# Chapter 10
# Anaerobic Metabolism

Under normal conditions glucose and fat oxidation within the metabolic pathways "fuels" ATP resynthesis (protein plays a limited role as a fuel). Glucose is unique because unlike fat its energy content is exploited by anaerobic metabolism in addition to aerobic metabolism. Fat oxidation is completely aerobic. Anaerobic metabolism, as its name implies, does not involve oxygen. However, the products of anaerobic metabolism – pyruvate and lactate – can undergo complete oxidation aerobically; but that is a later story (Chap. 11). In fact, biochemistry was a borne anaerobic baby and grew to become an aerobic adult. Let us start from the beginning . . .

## 10.1 A Brief History of Anaerobic Biochemistry

For literally thousands of years, prevailing thought held that heat was a prerequisite for life. It was Jean Baptiste van Helmont (1577–1644) who declared things the other way around: life produced heat. The warmth rising from the fermentation of wine – the splitting of glucose to form alcohol – was cited as part of his proof. Louis Pasteur (1822–1895) directed his talents toward a more complete investigation of alcohol production. Pasteur recognized anaerobic fermentation as an organized biological process dedicated to living organisms, not an act of chemical disintegration as thought by others at the time. Eduard Buchner (1860–1917) later demonstrated that fermentation could take place within both living yeast and the lifeless test tube (he was awarded the 1907 Nobel Prize for this). It became understood that glucose breakdown involved the actions of several enzymes, the catalysts of life's biochemical reactions; multiple reactants and products were produced along the metabolic pathway of glucose breakdown . . . but that was not all. A rather monumental understanding of metabolism was presented by Arthur Harden (1865–1940) and William Young (1878–1942), who first recognized the importance of inorganic phosphate (Pi) not as a part of ATP, but as part of the fermentation process.

In organisms other than yeast, it became recognized that a similar type of fermentation took place, but clearly, animal cells did not produce alcohol! The biochemical

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degradation of glucose in animal cells stopped one step short of alcohol production, instead the end product was found to be lactic acid. Suffice to say that once completed, the elucidation of glucose splitting – termed *glycolysis* (glyco = glucose; lysis = to split) – had not only made history but initiated the science of biological chemistry. As stated by the author of one well-known biochemistry text, "... the development of biochemistry and the delineation of this pathway went hand in hand" (1). Biochemistry indeed had an anaerobic birth. The anaerobic breakdown of glucose within your body's cells is now recognized with names that include substrate-level phosphorylation, anaerobic metabolism, and the Embden–Meyerhoff–Parnas pathway (Gustav Embden, 1874–1933; Otto Meyerhoff, 1884–1951; Jacob Parnas, 1884–1949).

## 10.2 The Glycolytic Gradient

Glycolysis with its associated phosphate (Pi) shifts and transfers takes place in the cellular cytoplasm as a 10- and 11-step chemical-to-chemical energy exchange (1, 2). The gradient begins with one molecule of glucose and ends with two molecules of pyruvate or lactate (1glucose → 2pyruvate or 2lactate). Two ATPs are needed to prep the reaction as it takes place within a cell. Ten enzymatic steps result in pyruvate formation (also known as "aerobic" glycolysis); an additional eleventh enzyme leaves lactate as the final product (also known as anaerobic glycolysis). With glucose as substrate, glycolysis, whether aerobic or anaerobic, resynthesizes four ATPs overall, resulting in a net production of two ATPs per glucose molecule or moiety.

Muscle stores glucose in the form of glycogen (glycogen being the predominately used carbohydrate substrate within working skeletal muscle). Glycogen degradation is termed *glycogenolysis*, resulting in the resynthesis of three ATPs. The starting points of glucose and glycogen degradation differ but the end products are the same. The glycolytic pathway is shown in Fig. 10.1.

Many biochemistry texts, when reporting the individual thermodynamic properties of each of the steps of glycolysis, have a tendency to report only Gibbs energy changes and for good reason (as opposed to enthalpy and entropy changes). Gibbs energy changes indicate the spontaneity and the amount of available energy from a given reaction, both of which represents invaluable information (1). Glycolytic Gibbs energy changes (of the system) under standard conditions ($\Delta G^{\circ\prime}$) and physiological conditions ($\Delta G$) are typically displayed to portray the glycolytic gradient; from concentrated energy as glucose, to more distributed energy as pyruvate.

As stated over 30 years ago by Minakami and de Verdier, "Data on enthalpy changes of [individual] glycolytic reactions are difficult to find in the literature" (3); the same was true regarding entropy. Such difficulty apparently served to motivate these investigators to measure and calculate the respective enthalpy ($\Delta H$), entropy ($\Delta S$), and Gibbs energy ($\Delta G$) changes of all the independent glycolytic reactions under physiological conditions (see Table 10.1). It needs to be noted that the values

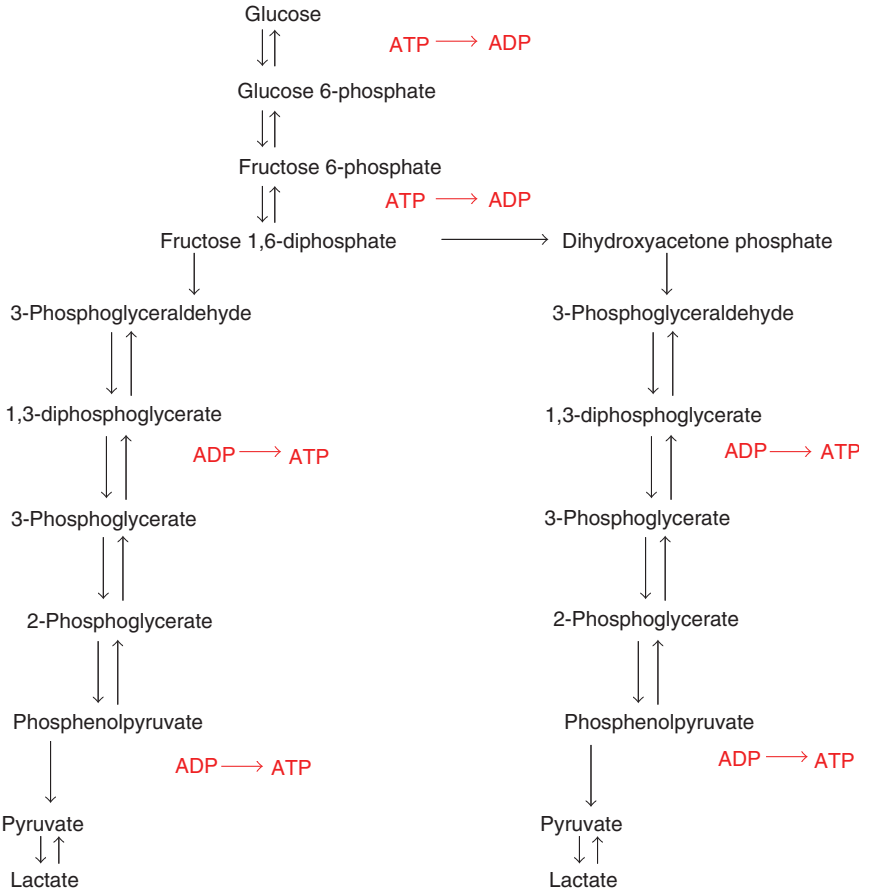


**Fig. 10.1** The *arrows* in the figure (in fact most figures where a metabolic pathway is represented) usually indicate forward and/or reverse directional capabilities of a given reaction. In addition, the overall start-to-finish dynamics of an active metabolic pathway also needs consideration. Reactants and products of each individual reactions are provided (enzymes are not shown). Glucose itself is split into two at reaction no. 4. Phosphate (Pi) shifts and transfers occur throughout much of the glycolytic pathway

listed in Table 10.1 represent a compilation of individual closed-system reactions (using red blood cells, erythrocytes) (also, Table 10.1 starts with one-half glucose and ends with one pyruvate or lactate molecule).

Instead of piecing together individual reaction steps, an over-all start to finish measure also can be taken to determine the molar enthalpy changes ($\Delta H$) for glucose-to-lactate conversion, that is, the overall glycolytic reaction. For example, Gnaiger reported glucose-to-lactate enthalpy changes ranging from $-70$ to $-80\,\mathrm{kJ\,mol^{-1}}$; with glycogen as a starting point the overall enthalpy change ranged from $-71$ to $-81\,\mathrm{kJ\,mol^{-1}}$ (3) (the value of $-80$ to $-81\,\mathrm{kJ\,mol^{-1}}$ exquisitely matches that of Table 10.1 when doubling the enthalpy change of one-half the

**Table 10.1** Closed-system thermodynamic parameters of one-half glycolysis

| Reaction | Enzyme | $\Delta$H (kJ mol$^{-1}$) | $\Delta$G (kJ mol$^{-1}$) | $\Delta S$ (kJ mol$^{-1}$) |
|---|---|---|---|---|
| 1. 0.5glucose + 0.5ATP → 0.5glucose 6-phosphate + 0.5ADP + 0.5H$^+$ | Hexokinase | −13.8 | −20.9 | +7.4 |
| 2. 0.5glucose 6-phosphate → 0.5fructose 6-phosphate | Phosphoglucose isomerase | +4.8 | +0.2 | +4.6 |
| 3. 0.5fructose 6-phosphate + 0.5ATP → 0.5fructose 1,6-bisphosphatase + 0.5ADP + 0.5H$^+$ | Phosphofructokinase | −13.8 | −13.0 | −0.8 |
| 4. 0.5fructose 1,6-bisphosphate → glyceraldehyde 3-phosphate | Aldolase | +20.9 | −0.4 | +21.3 |
| 4. 0.5fructose 1,6-bisphosphate → dihydroxyacetone phosphate | Aldolase | ? | ? | ? |
| 5. dihydroxyacetone phosphate → glyceraldehyde 3-phosphate | Triose phosphate isomerase | ? | −2.5 | ? |
| 6. glyceraldehyde 3-phosphate + Pi + NAD$^+$ → 1,3-bisphosphoglycerate + NADH + H$^+$ | Glyceraldehyde 3-phosphate dehydrogenase | −2.9 | −1.0 | −1.9 |
| 7. 1,3-bisphosphoglycerate + ADP → 3-phosphoglycerate + ATP | Phosphoglycerate kinase | −5.4 | + 1.3 | ? |
| 8. 3-phosphoglycerate → 2-phosphoglycerate | Phosphoglyceromutase | 0.0 | −0.6 | +0.6 |
| 9. 2-phosphoglycerate → phosphoenolpyruvate + $H_2O$ | Enolase | +14.6 | −1.3 | +15.9 |
| 10. phosphoenolpyruvate + ADP + H$^+$ → pyruvate + ATP | Pyruvate kinase | 0.0/+4.4 | −15.5/−53.7 | +15.5/ +62.6 |
| 11. Pyruvate + NADH + H$^+$ → lactate + NAD$^+$ | Lactate dehydrogenase | −44.4/−40.0 | 0.0/-53.7 | −44.4/ +18.2 |

Adapted from (1, 3).

**Table 10.2** Thermodynamics of two overall glycolytic reactions (from (5))

| Reaction | $\Delta$H (kJ mol$^{-1}$) | $\Delta$G (kJ mol$^{-1}$) | $\Delta S$ (kJ mol$^{-1}$) |
|---|---|---|---|
| 0.5glucose → pyruvate ("aerobic" glycolysis) | +4.4 | −53.7 | +62.6 |
| 0.5glucose → lactate (anaerobic glycolysis) | −40.0 | −53.7 | +18.2 |

glycolytic reaction). The enthalpy changes of any reaction are heavily influenced by the different mediums or surroundings where the events are recorded: muscle cells, intestinal cells, nerve cells, liver cells, test tubes, Petri dishes, etc. In pursuit of a proper acid–base balance, heat exchanges for an identical reaction or series of reactions (glycolyisis) may differ between and even within cells. Imagine those acid–base differences occurring between resting and exercising skeletal muscle for example. Including the heat associated with acid neutralization, di Prampero et al. measured the glycogen-to-lactate enthalpy change at 76 kJ mol$^{-1}$; when acid–base changes were accounted for and eliminated from the overall enthalpy changes, the glycogen-to-lactate enthalpy change was −64 kJ mol$^{-1}$ (4).

Overall, entropy increases ($+\Delta S$) as glucose is degraded to pyruvate but the reduction of pyruvate to form lactate results in a negative entropy change ($-\Delta S$) (5) (Table 10.2). This suggests lactate as acquiring a greater structure or order as compared with pyruvate. The acknowledgement of entropy and enthalpy changes in addition to Gibbs energy allows for a more complete interpretation of biological energy expenditure. As will be demonstrated in the next section (Sect. 10.3), the enthalpy and entropy exchanges of glycolysis as glucose-to-pyruvate (aerobic glycolysis) and as glucose-to-lactate (anaerobic glycolysis) suggests the presence of two distinct overall reactions and, when recognized as such, provides a valuable tool in the estimation of aerobic and anaerobic contributions to whole-body energy expenditure.

As shown, Table 10.1 is incomplete and thus needs to be interpreted with caution (see missing thermodynamic values for reactions 4, 5, and 7). Beyond the absence of data, some glycolytic reactions appear to be nonspontaneous under physiological conditions. One such reaction, a phosphate transfer, is shown below, the seventh reaction of glycolysis:

$$1,3\text{-bisphosphoglycerate} + \text{ATP} \rightarrow 3\text{-phosphoglycerate} + \text{ATP} (\Delta G = +1.3)$$

While having a (nonspontaneous) $\Delta$G of + 1.3 kJ mol$^{-1}$, it must be concluded that the reaction does indeed take place within cells, otherwise no living organism on earth would be able to exploit glycolytic energy exchange, considered one of the oldest metabolic pathways on our planet (6). How, with a positive $\Delta$G, is a nonspontaneous reaction promoted into spontaneity? The input of energy is the logical solution. Recalling those forces and flows that are inherent to an open metabolic system, metabolic power likely promotes the reaction in the forward direction (Fig. 10.2).

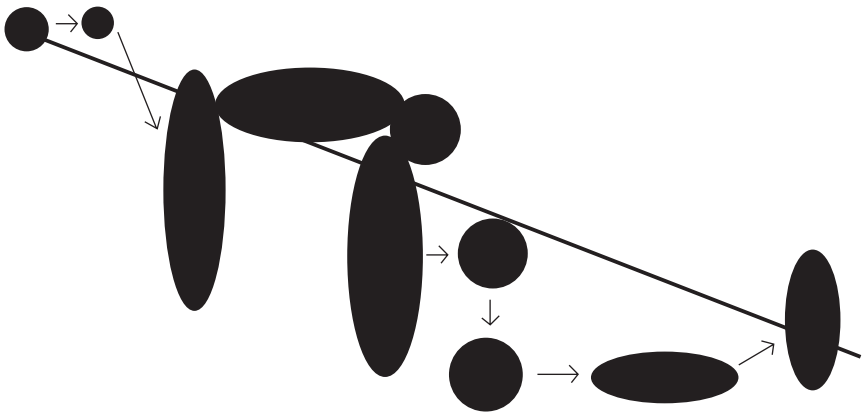

**Fig. 10.2** A depiction of anaerobic glycolytic enzyme–enzyme channeling and perfusion/streaming/convection as a composite of metabolic power (forces and flows). The *thick diagonal line* represents an actin filament, part of the cell's cytoskeleton. The *circles and ellipses* represent each of the ten glycolytic enzymes; some are attached to and affected by actin, while others are not. Enzymes in direct contact with each other represent enzyme–enzyme channeling (a metabolon). The *arrows* portray enzyme-to-enzyme perfusion/streaming. Glycolysis starts at hexokinase (*top left circle*) and ends at pyruvate kinase (*bottom right ellipse*) (modified from Bereiter-Hahn et al. (7)

## 10.3 Glycolytic Enthalpy and Entropy

Glycolysis is activated at the *onset* of muscle contraction, perhaps by the sudden increase of intracellular calcium ($Ca^{2+}$) (8, 9). Arguments have been made concerning kinetic (ADP concentration) vs. thermodynamic ($\Delta$G of ATP hydrolysis) control of the metabolic pathways (10). These arguments will not be settled here though it should suffice to say that metabolic control appears to fluctuate between sites of ATP utilization and ATP resynthesis: from actomyosin during low-to-moderate exercise to sites of ATP resynthesis at higher exercise intensities, respectively (11). Biochemical activity in working muscle may speed up some 3,000-fold upon the arrival of a muscle's action potential (9). So too must the impending enthalpy and entropy changes of this increase in metabolic activity. As mentioned earlier, the scientific literature reveals that the energy exchanges associated with glycolysis have mostly been interpreted in terms of lactate rather then pyruvate production: anaerobic glycolysis as opposed to "aerobic" glycolysis, respectively. This needs to be well recognized because the thermodynamics of anaerobic glucose-to-pyruvate formation are different from that of glucose-to-lactate formation (Tables 10.1 and 10.2).

If the thermodynamic parameters in Table 10.2 are correct, then the metabolic breakdown of glucose-to-pyruvate results in the net flux of heat (energy) to rather than from the system; the enthalpy change is small ($\Delta H = 4.4\,kJ\,mol^{-1}$). Note also the rather considerable change in overall entropy when pyruvate as opposed

to lactate is formed; $62.6\,\mathrm{kJ\,mol^{-1}}$ vs. $18.2\,\mathrm{kJ\,mol^{-1}}$, respectively. When lactate as opposed to pyruvate is the end product of glycolysis, a rather large change in enthalpy is seen: heat (energy) is exchanged from the system to the surroundings ($\Delta \mathrm{H} = -40.0\,\mathrm{kJ\,mol^{-1}}$). Entropy actually decreases ($\Delta S = -44.4\,\mathrm{kJ\,mol^{-1}}$) with the reduction of pyruvate to form lactate: glucose-to-pyruvate, $\Delta S = 62.6\,\mathrm{kJ\,mol^{-1}}$; glucose-to-lactate, $\Delta S = 18.2\,\mathrm{kJ\,mol^{-1}}$. Based on this information the largest amount of heat loss during glycolysis is with the reduction of pyruvate to form lactate, "justifying the interpretation that in most cases heat is produced only by lactate formation…" (3). Less heat (energy) is exchanged with the overall metabolic degradation of glucose-to-pyruvate. Moreover, the overall enthalpy change with pyruvate as the end product is positive, indicating heat (energy) flow toward the system, perhaps suggestive of the conversion of this energy to intracellular flow (perfusion/streaming/convection) with a concomitant increase in entropy. In Chap. 12, it will be demonstrated that both the enthalpy and entropy changes of the glucose-to-pyruvate and glucose-to-lactate reactions need to be taken into consideration when cellular and whole-body energy expenditure fueled by glucose oxidation is accounted for as a measurement of oxygen uptake.

Matter and energy are continuously moving into, through, and out of metabolic systems; energy is expended in the process. A working metabolism thus involves scalar fluxes that by definition entail units of quantity (e.g., mass or volume). The flux of heat in scalar (not vector – magnitude and direction) terms has been appropriated to the study of energy expenditure because biochemical reactions invoke the movement of energy (heat) throughout the metabolic (open) system (4, 12, 13). Both acts of ATP turnover, glycolytic ATP resynthesis along with the ATP hydrolysis of muscle contraction, produce heat (energy) that is irreversibly discarded (expended) from the system (14). However, in the context of metabolic power, in terms explicit to heat exchange (possible *conversion* into an organized intracellular current and *transfer* throughout the metabolic system or biochemical pathway), increases in entropy are the result (4, 12, 13).

Environmental heat and entropy increase as a result of biological energy exchange (15, 16). The reactions presented in Table 10.2 suggest that the overall breakdown of glucose-to-pyruvate is entropy driven ($\Delta S = +62.6\,\mathrm{kJ\,mol^{-1}}$). To the contrary, the overall breakdown of glucose-to-lactate appears to be driven primarily by a large overall enthalpy change ($\Delta \mathrm{H} = -40\,\mathrm{kJ\,mol^{-1}}$).

## 10.4 "High-Energy" Phosphate Buffering

At times energy demand within muscle exceeds that of glycolytic ATP supply. Under these conditions other "high-energy" phosphate molecules act as a buffer to rapidly resynthesize ATP, keeping ATP levels high (thereby preventing a drop in the $\Delta$G of ATP; "high energy" is placed in quotes because the energy availability depends on [ATP] and [ADP] concentrations; at equilibrium ATP has no available

energy; see Fig. 7.5). "High-energy" phosphate exchanges are considered a part of the anaerobic metabolic system. Creatine phosphate or phospho-creatine (PC), for example, instantaneously phosphorylates ADP via the enzyme *creatine kinase*:

$$CP + ADP + H^+ \rightarrow Cr + ATP$$

The reaction does not evolve heat to the extent that respiration and glycolysis do during ATP resynthesis (17, 18). It also is of interest that protons ($H^+$) are consumed in this reaction. Thus, at the very onset of intense muscle contraction when PC stores are heavily utilized, metabolic heat production is minimal and pH can momentarily rise as the muscle undergoes slight alkalosis (19).

Creatine phosphate is stored in muscle at about 3–4 times the concentration of ATP, but even so it is still not a lot. Muscle PC levels are high enough to fuel several seconds of all-out physical work. Power output drops precipitously as PC stores fall. As will be demonstrated later, the PC that is expended during exercise undergoes restoration exclusively via aerobic metabolism in the recovery from exercise (20). This must be taken into consideration when estimating energy expenditure during and after intense exercise (Chap. 16).

As intense muscle contraction continues and PC concentrations continue to approach depletion, the union of two ADP molecules also resynthesizes ATP:

$$2ADP \rightarrow ATP + AMP + heat$$

AMP is adenosine monophosphate. The enzyme for this reaction is *myokinase*.

The "high-energy" phosphates are a finite anaerobic energy source; when nearly depleted, fatigue sets in and muscle contraction ceases.

## 10.5 Anaerobic "Speed"

All of the ATP resynthesizing metabolic pathways appear to be activated upon the initiation of muscle contraction (21). It is important to note however that it takes time, several minutes, for aerobic metabolism to reach a maximum rate, while the high-energy phosphate PC achieves maximal rates of ATP resynthesis almost instantly and glycolytic rates take but a second or two to operate at maximum velocity. Maximal power output by muscle is accelerated only for about 10–20 s before declining, suggesting that the rate of anaerobic metabolism may be a potential factor in that decrease (see Fig. 10.3).

The rate of ATP resynthesis has been estimated at 60.0 μmol ATP per gram of wet tissue weight per minute for anaerobic glycolysis, and for the PC and ATP stores 96–360 μmol ATP per gram of wet weight per minute (in comparison the aerobic oxidation of fat and glucose resynthesizes ATP at 20 and 30 μmol ATP per gram of wet weight per minute, respectively) (23). The best experiments to induce rapid metabolic rates involve exercise with skeletal muscle undergoing contractions at high-power outputs. Some researchers have used electrical stimulation of muscle to

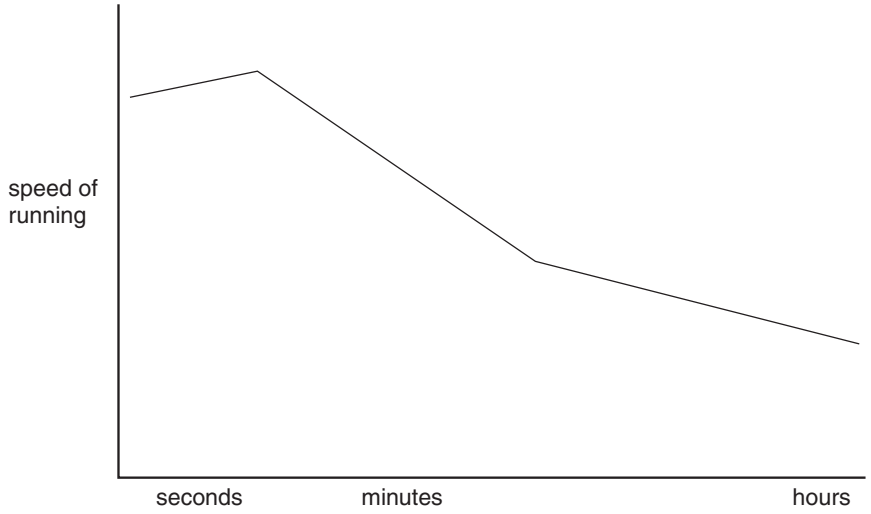


**Fig. 10.3** This logarithmic plot of running speed and time reveals three distinct linear slopes. It has been proposed that the three separate regression lines are indicative of ATP resynthesis contributions by the three metabolic systems: ATP, PC stores (seconds), anaerobic glycolysis (minutes), and aerobic respiration (minutes to hours). Adapted from McGilvery (22)

enhance the contractile effort. To obtain an estimation of anaerobic metabolic rates, a small biopsy is rapidly excised from the muscle that underwent contraction and is quickly frozen. The rapid freezing technique halts all biochemical reactions within the biopsy sample and the contents are then analyzed to determine the extent of their contribution during a given time period (see Table 10.3).

Clearly the greatest contributions to energy expenditure by anaerobic metabolism occur with intense and brief activity. The flow of metabolic reactants and products through the glycolytic metabolic pathway is nothing less than remarkable under these conditions, with net flux reported to be several hundred-fold greater than the rest (26). At such velocities however a compromise is reached because increases in power – including metabolic power – come at an expense. Keep in mind that anaerobic glycolysis generates only two ATPs when beginning with a single glucose moiety, so that many, many glucose moieties are needed (coming from

**Table 10.3** ATP resynthesis rates – millimoles of ATP per kilogram of dry muscle per second

| Exercise | Duration | PC | Glycolysis | Reference |
|---|---|---|---|---|
| Electrical stimulation | 1.28 s | 9.0 | 2.0 | (24) |
| | 2.56 s | 5.0 | 5.3 | (24) |
| | 3.0 s | 5.0 | 2.8 | (24) |
| Cycling (140 rpm) | 10 s | 4.4 | 9.3 | (25) |
| | 30 s | 0.7 | 6.5 | (25) |

glycogen) to provide a large amount of ATP. As will be shown in the next chapter, aerobic metabolism resynthesizes 17 times the ATP per molecule of glucose than does anaerobic glycolysis. A trade-off arises: efficient ATP supply or high-power output. Gnaiger has elegantly categorized this dichotomy in stating that, "maximal values of both (ATP supply and power output) cannot be achieved simultaneously" (27). In this regard, biological organisms have an apparent "metabolic choice" when attempting to sustain muscle contractions, accelerated glucose or glycogen breakdown coupled with rapid but limited anaerobic ATP supply (two ATPs per glucose molecule) or slower glucose or glycogen breakdown coupled to a more abundant aerobic ATP supply (36 ATPs per glucose molecule). Athletes, human or not, utilize anaerobic metabolism to supply the energy demands of strength-, speed- and power-related activities.

As fast as use of the ATP, PC stores and anaerobic glycolysis are it is apparent that working muscle never relies solely on a single metabolic system. At the initiation of any type of contraction, activation of both anaerobic metabolic pathways and the aerobic metabolic pathway takes place. Taking the concept that all metabolic systems operate simultaneously to the extreme, Shulman and Rothman have proposed the *glycogen shunt* hypothesis (28). Under the strict condition of easy to moderately intense rhythmic (pulsatile) muscle contraction, they propose that PC is broken down to resynthesize ATP within the first 15 ms of contraction. In the remainder of the contractile phase lasting from 15 to 100 ms, PC is resynthesized from glycogenolysis and glycolysis. The last phase of the glycogen shunt hypothesis takes place in the recovery phase of a rhythmic contraction and involves the rapid resynthesis of glycogen via glucose from the bloodstream and mitochondria's aerobic metabolic pathways (Chap. 11).

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