Mechanism of ATP loss in nonoxidative contracting muscle

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The transition from rest to intense exercise is a challenge to cellular energetics (11, 13, 15). The metabolic fuels, i.e., the sources of ATP to sustain muscular contraction, are creatine phosphate and glycogen. Two anaerobic metabolic pathways, leading to ATP generation, are catalyzed by creatine kinase and by the 12 enzymes of nonoxidative glycolysis, starting from glycogen. There is now general agreement that, unless replenished, creatine phosphate can sustain heavy muscle contraction for only 3–4 s. Thereafter, nonoxidative glycolysis becomes the main ATP source, until the onset of fatigue. This article aimed to relate the path of ATP generation during glycolysis utilization as a metabolic fuel with that of ATP breakdown in nonoxidative contracting muscle.

Correlation-type considerations. Table 1 shows the reactions of nonoxidative glycolysis, starting from glycogen. Since the product of each reaction becomes the substrate of the subsequent one, the 12 reactions constitute a catabolic pathway, whose summary equation, with all constituents and charges at physiological pH, is as follows:

\[
\text{Glycogen}_{(N)} + 3 \text{ADP}^3^- + 3 \text{P}_i^2^- + \text{H}^+ \rightarrow \text{glycogen}_{(N-1)} + 2 \text{lactate}^2^- + 3 \text{ATP}^4^- + 2 \text{H}_2\text{O} \tag{1}
\]

where \( N \) is the number of glycosyl units of the muscle glycogen macromolecule. It can be seen that nonoxidative glycolysis, starting from glycogen, not only does not generate protons, i.e., does not cause metabolic acidosis, but rather consumes one proton per glycosyl unit transformed into two lactate molecules. Metabolic acidosis occurs because the three ATP molecules are immediately hydrolyzed by the myosin ATPase to sustain contraction, as follows:

\[
3 \text{ATP}^4^- + 3 \text{H}_2\text{O} \rightarrow 3 \text{ADP}^3^- + 3 \text{P}_i^2^- + 3 \text{H}^+ \tag{2}
\]

Equation 3 is the summary equation of the conversion of one glycosyl residue of muscle glycogen into lactate and protons (Eq. 1 + Eq. 2):

\[
\text{Glycogen}_{(N)} + \text{H}_2\text{O} \rightarrow \text{glycogen}_{(N-1)} + 2 \text{lactate}^2^- + 2 \text{H}^+ \tag{3}
\]

Students should be aware that the two protons released in this equation are not generated by lactic acid dissociation, because lactate, not lactic acid, is formed in reaction 1. The two protons are generated by the production of the three protons released by reaction 2 and the consumption of one proton by reaction 1. Most important, the two reactions are strictly “coupled,” i.e., any ATP synthesized by anaerobic glycolysis (reaction 1) is hydrolyzed to ADP and P, (reaction 2), which are immediately converted back to ATP by anaerobic glycolysis. Moreover, the coupled Eqs. 1 and 2 imply that any increase in the rate of ATP synthesis to sustain an increased energy demand should be equal to that of ATP hydrolysis by myosin ATPase. Therefore, the muscular ATP pool (~8 mM in the intracellular water in humans) (3, 8) should remain unchanged during contraction. However, since the late 1970s (6, 9), ever-increasing experimental evidence has clearly shown that a considerable ATP loss occurs in heavy contracting muscle (16, 17, 19, 20). In humans, even a single 10-s sprint bout is sufficient to acutely deplete the muscular ATP pool by ~21% (1). After repeated bouts, a more marked reduction (~41%) has been reported (9). In horses, the ATP loss may reach a value of ~47% after 2 min of exercise at a speed of 12 m/s (16). It has also been shown that the muscular total adenine nucleotide ([ATP] + [ADP] + [AMP]) reduction caused by intense exercise is lower in women than in men (8). Lactate metabolism is strictly related to that of ATP in working muscle. The bulk of evidence suggests that lactate must not be considered as a dead-end waste product of nonoxidative glycolysis but as a particular mobile fuel for cellular, regional, and whole body aerobic metabolism (for a review, see Brooks (2)). In particular, lactate improves the excitability of depolarized rat skeletal muscle via mechanisms not related to a reduction of intracellular pH but by exerting a direct inhibitory effect on muscle Cl– channels (7). Finally, there is not a 1:1 correspondence between proton and lactate anion release from working muscle, and these releases can far exceed the net change in [ATP] (5).

A breakthrough on this important issue of muscle ATP metabolism was the finding that in contracting muscle inosine monophosphate (IMP), a nonadenine nucleoside monophosphate, accumulates intracellularly through AMP deamination followed by the extracellular release of inosine and hypoxanthine, two IMP catabolites (9, 14, 19).

Mechanisms of ATP loss during nonoxidative muscle contraction. When the rate of ATP hydrolysis exceeds the capacity of contracting muscle to phosphorylate ADP by anaerobic glycolysis, [ADP] and [AMP] rise, first leading to the deamination of AMP to IMP and subsequently to the dephosphorylation of IMP (19) (Table 2). The process of intracellular ATP breakdown in exhaustive contracting muscle may be divided into two stages (see Fig. 1, B and C). In the first stage, some of the ADP, generated by myosin ATPase (reaction 2) and escaping recycling, is transformed into AMP and ATP by the action of myokinase (reaction 4). AMP is then deaminated to generate IMP and NH3 by adenylate deaminase (reaction 5), as follows:

\[
\text{Myokinase: } 2 \text{ADP} \leftrightarrow \text{ATP} + \text{AMP} \tag{4}
\]

\[
\text{Adenylate deaminase: } \text{H}_2\text{O} + \text{AMP} \rightarrow \text{IMP} + \text{NH}_3 \tag{5}
\]

IMP may be reaminated to AMP by the successive action of adenylosuccinate synthetase and adenylate lyase (10), thus contributing in maintaining the intracellular concentration of...
purine rings at a constant level and favoring a rapid return of the ATP stores to the resting level during recovery. Most likely, this stage is responsible for the ATP loss during short anaerobic bouts, e.g., a 100-m sprint. However, during high-power anaerobic exercise, e.g., a 400-m sprint, or during repeated short-sprint bouts, IMP accumulates, thus increasing the rate of IMP dephosphorylation by 5′-nucleotidase and, consequently, the rate of inosine and hypoxanthine production (reactions 6 and 7) (4, 17). The second stage of the muscle ATP breakdown process is composed of the following two reactions:

\[
\text{IMP} + H_2O \rightarrow \text{inosine} + P_i \text{(enzyme: 5′-nucleotidase)} \quad (6)
\]

\[
\text{Inosine} + P_i \rightarrow \text{hypoxanthine} + \text{ribose-1-phosphate} \quad \text{(enzyme: purine nucleoside phosphorylase)} \quad (7)
\]

Inosine and hypoxanthine can diffuse into the bloodstream and are either excreted by the urine or imported into liver, where they are oxidized to urate (12) (Fig. 1). In summary, the path of intracellular ATP breakdown during exhaustive muscle contraction is ATP → ADP → AMP → IMP → inosine → hypoxanthine. The summary equation of this catabolic pathway (Table 2) shows that not only hypoxanthine but also ribose-1-phosphate, an important precursor for the synthesis of phosphoribosyl pyrophosphate (a sugar phosphate needed for the process of purine salvage) (18), and protons are among the final products of ATP breakdown. The level of the total urinary purine rings (inosine + hypoxanthine + urate) may be considered as a marker of ATP loss in contracting muscle (12, 17).

**Conclusions.** The aim of this article was to make it clear that during heavy muscular contraction a consistent aliquot of the ATP pool undergoing hydrolysis by myosin ATPase is not recycled by anaerobic glycolysis, with glycogen as the initial substrate. It is rather broken down by a tangential pathway composed of a series of five reactions, yielding inosine, hypoxanthine, and urate as the main final products.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**REFERENCES**


Fig. 1. Metabolic control of glycogen and ATP metabolism in anaerobic contracting muscle. In A (bold arrows), the rate of ATP synthesis by the glycolytic pathway, with glycogen as the metabolic fuel, matches the rate of ATP hydrolysis by myosin ATPase, to sustain contraction. As a consequence, intracellular [ATP] (~8 mM) remains constant. When the two processes are not tightly coupled, an aliquot of the ATP pool is broken down into inosine monophosphate (IMP), a purine nucleotide that cannot across the sarcolemma (B). During this stage, there is no loss of total purines from contracting muscle. During prolonged exhaustive muscle contraction, IMP accumulates and is broken down to inosine (Ino) and hypoxanthine (Hyp), which are either excreted as such by the urine or oxidized to urate in the liver (C). During this stage, there is a net loss of purine rings from contracting muscle. In the liver, lactate enters the gluconeogenic anabolic pathway. 1, ATPase (EC 3.6.4.1); 2, myokinase (EC 2.7.4.3); 3, adenylate deaminase (EC 3.5.4.6); 4, 5’-nucleotidase (EC 3.1.3.5); 5, purine nucleoside phosphorylase (EC 4.4.2.1); 6, xanthine (Xan) oxidase (EC 1.1.3.22).