 0014-4754/96/050396-08$1.50 + 0.20

Control of adenine nucleotide metabolism and glycolysis in vertebrate skeletal muscle during exercise

U. Krause and G. Wegener*

Institut für Zoologie, Johannes-Gutenberg-Universität, Saarstrasse 21, D-55099 Mainz (Germany), Fax + 49 6131 395112

Abstract. The turnover of adenosine triphosphate (ATP) in vertebrate skeletal muscle can increase more than a hundredfold during high-intensity exercise, while the content of ATP in muscle may remain virtually unchanged. This requires that the rates of ATP hydrolysis and ATP synthesis are exactly balanced despite large fluctuations in reaction rates. ATP is regenerated initially at the expense of phosphocreatine (PCr) and then mainly through glycolysis from muscle glycogen. The increased ATP turnover in contracting muscle will cause an increase in the contents of adenosine diphosphate (ADP), adenosine monophosphate (AMP) and inorganic phosphate (P_i), metabolites that are substrates and activators of regulatory enzymes such as glycogen phosphorylase and phosphofructokinase. An intracellular metabolic feedback mechanism is thus activated by muscle contraction. How muscle metabolism is integrated in the intact body under physiological conditions is not fully understood. Common frogs are suitable experimental animals for the study of this problem because they can readily be induced to change from rest to high-intensity exercise, in the form of swimming. The changes in metabolites and effectors in gastrocnemius muscle were followed during exercise, post-exercise recovery and repeated exercise. The results suggest that glycolytic flux in muscle is modulated by signals from outside the muscle and that fructose 2,6-bisphosphate is a key signal in this process.

Key words. Muscle metabolism; exercise; frog; phosphocreatine; fructose 2,6-bisphosphate; glycogen phosphorylase; phosphofructokinase.

Introduction

Motility, especially in the form of locomotion, is the most conspicuous physiological capacity of animals. Motion is brought about when muscles transform chemical into mechanical energy. Muscles have to function under very different conditions, from almost complete rest to very heavy exercise, and yet preserve their structural and metabolic integrity. This requires efficient regulatory mechanisms to maintain the metabolic homoeostasis of muscle cells despite large variations in metabolic rate. In this review we shall discuss some aspects of energy metabolism in white vertebrate muscle during exercise. White muscle is specialized for rapid and powerful contraction, which requires a high rate of ATP turnover. During heavy exercise ATP is regenerated almost exclusively from substrates that are stored in the muscle and can yield ATP anaerobically, i.e. PCr and glycogen. ATP, PCr and the regulation of the glycogenolysis and anaerobic glycolysis are hence pivotal for exercising white muscle.

Muscle work and ATP

Exercise requires a controlled alternation of muscular contraction and relaxation. Both processes are critically dependent on ATP and on the activity of ATPases such as myosin ATPase for powering contraction, and Ca^{2+}-ATPase and Na^+/K^+-ATPase for the control of contraction. Physiological activity of ATPases requires the ratio ATP/ADP in the cytosol to be maintained at a high level as can be seen from the observations that (a) ATPases can be inhibited by their reaction product ADP (K_i of myosin ATPase = 0.2 mM ADP); and (b) ATPases cease to function if the free energy available from the hydrolysis of ATP to ADP and inorganic phosphate (P_i) drops below a critical level[14].

In resting vertebrate muscle the content of ATP is typically around 5 μmol per gram wet muscle, whereas the content of ADP is much lower, only about 10% of the ATP content. Of physiological relevance, however, is not the total content of a substance but its concentration in the cytosol (the intracellular water space), because only free molecules can interact with enzymes. To estimate the free fraction of a metabolite is difficult if conventional biochemical methods are used. Nuclear magnetic resonance (NMR) spectroscopy of living tissue can contribute information because bound molecules remain invisible, i.e. they do not give rise to NMR signals[10]. Discrepancies between biochemical and NMR data can therefore be accounted for by the disintegration and extraction of tissues for biochemical analysis causing either solubilization of compounds or artefactual degradation of metabolites. Destructive biochemical methods and NMR spectroscopy in vivo yield similar results for some metabolites but strikingly differ-

* Corresponding author.

Table 1. Total contents of some metabolites (in μmol per g wet muscle, biochemical analysis) and their estimated free concentrations in the intracellular water space (cytosol) of frog skeletal muscle at rest and during high intensity exercise.
For metabolites that are fully soluble in the intracellular water space and are not degraded to a significant extent during tissue destruction, the concentration (in mM) has been calculated as content × 1.72 (assuming the intracellular water space to be 58% of wet weight). However, with the exception of ATP (which appears to be fully soluble in muscle and decreases by not more than a few percent during freeze-clamping), the free concentrations of the metabolites in this table cannot be calculated from the total contents directly because (1) freeze-clamping leads to a brief activation of muscle metabolism, and (2) tissue extraction will free metabolites from intracellular binding sites[12, 19] (for details, see text). As a further consequence of these artefacts the apparent changes in the contents of metabolites in muscle during exercise may appear small, while the changes in their concentrations can be much larger. For instance, the content of AMP in working muscle may not be significantly different from that in pre-exercise control muscle, while the free concentration of AMP in working muscle may be 27-fold that in resting muscle. The data in this table are representative of frog gastrocnemius muscle, pre-exercise control and after 10 s swimming, respectively[19].

	Pre-exercise muscle	Resting muscle	Working muscle	
	measured content (μmol/g)	calculated concentration	measured content (μmol/g)	calculated concentration
ATP	5.75	9.91 mM	5.67	9.78 mM
ADP	0.68	14.0 μM	0.73	73.3 μM
AMP	0.05	0.018 μM	0.06	0.49 μM
PCr	20.04	46.48 mM	12.24	25.24 mM
Creatine	11.68	8.21 mM	19.71	29.84 mM
P_i	15.17	2–4 mM	21.27	20.45 mM

ent results for others. ATP belongs to the first category and hence can be regarded as being in solution (i.e. mobile) in the cytosol. In contrast, no ADP and relatively little P_i can be detected in NMR spectra of resting live muscle. This is due to the fact that a major fraction of ADP in muscle is bound to proteins, particularly actin, whereas some phosphocreatine may be degraded and some P_i generated as a consequence of a brief activation of muscle ATPases and creatine kinase during freeze-clamping of muscle[24]. Some P_i seems also to be bound to cell structures in vivo[9, 39], but exactly how much is difficult to assess.

Estimates of total tissue metabolite contents and free concentrations of frog muscle are collated in table 1. Using these concentrations we can calculate the free energy, ΔG, available from the hydrolysis of ATP according to the formula

$$\Delta G = RT \ln \frac{[ADP][P_i]}{[ATP]} + \Delta G^\circ$$

where ΔG° refers to the standard conditions at a given pH (other concentrations are 1 mol · l^{-1}, the concentration of water is taken as unity). ΔG° and hence ΔG depend on temperature, pH and $[Mg^{2+}]$. We have assumed $[Mg^{2+}]_{free} = 10^{-3}$ M in frog muscle and used ΔG° values as determined by Rosing and Slater[33] for T = 25 °C (298 K) between 6.0 and 7.5 pH. It should, however, be noted that different values of ΔG°, which will result in different ΔG values, have been reported in the literature. At pH 7 and 37 °C, for instance, ΔG° was -28.4 kJ · mol^{-1} according to Rosing and Slater[33] but given as -36.8 kJ · mol^{-1} by Alberty[1]. The uncertainty is due to the fact that (a) ΔG depends on various factors in a complicated manner, and (b) the exact conditions under which ATP hydrolysis occurs in vivo are not known. This must be accounted for if ΔG values reported in the literature are to be compared.

The free energy, ΔG available from ATP hydrolysis is relatively high in resting muscle (about -60 kJ per mole of ATP) because of the low concentrations of free ADP and P_i, but is reduced to about -46 kJ per mole of ATP in fatigued muscle. During intense exercise the rate of ATP hydrolysis may increase several hundredfold. To maintain muscle work, ATP must be regenerated at virtually the same rate as it is split, because otherwise the decrease in ΔG and the massive increase in $[ADP]_{free}$ would halt muscle contraction. Thus muscle must be able to adjust the rate of ATP synthesis rapidly according to the work performed. In many animals, be they predators or those trying to avoid becoming prey, survival is critically dependent on this capacity.

Burst activity as required for 'fight or flight' can be sustained only briefly, because energy sources are rapidly exhausted, while lactate and H^+ ions are accumulated if ATP has to be regenerated anaerobically[50]. When ATP demand in working muscle cannot be met by ATP synthesis, a marked reduction of ATP will occur. Nearly half the ATP can thus rapidly be degraded (see fig. 1), via AMP to IMP (inosine monophosphate) plus NH_4^+, through a transient activation of AMP deaminase. This phenomenon has been studied for almost 70 years, but how the activity of AMP deaminase is regulated in working muscle is still not understood[19, 34].

Studies on isolated muscle and exercising animals

Much of what we know about muscle physiology and metabolism stems from work on isolated muscle, especially frog muscle, which has been the physiologists' favoured preparation for more than half a cen-

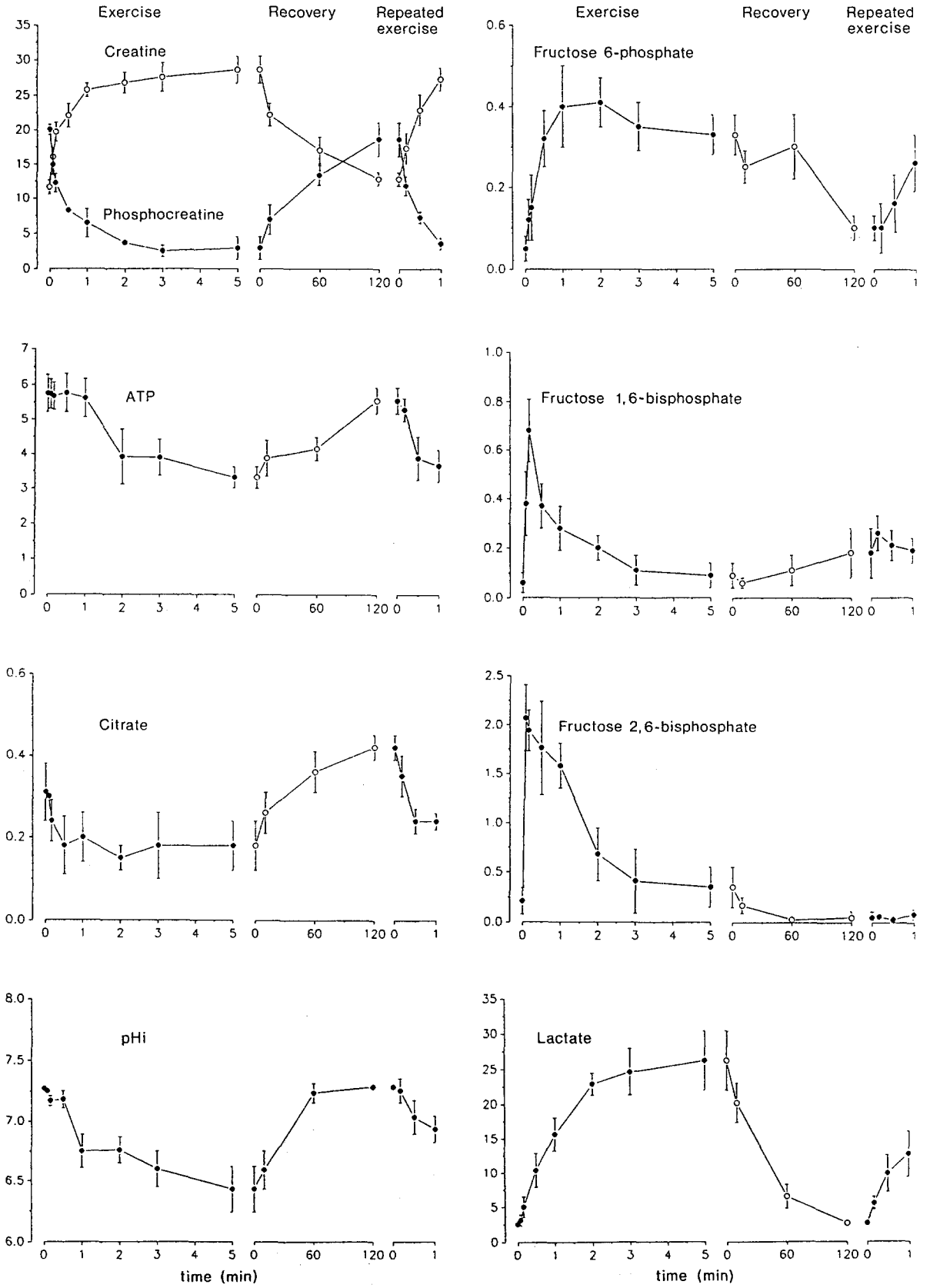

Figure 1. Effect of exercise, post-exercise recovery, and repeated exercise on the contents of metabolites and the intracellular pH in frog gastrocnemius muscle (μmol per g wet weight, nmol per g in case of $F2,6P_2$). The frogs were carefully rested before the exercise, which was swimming for up to 5 min (Exercise, intervals: 5, 10, 30 s and 1, 2, 3, 5 min). After 5 min swimming frogs were allowed to recover (Recovery, intervals: 10, 60 and 120 min). Some frogs that had recovered for 2 h were again induced to swim for up to 1 min (Repeated exercise, intervals: 10 s, 30 s and 60 s). For details see text and refs 17, 19, 20, 48.

tury[12, 22, 26]. Frog muscle can easily be prepared and remains viable and stable in vitro over a large range of temperatures (0 to 25 °C) under both aerobic and anaerobic conditions. The external parameters affecting muscle can be controlled, and the experimental conditions are thus well defined. But the advantages of using isolated muscle for experiments are limited by the fact that muscle is separated from the body and integrative aspects of muscle metabolism might be missed. Our goal has been to study muscle metabolism in intact animals under well-defined experimental conditions. This can, to a great extent, be achieved by choosing the frog as experimental animal and by analyzing metabolites in gastrocnemius muscle during short-term exercise (swimming)[19, 20, 48].

The gastrocnemius muscle of frog is a powerful muscle of the hindlimb, involved in swimming and jumping. The gastrocnemius is a white (pale) muscle consisting predominantly of fast twitch fibres with high glycolytic capacity (II b fibres). This type of muscle is specialized for burst activity; it is poorly capillarized and contains relatively few mitochondria. ATP regeneration during exercise must therefore be based on substrates that are stored in the muscle fibres themselves and can be used anaerobically, i.e., PCr and glycogen[50].

The exercise-related metabolism of PCr can be estimated because the products of PCr degradation are not lost from the muscle but reincorporated into PCr during post-exercise recovery (see fig. 1). Glycogen breakdown can easily be followed, because glycolytic intermediates are confined to the cytosol, and the end-product, lactate, is exported from amphibian muscle at a slow rate[6, 23]. The complete oxidation of glycogen to CO_2 and H_2O is quantitatively unimportant in frog gastrocnemius during exercise because of the poor oxygen provision. Thus, during short-term exercise frog gastrocnemius can be regarded as an almost closed metabolic system, while it is still fully integrated in the body with respect to neuronal and hormonal signals.

Phosphocreatine and adenine nucleotides in muscle during exercise and recovery

PCr represents a store of energy-rich phosphate, and its main function in white muscle is to buffer the ATP/ADP ratio during intervals of high ATP turnover. PCr can serve this function because its content in white muscle exceeds that of ATP and the catalytic capacity of creatine kinase is several fold higher than the maximal rate of ATP hydrolysis in working muscle[18, 28, 50]. As a result the system is maintained close to equilibrium according to

$$PCr^{2-} + ADP^{3-} + H^+ \leftrightarrows ATP^{4-} + \text{creatine};$$

$$\text{with } K = \frac{[ATP][\text{creatine}]}{[PCr][ADP][H^+]}.$$

The apparent equilibrium constant has been determined:

$K_{obs} = 233 \times 10^7\ M^{-1}$ (at 20 °C)[40].

The $[ADP]_{free}$ in muscle can hence be calculated from [PCr], [creatine] and [ATP] if the intracellular pH is known (see table 1). The $[ADP]_{free}$ thus calculated can be used to estimate the $[AMP]_{free}$ because ATP, ADP and AMP will be maintained in near-equilibrium in muscle by adenylate kinase:

$$ADP + ADP \leftrightarrows ATP + AMP; \qquad K = \frac{[ATP][AMP]}{[ADP]^2}$$

with $K_{obs} = 0.89$[46].

Some properties of the creatine kinase and adenylate kinase reactions deserve mentioning: (1) Regeneration of ATP from PCr consumes H^+ such that the pH of working muscle may slightly increase if PCr is the main substrate for the regeneration of ATP. (2) Degradation of PCr gives rise to P_i which functions as a substrate in glycogenolysis and glycolysis and is an activator of the glycolytic key enzyme phosphofructokinase (see next section). (3) Changes in the concentration of ADP will be accompanied by larger fractional changes in [AMP] because of the adenylate kinase reaction (see above). Thus, an exercise-induced fivefold increase in [ADP] corresponds to a 27-fold increase in [AMP] (see table 1). This amplification is important since AMP is a potent activator of glycogen phosphorylase and phosphofructokinase.

Changes in PCr and P_i have been followed in electrically stimulated, isolated frog muscle by means of ^{31}P NMR spectroscopy[11]. From these data, changes in AMP and ADP have been calculated for a 3 s tetanus. NMR spectroscopy has also been applied to isolated mammalian fast twitch muscle[24]. The main results are similar and indicate that (1) the content of ATP in muscle is kept virtually constant during brief intervals of muscle work; (2) the free concentrations of P_i, ADP and AMP in resting muscle are much lower than the total contents; and (3) the concentrations of ADP and AMP increase almost instantaneously at the initiation of muscle work while $[P_i]$ increases gradually as PCr is degraded.

Experiments in which we induced intact, well-rested frogs to perform exercise (swimming) and followed the changes in metabolites by biochemical methods gave lower PCr and higher P_i values in pre-exercise muscle than would have been expected from the NMR studies. This indicates that freeze-clamping caused an artefactual degradation of PCr which was obviously caused by a brief activation of muscle[24]. If this is taken into account, the data on PCr and adenosine nucleotides appear very similar, irrespective of the analytical method used and of how contraction was brought

about (by electrical stimulation of isolated muscle or by exercise in intact animals).

Regulation of glycolytic flux in muscle during exercise

Glycogen mobilization during exercise

Bursts of activity can be powered exclusively at the expense of muscle PCr, but glycolytic ATP production must be rapidly activated if exercise is to be sustained for more than a few seconds[18, 48, 50]. Because of the poor blood (and hence oxygen and glucose) supply white muscle can support high rates of ATP turnover only by anaerobic glycolysis from muscle glycogen. The rate of glycogen breakdown is controlled by the activity of glycogen phosphorylase. Glycogenolysis and muscle work are coordinated via an increase in cytosolic [Ca^{2+}] which triggers both muscle contraction and the phosphorylation of glycogen phosphorylase into an active form. Glycogen phosphorylase is a dimeric molecule made up of identical subunits which can be phosphorylated at a specific serine amino acid residue. The structural and catalytic properties of both forms, the phosphorylated *a* form and the unphosphorylated *b* form have been thoroughly studied in the rabbit muscle enzyme[37].

The *b* form is hardly active under physiological conditions, but can be fully activated in vitro by high AMP concentrations. The *a* form shows significant activity even in the absence of AMP, and is fully active at physiological concentrations of AMP.

In muscle of some vertebrates[35] and invertebrates[7, 43] these two forms of phosphorylase have been shown to coexist with a hybrid form *ab*, in which one subunit of phosphorylase is phosphorylated while the other is not. With respect to its kinetics the hybrid form is intermediate, but resembles more the fully phosphorylated *a* form than the *b* form. The insertion of a partly phosphorylated form is thought to provide the system with a greater flexibility and sensitivity towards metabolic effectors[21, 35].

Muscle work will increase the proportion of phosphorylated (active) phosphorylase and, at the same time, also the concentrations of its activator AMP and its substrate P_i (from degradation of PCr). Thus, initiation of exercise will cause the phosphorolysis of glycogen to glucose 1-phosphate which will be equilibrated with glucose 6-phosphate and fructose 6-phosphate (F6P). F6P is the substrate of phosphofructokinase (PFK), the key regulatory element of glycolysis. Unlike glycogen phosphorylase, PFK in vertebrate muscle is not regulated by reversible phosphorylation, and Ca^{2+} has no effect on the activity of PFK or any of the glycolytic enzymes in muscle. How muscle work and glycolytic flux are coordinated is still not fully understood, although this problem has been intensively studied for several decades[13, 48, 51].

Regulation of phosphofructokinase activity in muscle during exercise

PFK has long been recognized as a prototype of a multimodulated enzyme. Its activity is influenced by a multitude of effectors and very complex kinetics have been demonstrated in vitro[15, 36]. The basis of PFK regulation is the inhibition of its activity by physiological concentrations of ATP. ATP is not only a substrate of PFK but also a potent allosteric inhibitor. In addition to its active site, PFK contains a regulatory site for ATP[30]. Binding of ATP to this site will decrease the affinity of the enzyme for its second substrate F6P, thus shifting the $S_{0.5}$ value ($S_{0.5}$ = the concentration of substrate required to bring about half maximum activity) out of the physiological concentration range which is, in resting frog muscle, about 0.1 mM (0.06 $\mu mol \cdot g^{-1}$ wet muscle[18]). The inhibition by ATP is reinforced by citrate and this property is thought to make PFK an important element of control in fuel selection[29]. According to this hypothesis the availability of substrate other than carbohydrate will increase the concentration of citrate which in turn will reduce PFK activity and hence glycolytic flux. Thus, carbohydrate reserves could be spared in muscle, provided that ATP demand can be met by aerobic ATP production, as would be the case in white muscle at rest or, in more aerobic muscles, during moderate work load. PFK is also sensitive to H^+ so that its activity is decreased if muscle pH decreases due to the accumulation of lactic acid.

PFK in working muscle cannot be activated by a decrease in the concentration of its inhibitors ATP or citrate because the fractional changes in these metabolites are too small (fig. 1) to account for a more than hundredfold increase in PFK activity (see above section). Neither can the exercise-induced increase in the concentration of F6P account for the activation of PFK. The breakdown of glycogen in working muscle causes a marked increase in [F6P], but [F6P] and PFK activity (as indicated by changtes in [F1,6P_2]) follow different time courses (see fig. 1). PCr, the concentration of which decreases with exercise, is not an inhibitor of PFK, although this has repeatedly been claimed. Consequently, the relief of PFK from its inhibition by ATP and citrate requires potent activators, which can be grouped in two categories.

(1) Activators derived from 'high-energy' phosphates. These are produced as a direct consequence of the increased ATP turnover and the concomitant degradation of PCr in working muscle. This applies to AMP and P_i (see above), which can therefore be considered as parts of an intracellular feedback mechanism by which information on the rate of ATP hydrolysis is transferred to PFK (and other regulatory enzymes[11, 22, 49]). But it has repeatedly been questioned whether such feedback mechanisms are sufficient to bring about the rapid and massive increase in glycolytic flux in contracting muscle[48, 51].

(2) Hexosebisphosphates. Fructose 1,6-bisphosphate, glucose 1,6-bisphosphate and fructose 2,6-bisphosphate are activators of PFK in vitro, yet their physiological significance is highly controversial[2, 3, 38, 44]. There is evidence that the muscle content of $F2,6P_2$ and possibly of $G1,6P_2$ can be affected by hormones and neurotransmitters[5, 18]. Therefore, some of the controversy could be due to the fact that experimental intervention, such as animal handling, anaesthesia, and/or electrical stimulation, may have interfered with mechanisms controlling and integrating muscle metabolism.

Our studies on exercising frogs have indicated that the simultaneous increase in PFK activity and $[F1,6P_2]$ in muscle is of little regulatory significance but merely reflects the fact that $F1,6P_2$ is the product of the PFK reaction. This notion is corroborated by the observation that the activation by $F1,6P_2$ is almost completely eliminated by near-physiological concentrations of citrate[20, 41] (see fig. 2). However, $F2,6P_2$ appears to be an important modulator of PFK activity in muscle in vivo (see below). $G1,6P_2$ could be an activator of PFK in muscle during repeated exercise in muscle that has not fully recovered from previous exercise[20].

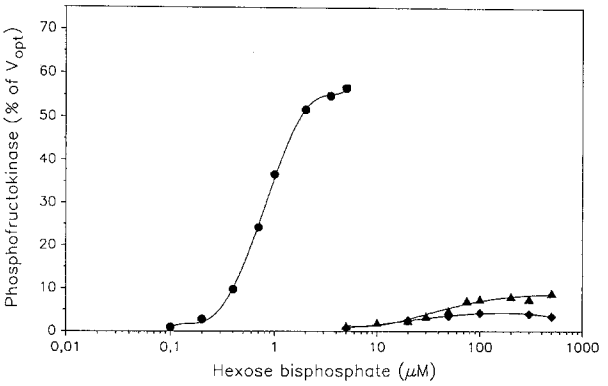

Figure 2. Hexosebisphosphates and the activity of 6-phosphofructokinase from frog skeletal muscle in vitro at pH 7.2 (see ref. 16). Fructose 1,6-bisphosphate (◆), glucose 1,6-bisphosphate (▲), and fructose 2,6-bisphosphate (●). The assays contained substrate concentrations (0.1 mM fructose 6-phosphate, 7.5 mM ATP) in the physiological range for resting muscle[17]. The same applies to the activator inorganic phosphate (2 mM NaP_i) and the inhibitor citrate (0.5 mM), whereas [AMP] in the assay was 10 µM, which is higher than the $[AMP]_{free}$ in the cytosol of resting muscle. V_{opt} refers to PFK activity under optimum conditions[20]. Under the given conditions, of the three hexose bisphosphates only fructose 2,6-bisphosphate has a significant activatory effect on PFK.

Fructose 2,6-bisphosphate and the regulation of PFK activity in vertebrate muscle

$F2,6P_2$ is a metabolic signal, not an intermediate of glycolysis or any other metabolic pathway. The substance was discovered in rat liver in 1980 by Van Schaftingen, Hue and Hers[45]. $F2,6P_2$ is a potent activator of phosphofructokinase and an inhibitor of the gluconeogenic enzyme fructose 1,6-bisphosphatase. $F2,6P_2$ is synthesized and degraded by the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2), which carries both enzyme activities on the same polypeptide. The liver enzyme can be phosphorylated by the cAMP-dependent protein kinase (for instance, as consequence of the action of glucagon) and will then act as a phosphatase specific for the degradation of $F2,6P_2$. The level of $F2,6P_2$ is decisive for directing glucose metabolism in the liver, favouring either glycolysis or gluconeogenesis (the latter at low levels of $F2,6P_2$). Hence the function of $F2,6P_2$ in liver is well established.

$F2,6P_2$ has been found in all animal tissues tested so far, but in many organs its function is not well understood. No agreement has yet been reached as to the physiological functions of $F2,6P_2$ in skeletal muscle. In rat gastrocnemius muscle electrically stimulated in situ, $F2,6P_2$ was twofold increased upon stimulation at low frequency, but during tetanus its content did not correlate with the rate of lactate accumulation[25]. In sharp contrast, brief exercise (a few seconds swimming) triggered a rapid increase in the content of $F2,6P_2$ in gastrocnemius muscle of frog (up to fortyfold from less than 0.1 to about 2 nmol · g^{-1}muscle)[48]. The difference between rat and frog is mainly due to the fact that, in unstimulated rat muscle, levels of $F2,6P_2$ were much higher than in muscle from non-exercised frogs, as long as the frogs had been carefully rested before the experiment. Recently, Pette and coworkers[8] have shown a very rapid, transient increase (fourfold within 1 s) in $F2,6P_2$ in contracting fast-twitch rabbit muscle.

The observations suggest that the $F2,6P_2$-system in skeletal muscle is affected by the physiological state of the animal, i.e. by signals from outside the muscle cell, such as hormones or neurotransmitters[18]. This notion is supported by experiments that have shown $F2,6P_2$ in muscle to respond very flexibly to exercise, depending on the amount of previous exercise and the degree of recovery. Frogs, fatigued by swimming for 5 min (*exercise*), were allowed to recover for 2 h, which is sufficient for most metabolites to return to their pre-exercise control levels (fig. 1). When the frogs were then induced to swim again for up to one minute (*repeated exercise*), a striking difference was seen with respect to $F2,6P_2$ between *exercise* and *repeated exercise*. The content of $F2,6P_2$, which had fallen to almost zero during recovery, remained at this level and did not increase upon *repeated exercise* (fig. 1 and ref. 20). Glycolytic flux was lower during *repeated exercise* than during *exercise* in rested frogs, and this was partially compensated for by an increased rate of PCr breakdown and an earlier degradation of ATP (see fig. 1 and ref. 19) to IMP. It required many hours of recovery before the $F2,6P_2$-system regained its reactivity to exercise (Krause and Wegener, unpublished results).

Obviously, different means for the activation of phosphofructokinase can be recruited in frog muscle depend-

ing on the physiological state and previous experience of the animals. The molecular mechanisms underlying this phenomenon are not yet known. PFK-2/FBPase-2 from frog muscle has recently been isolated and its properties studied[31]. The enzyme is more a kinase than a phosphatase (in contrast to the rat muscle enzyme), but from its kinetic properties no consistent hypothesis can be proposed to account for the different responses of $F2,6P_2$ to *exercise* and *repeated exercise*. The $F2,6P_2$-system in skeletal muscle appears to have regulatory properties yet to be discovered. Finally, there remains the question to be answered of whether or not similar effects of *exercise* and *repeated exercise*, as seen in muscle of frogs, are also present in skeletal muscle from other vertebrates.

Conclusions and perspectives

The regulation of energy metabolism, and particularly of glycolysis, in muscle is much more complex than had previously been envisaged. Studies of muscle by non-destructive methods such as ^{31}P NMR spectroscopy have contributed much to a better understanding of intracellular regulation based on energy-rich phosphates and related metabolites[32]. But NMR in vivo cannot detect compounds that are present at very low concentrations, such as metabolic signals (second messengers, transmitters, hormones). In this respect much progress has been made by using techniques of molecular biology on isolated tissue and cells. Both approaches, if combined and complemented by experiments on intact animals and/or humans exercising under physiological conditions, will further advance our understanding of how muscle metabolism is coordinated and integrated in the exercising body[4, 27, 42, 47].

Acknowledgement. Our work was supported by grants from the Deutsche Forschungsgemeinschaft, D-53175 Bonn, Germany.

1 Alberty, R. A., Effect of pH and metal ion concentration on the equilibrium hydrolysis of adenosine triphosphate to adenosine diphosphate. J. biol. Chem. *243* (1968) 1337–1343.
2 Bassols, A. M., Carreras, J., and Cusso, R., Changes in glucose 1,6-bisphosphate content in rat skeletal muscle during contraction. Birochem. J. *240* (1986) 747–751.
3 Beitner, R., Regulation of carbohydrate metabolism by glucose 1,6-bisphosphate in extrahepatic tissues; comparison with fructose 2,6-bisphosphate. Int. J. Biochem. *22* (1990) 553–557.
4 Blau, C., and Wegener, G., Metabolic integration in locust flight: the effect of octopamine on fructose 2,6-bisphosphate content of flight muscle in vivo. J. comp. Physiol. B *164* (1994) 11–15.
5 Bosca, L., Challiss, R. A. J., Newsholme, E. A., The effect of fructose 2,6-bisphosphate on muscle fructose-1,6-bisphosphatase activity. Biochim. biophys. Acta *828* (1985) 151–154.
6 Boutilier, R. G., Emilio, M. G., and Shelton, E., Aerobic and anaerobic correlates of mechanical work by gastrocnemius muscles of the aquatic amphibian *Xenopus laevis*. J. expl. Biol. *122* (1986) 223–235.
7 Burkhardt, G., and Wegener, G., Glycogen phosphorylase from flight muscle of the hawk moth, *Manduca sexta:* purification and properties of three interconvertible forms and the effect of flight on their interconversion. J. comp. Physiol. B *164* (1994) 261–271.
8 Cadefau, J. A., Parra, J., Cusso, R., Heine, G., and Pette, D., Responses of fatiguable and fatigue-resistant fibres of rabbit muscle to low-frequency stimulation. Pflügers Arch. *424* (1993) 529–537.
9 Challiss, R. A. J., Blackledge, M. J., Shoubridge, E. A., and Radda, G. K., A gated ^{31}P-n.m.r. study of bioenergetic recovery in rat skeletal muscle after tetanic contraction. Biochem. J. *259* (1989) 589–592.
10 Gadian, D. G., Nuclear magnetic resonance and its applications to living systems. Oxford University Press, Oxford 1982.
11 Gadian, D. G., Radda, G. K., Brown, T. R., Chance, E. M., Dawson, M. J., and Wilkie, D. R., The activity of creatine kinase in frog skeletal muscle studied by saturation-transfer nuclear magnetic resonance. Biochem. J. *194* (1981) 215–228.
12 Godt, R. E., and Maughan, D. W., On the composition of the cytosol of relaxed skeletal muscle of the frog. Am. J. Physiol. *254* (1988) C591–C604.
13 Helmreich, E., and Cori, C. F., Regulation of glycolysis in muscle. Adv. Enzyme Reg. *3* (1965) 91–107.
14 Kammermeier, H., High energy phosphate of the myocardium: concentration versus free energy change. Basic Res. Cardiol. *82* (1987) 31–36.
15 Kemp, R. G., and Foe, L. G., Allosteric regulatory properties of muscle phosphofructokinase. Molec. Cell Biochem. *57* (1983) 147–154.
16 Krause, U., and Wegener, G., 6-phosphofructokinase from frog skeletal muscle: purification and properties. Biochem. Soc. Trans. *18* (1990) 592–593.
17 Krause U., and Wegener, G., Metabolic changes in skeletal muscle of frog during exercise and recovery. Biochem. Soc. Trans. *19* (1991) 137S.
18 Krause, U., and Wegener, G., Regulation of fructose 2,6-bisphosphate content in skeletal muscle of the common frog: effects of muscle work and drugs. Verh. Dtsch. Zool. Ges. *88.1* (1995) 113.
19 Krause, U., and Wegener, G., Exercise and recovery in frog muscle: metabolism of PCr, adenine nucleotides, and related compounds. Am. J. Physiol. *270* (Regulatory Integrative Comp. Physiol. *39*) (1996) R811–R820.
20 Krause, U., and Wegener, G., Control of glycolysis in vertebrate skeletal muscle during exercise. Am. J. Physiol. *270* (Regulatory Integrative Comp. Physiol. *39*) (1996) R821–R829.
21 Krebs, E. G., Phosphorylation and dephosphorylation of glycogen phosphorylase: a prototype of reversible covalent modification. Curr. Top. Cell. Reg. *18* (1981) 401–419.
22 Kushmerick, M. J., Energetics of muscle contraction, in: Handbook of Physiology, pp. 189–236. Eds L. D. Peachey, R. H. Adrian, and S. R. Geiger. Bethesda, Maryland: Am. Physiol. Soc., Section 10: Skeletal muscle 1983.
23 Mainwood, G. W., and Worsley-Brown, P., The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. J. Physiol. *250* (1975) 1–22.
24 Meyer, R. A., Brown, T. R., and Kushmerick, M. J., Phosphorus nuclear magnetic resonance of fast- and slow-twitch muscle. Am. J. Physiol. *248* (Cell Physiol. *17*) (1985) C279–C287.
25 Minatogawa, Y., and Hue, L., Fructose 2,6-bisphosphate in rat skeletal muscle during contraction. Biochem. J. *223* (1984) 73–79.
26 Needham, D. M., Machina carnis – the Biochemistry of Muscular Contraction and its Historical Development. Cambridge University Press 1971.
27 Newsholme, E. A., and Blomstrand, E., The plasma level of some amino acids and physical and mental fatigue. Experientia *52* (1996) 413–415.
28 Newsholme, E. A., Beis, I., Leech, A. R., and Zammit, V. A., The role of creatine kinase and arginine kinase in muscle. Biochem. J. *172* (1978) 533–537.
29 Newsholme, E. A., and Leech, A. R., Biochemistry for the Medical Sciences. John Wiley and Sons, Chichester, New York 1983.
30 Poorman, R. A., Randolph, A., Kemp, R. G., and Heinrikson, R. L., Evolution of phosphofructokinase-gene duplication and

creation of new effector sites. Nature, Lond. *309* (1984) 467–469.

31 Pyko, M., Rider, M. H., Hue, L., and Wegener, G., 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase from frog skeletal muscle: purification, kinetics and immunological properties. J. comp. Physiol. B *163* (1993) 89–98.

32 Radda, G. K., Control, bioenergetics, and adaptation in health and disease: noninvasive biochemistry from nuclear magnetic resonance. FASEB J. *6* (1992) 3032–3038.

33 Rosing, J., and Slater, E. C., The value of ΔG° for the hydrolysis of ATP. Biochim. biophys. Acta *267* (1972) 275–290.

34 Sahlin, K., and Katz, A., Adenine nucleotide metabolism, in: Principles of Exercise Biochemistry (2nd ed), pp. 137–157. Ed. J. R. Poortmans. Karger, Basel 1993.

35 Schmidt, H., and Wegener, G., Glycogen phosphorylase in fish muscle: demonstration of three interconvertible forms. Am. J. Physiol. *258* (Cell Physiol. *27*) (1990) C344–C351.

36 Sols, A., Castaño, J. G., Aragón, J. J., Domenech, C., Lazo, P. A., and Nieto, A., Multimodulation in phosphofructokinases in metabolic regulation, in: Metabolic Interconversion of Enzymes, pp. 111–123. Ed. H. Holzer. Springer, Berlin, Heidelberg, New York 1981.

37 Sprang, S. R., Acharya, K. R., Goldsmith, E. J., Stuart, D. I., Varvill, K., Fletterick, R. J., Madsen, N. B., and Johnson, L. N., Structural changes in glycogen phosphorylase induced by phosphorylation. Nature, Lond. *336* (1988) 215–221.

38 Stanley, W. C., and Connett, R. J., Regulation of muscle carbohydrate metabolism during exercise. FASEB J. *5* (1991) 2155–2159.

39 Taylor, D. J., Styles, P., Matthews, P. M., Arnold, D. A., Gadian, D. G., Bore, P. J., and Radda, G. K., Energetics of human muscle: exercise-induced ATP depletion. Magn. Res. Med. *3* (1986) 44–54.

40 Teague, W. E., and Dobson, G. P., Effect of temperature on the creatine kinase equilibrium. J. biol. Chem. *267* (1992) 14084–14093.

41 Tornheim, K., Activation of muscle phosphofructokinase by fructose 2,6-bisphosphate and fructose 1,6-bisphosphate is differently affected by other regulatory metaboites. J. biol. Chem. *260* (1985) 7985–7989.

42 Ulmer, H.-V., Concept of an extracellular regulation of muscular metabolic rate during heavy exercise of humans by psychological feedback. Experientia *52* (1996) 416–420.

43 Vaandrager, S. H., Van Marrewijk, W. J. A., and Beenakkers, A. M. T., Glycogen phosphorylase activity in flight muscles of *Locusta migratoria* at rest and during flight. Insect Biochem. *16* (1986) 749–756.

44 Van Schaftingen, E., D-fructose 2,6-bisphosphate, in: Adv. Enzymol. relat. Areas Mol. Biol, pp. 315–395. Ed. A. Meister. John Wiley and Sons, New York 1987.

45 Van Schaftingen, E., Hue, L., and Hers, H.-G., Fructose 2,6-bisphosphate, the probable structure of the glucose- and glucagon-sensitive stimulator of phosphofructokinase. Biochem. J. *192* (1980) 897–901.

46 Veech, R. L., Lawson, J. W. R., Cornell, N. W., and Krebs, H. A., Cytosolic phosphorylation potential. J. biol. Chem. *245* (1979) 6538–6547.

47 Wegener, G., Flying insects: model systems in exercise physiology. Experientia *52* (1996) 404–412.

48 Wegener, G., Krause, U., and Thuy, M., Fructose 2,6-bisphosphate and glycolytic flux in skeletal muscle of swimming frog. FEBS Lett. *267* (1990) 257–260.

49 Wegener, G., Bolas, N. M., and Thomas, A. A. G., Locust flight metabolism studied in vivo by ^{31}P NMR spectroscopy. J. comp. Physiol. B *161* (1991) 247–256.

50 Wegener, G., and Krause, U., Environmental and exercise anaerobiosis in frogs, in: Surviving Hypoxia: Mechanisms of Control and Adaptation, pp. 217–236. Eds P. W. Hochachka, P. L. Lutz, T. Sick, M. Rosenthal, and G. van den Thillart. CRC Press, Boca Raton 1993.

51 Wilkie, D. R., The control of glycolysis in living muscle studied by nuclear magnetic resonance and other techniques. Biochem. Soc. Trans. *11* (1983) 244–246.