC	100.6 (4.7)	37.6 (5.1)	31.2 (7.2)	33.8 (7.5)
ATP	29.7 (2.3)	20.1 (2.2)	11.0 (1.2)	11.6 (1.2)
ADP	4.3 (0.2)	5.0 (0.2)	4.0 (0.7)	4.3 (0.3)
IMP	<1	9.9 (2.1)	18.6 (1.2)	17.8 (1.7)
Lactate	8.4 (2.4)	54.6 (10.1)	56.7 (5.1)	57.2 (6.5)

Mean data (SD) are given in μmol/gdw

It can be hypothetized therefore that severe depletion of glycogen would result in an increased formation of IMP, which compound would inhibit contractile activity. This hypothesis is supported by recent in vitro experiments which showed that IMP inhibits actin-stimulated myosin ATPase activity (Westra et al. 1992).

In conclusion, it is suggested that effects of glycogen depletion may play a role in fatigue during short-duration high-intensity exercise. Further, it is suggested that depletion of glycogen may lead to increased IMP levels, which would inhibit contractile function and thus lead to fatigue.

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