Fueling Normal and Diseased Hearts: Myocardial Bioenergetics

21

Arthur H.L. From and Robert J. Bache

Abstract

Cardiac contractile performance depends upon: (1) the delivery of carbon substrates and oxygen present in the blood to the cardiac extracellular space (via the coronary circulation), (2) the ability of the cardiomyocytes to efficiently extract these substrates from the extracellular space, and (3) the pathways via which the chemical energy stored within the carbon substrates is transferred to adenosine triphosphate (ATP), an energy storage molecule that can be directly utilized by most chemical energy driven processes. Importantly, ATP synthetic capacity must be sufficient to support a wide range of energy demands with high rates of ATP generation and must not be associated with destabilization of cytosolic and intraorganelle chemical milieus. The latter characteristic is crucial if the performance of the contractile apparatus and intracellular organelles is to remain optimal over the broad range of cardiac work states required by a physically active organism. Hence, even a high rate of myocardial energy expenditure must not induce the fatigue that is known to develop in heavily working skeletal muscle. This chapter describes the ways in which the chemical energy stored in ingested carbon substrates (glucose, fatty acids, and, to a modest extent, proteins) is transferred to ATP and reviews some of the regulatory systems which integrate the function of these pathways and make them responsive to changes in ATP demand without destabilizing the intracellular chemical milieu. The generation of toxic by-products of the metabolic processes and mechanisms that limit their adverse effects are also reviewed. Last of all, the effects of several physiological states and diseases on these processes are briefly discussed, and the concept that the diseased heart may be energy limited is presented.

Keywords

Adenosine triphosphate • Myocardial blood flow • Glucose metabolism • Fatty acid metabolism • Electron transport • Oxidative phosphorylation • Regulatory processes • Stable intracellular chemical milieu reactive oxygen species

Abbreviations

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated kinase
ATP	Adenosine triphosphate
$FADH_2$	Flavin adenine dinucleotide
LDH	Lactic acid dehydrogenase
NADH	Nicotinamide adenine dinucleotide
NEFA	Nonesterified free fatty acids

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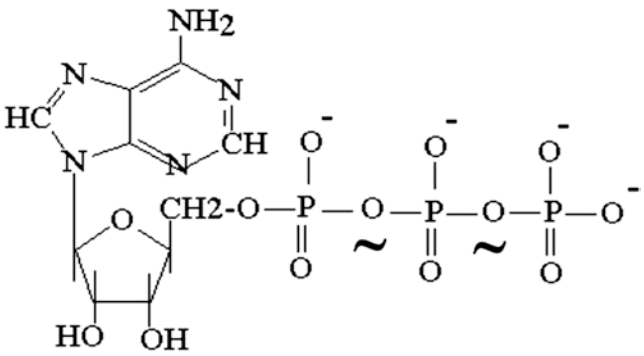
PDH	Pyruvate dehydrogenase
ROS	Reactive oxygen species
TCA	Tricarboxylic acid
VLCAD	Very long-chain acyl-CoA dehydrogenase

21.1 Introduction

In the interest of putting the subject of *myocardial metabolism* into a broader perspective, it should be understood that the heart is ultimately a *solar-powered organ* (as are all other organs). Virtually, all energy-driven biological processes in our biosphere are ultimately dependent upon energy radiated by the sun (in the form of photons). Photons are captured by plants, and their energy is used to synthesize energy-rich carbon molecules that are then used to fuel many biological processes. In green plants, a portion of the solar photon energy is first trapped by chlorophyll and then stored in the form of highly energetic chemical bonds resident in adenosine triphosphate (ATP) and NADPH (Fig. 21.1); the equation for this process is as follows:

$$2H_2O + 2NADP^+ + 3ADP + 3P_i + \text{light} \rightarrow 2NADPH + 2H^+ + 3ATP + O_2$$

The distal phosphate bonds of ATP store high levels of chemical energy. In plants, the energy released by breakdown of the terminal high-energy phosphate bond of ATP and the reduction of NADPH is used to drive the synthesis of simple carbohydrates and ultimately glucose. The chemical energy stored in glucose can be released in a controlled fashion by enzyme-catalyzed reactions and drives the synthesis of all other species of biomolecules including fatty acids (another convenient storage molecule for chemical energy) and amino acids, as well as for resynthesis of ATP. Hence, because animals are unable to directly convert solar photon energy to a storage form of chemical energy, simple and complex animal life is ultimately powered by plants that carry out photosynthesis. The ingestion of plants and (other) animals containing energy-rich molecules supplies animals with carbohydrates, fatty acids, and amino acids that can be metabolized to support the generation of ATP.



ATP

Fig. 21.1 Adenosine triphosphate (*ATP*). ATP is the major source of chemical energy used to power the reactions that support contractile and other processes in myocardium and all other living tissues. The ~ symbol is used to designate a phosphate bond which has a very high level of stored chemical energy. Although ATP contains two ~ phosphate bonds, it is the hydrolysis of the terminal phosphate bond that releases the energy that directly powers cellular processes

In the myocardium, as in other biologic tissues, most energy-driven processes use ATP as the immediate source of energy. Hydrolysis of the terminal phosphate bond of ATP releases energy that can be captured and used to drive (energy-dependent) processes such as protein synthesis, muscle contraction, ion transport, etc. Therefore, the ability of the heart to pump blood to the pulmonary and systemic circulations is dependent on the presence of adequate concentrations of ATP. Unfortunately, myocardial stores of ATP are modest in relation to the rate of expenditure, and continuous ATP synthesis is required to support energy-requiring processes. Importantly, most of the energy required for ATP synthesis is derived from the controlled breakdown of the chemical bonds in carbohydrates (glucose) and fatty acids; body protein-derived amino acids are used to fuel ATP generation only when supplies of the major fuels are compromised, as would be the case during a period of starvation.

To summarize, this chapter will describe: (1) how carbon substrates and oxygen are delivered to the heart, (2) the biochemical pathways within the heart (most of the same pathways are present in all tissue types) that transfer the chemical bond energy stored in carbon substrates to ATP, (3) some of the ways that these pathways are regulated such that they are capable of responding to the increased ATP demand associated with increased cardiac work states, and (4) some of the alterations in these processes that occur under changing physiological states in normal and diseased hearts. The reader should understand that this relatively brief summary of myocardial metabolism is, by necessity, an extremely superficial overview of the individual topics. For those readers wishing to review some of the major topics in greater detail, a list of recent references—primarily current topical reviews, research reports, and a standard biochemistry textbook—is provided at the end of the chapter.

21.2 Myocardial Blood Flow: Carbon Substrate and Oxygen Delivery to the Heart

Blood containing carbon substrates and oxygen is delivered into human and animal hearts (see Chap. 6) by coronary arteries that originate from the proximal aorta. These arteries then subdivide to form progressively smaller branches that arborize inward throughout the ventricular wall and

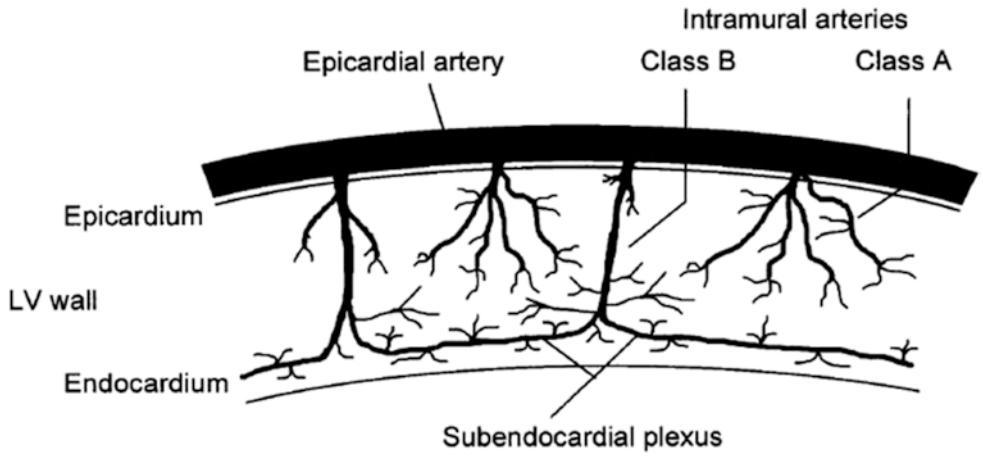


Fig. 21.2 Transmural distribution of the coronary arterial system. The large epicardial conductance arteries supply shallow and deep branches to the subepicardium and subepicardium, respectively. These perforating vessels arborize to create the arteriolar network that supplies the myocardial capillary bed. *LV* left ventricle. Reprinted from Duncker DJ and Bache RJ, Regulation of coronary vasomotor tone under normal conditions and during acute myocardial hypoperfusion, Pharmacol Ther, Vol. 86, 87–110, 2000. Pharmacology and Therapeutics (by International Union of Pharmacology). Reproduced with permission of Elsevier Inc., in the format Book via Copyright Clearance Center

supply blood to the myocardium (Fig. 21.2). The left ventricular wall (for descriptive purposes) is arbitrarily subdivided into transmural layers termed the subepicardium (outermost layer), the midmyocardium, and the subendocardium (innermost layer). The coronary arterial tree terminates in muscular vessels 60–150 mm in diameter termed *arterioles*. The arterioles are the major locus of resistance to blood flow, and contraction or relaxation of the smooth muscle in the walls of the arterioles (vasomotion) provides the mechanism for control of the rate of blood flow into the myocardium. Each arteriole supplies an array of capillaries, thin-walled tubes comprised of a single layer of endothelial cells, across which most of the exchange of nutrients, oxygen, and metabolic waste products occurs. As their terminal end capillaries coalesce into venules, the initial component of the cardiac venous system then conducts most postcapillary blood back into the cardiac chambers, primarily through the coronary sinus that drains into the right atrium. For more details on the coronary circulation, see Chap. 8.

21.2.1 Regulation of Myocardial Blood Flow

Coronary blood flow is highly regulated and falls or rises appropriately in response to subtle or large changes in the rate of myocardial energy expenditure. The principal purpose of the heart is to generate the pressure that forces blood from the ventricles into the pulmonary and systemic circulations. Elevation of the cardiac rate of ATP expenditure during exercise or other stresses increases myocardial demand for oxygen and carbon substrates. In clinical practice, there is often a need to assess the effects of changes of the rate of myocardial energy expenditure on cardiac performance (e.g., during exercise stress testing). Since routine direct measurement of myocardial oxygen consumption is not practical, a rough estimate of the change in the energy demands of the heart is afforded by the product of systolic blood pressure multiplied by the times per minute that pressure generation occurs (heart rate); this is termed the *rate-pressure product*. This measurement provides a simple estimate of changes in the metabolic requirement of the heart resulting from different cardiac work states in animals and humans.

The coronary circulation operates on the principle of "just-in-time" delivery of oxygen and carbon substrates. In other words, coronary blood flow is regulated to be only minimally greater than required to meet the instantaneous metabolic demands of the heart. Furthermore, the heart extracts 70–80 % of the O_2 from the blood as it flows through the coronary capillaries. Because of this high level of basal oxygen extraction, there is little ability to increase oxygen uptake by means of increased extraction of oxygen from the blood. As a result, increases in myocardial energy requirement during exercise or other stresses must be satisfied by concomitant increases of coronary blood flow. As noted, ATP and oxygen stores in the myocardium are relatively small. Therefore, the response time for the increase in coronary flow following an increase in cardiac work must be rapid (i.e., a few seconds). From these considerations, it is clear that highly responsive signaling systems exist which link the rate of myocardial energy expenditure to vasomotor activity in the resistance vessels that control coronary blood flow. Interestingly, these signaling systems are not fully understood despite intense study over the last 100 years.

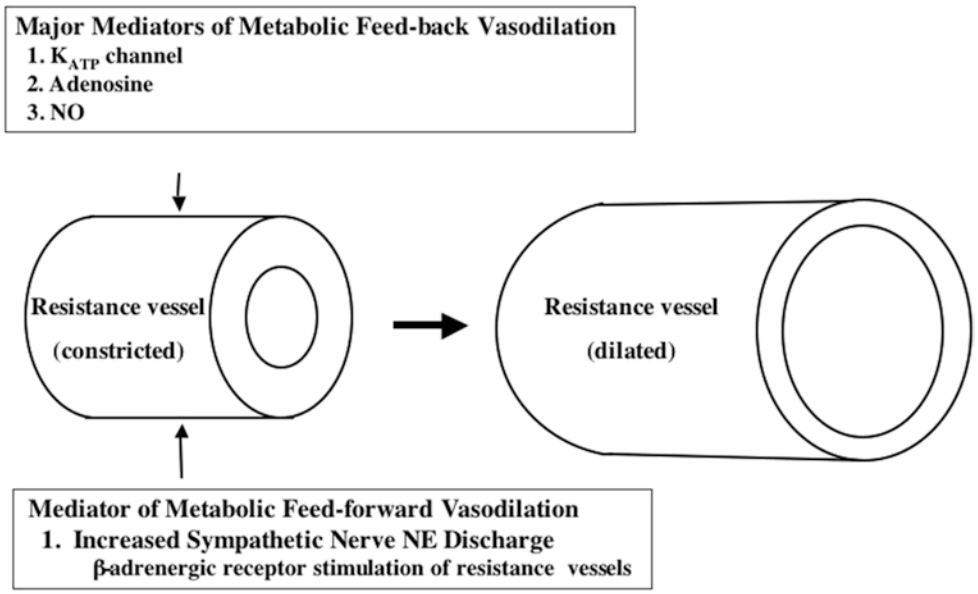


Fig. 21.3 Major feedback and feed-forward mechanisms underlying metabolic vasodilatation of resistance vessels are depicted (see text for discussion). *K_{ATP} channel* ATP-inhibited potassium channel, *NO* nitric oxide

21.2.2 Signaling Pathways Regulating the Coronary Circulation

The regulatory signals that increase blood flow in response to increases/decreases of cardiac work can be classified as having feedback or feed-forward characteristics; the final common response to these signals is relaxation/contraction of vascular smooth muscle cells within the arteriolar resistance vessels that control coronary blood flow (Fig. 21.3). Several major feedback mechanisms resulting from increased cardiomyocyte metabolism (including adenosine, nitric oxide, ATP, H_2O_2, and others) cause opening of ATP-sensitive potassium channels (K_{ATP}) located within the sarcolemma of arteriolar smooth muscle cells. Opening of these channels allows potassium to escape from the cytosol of the smooth muscle cells, resulting in hyperpolarization (increased negativity) of the arteriolar smooth muscle cell membrane. The increased negativity of the membrane causes sarcolemmal voltage-dependent calcium channels to close; as a result, calcium entry into the smooth muscle is reduced, the vessel relaxes (i.e., dilates), and coronary blood flow increases. In addition to effects on the K_{ATP} channels, adenosine (a product of ATP utilization in the cardiomyocyte) also has potent, direct dilator effects on arteriolar smooth muscle. Another metabolic feedback signal is nitric oxide generated by the vascular endothelium. Mechano-transduction of flow-induced shear forces exerted on the endothelial cells augments nitric oxide synthesis. In addition to causing potassium channel opening, nitric oxide also initiates direct relaxation processes in vascular smooth muscle. It is important to note that this brief discussion does not include many of the known feedback mechanisms involved in regulation of coronary blood flow.

Notably, increased cardiac sympathetic nerve activity (i.e., during exercise) activates a feed-forward mechanism for control of coronary blood flow which augments the local metabolic vasodilator influences. The sympathetic neurotransmitter norepinephrine activates α- and β-adrenergic receptors located within the sarcolemma of arteriolar smooth muscle cells. Activation of similarly located α-adrenergic receptors causes modest constriction of the large coronary arteries; since these arteries function as conduit vessels that offer little resistance to blood flow, this has little effect on coronary flow. However, activation of arteriolar β-adrenergic receptors results in relaxation (vasodilation) of these small resistance vessels; the resultant decrease of coronary resistance causes a feed-forward increase in blood flow that is independent of local metabolic mechanisms and augments the increase of coronary blood flow during exercise.

Pharmacologic studies have shown that simultaneous blockade of the coronary K_{ATP} channels, adenosine, and nitric oxide pathways significantly decreases myocardial blood flow in the resting animal. Moreover, the increase of coronary flow that normally occurs during exercise is severely blunted, resulting in a perfusion-metabolism mismatch that is accompanied by evidence of ischemia even in the normal heart. Hence, activation of these three pathways appears to be the primary means by which metabolic vasodilatation is achieved in the heart. However, in the healthy heart, blockade of any one of these mechanisms for smooth muscle relaxation will elicit compensatory (i.e., increased) activation of the other pathways to minimize changes in coronary blood flow. Lastly, other (not discussed) circulatory regulatory mechanisms may also be of biological significance.

21.2.3 Blood Flow in the Diseased Heart

Coronary blood flow in the diseased heart can be limited by: (1) partial or complete obstruction of the large coronary arteries (e.g., atherosclerotic disease), (2) decreased responsiveness of the signaling systems relating blood flow to energy requirements, and/or (3) increases in extravascular mechanical forces acting to compress the small vessels in the wall of the left ventricle. In the case of obstructive (generally atherosclerotic) disease of the epicardial coronary arteries, a moderately narrowed vessel may only restrict blood flow during periods of increased blood flow demand (i.e., it reduces vasodilator reserve), while a severely narrowed vessel may limit blood flow even when the subject is at rest. In the presence of a moderate coronary obstruction, the arteriolar bed can maintain adequate blood flow by metabolic signaling-based arteriolar vasodilatation. That is, a decrease in small vessel resistance can compensate for the increased resistance caused by a proximal coronary artery stenosis. However, when the capacity for vasodilation of the arterioles has been exhausted, any further increase in cardiac work cannot induce an increase of blood flow, and the myocardium supplied by the narrowed epicardial vessel will become ischemic. There is also considerable evidence that malfunction of metabolic signaling pathways in the arteriolar resistance vessels (e.g., in the absence of obstructed large coronary arteries) can cause myocardial ischemia in certain patients.

In the normal heart, blood flow to the inner layers of the left ventricle occurs principally during diastole. This is because tissue pressures in the wall of the left ventricle during systole are so great that inner layer arterioles are squeezed shut by the extravascular compressive forces produced by cardiac contraction. Diastolic left ventricular tissue pressures are also greatest in the subendocardium. When the heart fails and/or becomes hypertrophied, these extravascular compressive forces increase as left ventricular filling pressure increases. In the hypertrophied or failing heart, slowing of myocyte relaxation also shortens the duration of the diastolic interval and thus limits coronary flow reserve in the inner cardiac layers. In the normal heart, autoregulatory (i.e., metabolic vasodilatation) processes cause enough arteriolar vasodilation in the subendocardium to compensate for systolic underperfusion. However, increased left ventricular diastolic pressure (and myocardial tissue pressure) in the failing heart may compress the arteriolar bed in the inner myocardial layers sufficiently to overwhelm autoregulatory mechanisms, particularly those that normally maintain adequate subendocardial blood flow. Since subendocardial blood flow occurs almost exclusively during diastole, tachycardia also acts to impede blood flow in the subendocardium by shortening of the diastolic interval. Thus, even in the absence of obstructive coronary artery disease or intrinsic arteriolar abnormalities, increased arteriolar compression can limit blood flow to the inner myocardial layers of the diseased heart. Importantly, if these abnormalities limit substrate delivery to the inner myocardial layers, they will disrupt the balance between ATP synthetic capacity and ATP utilization in this region of the ventricular wall. Thus, the extravascular forces cause the subendocardium to be the region of the ventricular wall that is most vulnerable to hypoperfusion and ischemia.

21.3 Intermediary Metabolism and Bioenergetics in the Normal Heart

Glucose and fatty acids are the main substrates consumed by the heart, with fatty acid consumption predominating under most circumstances (Fig. 21.4). Exceptions to this statement will be discussed later.

21.3.1 Glucose Metabolism

Figure 21.5 shows a flowchart for glucose metabolism. Glucose enters the cardiomyocyte via the sarcolemmal glucose transport proteins, GLUT 1 (that is insulin independent) and GLUT 4 (that is insulin dependent). Once in the cell, glucose is phosphorylated to glucose-6-phosphate by the enzyme hexokinase. Since glucose-6-phosphate is membrane impermeable, this effectively traps glucose within the cell. Glucose-6-phosphate can enter the glycogen synthesis pathway (glycogen is a macromolecular polymeric storage form of glucose), the pentose monophosphate shunt (that generates NADPH and ribose, moieties utilized in many important cellular processes), or it can undergo molecular rearrangement via the enzyme phosphohexose isomerase to form fructose-6-phosphate and continue on through the glycolytic series of reactions. A second phosphorylation of fructose-6-phosphate via phosphofructokinase generates fructose-1,6-bisphosphate. Each of these phosphorylations consumes one molecule of ATP. Fructose-1-6-bisphosphate is next split into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate by the enzyme aldolase. These two molecules are in constant exchange with each other via the enzyme phosphotriose isomerase. Glyceraldehyde-3-phosphate is then phosphorylated to form 1,3-bisdiphosphoglycerate by the enzyme glyceraldehyde-3-phosphate dehydrogenase. The phosphate bond in the 1 position is a high-energy-containing bond (signified by the ~P symbol); this reaction also simultaneously reduces NAD^+ to NADH. Cytosolic oxidation of NADH and transport of the two removed electrons and H^+ into the mitochondrial matrix then occur in cardiac muscle via the malate-aspartate shuttle (not shown in Fig. 21.5). In the mitochondrial matrix, NAD^+ is then reduced back to NADH which can be utilized by the mitochondria to generate ATP (to be discussed later). In the next cytosolic reaction, the high-energy phos-

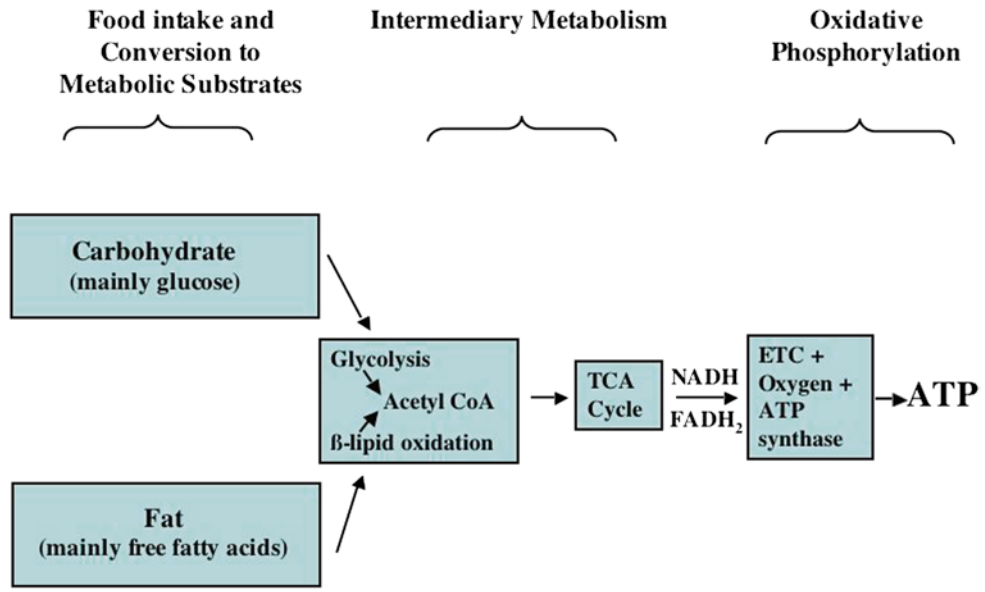


Fig. 21.4 A general overview of carbon substrate metabolism in the heart. First, ingested food is broken down into usable carbon substrates, primarily amino acids (not major contributors to ATP synthesis), glucose, and fatty acids. The pathways which convert amino acids and other molecules to glucose are not shown. Glucose and fatty acids are processed (via intermediary metabolic processes) to yield the reducing equivalents, NADH and $FADH_2$, which supply the energy necessary to power oxidative phosphorylation. The latter process, which occurs within the mitochondria in the presence of oxygen, supplies almost all of the ATP synthesized and utilized in the heart. *ATP* adenosine triphosphate, *ETC* electron transport chain, *$FADH_2$* flavin adenine dinucleotide, *NADH* nicotinamide adenine dinucleotide, *TCA* tricarboxylic acid

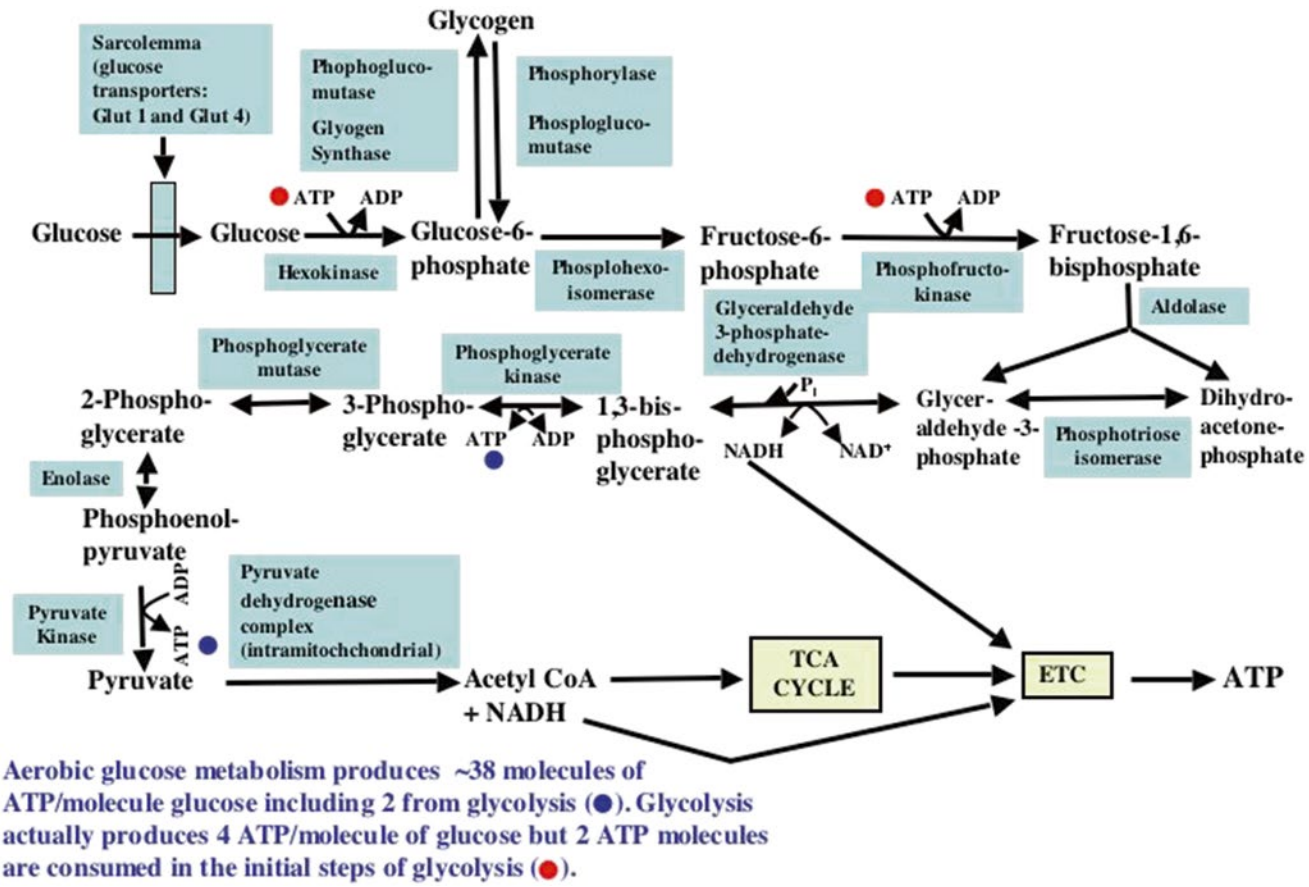


Fig. 21.5 Flowchart of cellular uptake of glucose and the pathways through which glucose metabolism proceeds. See text for discussion. *ADP* adenosine diphosphate, *ATP* adenosine triphosphate, *ETC* electron transport chain, *NADH* nicotinamide adenine dinucleotide, *TCA* tricarboxylic acid

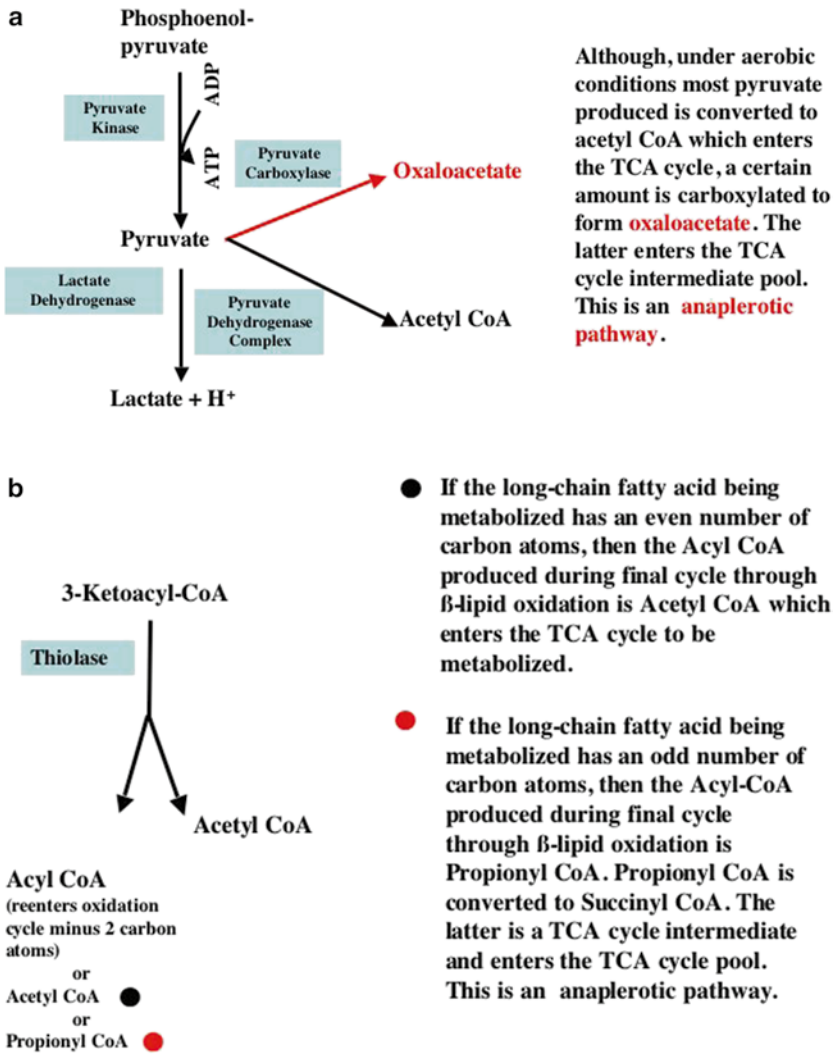


Fig. 21.6 Anaplerotic and cataplerotic processes. Anaplerotic processes are those which supply substrate used to maintain the TCA cycle intermediate pool size. In contrast, cataplerotic processes remove substrates from the TCA cycle intermediate pool and decrease its size. The balance between these two processes determines the TCA cycle pool size. (**a**) Shows how glucose (via its metabolic product, pyruvate) contributes to anaplerosis. (**b**) Shows how the β-oxidation of odd-numbered carbon chain fatty acids contributes to anaplerosis. (Note that readers may want to review Fig. 21.13, a flowchart for β-lipid oxidation, before examining (b) which depicts the terminal reaction of β-lipid oxidation.) *ADP* adenosine diphosphate, *ATP* adenosine triphosphate, *TCA* tricarboxylic acid

phate bond in 1,3-bisdiphosphoglycerate is transferred to adenosine diphosphate (ADP) to form ATP and 3-phosphoglycerate via the enzyme phosphoglycerate kinase. The latter molecule is converted to 2-phosphoglycerate by the enzyme phosphoglycerate mutase. Enolase, another cytosolic enzyme, then converts 2-phosphoglycerate to the ~P-containing molecule phosphoenolpyruvate. The latter is converted to pyruvate by the enzyme pyruvate kinase. During this reaction, the ~P in phosphoenolpyruvate is transferred to ADP to form a second ATP molecule. Pyruvate can then enter the mitochondria to be further metabolized by the pyruvate dehydrogenase (PDH) complex of enzymes. Both the pyruvate dehydrogenase complex that converts pyruvate to acetyl-CoA (and NAD^+ to NADH), and the tricarboxylic acid (TCA) cycle that metabolizes acetyl-CoA are located within the mitochondria (Figs. 21.5, 21.6a, and 21.7). Within the mitochondria another metabolic pathway for pyruvate metabolism also exists. The enzyme, pyruvate carboxylase, converts pyruvate to oxaloacetate, which is a TCA cycle intermediate (Figs. 21.6a and 21.8). The significance of the latter reaction will be discussed later.

Within the cytosol, pyruvate can be converted to lactic acid by lactic acid dehydrogenase (LDH). Lactate and a hydrogen ion are then exported from the cell via the monocarboxylic acid transporter (Figs. 21.5, 21.6a, and 21.9). This pyruvate to lactic acid reaction is associated with the oxidation of NADH to NAD^+ and, as will be shown, is critical to maintaining glycolysis when the availability of oxygen to the cardiomyocyte is limited. Conversely, under aerobic conditions, lactate in the blood can be transported into the cardiomyocyte by the monocarboxylic acid transporter, to be converted to pyruvate by LDH. The LDH-catalyzed reaction also reduces NAD^+ to NADH, and the reducing equivalents from NADH can be transferred into the mitochondrial matrix by the malate-aspartate shuttle, and the pyruvate generated is available for processing by PDH.

During glycolysis of one glucose molecule, two pyruvate molecules, four ATP molecules, and two NADH molecules are produced. However, because two ATP molecules are consumed early in the glycolytic pathway, the net production of ATP in the cytosol is two molecules/glucose molecule. As previously indicated, pyruvate and NADH are utilized in the

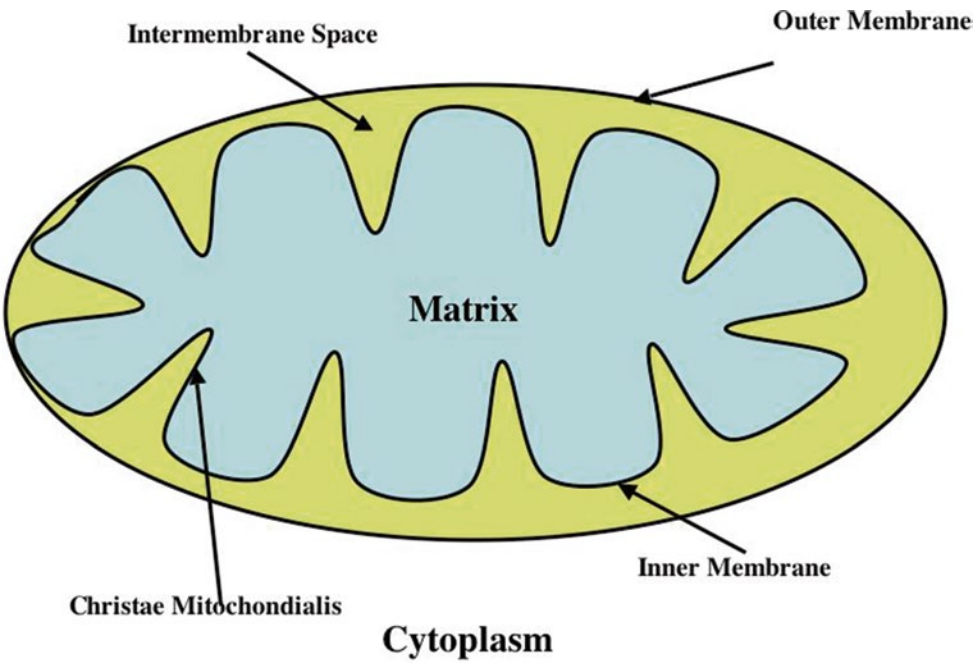


Fig. 21.7 Major features of mitochondrial morphology. See text for discussion

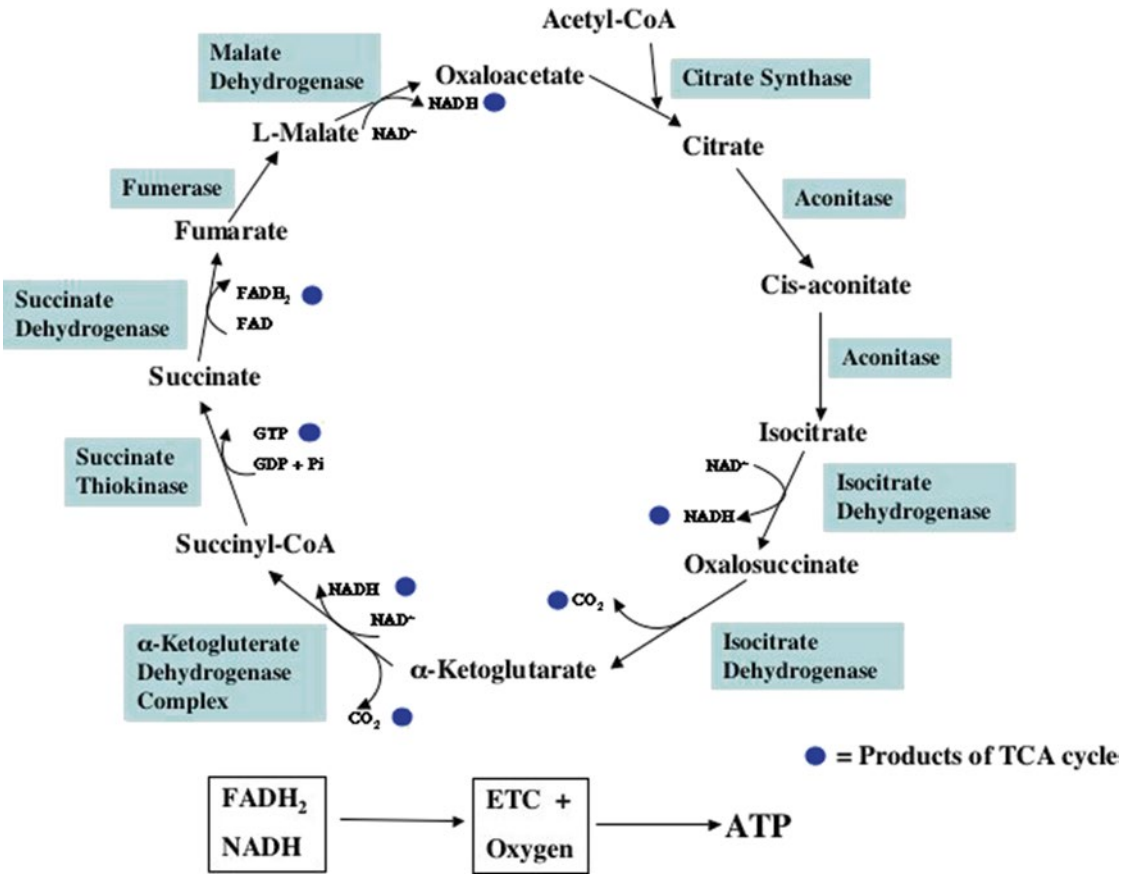


Fig. 21.8 Tricarboxylic acid (*TCA*) cycle. The filled blue circles are the products resulting from one turn of the TCA cycle. See text for discussion. *ATP* adenosine triphosphate, *ETC* electron transport chain, *$FADH_2$* flavin adenine dinucleotide, *NADH* nicotinamide adenine dinucleotide, *TCA* tricarboxylic acid

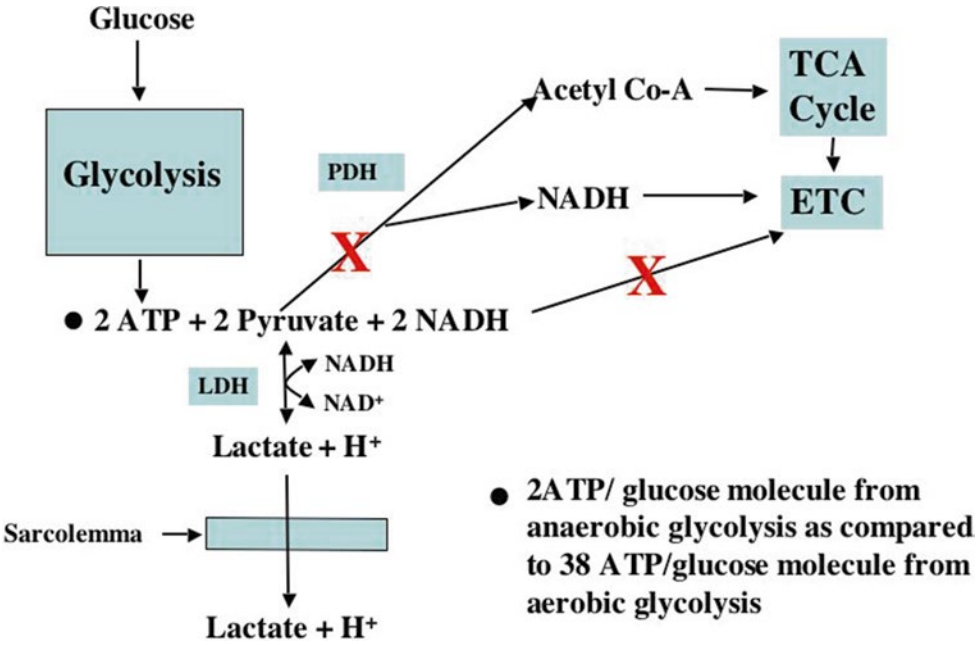


Fig. 21.9 Flowchart for anaerobic glucose metabolism. A *large red X* indicates metabolic pathways blocked during ischemia. See text for discussion. *ATP* adenosine triphosphate, *ETC* electron transport chain, *LDH* lactic acid dehydrogenase, *NADH* nicotinamide adenine dinucleotide, *PDH* pyruvate dehydrogenase, *TCA* tricarboxylic acid

mitochondria for oxidative generation of ATP. Complete metabolism of one glucose molecule (i.e., including oxidation of the products of glycolysis in mitochondria) results in the formation of many additional ATP molecules (30–36, depending on the literature cited) than does glycolysis alone. However, under conditions when mitochondrial function is severely oxygen limited, the oxidative contribution to ATP synthesis is lost, and only two ATP molecules can be formed from each glucose molecule.

21.3.2 The Mitochondrion

Mitochondria, which are the primary site of ATP synthesis in most mammalian cells, contain the β-lipid oxidation pathway enzymes, the TCA cycle enzymes, the electron transport chain, and the F_1F_0-H^+-ATPase (also called F_1F_0-ATP synthase or ATP synthase). To better understand the location of these systems, mitochondrial structure will be briefly reviewed (Fig. 21.7). The inner mitochondrial membrane contains the electron transport chain, F_1F_0-H^+-ATPase, the adenine nucleotide translocase, and other transporters. The mitochondrial matrix contains the TCA cycle enzymes, β-lipid oxidation enzymes, and many other enzymes and reactants. The cristae are invaginations that markedly expand the surface area of the inner membrane and, thereby, the quantity of energy generation-associated proteins that can be contained within the inner membrane. The inner membrane-bound components of the metabolic pathways are positioned to optimize the flow of substrates through their reaction sequences. The mitochondrial outer membrane forms a boundary between the cellular cytoplasm and the mitochondrial intermembrane space. The intermembrane space contains creatine kinase which is important for high-energy phosphate transport out of mitochondria (Figs. 21.7, 21.10, and 21.11) and cytochrome c, a component of the electron transport chain. The importance of the intermembrane space to oxidative ATP synthesis will be discussed subsequently. The outer mitochondrial membrane also contains voltage dependent anion channels (VDAC) through which ATP and other moieties exit the intermembrane space and through which ADP and many other molecules enter the intermembrane space.

21.3.3 Fatty Acid Metabolism

Figure 21.12 presents a flowchart for the β-lipid oxidation pathway. Dietary long-chain, nonesterified, free fatty acids (NEFAs) are generally the predominant carbon substrate in normal myocardium. They are transported in the blood bound to plasma albumin, lipoprotein moieties, or in the form of triacylglycerol which is also bound to albumin. The latter can be broken down to release NEFAs by an enzyme present in the plasma and at the surface of the capillary and cardiomyocyte. After dissociating from albumin, NEFAs are transported accross capillary walls and into cardiomyocytes by fatty acid transport proteins. Within the cell, NEFAs are bound to fatty acid-binding proteins which provide solubility and intracellular transport. Once in the cell, NEFAs are either reesterified and stored as triglycerides or activated by acyl-CoA synthetase (which requires the presence of free CoA and ATP) to form a long-chain acyl-CoA. Because long-chain acyl-CoA cannot readily diffuse through the mitochondrial inner membrane, it is converted to long-chain acyl carnitine by carnitine palmitoyltransferase 1 at the outer surface of the inner mitochondrial

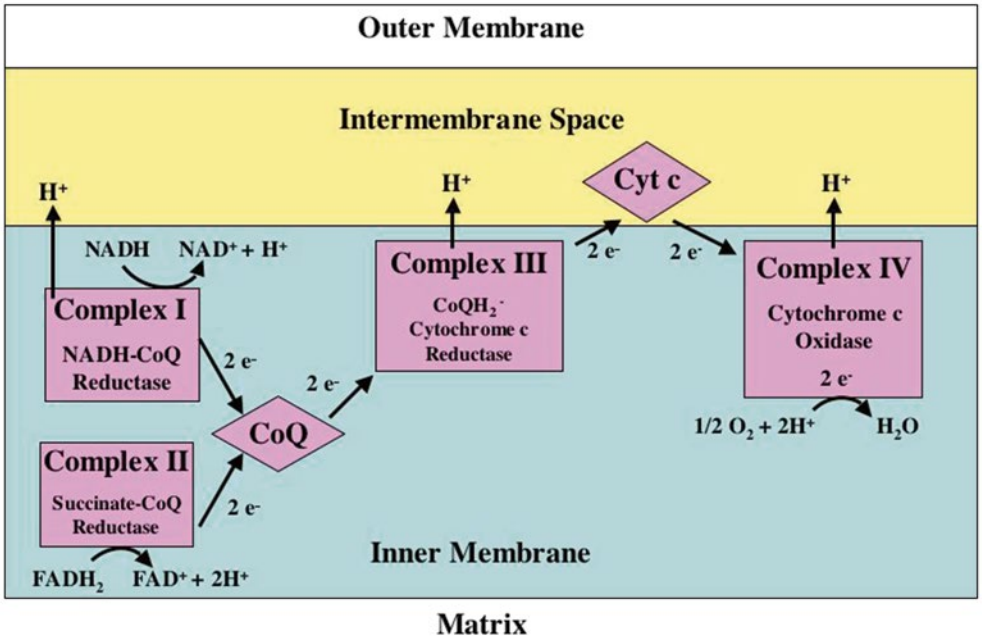


Fig. 21.10 Electron transport chain (*ETC*). See text for discussion. *$FADH_2$* flavin adenine dinucleotide, *NADH* nicotinamide adenine dinucleotide

membrane and then transported across the inner membrane by carnitine-acylcarnitine translocase (which also transports free carnitine liberated by carnitine palmitoyltransferase 2 back into the intermembrane space; see below). The long-chain acyl carnitine is next converted back to acyl-CoA at the inner surface of the mitochondrial inner membrane by carnitine palmitoyltransferase 2. Long-chain acyl-CoA is then processed by a sequence of enzyme-catalyzed reactions that comprise the β-lipid oxidation pathway. If the long-chain acyl-CoA has an even number of carbon atoms, then the final products of the last cycle (i.e., the metabolism of a four-carbon acyl-CoA molecule) through the β-oxidation sequence are two acetyl-CoA molecules (Fig. 21.6b). However, if the last long-chain acyl-CoA has an odd number of carbon atoms (i.e., 5), then the products of the last cycle through β-oxidation are one acetyl-CoA and one propionyl CoA. Unlike acetyl-CoA, propionyl-CoA cannot enter the TCA cycle. However, propionyl-CoA is readily converted to succinyl-CoA, a TCA cycle intermediate (Fig. 21.6b). Hence, this is another pathway that contributes to the maintenance of the TCA cycle intermediate pool size. Both pyruvate molecules produced from glucose and odd-numbered fatty acid molecules that undergo complete β-oxidation contribute to maintenance of the TCA intermediate pool. Processes that add molecules to the TCA intermediate pool are termed *anaplerotic* (Fig. 21.6a, b), and those that remove intermediates from the pool are called *cataplerotic*. The importance of these processes to TCA cycle function will be illustrated in a clinical example of an inborn metabolic abnormality to be presented later.

The products of complete β-oxidation of a fatty acid are acetyl-CoA (and propionyl-CoA if the chain has an odd number of carbons), NADH, and flavin adenine dinucleotide ($FADH_2$). However, consumption of these products by the TCA cycle and the electron transport chain cannot occur in the absence of oxygen. Thus, β-oxidation cannot occur under anoxic or ischemic conditions, and markedly ischemic myocardium does not support either pyruvate- or fatty acid-derived ATP synthesis. Hence, during ischemia, the only source of ATP synthesis is anaerobic glycolysis.

21.3.4 Regulation of Carbon Substrate Metabolic Pathways

Myocardial glycolysis is regulated at several levels (some of which are shown in Fig. 21.13a); the first is entry of glucose into the cell. In the heart and skeletal muscle, this process is largely dependent on the activity of the GLUT 4 glucose transporter. The quantity of the GLUT 4 transporter in the plasma membrane is determined by the action of insulin on the sarcolemmal insulin receptor, the activation of which begins a sequence of biochemical events that ultimately triggers migration of GLUT 4 molecules to the plasma membrane from cytoplasmic storage sites. Increased levels of fatty acid metabolites in cardiomyocytes inhibit insulin receptor activation and the consequent transport of GLUT 4 to the plasma membrane. Blood glucose levels are generally well defended by an organism, so glucose availability is not usually limiting to transport. Rather, the number of GLUT 4 molecules present in the plasma membrane usually determines the rate of glucose transport. In addition to insulin-associated increases, plasma membrane GLUT 4 levels are also enhanced during

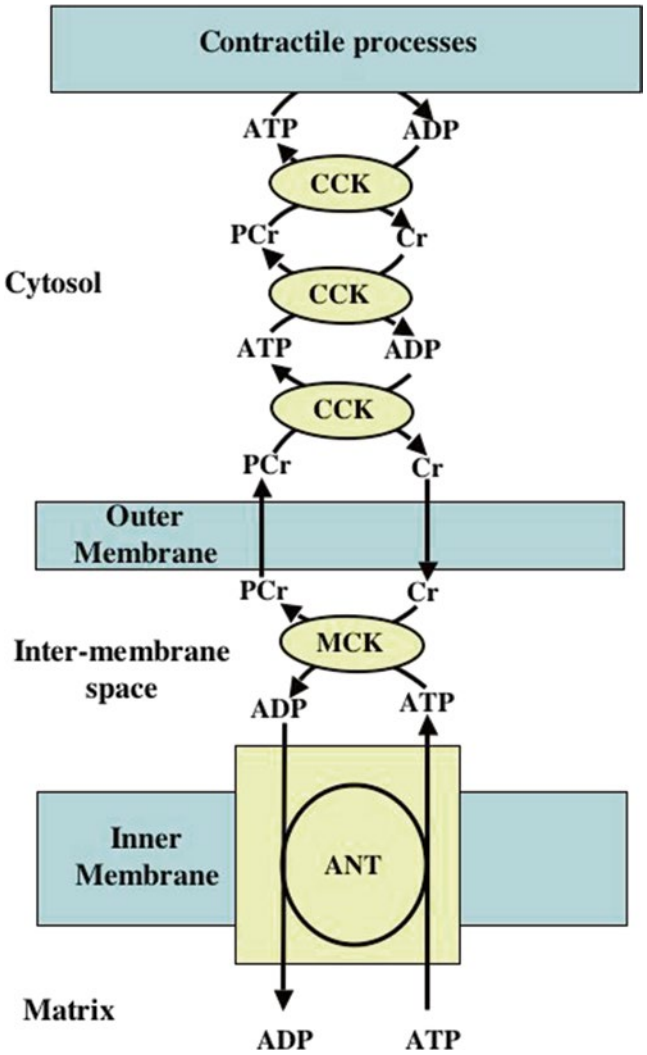


Fig. 21.11 Creatine kinase shuttle hypothesis. The diffusion rates of ATP out of the intermembrane space and through the cytosol and ADP through the cytosol and into the intermembrane space are considered to be relatively slow compared to those of creatine and creatine phosphate. The creatine kinase shuttle is thought to facilitate the transfer of ADP from sites of ATP utilizing reactions to the mitochondrial intermembrane space and also to facilitate the transfer of ATP to the sites of utilization. The way the shuttle is proposed to function is described below. The adenine nucleotide transporter (*ANT*), which is not rate limiting, transports ADP from the mitochondrial intermembrane space to the mitochondrial matrix where it is rephosphorylated back to ATP. The ANT simultaneously transports newly synthesized ATP from the matrix to the intermembrane space. Within the intermembrane space, the mitochondrial isozyme of creatine kinase (*MCK*) transfers the terminal high-energy phosphate of ATP to creatine to form phosphocreatine. Phosphocreatine then diffuses into the cytosol, and the ADP produced by this reaction in the intermembrane space is returned to the mitochondrial matrix by ANT, where it is rephosphorylated. The phosphocreatine which has diffused into the cytosol is then used as a high-energy phosphate donor for the rephosphorylation of cytosolic ADP by the cytosolic isozyme of creatine kinase. Because the isozymes of creatine kinase have extremely rapid turnover rates, they can facilitate transport of ATP (by, in effect, shuttling the terminal high energy phosphate bonds) from the mitochondria to the sites of ATP hydrolysis and of ADP from ATP hydrolysis sites back to the mitochondrial outer membrane. There, phosphocreatine leaving the mitochondria is used to rephosphorylate ADP, and the creatine liberated from phosphocreatine diffuses into the mitochondrial intermembrane space to be rephosphorylated. Obviously, ATP and ADP also pass through the VDAC, but at slower rates than creatine and phosphocreatine. As a result, cytosolic ADP levels (including those in proximity to the points of ATP utilization) are kept at low levels even if the rate of ATP utilization increases. The stability of ADP levels in the face of increasing ATP utilization permits ATP to maintain a high level of free energy that can be transferred to energy-requiring cellular processes. *ADP* adenosine diphosphate, *ANT* adenine nucleotide transporter, *ATP* adenosine triphosphate, *CCK* cytosolic creatine kinase, *Cr* creatine, *MCK* mitochondrial creatine kinase, *PCr* phosphocreatine

increased myocardial work states via the associated elevation of cytosolic adenosine monophosphate (AMP) and ADP. Increased AMP levels activate AMP-activated protein kinase (AMPK), which causes increased GLUT 4 trafficking to the plasma membrane. The subsequent reactions of the glycolytic sequence (and glycogen breakdown) are also activated by a number of factors including increased cytosolic Ca^{++}, AMPK, protein kinases A and C, PI3K, and fructose 2,6-bisphosphate, as well as AMP and ADP. These reactions are inhibited by increased levels of ATP and increased cytosolic levels of H^+ and/or citrate and other factors as well. For example, increased free fatty acid metabolism increases mitochondrial citrate synthesis, and increased citrate exiting the mitochondria inhibits glycolysis. The first step of oxidative glucose metabolism (the decarboxylation of pyruvate to form acetyl-CoA) by PDH is also highly regulated by PDH kinases and phosphatases, and a high level of β-lipid oxidation causes inactivation of PDH by stimulating its phosphorylation by specific kinases. The regulatory pathways are complex and discussed in more detail in several references cited at the end of the chapter.

Fatty acid metabolism has two major regulatory sites (Fig. 21.13b). The first is at the level of fatty acid transport through the plasma membrane; transport occurs by means of specific proteins located within the membrane (major path) and via free diffusion through the plasma membrane (minor path). This process is mainly regulated by the blood concentrations of fatty acids, i.e., fatty acid uptake is blood level dependent. This means that if the rate of fatty acid utilization is slower than the uptake rate, cardiomyocytes will accumulate fatty acids. The latter are mainly stored as triglycerides. Excess lipid accumulation can be damaging to cardiomyocytes (and other cells as well). A second regulatory site is at the level of long-chain fatty acid transport into mitochondria. As discussed above, long-chain acyl-CoA must be converted to long-chain acylcarnitine at the outer mitochondrial membrane by the enzyme carnitine palmitoyltransferase 1. This enzyme is inhibited by malonyl-CoA, a molecule produced by cytosolic acetyl-CoA carboxylase in response to increased cytosolic levels of acetyl-CoA. The latter occurs as a result of increased fatty acid or pyruvate oxidation by the mitochondria; this overall signaling pathway is complex. Hence, in nonischemic myocardium, increased fatty acid

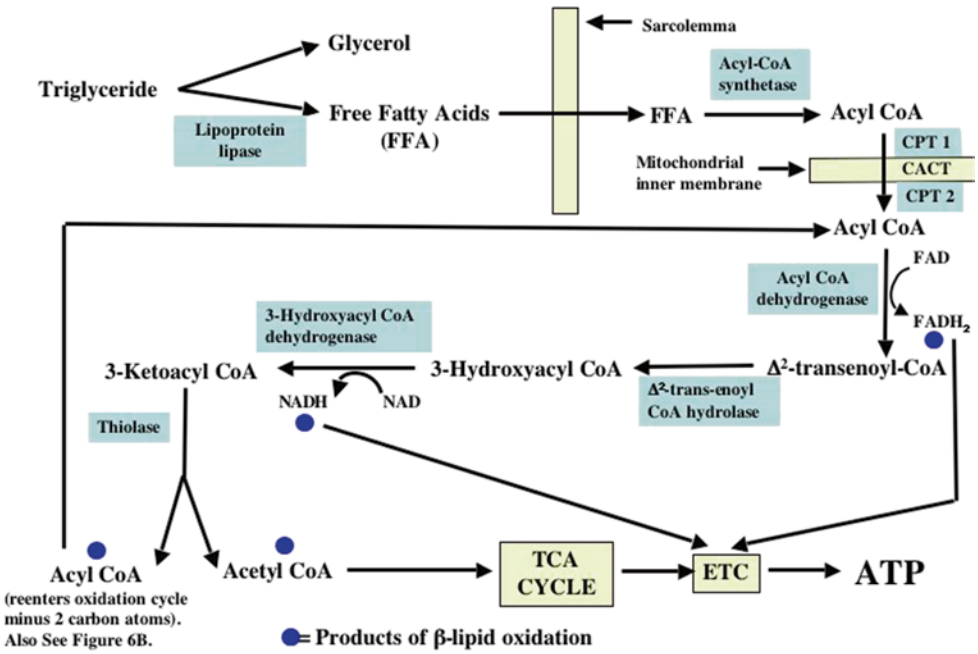


Fig. 21.12 Flowchart depicting the cellular uptake of free fatty acids and the pathways through which their metabolism proceeds. See text for discussion. *ATP* adenosine triphosphate, *CPT 1* carnitine palmitoyltransferase 1, *CPT 2* carnitine palmitoyltransferase 2, *ETC* electron transport chain, $FADH_2$ flavin adenine dinucleotide, *FFA* free fatty acids, *NADH* nicotinamide adenine dinucleotide, *TCA* tricarboxylic acid

oxidation or high blood concentrations of lactate (the latter, by virtue of increasing cytosolic pyruvate levels) will increase cytoplasmic citrate levels, and this increases malonyl-CoA synthesis and thereby limits fatty acid uptake and utilization by mitochondria. This explains why lactate (and exogenously supplied pyruvate; see below) is able to compete successfully with fatty acids for oxidation by mitochondria. Activation of AMPK by an increased cardiac work state can directly inhibit acetyl-CoA carboxylase and thereby reduce malonyl-CoA levels. This will relieve inhibition of carnitine palmitoyltransferase 1 and facilitate fatty acid entry into mitochondria. The rate of mitochondrial fatty acid or pyruvate oxidation (assuming no limitation of transport into mitochondria) is, of course, ultimately controlled by the rate of consumption of the products of these metabolic pathways. The latter is determined by the rate at which the cell utilizes ATP (see below for discussion of this point). A more detailed discussion of the regulation of fatty acid metabolic pathways and of how malonyl-CoA levels are controlled is available in references cited at the end of this chapter.

21.3.5 Myocardial Carbon Substrate Selection

Although the primary carbon substrates (Fig. 21.4) taken up and metabolized by the myocardium are free fatty acids and glucose, the heart also readily takes up and metabolizes pyruvate, lactate, ketone bodies, and amino acids (that are converted to pyruvate if availabilities of glucose and fatty acids are limited). However, the relatively low blood concentrations of the latter substrates and the normal availability of glucose and fatty acids limit their utilization. In contrast, during intense exercise that is associated with marked elevation of blood lactate content or during periods when blood levels of ketone bodies are elevated, the utilization of these substrates increases markedly, and glucose and fatty acid utilization is decreased. A more detailed discussion of the regulation of blood levels of these substrates exceeds the scope of this chapter, yet a few orienting comments are appropriate with regard to glucose and fatty acid utilization patterns. Blood glucose levels are maintained within a narrow range (~4–5 mM) in nondiabetic subjects. Glucose homeostasis reflects a balance between the alimentary uptake of glucose, glucose release from the liver (which can synthesize glucose or release glucose from the glycogen storage pool), and removal of glucose from the blood by various organs. As noted, glucose uptake in both cardiac and skeletal muscle is stimulated by insulin, a hormone secreted by specialized cells in the pancreas in response to increased blood glucose levels.

The heart has often been called an *omnivore* because of its capacity to consume virtually any available carbon substrate (either directly or following processing). As already noted, glucose is transported into the cardiomyocyte by a family of sarcolemmal glucose transport proteins and the predominant transporter (GLUT 4) is insulin dependent. Fatty acids enter myocytes via sarcolemmal fatty acid transport proteins, while lactate and pyruvate are taken up by the sarcolemmal monocarboxylic acid transporter. Utilization of glucose by

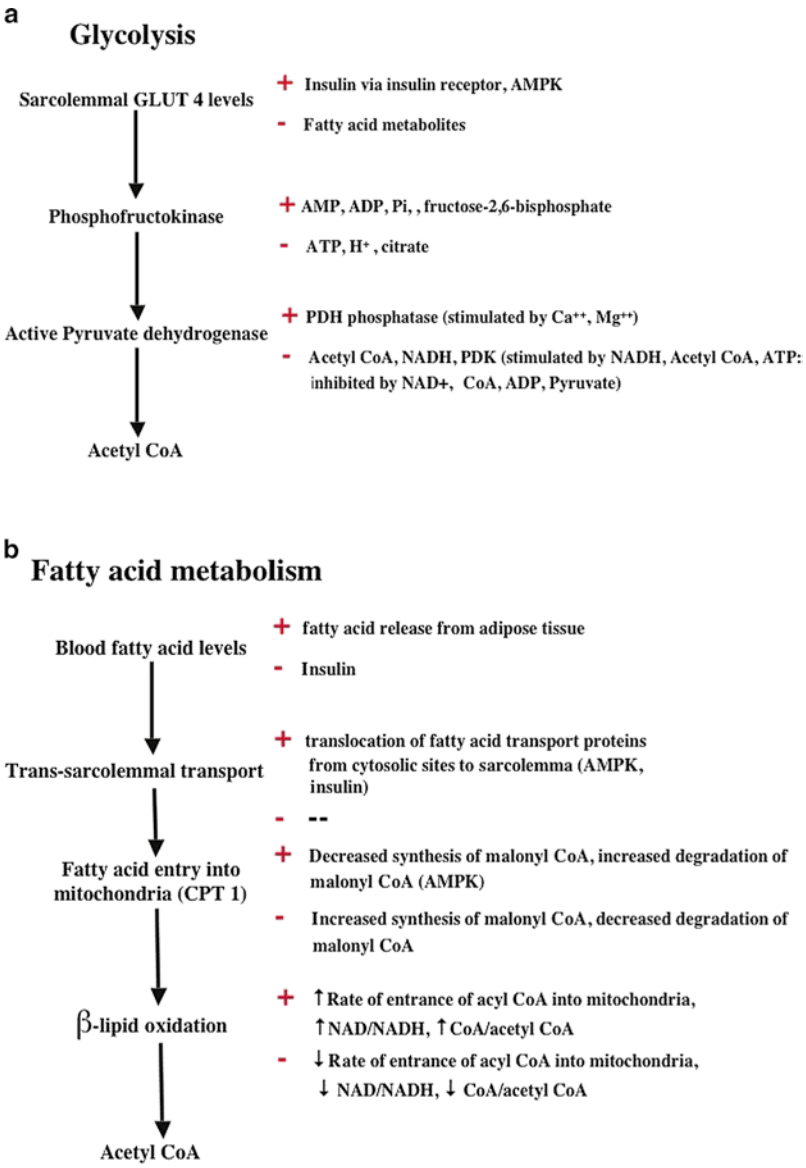


Fig. 21.13 (**a**) Major regulatory sites in the glycolytic pathway (including pyruvate dehydrogenase which is immediately distal to the glycolytic sequence). (**b**) Major regulatory sites in the fatty acid metabolic pathway (including β-lipid oxidation). *ADP* adenosine diphosphate, *AMP* adenosine monophosphate, *AMPK* adenosine monophosphate-activated kinase, *ATP* adenosine triphosphate, *CPT 1* carnitine palmitoyltransferase 1, *NADH* nicotinamide adenine dinucleotide, *PDH* pyruvate dehydrogenase

the heart is largely regulated by the availability of fatty acids, glucose, and insulin in the blood. In the fasted state, glucose levels are generally normal, but insulin secretion is modest, and blood fatty acid levels (released from the liver and adipose tissue) are high. Hence, fatty acids are the predominant cardiac substrate despite normal blood glucose levels. In contrast, during vigorous exercise, blood lactate levels can rise markedly (remember, they are a by-product of skeletal muscle glycolysis which is markedly enhanced by exercise). Lactate competes favorably with the myocardial metabolism of fatty acids, and glucose despite the presence of substantial blood levels of the latter substrates and much more lactate is consumed by the heart.

In contrast, after a high-carbohydrate meal, blood glucose levels rise, and this elicits insulin secretion. Increased insulin levels stimulate glucose uptake by the heart, skeletal muscle, and certain other tissues and also cause blood fatty acid levels to decrease by stimulating their uptake by the liver and adipose tissue. As a result, myocardial glucose consumption increases, and fatty acid consumption decreases. Of interest, lactate (and pyruvate) are the most favored substrates when the relative blood concentrations of all of the substrates are experimentally equalized. Of major importance, under physiological conditions, the switching of carbon substrates *does not* affect myocardial performance.

21.3.6 The TCA Cycle

Acetyl-CoA is the carbon substrate consumed by the (tricarboxylic acid) TCA cycle. As already discussed, when oxygen is available, acetyl-CoA is produced (within mitochondria)

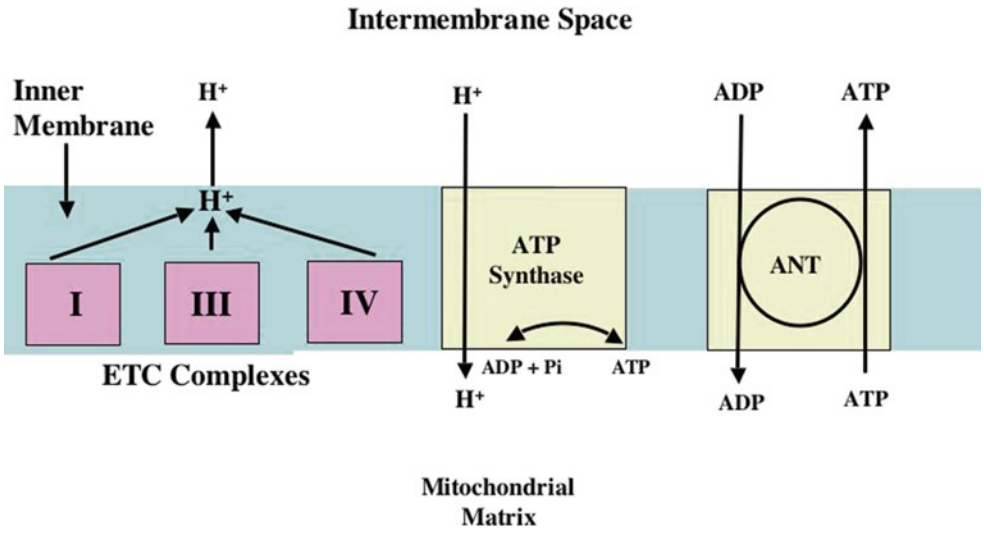


Fig. 21.14 Mechanism of oxidative phosphorylation and the transport of ATP from the mitochondrial matrix to the intermembrane space. See text for discussion. *ADP* adenosine diphosphate, *ANT* adenine nucleotide transporter, *ATP* adenosine triphosphate, *ETC* electron transport chain

from glycolysis derived pyruvate and by β-oxidation of fatty acids. Figure 21.8 shows the individual reactions of the TCA cycle. In the first step of the cycle, acetyl-CoA is combined with oxaloacetate to form citrate and free CoA; this reaction is catalyzed by the enzyme citrate synthase. Citrate then enters a sequence of reactions that ultimately generate two molecules of CO_2 and four reducing equivalents in the form of NADH (3) and $FADH_2$ (1). One high-energy phosphate molecule (GTP) is also produced, which can be directly utilized or converted to ATP. During this process, citrate (a six-carbon molecule) is stepwise decarboxylated and ultimately converted back to oxaloacetate, the four-carbon molecule which, when condensed with a new acetyl-CoA, reinitiates the sequence of reactions just described. The reducing equivalents generated (NADH and $FADH_2$) deliver electrons to the electron transport chain. Rate-limiting enzymes of the TCA cycle are the pyruvate dehydrogenase complex (which precedes, but is not really a component of the TCA cycle), isocitrate dehydrogenase, and α-ketoglutarate; these enzymes are highly regulated as will be discussed later. For elucidating the TCA cycle (also known as the Krebs cycle), Sir Hans Krebs was awarded the Nobel Prize in Medicine or Physiology in 1953.

21.3.7 The Electron Transport Chain and Oxidative Phosphorylation

The two substrates of the electron transport chain are NADH and $FADH_2$. These molecules are produced by glycolysis, β-lipid oxidation, and the TCA cycle as previously discussed; in this context, they transfer electrons to the electron transport chain. The electron transport chain (Fig. 21.10) is comprised of: (1) two freely diffusible compounds, ubiquinone (also known as coenzyme Q) which is confined to the mitochondrial inner membrane and cytochrome c which is located in the intermembrane space, and (2) four multi-protein functional complexes (I, II, III, and IV) that are contained within the mitochondrial inner membrane. NADH interacts with the electron transport chain by transferring electrons to (and thereby reducing) complex I; this, in turn, reduces coenzyme Q (ubiquinone). $FADH_2$ interacts with the electron transport chain by transferring electrons to complex II that, like complex I, also transfers its electrons to coenzyme Q. It should be noted that complex II is also a component of the TCA cycle (it is succinate dehydrogenase) and, thereby, directly links the TCA cycle to the electron transport chain by virtue of its coidentity as complex II. Reduced coenzyme Q then diffuses to complex III and transfers its electrons. Next, complex III reduces cytochrome c, and this molecule diffuses to complex IV and transfers electrons to this complex. The latter, when reduced by four electrons, reduces O_2 to two O^{-2} ions. Each O^{-2} ion combines with two H^+ ions to form H_2O in an irreversible reaction. In recent years, it has become apparent that the individual complexes of the electron transport chain (many of which contain multiple proteins) are organized to form "super-complexes" (see references for additional information if desired). These super-complexes appear to increase the efficiency of oxidative phosphorylation and to reduce the generation of toxic by-products of the oxidative phosphorylation process such as reactive oxygen species (ROS), etc. Toxic metabolites generated by metabolic processes such as ROS and the cellular defenses against them will be discussed later.

During the process of electron transport, energy is released as electrons pass sequentially through the complexes of the electron transport chain. The purpose of electron transport chain complexes I, III, and IV is to capture this released energy and use it to pump H^+ from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space (Fig. 21.10). These pumps create an electrochemical gradient ($\Delta\mu H^+$) comprised of an electrical potential and a chemical potential (the latter reflected by

ΔpH) across the inner mitochondrial membrane. As a consequence of proton transport, the mitochondrial matrix is more negative than the intermembrane space. The F_1-F_0-ATPase, which is also located in the inner mitochondrial membrane (Fig. 21.14), is the major pathway of H^+ return from the intermembrane space into the matrix. The energy released by the passage of H^+ down this electrochemical potential gradient is captured by the F_1F_0-ATPase and used to drive the next reaction [**inorganic phosphate (Pi) + ADP → ATP**]. In this way, much of energy released by the metabolism of glucose and fatty acids is transferred to the terminal high-energy phosphate bond of ATP. The concept of proton pumping into the intermembrane space, and the use of the electrochemical gradient thus formed to synthesize ATP, is known as the chemiosmotic theory. In recognition of his insight into how these processes function (ideas initially considered to be quite controversial), Sir Peter Mitchell was awarded the Nobel Prize in Chemistry in 1978. The production of ATP by mitochondria is termed oxidative phosphorylation; in virtually every human tissue (heart, brain, kidney, but not red blood cells), oxidative phosphorylation is the primary source of ATP generation.

To date, the precise mechanism by which the F_1F_0-H^+-ATPase generates ATP from its substrates (ADP and Pi) remains under investigation. However, a number of characteristics of the process are well established. For example, the F_1F_0-H^+-ATPase has been shown to be a near-equilibrium enzyme. In other words, the reaction can occur in both directions. However, it has also been shown, both in isolated mitochondria (which generate ATP under conditions of carbon substrate, ADP, and oxygen excesses) and in the perfused rat heart that the F_1F_0-H^+-ATPase operates far out of equilibrium so that virtually all fluxes are in the ADP + Pi -> ATP direction. In the presence of abundant oxygen, the F_1F_0-H^+-ATPase is kinetically controlled by the concentrations of its immediate substrates (ADP and Pi) and the magnitude of the proton electrochemical potential gradient. Hence, in principle, ADP and Pi levels can regulate the rate of ATP synthesis *as long as oxygen and carbon substrates are not limiting and the proton gradient is large*. However, in vivo regulatory mechanisms are far more complicated than those described by this relatively simple scheme first presented by B. Chance and G.R. Williams in 1955.

21.3.8 Regulation of the TCA Cycle, Electron Transport Chain, and Oxidative Phosphorylation

As pointed out by Chance and Williams, a simple kinetic regulatory scheme for oxidative phosphorylation with ADP (and Pi) availability controlling the ATP synthetic rates is valid when both oxygen and reducing equivalents are in excess. Under these conditions, the high proton electrochemical gradient will drive ATP synthesis until ADP and/or Pi fall to levels that are rate limiting to ATP synthesis. From this point on, ADP availability (i.e., the rate of ADP production) determines the steady-state rate of ATP synthesis. Therefore, because ADP is a product of ATP hydrolysis, the rate of ATP synthesis is determined by the rate of ATP hydrolysis (i.e., the rate of ATP utilization). This simple feedback regulation of ATP synthesis can be demonstrated in the perfused rat heart by providing a perfusate containing unphysiologically high concentrations of pyruvate or octanoate. Under these experimental conditions, mitochondrial NADH levels are very high, and it appears that extramitochondrial ADP and/or Pi levels become low enough to kinetically regulate oxygen consumption. In this case, increased ATP utilization will cause ADP and Pi levels to rise and the rate of ATP synthesis to speed up, in association with an increase in the rate of oxygen consumption. In this construct, the increased rate of delivery of ADP and Pi to the F_1F_0-ATPase increases the rate of ATP synthesis. In other words, ADP levels *must increase concordantly* with the increased rate of ATP synthesis.

In the in vivo mammalian heart (e.g., rat, dog, swine, human, etc.), however, free ADP levels in the cytosol (as estimated by means of ^{31}P magnetic resonance spectroscopy) are higher than those in the isolated perfused rat heart and are well above the usual kinetic regulatory range described in studies of isolated mitochondria or perfused rat hearts. Further, in the in vivo animal heart, ADP levels actually remain fairly constant even when increased rates of ATP utilization (increased cardiac work) drive substantial increases of myocardial oxygen consumption. However, this does not mean that ADP availability is not crucial to the rate of ATP synthesis. When the rate of ATP expenditure is increased in normal myocardium, there is an obligatory identical increase in the rate of ADP delivery to mitochondria (once a new steady state is reached). To emphasize this point, the increase in the *rate of ADP delivery* to the ATP synthase (e.g., as myocardial work increases) is obligatory, despite the fact that a change in *steady-state ADP levels* is not.

The observation that ADP levels did not increase in the in vivo myocardium as the rates of ATP utilization and synthesis increase led to the recognition that additional regulatory systems are required to explain the data obtained in the in vivo heart. Further, these overall regulatory processes must have very rapid response times with regard to facilitation of ATP synthesis. The latter is required because myocardial contents of ATP and creatine phosphate (another high-energy phosphate bond storage molecule which serves to buffer ATP levels) are only sufficient to support a few seconds of ATP expenditure in the absence of continuing ATP synthesis. The reaction converting cytosolic ATP to phosphocreatine is rapid and near equilibrium via the enzyme creatine kinase as discussed earlier. See Fig. 21.11 provides a brief overview of the ~P shuttle function of phosphocreatine.

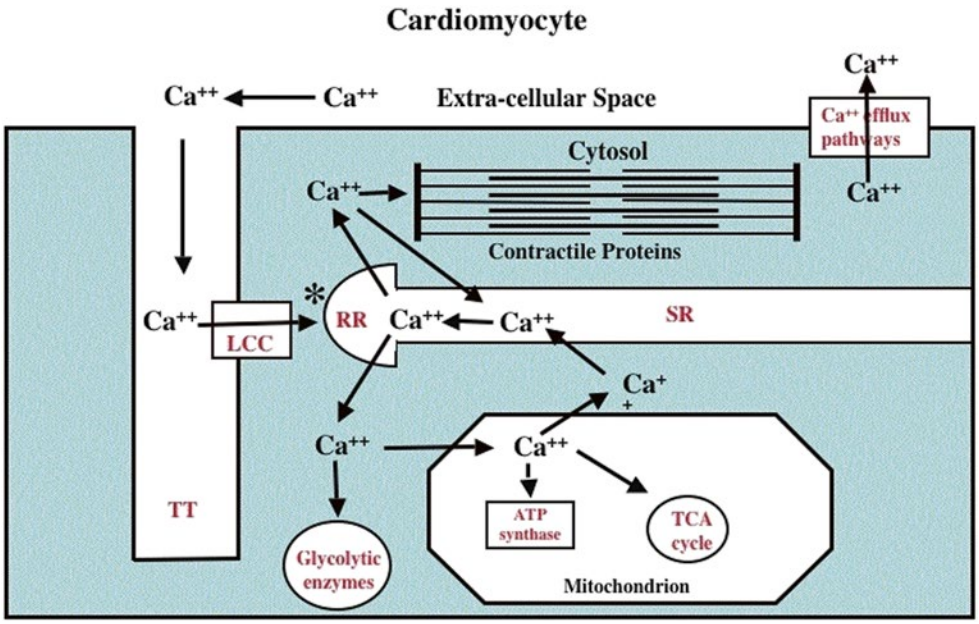


Fig. 21.15 Pathways of Ca^{++} entry and exit in the cardiomyocyte and several intracellular organelles relevant to excitation-contraction coupling and energy metabolism. The strong linkage between the stimulatory effects of intracellular [Ca^{++}] on contraction and ATP generation is also shown. See text for discussion. *ATP* adenosine triphosphate, *LCC* l-type voltage-activated Ca^{++} channel, *RR* ryanodine receptor, *SR* sarcoplasmic reticulum, *TCA* tricarboxylic acid, *TT* t-tubule

It is now known the overall regulation of metabolism in cardiomyocytes is a complex process that is quite far from being fully understood. It is comprised of a large number of parallel, sequential, and massively interacting feedback and feed-forward regulatory pathways, the effects of which combine to allow the cell to alter ATP synthetic rates sufficiently to meet changing demands without destabilizing the cellular chemical milieu and inducing fatigue. In contrast, ATP demand can exceed synthetic capacity in tissues such as skeletal muscle, and destabilization of the cellular chemical milieu and fatigue develop under these circumstances. To offer the reader a feel for such regulatory processes, a discussion of several (but far from all) Ca^{++}-based mechanisms for regulation of oxidative phosphorylation will be presented next. Readers can review the discussion of Ca^{++}-based contraction and relaxation mechanisms presented elsewhere in this book and also briefly in Fig. 21.15 to refresh their understanding of cardiomyocyte Ca^{++} dynamics.

During exercise, increased norepinephrine release from sympathetic nerve fibers and increased epinephrine from the adrenal glands activate β-adrenergic receptors in cardiomyocytes. This receptor activation causes an increase of Ca^{++} entry into the cytosol (per beat) via sarcolemmal voltage-dependent Ca^{++} channels. Cytosolic Ca^{++} levels are further augmented by the increased heart rate (which reflects an increased number of Ca^{++} channel openings/min). The increased Ca^{++} entering the cytosol, together with β-adrenergic receptor-mediated activation of sarcoplasmic reticulum Ca^{++} sequestration, increases the sarcoplasmic reticulum Ca^{++} store. The more fully loaded sarcoplasmic reticulum (which is the predominant source of "contraction activation" Ca^{++}) can then release more Ca^{++} per beat (via its Ca^{++} release site, the ryanodine receptor) into the cytosol. The larger systolic cytosolic Ca^{++} transient present following adrenergic stimulation and/or heart rate increase then generates increased force and shortening. The ryanodine receptor is also activated by β-adrenergic receptor stimulation and other factors. Concomitantly, the increased frequency of Ca^{++} transients (heart rate) and their larger size increase the "average" Ca^{++} level in the cytosol. Mitochondria normally transport Ca^{++} both in and out of their matrix and maintain a "steady-state" matrix Ca^{++} level that is related to average cytosolic Ca^{++}. In response to higher average cytosolic Ca^{++} levels, mitochondrial Ca^{++} uptake increases, resulting in an increase in the steady-state level of matrix Ca^{++}. It is important to note that increased mitochondrial matrix Ca^{++} has multiple effects on oxidative phosphorylation. First, as already mentioned, the TCA cycle has three rate-limiting enzymes (PDH, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase), and the activities of these enzymes are regulated, in part, by mitochondrial matrix Ca^{++} levels. Specifically, when these enzymes interact with Ca^{++}, their sensitivity to respective substrates is increased. This accelerates their reaction rates and increases the rate of acetyl-CoA entry into and flux through the TCA cycle without requiring pyruvate, acetyl-CoA levels, or immediate substrate levels of the TCA cycle pool to rise, provided that the rates of delivery of acetyl-CoA to the TCA cycle (and anaplerotic processes) are increased sufficiently to support the increased TCA cycle flux rate. Therefore, glucose uptake and glycolysis-mediated delivery of pyruvate to (and its rate of metabolism by) PDH must increase, and/or the rates of fatty acid uptake and β-oxidative delivery of acetyl-CoA to the TCA cycle must increase to support the increased rate of TCA cycle flux.

As noted above, the rates of cellular substrate uptake, glycolytic flux, and β-oxidation also respond to metabolic signaling to produce the required increases in the rate of acetyl-CoA production.

A major consequence of an increased TCA cycle flux is the more rapid generation of both mitochondrial NADH and $FADH_2$; this results in maintenance or increases in their steady-state levels despite the increased rate of electron transfer to the electron transport chain. Hence, stimulation of the electron transport chain fluxes is facilitated by an increased rate of reducing equivalent generation and also by direct stimulation of electron transport chain complex activities by other metabolic signals which are present during periods of increased ATP demand. Increased rates of electron transport chain fluxes will then result in an increased rate of H^+ pumping by the electron transport chain, which serves to maintain the electrochemical gradient across the inner mitochondrial membrane at a level adequate to support the increased rate of ATP synthesis despite the fact that the rate of H^+ passage (through the ATP synthase) is markedly increased to support the increased ATP synthesis rate. Additionally, the F_1F_0-ATPase is also regulated in that the fraction of this enzyme that is in an active state is increased by elevations of mitochondrial matrix Ca^{++}. An increased fraction of this enzyme in the active state is beneficial during periods of increased ATP synthesis, because an increased amount of active enzyme increases the rate of ATP synthesis without requiring ADP levels to rise. Hence, so long as the rate of ADP delivery to mitochondria increases appropriately (which it does when the rate of ATP utilization increases), ADP levels can remain reasonably stable. This is crucial because elevated ADP levels have direct effects on the contractile protein interactions and also decrease the amount of energy that hydrolysis of the terminal phosphate bond of ATP can release to drive energy-dependent processes. Nevertheless, it should be realized that although coordinated activation of carbon substrate metabolic pathways, the TCA cycle, the electron transport chain, and ATP synthase are all crucial to meeting work increase-associated ATP synthetic requirements, control of respiration is equally linked *to the rate of ATP utilization and the resulting rate of delivery of ADP* to the mitochondrial matrix. Hence, the newer, more complex model of respiratory regulation still includes aspects of the simpler Chance and Williams model.

To summarize, these observations support the concept that increases of average cardiomyocyte cytosolic Ca^{++} levels (e.g., that occur during exercise) act as an important feedforward signal for oxidative phosphorylation. This signal stimulates, in a parallel manner, ATP utilization by the contractile processes and ATP synthesis by the F_1F_0-ATPase as well as many preceding reactions in the energy-generating scheme (Fig. 21.15). The net effects of these regulatory mechanisms (and additional feedback regulatory mechanisms which are not discussed) are to facilitate fluxes of basic food-derived carbon substrates through the metabolic sequences without requiring the cytosolic concentrations of the initial and intermediate substrates (including ADP) to increase. The rapid response time of this entire system also allows ATP levels to remain relatively constant despite wide and rapid fluctuations in the rate of ATP utilization. Since these mechanisms allow both ADP and ATP levels and the ATP/ADP ratio to remain stable during increased ATP synthetic rates, the free energy (ΔG ATP) that can be released by hydrolysis of the terminal high-energy phosphate bond of ATP (and transferred to the reactions it drives) is maintained constant despite the increased rate of ATP expenditure. Metabolic control theory as applied to the regulation of oxidative phosphorylation, glycolysis, β-lipid oxidation, and the TCA cycle has been the subject of detailed study; for further discussion of this topic, refer to the references listed at the end of this chapter.

21.3.9 Toxic By-Products Generated by Mitochondria and Other Cellular Moieties that Impact Energy Generation and Contraction-Associated Processes

Carbon substrate metabolism, oxidative phosphorylation, and a number of other cellular processes produce potentially toxic by-products (Fig. 21.16). The descriptor "potentially" is employed because a number of these by-products (when generated at low levels) can exert useful influences on intracellular signaling pathways; in contrast, when generated at high levels, they are highly toxic. As a generalization, these moieties can induce modifications of proteins, lipids, and carbohydrates which can alter the performance of these molecules in diverse ways including the stimulation and/or disruption of their normal function. Some examples of the "toxic by-products" included in this category are highly reactive oxygen and highly reactive nitrogen species (ROS and RNS), hydrogen sulfide (H_2S), carbon monoxide (CO), nitric oxide (NO), and others. Consequently, the heart and all other tissues have evolved strong defense systems at sites of generation of these reactive species (i.e., in the cytosol, mitochondria, and other organelles). These systems are capable of maintaining the concentration of reactive molecules below levels that can adversely affect cardiomyocyte structure and function while preserving their physiological signaling roles. Discussion of all of these systems is far beyond the scope of this survey chapter, but several reactive species generation and degradation systems will be briefly described to convey the concept.

The most often cited ROS species produced by the mitochondrial electron transport chain (complexes 1, 2, and 3)

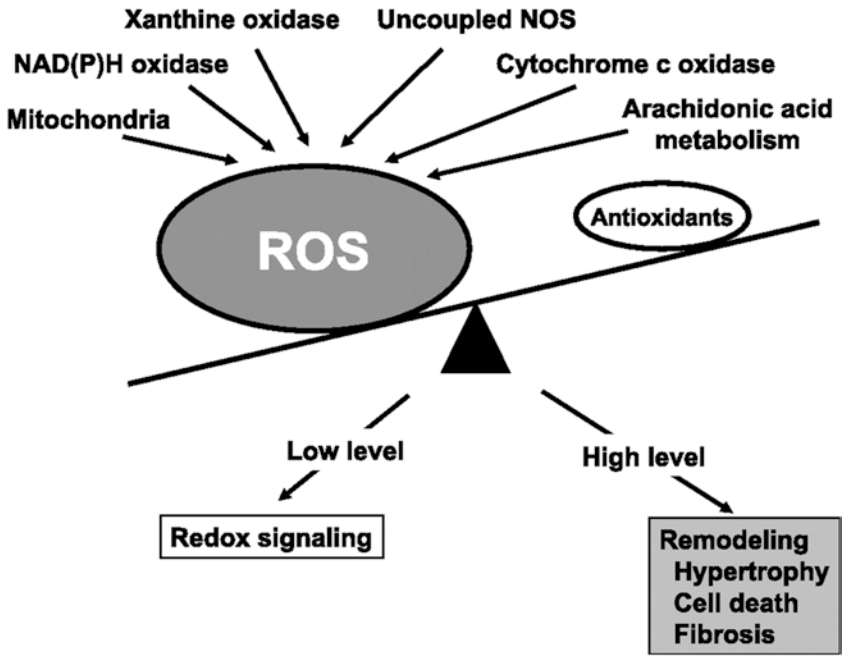


Fig. 21.16 Sources of reactive species in myocardium. These sources are located in various cellular compartments including the plasma membrane, cytosol, and subcellular organelles. Low levels of ROS generation stemming from physiological activation of these mechanisms serve as intracellular signals, while high levels of ROS activate adverse signaling pathways that induce pathological remodeling of the myocardium. Antioxidant defenses limit accumulation of ROS from the various sources shown. *ROS* reactive oxygen species, *NOS* nitric oxide synthase. Reprinted from Tsutsui H, Kinugewa S, and Matsusima S, Oxidative stress and heart failure, Am J Physiol Heart Circ Physiol, Vol. 301, H2181-H2190, 2011. Reprinted from American Journal of Physiology Heart and Circulatory Physiology (by American Physiological Society). Reproduced with permission of American Physiological Society, in the format of Republish in a book via Copyright Clearance Center

and by NADPH oxidases located in the plasma membrane, sarcoplasmic reticulum, and peroxisomes (as well as other intracellular sources) is the superoxide radical ($^{-}O_2$). $^{-}O_2$ in itself is not highly toxic, and its mobility (i.e., diffusivity) is limited. However, $^{-}O_2$ is readily converted to a more diffusible ROS such as hydrogen peroxide (H_2O_2). The latter can then be rapidly converted the hydroxyl radical, a highly toxic entity. Additionally, $^{-}O_2$, by interacting with NO, forms peroxynitrite, another highly toxic molecule. Somewhat paradoxically, low concentrations of H_2O_2 (and other reactive molecules) can act to modulate a number of intracellular molecular signaling pathways. However, at high concentrations, these reactive species are quite toxic and interfere with many important processes required for cellular homeostasis. For example, in the heart ROS are generated in huge quantities during the postischemic reperfusion period and adds substantially to the injury accrued during the ischemic period (see later discussion of effects of ischemia on myocardium). ROS are also produced in normal working myocardium at low non-toxic levels that have signaling functions. Somewhat surprisingly, recent experimental work suggests that in *normal* working cardiac and skeletal muscle, reactive species originate mainly from non-mitochondrial sources. In the heart, the levels reached during endurance exercise do not significantly decrease myocardial contractile performance. In contrast, in working skeletal muscle, exercise-induced ROS generation has been considered to be one of the many contributing causes of the fatigue associated with substantial levels of work.

Fortunately, cardiomyocytes and skeletal muscle cells (and most other cell types) have a number of defenses against accumulation of excessive levels of $^{-}O_2$ and its toxic derivatives. The enzyme superoxide dismutase, isoforms of which are located in the interstitial space, cytoplasm, and within mitochondria, converts $^{-}O_2$ into H_2O_2. H_2O_2, in turn, is converted to water by catalase, another protective enzyme that is widely distributed within cells. The destruction of H_2O_2 limits the formation of the highly reactive (and thereby toxic) hydroxyl radical. In experimental models, augmenting ROS defenses by transgenic techniques or by administration of small molecule scavengers of ROS has been shown to protect against ROS-induced damage occurring during the postischemic reperfusion period. However, it should be noted that large clinical trials involving ingestion of substantial doses of antioxidants (e.g., vitamin E) in an attempt to prevent age-associated degenerative processes have been negative, possibly because the antioxidant molecules do not reach the intracellular site of ROS generation and/or because they may ablate the effects of beneficial signaling induced by low levels of ROS generation. There are also small molecules present in the cytosol and mitochondria which can nonenzymatically scavenge the reactive species.

Another defense against the deleterious effects of reactive species (in addition to their destruction) is the reversal of the chemical "damage" they induce. There are a number of coupled enzyme/substrate systems that do this, and the interested reader can review a reference listed at the end of the chapter for more insight. One cogent example of ROS-induced damage is the sequential oxidation of the sulfur atoms contained in some of the amino acids that form a protein. Oxidation of these sulfur atoms can disrupt the functional properties of the protein. Under these circumstances, another enzyme, glutaredoxin, uses glutathione, an SH group-containing molecule that is abundant in cardiomyocytes (and many other cell types), as a cofactor and reduces the oxidized sulfur atoms on the protein at the cost of the oxidation of glutathione molecules. The oxidized glutathione is then reduced back to its native form by another enzyme, glutathione reductase. Notably, if the sulfur atom undergoes multistage oxidation as a result of the interaction with ROS, the process may then not be reversible by antioxidative defenses.

Within limits, irreversibly modified proteins and other molecules can be replaced by cellular synthetic processes. However, if large numbers of these molecules are damaged by an oxidative insult, then repair capacity will be exceeded, and the cell will die. Taken together, the oxidant defenses of the *normal* cardiomyocyte are extremely effective and are highly protective over a wide range of myocardial work states. However, they are clearly inadequate to protect diseased myocardium which is often subjected to quite severe oxidant stresses induced by various types of pathological processes.

21.4 Metabolism in Diseased Myocardium

21.4.1 Ischemic Myocardium

The most common cause of inadequate myocardial ATP synthesis is ischemia, and the most frequent cause of myocardial ischemia is occlusive disease of the major coronary arteries. *Demand ischemia* occurs when: (1) a narrowed coronary artery can conduct sufficient blood to sustain aerobic metabolism in the portion of myocardium that it supplies during basal levels of physical activity and (2) the degree of narrowing is sufficient to limit increases of blood flow that normally (and seamlessly) occur during the increased demands for ATP production that are present during exercise or other stress. This phenomenon is the basis of exercise- or stress-induced angina pectoris (i.e., heart-associated chest pain). Patients with this pathophysiology are asymptomatic at rest or during mild exertion, but when the work of the heart exceeds the ability of a narrowed coronary artery to meet the increased blood flow required to support the increased rate of ATP demand, ischemia ensues, and the patient experiences chest pain. In response to the symptom of chest pain, the patient will typically discontinue exercise, and during the rest period, the cardiac work and ATP synthetic needs fall to levels where the narrowed coronary artery can meet the blood flow demand; hence, ischemia is relieved.

In contrast, *supply ischemia* occurs when the coronary artery narrowing is so severe that it limits blood flow in the absence of increased ATP demand, i.e., ischemia occurs even when the patient is at rest. Supply ischemia is generally caused by an acute narrowing or abrupt complete closure of coronary vessels and may cause myocardial infarction. Less commonly, supply ischemia can occur as the result of coronary artery spasm, in which inappropriate (pathologic) vasoconstriction of a major coronary artery causes transient total (or near total) occlusion of blood flow. Although the nature of the myocardial ischemia is qualitatively similar in supply and demand ischemia, supply ischemia is generally more clinically severe. Because of the greater vulnerability of the inner myocardial layers of the left ventricle to blood flow reduction, mild ischemia is usually confined to the subendocardium. However, as the coronary obstruction becomes more severe, ischemia proceeds as a wave front from subendocardium to subepicardium until the full myocardial wall is involved.

When myocardial blood flow is inadequate, the limited availability of both oxygen and carbon substrate may limit the ATP synthetic rate, but it is usually the oxygen deficiency that is most critical. This is because the myocyte has substantial glucose reserves (i.e., in the form of glycogen that can be converted to glucose); the glucose released from glycogen can then undergo anaerobic glycolysis and generate modest amounts of ATP. In contrast, myocardial oxygen stores are very small and can only support a short period of oxidative metabolism. Consequently, when coronary blood flow is limited, the severity of the induced oxygen deficit determines the extent of limitation of oxidative phosphorylation and secondarily enhances the rate of glycogen breakdown and glycolysis. As indicated, the early effects of oxygen limitation are confined to pathways supporting oxidative phosphorylation (Figs. 21.5 and 21.17). During hypoxia or anoxia (i.e., when blood oxygen content is moderately or markedly reduced, but blood flow is maintained) or during ischemia (where blood flow is restricted), electron transport within the electron transport chain slows or stops depending on the severity of the oxygen deficit. This is because electrons cannot be transferred from cytochrome oxidase to molecular oxygen (the terminal electron acceptor). In consequence, the pumping of H^+ from the mitochondrial matrix into the intermembrane space stops because it depends on energy liberated as electrons pass through the electron transport chain on their way to interacting with oxygen within cytochrome oxidase. As a result, the proton-motive force across the inner

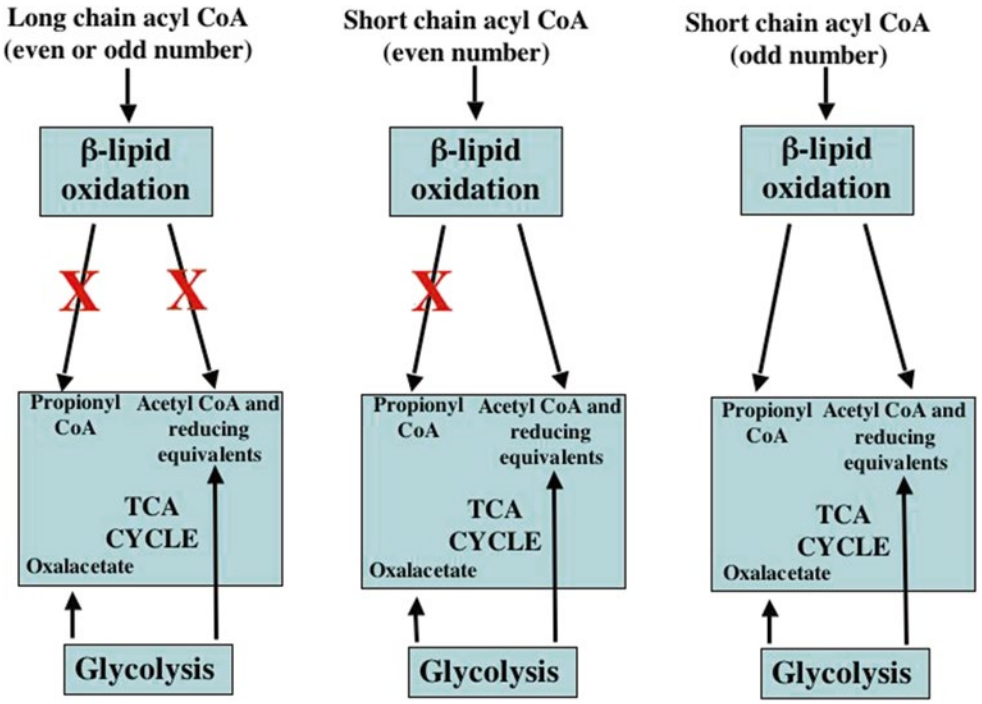


Fig. 21.17 Effects of very long-chain acyl-dehydrogenase deficiency on the metabolism of long-chain fatty acids (even and odd numbered), short-chain fatty acids (even numbered), and short-chain fatty acids (odd numbered). Only the last one can also supply anaplerotic substrate to the TCA cycle. A *large red X* indicates metabolic pathways which cannot be used by a specific type of free fatty acid. See discussion in text. *TCA* tricarboxylic acid

mitochondrial membrane begins to dissipate, and mitochondrial ATP synthesis fails. If the oxygen deficiency is severe, the dissipation of the H^+ gradient is marked, and the F_1F_0-H^+-ATPase begins to operate in the reverse direction, i.e., it converts ATP to ADP and uses energy liberated by ATP hydrolysis to pump H^+ back into the intermembrane space. Because the H^+ electrochemical potential is also used to energize other mitochondrial functions in addition to supporting ATP synthesis, mitochondrial degradation of ATP during periods of oxygen limitation may serve to preserve nonoxidative mitochondrial function and prolong the time when mitochondrial oxidative function can respond to resumption of blood flow. However, this mechanism of mitochondrial preservation occurs at the expense of hastening the fall in cytoplasmic ATP levels, and the latter limits all other cardiomyocyte functions that are ATP dependent.

Glycolysis can, in principle, proceed normally in the absence of oxygen, but it is also limited when blood flow is reduced or stops. Oxygen limitation causes cessation of forward electron transport chain function and, thereby, stops the mitochondrial oxidation of NADH generated by the TCA cycle and/or transferred from cytosolic NADH into mitochondria. Cytosolic NAD^+ is a substrate of glyceraldehyde phosphate dehydrogenase, a key enzyme of the glycolytic sequence, and if the NADH generated by that reaction is not oxidized back to NAD^+ by the transfer of reducing equivalents into the mitochondria by the malate-aspartate shuttle or into cytosolic lactate by LDH (see below), the lack of NAD^+ will then inhibit glyceraldehyde phosphate dehydrogenase. Other factors limiting the rates of glycolytic ATP synthesis during ischemia are cytosolic accumulations of lactate and hydrogen ions (Figs. 21.5 and 21.9). Under conditions of normal coronary flow, the myocardium can rapidly export both of these ions even when the glycolytic rate is augmented. Thus, in a model in which low oxygen content of coronary arterial blood is associated with preserved blood flow, lactate and H^+ export are not inhibited. But, when the myocardium is ischemic, the capacity to export lactate and protons decreases in proportion to the severity of the blood flow reduction. Furthermore, the increase in cytosolic lactate concentration during ischemia can inhibit the conversion of pyruvate to lactate via LDH, so that pyruvate generated by glycolysis may have limited exit possibilities, since pyruvate oxidation is also inhibited. Because the conversion of pyruvate to lactate results in the oxidation of cytosolic NADH, the availability of NAD^+ to glyceraldehyde phosphate dehydrogenase is further reduced when rising cytosolic levels of lactate slow this reaction (Figs. 21.5, 21.9, and 21.13a). In contrast, during hypoxia, if coronary flow is not restricted, glycolysis proceeds at a rapid rate because metabolic signaling activates the rate-limiting glycolytic reactions, the transport of glucose into the myocyte and lactate export. During moderate ischemia, when both glucose and oxygen deliveries are impaired, myocyte glycogen stores are the main source of glucose for glycolysis. When glycogen stores are exhausted, the limited rates of glucose delivery to (and lactate clearance from) the ischemic myocyte control the rate of glycolysis.

These concepts have specific importance in patients with coronary artery disease, because they typically define the

temporal boundary for viability of the ischemic myocardium. For example, in the moderately ischemic myocardium, contractile function rapidly decreases in response to: (1) decreased ATP availability, (2) accumulation of H^+ ions, and (3) many other factors. However, if residual coronary blood flow during ischemia is sufficient to permit some glycolysis (by allowing the export of some lactate and H^+ and the import of some glucose), then modest (glycolytic) ATP production may preserve tissue viability for some time. In contrast, following acute total coronary occlusion, glycolysis is rapidly inhibited, and myocyte death will occur much more rapidly. In other words, a level of coronary blood flow that is insufficient to maintain oxidative phosphorylation and contractile function may support glycolytic ATP production at a rate sufficient to sustain myocardial viability until appropriate medical or surgical treatment can restore blood flow to the ischemic myocardium.

Another adverse biochemical event that occurs during the ischemic period, and especially during a subsequent period of reperfusion, is a markedly enhanced rate of generation of highly chemically reactive species (ROS and others) by mitochondria and extramitochondrial enzymes. The interaction of these species with myocardial lipids, proteins, and carbohydrates further damages the structural and functional capabilities of cardiomyocytes. Cardiomyocyte endogenous protective mechanisms (see earlier discussion) offer a partial defense against the effects of the high levels of reactive species generated during and following a relatively short period of ischemia, but they are inadequate to forestall the injury consequent to a long period of ischemia. Reactive species-induced damage contributes to both ischemic and post-ischemic cardiomyocyte loss. In experimental models of cardiac ischemia/reperfusion, augmentation of defenses against reactive species has been shown to decrease cardiomyocyte loss.

21.4.2 Metabolism in Hypertrophied and Failing Hearts

During fetal life, the myocardium primarily metabolizes glucose and not fatty acids. This pattern of metabolism is well suited to the low oxygen levels in fetal arterial blood; glucose metabolism requires less oxygen than fatty acid metabolism. Additionally, the lower cardiac work levels (lower blood pressure) in the fetus are adequately supported by ATP production through glycolysis and pyruvate oxidation. However, after birth substrate preference of the heart (now in a higher oxygen environment and operating at a higher work load) rapidly shifts to the adult metabolic pattern, which is predominantly dependent on fatty acid oxidation. This change of substrate preference is the result of upregulated expression of genes controlling the transport of fatty acids into the myocyte and their subsequent metabolism in the cytosol and mitochondria. This upregulation in the expression of genes involved in fatty acid metabolism and oxidative phosphorylation is commonly referred to as the shift from a fetal to an adult gene expression pattern.

A chronic mechanical overload produced by hypertension, heart valve abnormalities, and/or ischemic destruction of a portion of the left ventricle can result in left ventricular dilation as well as cellular hypertrophy. The severity of the dilation is dependent on the excess stress placed upon the viable myocardium. In general, left ventricular wall tension (systolic and diastolic) increases as ventricular dimensions increase; this is a consequence of the law of LaPlace that relates the internal chamber pressure and chamber radius (and wall thickness) to the level of chamber wall tension. The increased left ventricular wall tension (this principle applies to all cardiac chambers) can activate changes in the expression patterns of a number of genes involved in control of myocyte growth (hypertrophy), energy metabolism, and many of the processes that support cardiomyocyte contraction. It does this, in part by directly activating sarcolemmal receptors (e.g., angiotensin type 2 receptors) that are responsive to stretch. Among the adverse consequences of activation of these receptors is the stimulation of ROS generation and increased levels of reactive species that may overwhelm antioxidant capacity and cause oxidant damage to many important structural and functional molecules. This damage then limits a variety of cardiomyocyte functions (i.e., metabolism, ion pumping, and contraction).

Initially, the stress-activated growth stimulation pathways stimulate cardiomyocytes to grow (i.e., hypertrophy), and this can increase chamber wall thickness. Because the increased wall thickness can reduce systolic wall stress toward normal (via the LaPlace relationship), this may result in a prolonged period of stable compensated myocardial hypertrophy. However, chronic (*pathological*, *not exercise induced*) myocardial hypertrophy is usually associated with important changes in energy metabolism, including reductions of ATP and creatine phosphate levels. This shift in the myocardial gene expression is often referred to as a reversion to the fetal gene expression pattern. Subsequently, reductions also occur in the level of molecules participating in energetic processes such as oxidative phosphorylation. Although the increased glucose metabolism and decreased fatty acid metabolism associated with this reversion (to a fetal gene expression pattern) may have short-term energetic benefits (perhaps by modestly decreasing the oxygen cost of ATP synthesis), the overall ATP synthetic capacity of severely hypertrophied and failing myocardium is significantly reduced. The period of compensated hypertrophy in the chronically overloaded heart is often followed by a significant contractile dysfunction of the ventricular myocytes with ultimate progressive left heart dilation and dysfunction and

mild to severe symptoms such as marked fatigue and shortness of breath. At this point, the clinical syndrome known as *heart failure* is present.

The question arises as to whether the decreased ATP synthetic capacity observed in hypertrophied and/or failing cardiomyocytes *induces* the decompensated state. It has been shown that all of the operational subsystems in the failing cardiomyocyte (i.e., energetic, contractile, and electrical) are severely dysfunctional and that this dysfunction occurs in a parallel manner. However, whether contractile dysfunction in the failing heart is caused primarily by a limited ability of the abnormal contractile apparatus *to consume ATP* or whether the reduced ability *to produce ATP* (i.e., the concept of the energy-starved heart) is also causal is still unclear.

In animal models, molecular and other interventions that have been specifically directed toward improving *either* disordered energetic functions or contractile processes have often attenuated the development of *both* contractile and energetic dysfunctions. Hence development of malfunctions in both systems appear to be inter-dependent. As discussed elsewhere in this book, interventions that block the adverse effects of the neurohumoral activation present in patients and animals with heart failure are also beneficial. This is because neurohumoral activation is associated with stimulation of many of adverse intracellular signaling pathways.

Lastly, significant abnormalities of left ventricular chamber function can add an ischemic component to the intrinsic metabolic abnormalities of hypertrophied and failing myocardium, even when occlusive coronary artery disease is not present. Increased ventricular filling pressures, impaired diastolic relaxation, and tachycardia present in the abnormal heart can limit the ability of myocardial blood flow (especially in the subendocardium) to increase normally in response to an increased metabolic rate. Hence, in the chronically overloaded heart, acquired intrinsic abnormalities of the cardiomyocytes and superimposed limitations of blood flow can act together to compromise cardiomyocyte ATP synthetic capacity and thus further impair overall cardiac function.

21.4.3 Primary (Genetic) Myocardial Metabolic Abnormalities

As described above, changes in gene expression in the hypertrophied or failing cardiomyocyte can cause various abnormalities of myocardial metabolism that, in turn, can act to constrain contractile function. However, "primary" (genetically determined) abnormalities of carbon substrate metabolism or oxidative phosphorylation can also cause myocyte hypertrophy and/or failure. Interested readers can refer to the references at the end of this chapter for more information on such defects. A dramatic example of one abnormality and a successful therapeutic approach to treat this metabolic defect will be discussed below.

An inherited deficiency of the enzyme very long-chain acyl-CoA dehydrogenase (VLCAD) is known to be associated with cardiomyopathy and skeletal muscle myopathy (Fig. 21.17). This enzyme is the first component of the β-lipid oxidation sequence. Because most dietary fatty acids are of the long-chain type, the deficiency of VLCAD results in a markedly increased cardiac dependence on glucose for ATP generation. The fact that even upregulated glucose metabolism cannot adequately support cardiac function is indicated by the development of progressive myopathy (although the cytosolic accumulation of long-chain fatty acids, which cannot be fully metabolized, also contributes to the myopathy). The classical treatment for this condition has been to replace the long-chain fatty acids in an individual's diet with even-numbered short- or medium-chain length fatty acids such as octanoate or decanoate. Importantly, the short-chain fatty acids bypass the metabolic roadblock, because the medium- and short-chain acyl-CoA dehydrogenases are normal in these patients. This therapeutic strategy induces increased fatty acid utilization and causes clinical improvement, but unfortunately cardiac and skeletal muscle myopathies may persist in many patients treated in this manner.

The question arose as to why feeding even-numbered short-chain length fatty acids did not fully correct the muscle defects in these patients despite the augmentation of acetyl-CoA delivery to the TCA cycle. The answer was that VLCAD deficiency caused limitation of metabolism of both even- and odd-numbered long-chain fatty acids; hence, both acetyl-CoA and propionyl-CoA deliveries (i.e., from beta oxidation of fatty acids) to the TCA cycle were limited by this inherited defect. Although the metabolism of even-numbered short-chain fatty acids supplied acetyl-CoA, it did not supply the anaplerotic substrate propionyl-CoA. Consequently, even-numbered short-chain fatty acids could not correct the defect in the anaplerotic delivery of substrate to the TCA cycle and, therefore, were unable to correct a possible decrease in TCA intermediate pool size resulting from propionyl-CoA deficiency (Fig. 21.6b). Following this logic, dietary supplementation with an odd-numbered short-chain fatty acid would be expected to permit β-lipid oxidation to supply both acetyl-CoA and propionyl-CoA. In fact, when patients were treated with heptanoate, a seven-carbon fatty acid, they showed marked improvement in both skeletal muscle and cardiac abnormalities. Hence, this disorder was effectively managed by defining all of the biochemical defects and prescribing a biochemical treatment strategy that compensated for these defects. Unfortunately, most inborn abnormalities of energy-generating systems in the heart and other organs are not amenable to such a simple dietary manipulation. Over the long term, work in the molecular biology of genetic manipulation shows promise in remedying these diseases.

21.5 Summary

This chapter summarized how chemical energy stored in ingested carbon substrates is transferred to ATP, as well as some of the regulatory systems which integrate the function of these pathways and make them responsive to changes in ATP demand without destabilizing the intracellular chemical milieu. Also reviewed were the generation of toxic by-products of the metabolic processes and mechanisms that limit their adverse effects and the effects of several physiological states and diseases on these processes.

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