Glycogen as a fuel: metabolic interaction between glycogen and ATP catabolism in oxygen-independent muscle contraction

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ABSTRACT

The main role of muscular oxygen-independent glycolysis, starting from glycogen as the initial substrate, is the production of 3 ATP molecules from ADP and P_i per glucosyl moiety transformed into 2 lactate molecules. During this catabolic process not only there is no proton release, but one proton is consumed. Metabolic acidosis occurs because the three ATP molecules are immediately hydrolysed by myosin ATPase back to 3 P_i and 3 ADP, to sustain contraction. As a consequence of this ATP turnover, the ATP pool (~ 5 mmol kg^{-1} wet weight) should remain constant. However, a bulk of experimental evidence has clearly shown that depletion of the muscular ATP pool, and accumulation of ATP catabolites occur even during short sprint bouts. In the present article the interrelationship between glycogen and ATP catabolism in anaerobic contracting muscle is discussed. It is shown how myosin ATPase plays a role not only in the mechanisms of ATP recycling through glycogen anaerobic catabolism, but also in the process of ATP depletion.

Keywords: lactic acidosis; myosin ATPase; glycogen catabolism; ATP breakdown; anaerobic muscle contraction

Introduction. When addressing the metabolic response of skeletal muscle to anaerobic contraction, we are faced with three intriguing problems, still a subject of debate: i) where do the released protons come from? ii) Does the "lactic acid hypothesis" explain metabolic acidosis? iii) Does the ATP level remain constant during contraction? (see, for instance, Robergs et al 2004; Robergs et al. 2005; Lindinger et al. 2005; Robergs et al. 2006; Kemp et al. 2006; Boning and Maassen 2008; Marcinek et al. 2010; Ipata 2011). In this article we tried to give convincing answers to these questions, based on our present knowledge on the interconnected metabolic networks of glycogen and ATP in oxygen-independent contracting muscle.

Anaerobic glycolysis, starting with glucose. The familiar summary equation of the 11 reactions of anaerobic glycolysis, starting from glucose, is the following:

$$\text{glucose} + 2\,\text{MgADP}^{1-} + 2\,\text{P}_i^{2-} \rightarrow 2\,\text{lactate}^{1-} + 2\,\text{MgATP}^{2-} + 2\,\text{H}_2\text{O} \quad (1)$$

Metabolic acidosis occurs, when the two ATP molecules are hydrolyzed, to drive the cellular anabolic processes in body districts devoid of mitochondria, such as red blood cells, or in proliferating cells, when there is an increased oxygen independent energy demand:

$$2\,\text{MgATP}^{2-} + 2\,\text{H}_2\text{O} \rightarrow 2\,\text{MgADP}^{1-} + 2\,\text{P}_i^{2-} + 2\,\text{H}^{1+} \quad (2)$$

Equation (3) presents the summary equation of the conversion of glucose into lactate and H^+ (eq.(1) + reaction (2))

$$\text{glucose} \rightarrow 2\,\text{lactate}^{1-} + 2\,\text{H}^{1+} \quad (3)$$

It is important to point out that the protons are not generated through lactic acid dissociation (pK_a = 3.87) (Robergs et al. 2004), as it might appear from equation (3), but from ATP hydrolysis. As discussed by Lane et al. (2009), "this is a long-standing error, that leads to incorrect biochemical interpretations".

Anaerobic glycolysis, starting from glycogen. Transition from rest to intense exercise is a challenge to muscle cell energetics (Jones et al. 2009; Sahlin et al. 1998). Glucose catabolism cannot sustain intense oxygen-independent muscle contraction, thus more convenient fuels, stored phosphocreatine and glycogen synthesized in the muscle in resting conditions, are used as ATP sources. In short-term intense exercise the ATP demand depends almost exclusively on the phosphocreatine fuel. The muscle contraction is powered by the following two reactions, catalyzed by creatine kinase and myosin ATPase, respectively:

$$H^{1+} + \text{phosphocreatine}^{2-} + MgADP^{1-} \rightarrow \text{creatine} + MgATP^{2-} \quad (4)$$

$$MgATP^{2-} + H_2O \rightarrow MgADP^{1-} + P_i^{-2} + H^{1+} \quad (5)$$

Net reaction: $\text{phosphocreatine}^{2-} + H_2O \rightarrow \text{creatine} + P_i^{2-}$

There is now general agreement that phosphocreatine can sustain muscle contraction for ~5 s of the onset of maximal exercise. Thereafter, the stores of phosphocreatine are largely depleted, due to the exponential path of its degradation (Walter et al., 1997), and a gradual switch to glycogen as the main metabolic fuel occurs.

Table 1 shows the conversion of one glycosyl moiety of glycogen into lactate and ATP. Each reaction is presented with all constituents and charges, to better follow the release and consumption of protons. After the intervention of glycogen phosphorylase and phosphoglycomutase (reactions 1 and 2 of Table 1), glucose-6-phosphate enters the anaerobic glycolysis. Reaction 3-12 are common to anaerobic glycolysis, starting with glucose. Thus, the summary equation is the following:

$$\text{glycogen}_{(N)} + 3\,MgADP^{1-} + 3\,P_i^{2-} + H^{1+} \rightarrow \text{glycogen}_{(N-1)} + 2\,\text{lactate}^{1-} + 3\,MgATP^{2-} + 2\,H_2O \quad (6)$$

where N is the number of glycosyl units of the muscle glycogen. It can be seen that i) protons are consumed, rather than released, during this anaerobic process, (3 protons are released through reactions 4 and 7 of Table 1, and 4 protons are consumed trough reactions 11 an 12) and ii) three ATP molecules are produced, corresponding to a 50 % increase in ATP generation when glycogen

is the metabolic fuel, instead of glucose. Moreover, ATP must be immediately hydrolyzed, to sustain contraction:

$$3\ MgATP^{2-} + 3\ H_2O \rightarrow 3\ MgADP^{1-} + 3\ P_i^{2-} + 3\ H^{1+} \quad (7)$$

Equation (8) presents the summary equation of equation (6) and reaction (7):

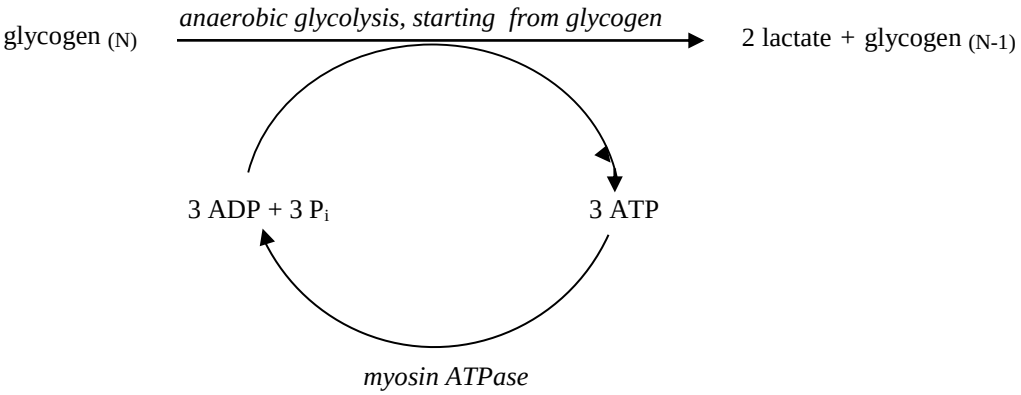
$$glycogen_{(N)} + H_2O \rightarrow glycogen_{(N-1)} + 2\ lactate^{1-} + 2\ H^{1+} \quad (8)$$

When using the term "lactic acidosis", it should be recalled that protons are released by the consumption of one proton through equation (6), followed by the release of three protons by reaction (7). Lactic acid is not involved.

The intracellular pH in resting and exercising human muscle, and the role of the P_i produced in the ATPase reaction (5) as a potential buffer of the free proton that is released seem to be controversial. In this regard, the following strictly interrelated points should be considered: i) in vivo NMR studies by several groups (Pan et al., 1988; Chen et al., 2001) indicate pH values near 7 in resting/recovery condition, and significantly lower during vigorous exercise; ii) the three single bonded oxygen atoms of P_i have the following p*K* values: 2.15, 6.82, and 12.38 (Robergs et al., 2004; Lane et al., 2009), thus one oxygen atom is able to become protonated when the pH falls well below 6.82; iii) however, as discussed by Robergs et al. (2004), the buffering potential of P_i does not decrease the importance of ATP hydrolysis as a source of protons, because the P_i produced functions as a substrate for glycolysis to produce ATP during contraction, leaving free protons to accumulate. Moreover, P_i is also a substrate for the very active glycogenolysis.

Correlation-type considerations The strictly coupled equations (6) and (7) imply that any increase in the rate of ATP synthesis by oxygen-independent glycolysis to sustain an increased energy demand should be equal to that of ATP hydrolysis by myosin ATPase, to sustain muscle

contraction. This “coupling” concept is better illustrated by the following scheme:



It can be seen that, as far as muscular glycogen is consumed through oxygen-independent glycolysis and lactate accumulates, ATP is continuously turned over by the myosin ATPase. Thus, the ~ 5 mM muscular ATP pool (Berg et al., 2007) should remain unchanged during contraction. This explanation of the process of muscle ATP turnover is based on the assumption that all the ADP and P_i, generated by the myosin ATPase are used exclusively to re-generate ATP by glycolysis. For many years this has been an important tenet in muscle physiology. However, since the late 1970s an ever increasing experimental evidence has clearly shown that a strong correlation exists between force development and a reduction in ATP skeletal muscle content (see, for instance, Dawson et al. 1978; Dudley and Terjung 1985; Norman et al 1987; Sewell and Harris, 1992; Stathis et al. 1999; Zaho e t al. 2000; Esbjernsson-Liljedahl al. 2002). In other words, the two processes of ATP generation by anaerobic glycolysis and ATP hydrolysis by myosin ATPase are not always strictly coupled. Some of the ADP generated by the myosin ATPase is not phosphorylated back to ATP through the process of anaerobic glycolysis, and undergoes a tangential breakdown process during contractions, to yield IMP, inosine and hypoxanthine as degradation products.

The metabolic interaction between glycogen and ATP breakdown in contracting muscle may be divided into three stages (Fig.1). In stage A, the muscle ATP pool is maintained at a constant level by equation (6) and reaction (7). In stage B, IMP accumulates (Sahlin et al. 1978; Tullson and

Terjung, 1990; Gaitanos et al., 1993) via myosin ATPase, myokinase, and adenylate deaminase (reactions (9)-(11)). IMP is unable to across the sarcolemma, and remains inside the myocyte, thus favouring a rapid ATP re-synthesis during recovery. Most likely, this stage is responsible of the ATP loss during short anaerobic bouts, e.g. during the 100 meter sprint race:

$$2\ MgATP^{2-} + 2\ H_2O \rightarrow 2\ MgADP^{1-} + 2P_i^{2-} + 2\ H^{1+} \quad \textit{myosine ATPase} \quad (9)$$

$$2\ MgADP^{1-} \rightarrow MgATP^{2-} + AMP^{2-} + Mg^{2+} \quad \textit{myokinase} \quad (10)$$

$$H_2O + AMP^{2-} + H^{1+} \rightarrow IMP^{2-} + NH_4^{1+} \quad \textit{adenylate deaminase} \quad (11)$$

IMP may be re-aminated (Tullson et al, 1996) by the successive action of adenylosuccinate synthetase and adenylate lyase:

$$IMP^{2-} + aspartate^{1-} + MgGTP^{2-} \rightarrow adenylosuccinate^{4-} + MgGDP^{1-} + P_i^{2-} + 2\ H^{1+} \quad \textit{adenylosuccinate synthetase} \quad (12)$$

$$adenylosuccinate^{4-} \rightarrow AMP^{2-} + fumarate^{2-} \quad \textit{adenylosuccinate lyase} \quad (13)$$

During this stage the purine nucleotide cycle, composed of enzymes (11), (12), and (13) (see also Fig 1) contributes in maintaining the intracellular concentration of the purine rings at a constant level in muscle cell during contraction, at the expense of GTP and aspartate, thus favouring a rapid return of the ATP stores to the resting level during recovery. It should be noted that the adenylosuccinate synthetase reaction liberates two protons. The summary equation of ATP breakdown to IMP, followed by IMP re-amination (reactions (9)- (13))

$$MgATP^{2-} + MgGTP^{2-} + aspartate^{1-} + 3\ H_2O \rightarrow AMP^{2-} + MgGDP^{1-} + fumarate^{2-} + NH_4^{+} + 3\ Pi^{2-} + 3\ H^{1+} + Mg^{2+} \quad (14)$$

generates 3 protons, and might contribute in part to metabolic acidosis. Since the return to ATP stores to the resting level require several minutes (Graham et a., 1990, Stathis et al., 2004), it is likely that IMP accumulates during intense muscle contraction, thus increasing the activity of

cytosolic 5'-nucleotidase, and consequently the rate of inosine and hypoxanthine production (Stathis et al., 1999). Step C of muscular ATP breakdown is composed of two reactions:

$IMP^{2-} + H_2O \rightarrow$ inosine $+ P_i^{2-}$ *5'-nucleotidase* (15)

inosine $+ P_i^{2-} \rightarrow$ hypoxanthine + ribose-1-phosphate^{2-} *purine nucleoside phosphorylase* (16)

The summary equation of ATP breakdown to hypoxanthine and ribose-1-phosphate via equations (9)-(11), (15), and (16) is:

$MgATP^{2-} + 4\ H_2O \rightarrow$ hypoxanthine + ribose-1-phosphate^{2-} $+ 2\ P_i^{2-} + NH_4^+ + H^+ + Mg^{2+}$

Ribose-1-phosphate cannot across the sarcolemma, and acts as a precursor of 5-phosphoribosyl-1-pyrophosphate, an important precursor of *de novo* and salvage synthesis of nucleotides. Hypoxanthine and inosine can diffuse into the blood stream, and are either excreted by the urine as such, or imported into liver, to be oxidized to urate. The level of the total urinary purine rings (inosine + hypoxanthine + urate) may be considered as a marker of ATP loss in exhaustive contracting muscle (Stathis et al., 1999; Macedo et al., 2009).

Conclusions Much of our present understanding on glycogen utilization as a fuel via anaerobic glycolysis stands on our present knowledge of muscular biochemistry. The aim of this article was to give a contribute to the understanding of muscle contraction mediated metabolic acidosis. A strict interrelation exists between glycogen and ATP catabolic pathways in contracting muscle. If the rate of ATP hydrolysis by myosin does not match the rate of ATP synthesis by the anaerobic glycolytic pathway, with glycogen as the metabolic fuel, an aliquot of the ATP pool is broken down by a tangential catabolic pathway, yielding inosine and hypoxanthine, which are excreted by the urine.

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Disclosures

The Authors have no disclosures to report.

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LEGEND TO FIGURE 1

Metabolic interaction between glycogen and ATP catabolism in muscle cells. In A) the rate of ATP synthesis by the oxygen-independent glycogen catabolism matches the rate of ATP hydrolysis by myosin ATPase, thus the muscle ATP pool remains unchanged. In B, an increased rate of ATP hydrolysis, caused by a more vigorous muscle contraction, does not match the rate of ATP synthesis via glycogen anaerobic breakdown, and an aliquot of the ATP pool is broken down to IMP, a non adenine nucleotide. The purine nucleotide cycle re-converts IMP to AMP via enzymes 3 and 4. In strenuous muscle contraction, IMP is irreversibly broken down to inosine and hypoxanthine, which are excreted by urine. 1: myokinase (EC 2.7.4.3); 2:. adenylate deaminase (EC 3.5.4.6); 3: adenylosuccinate synthetase (EC 6.3.4.4); 4: adenylosuccinate lyase (EC 4.3.2.2); 5: 5'-nucleotidase (EC 3.1.3.5); 6: purine nucleoside phosphorylase (EC 4.4.2.1). SAMP = adenylosuccinate; Hyp = hypoxanthine; Ino = inosine.

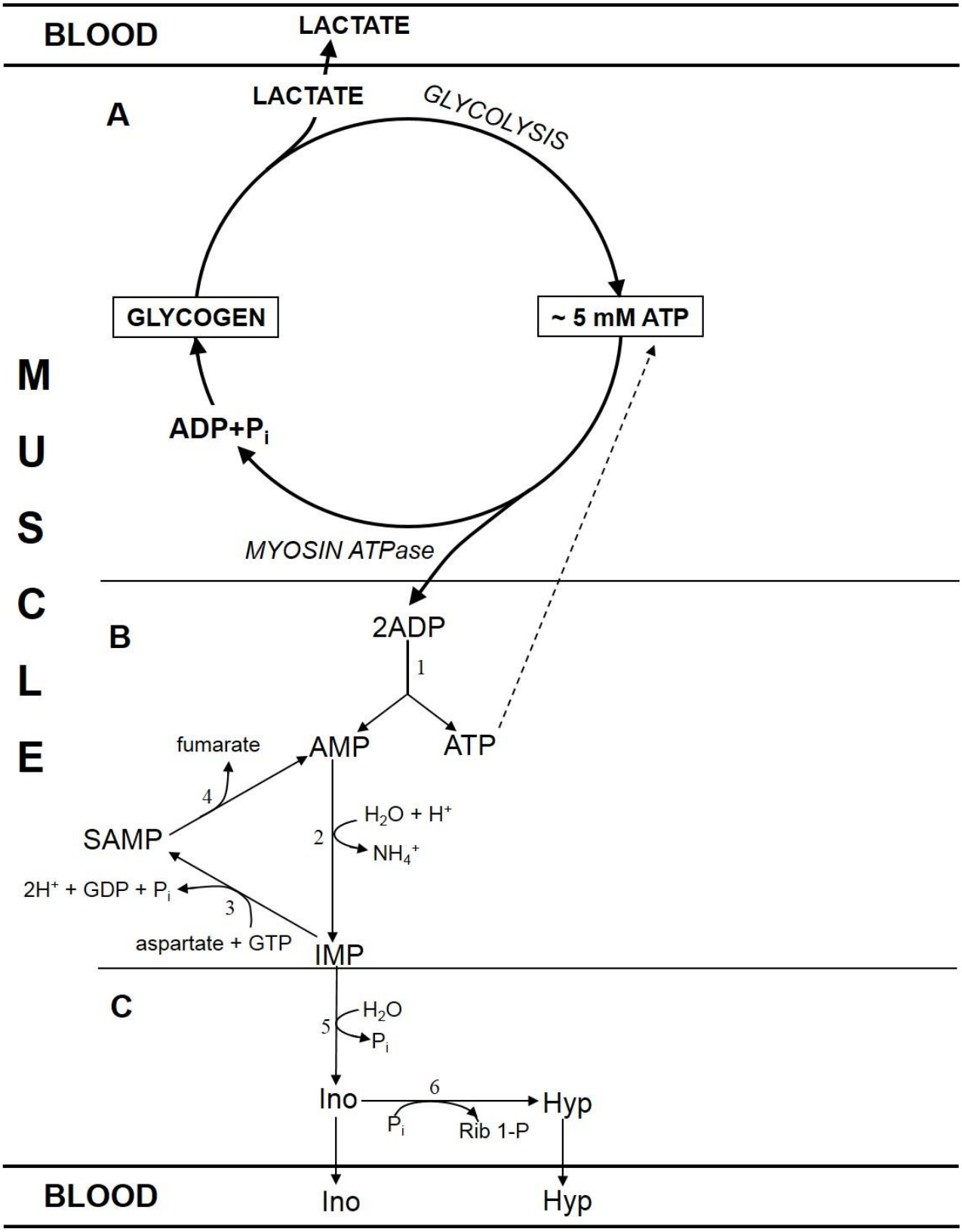
BLOOD
LACTATE
LACTATE
A
GLYCOLYSIS
GLYCOGEN
~ 5 mM ATP
ADP+Pi
MUSCLE
MYOSIN ATPase
B
2ADP
1
fumarate
AMP
ATP
4
SAMP
2
H2O + H+
NH4+
2H+ + GDP + Pi
3
aspartate + GTP
IMP
C
5
H2O
Pi
Ino
6
Pi
Rib 1-P
Hyp
BLOOD
Ino
Hyp

Figure 1

Table I. *Reactions and enzymes of oxygen- independent glycolysis, starting from glycogen*

	Reaction	Enzyme
1	$glycogen_{(N)} + P_i^{2-} \rightarrow glycogen_{(N-1)} + glucose\text{-}1\text{-}P^{2-}$	*Phosphorylase*
2	$glucose\text{-}1\text{-}P^{2-} \rightarrow glucose\text{-}6\text{-}P^{2-}$	*Phosphoglucomutase*
3	$glucose\text{-}6\text{-}P^{2-} \rightarrow fructose\text{-}6\text{-}P^{2-}$	*Glucose 6-phosphate isomerase*
4	$fructose\text{-}6\text{-}P^{2-} + MgATP^{2-} \rightarrow fructose\text{-}1,6\text{-}bis\text{-}P^{4-} + MgADP^{1-} + H^{1+}$	*6-Phosphofructokinase*
5	$fructose\ 1,6\text{-}bis\text{-}P^{4-} \rightarrow dihydroxyacetone\text{-}P^{2-} + glyceraldehyde\text{-}3\text{-}P^{2-}$	*Aldolase*
6	$dihydroxyacetone\text{-}P^{2-} \rightarrow glyceraldehyde\text{-}3\text{-}P^{2-}$	*Triose phosphate isomerase*
7	$2\ glyceraldehyde\text{-}3\text{-}P^{2-} + 2\ NAD^{+} + 2\ P_i^{2-} \rightarrow 2\ 1,3\text{-}bis\text{-}phosphoglycerate^{4-} + 2\ NADH + 2\ H^{1+}$	*Glyceraldehyde-3- phosphatedehydrogenase*
8	$2\ 1,3\text{-}bis\text{-}Phosphoglycerate^{4-} + 2\ MgADP^{1-} \rightarrow 2\ 3\text{-}phosphoglycerate^{3-} + 2\ MgATP^{2-}$	*Phosphoglycerate kinase*
9	$2\ 3\text{-}phosphoglycerate^{3-} \rightarrow 2\ 2\text{-}phosphoglycerate^{3-}$	*Phosphoglycerate mutase*
10	$2\ 2\text{-}phosphoglycerate^{3-} \rightarrow 2\ phosphoenolpyruvate^{3-} + 2\ H_2O$	*Enolase*
11	$2\ phosphoenolpyruvate^{3-} + 2\ MgADP^{1-} + 2\ H^{1+} \rightarrow 2\ pyruvate^{1-} + 2\ MgATP^{2-}$	*Pyruvate kinase*
12	$2\ pyruvate^{1-} + 2\ NADH + 2\ H^{1+} \rightarrow 2\ lactate^{1-} + 2\ NAD^{1+}$	*Lactate dehydrogenase*

Summary equation: $glycogen_{(N)} + 3\ MgADP^{1-} + 3\ P_i^{2-} + H^{1+} \rightarrow glycogen_{(N-1)} + 2\ lactate^{1-} + 3\ MgATP^{2-} + 2\ H_2O$