# Mitochondria in Health and Disease

edited by

Carolyn D. Berdanier

# Mitochondria in Health and Disease

## OXIDATIVE STRESS AND DISEASE

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Carolyn D. Berdanier

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# Series Introduction

Oxygen is a dangerous friend. Through evolution, oxygen — itself a free radical — was chosen as the terminal electron acceptor for respiration. The two unpaired electrons of oxygen spin in the same direction; thus, oxygen is a biradical. Other oxygen-derived free radicals, such as superoxide anion or hydroxyl radicals, formed during metabolism or by ionizing radiation are stronger oxidants, i.e., endowed with a higher chemical reactivity. Oxygen-derived free radicals are generated during oxidative metabolism and energy production in the body and are involved in regulation of signal transduction and gene expression, activation of receptors and nuclear transcription factors, oxidative damage to cell components, the antimicrobial and cytotoxic action of immune system cells, neutrophils and macrophages, as well as in aging and age-related degenerative diseases. Overwhelming evidence indicates that oxidative stress can lead to cell and tissue injury. However, the same free radicals that are generated during oxidative stress are produced during normal metabolism and, as a corollary, are involved in both human health and disease.

In addition to reactive oxygen species, research on reactive nitrogen species has been gathering momentum to develop an area of

enormous importance in biology and medicine. Nitric oxide or nitrogen monoxide (NO) is a free radical generated by nitric oxide synthase (NOS). This enzyme modulates physiological responses in the circulation such as vasodilation (eNOS) or signaling in the brain (nNOS). However, during inflammation, a third isoenzyme is induced, iNOS, resulting in the overproduction of NO and causing damage to targeted infectious organisms and to healthy tissues in the vicinity. More worrisome, however, is the fact that NO can react with superoxide anion to yield a strong oxidant, peroxynitrite. Oxidation of lipids, proteins, and DNA by peroxynitrite increases the likelihood of tissue injury.

Both reactive oxygen and nitrogen species are involved in the redox regulation of cell functions. Oxidative stress is increasingly viewed as a major upstream component in the signaling cascade involved in inflammatory responses and stimulation of adhesion molecule and chemoattractant production. Hydrogen peroxide decomposes in the presence of transition metals to the highly reactive hydroxyl radical, which by two major reactions — hydrogen abstraction and addition — accounts for most of the oxidative damage to proteins, lipids, sugars, and nucleic acids. Hydrogen peroxide is also an important signaling molecule that, among others, can activate NF-kB, an important transcription factor involved in inflammatory responses. At low concentrations, hydrogen peroxide regulates cell signaling and stimulates cell proliferation; at higher concentrations it triggers apoptosis and, at even higher levels, necrosis.

Virtually all diseases thus far examined involve free radicals. In most cases, free radicals are secondary to the disease process, but in some instances free radicals are causal. Thus, there is a delicate balance between oxidants and antioxidants in health and disease. Their proper balance is essential for ensuring healthy aging.

The term oxidative stress indicates that the antioxidant status of cells and tissues is altered by exposure to oxidants. The redox status is thus dependent on the degree to which a cell's components are in the oxidized state. In general, the reducing environment inside cells helps to prevent oxidative damage. In this reducing environment, disulfide bonds (S–S) do not spontaneously form because sulfhydryl groups are maintained in the reduced state (SH), thus preventing protein misfolding or aggregation. This reducing environment is maintained by oxidative metabolism and by the action of antioxidant enzymes and substances, such as glutathione, thioredoxin, vitamins E and C, and enzymes such as superoxide dismutases, catalase, and the selenium-dependent glutathione

reductase and glutathione and thioredoxin hydroperoxidases, which serve to remove reactive oxygen species (hydroperoxides).

Changes in the redox status and depletion of antioxidants occur during oxidative stress. The thiol redox status is a useful index of oxidative stress mainly because metabolism and NADPH-dependent enzymes maintain cell glutathione (GSH) almost completely in its reduced state. Oxidized glutathione (glutathione disulfide, GSSG) accumulates under conditions of oxidant exposure and this changes the ratio GSSG/GSH; an increased ratio is usually taken as indicating oxidative stress. Other oxidative stress indicators are ratios of redox couples such as NADPH/NADP, NADH/NAD, thioredoxin$_{reduced}$/thioredoxin$_{oxidized}$, dihydrolipoic acid/α-lipoic acid, and lactate/pyruvate. Changes in these ratios affects the energy status of the cell, largely determined by the ratio ATP/ADP + AMP. Many tissues contain large amounts of glutathione, 2–4 mM in erythrocytes or neural tissues and up to 8 mM in hepatic tissues. Reactive oxygen and nitrogen species can oxidize glutathione, thus lowering the levels of the most abundant nonprotein thiol, sometimes designated as the cell's primary preventative antioxidant.

Current hypotheses favor the idea that lowering oxidative stress can have a health benefit. Free radicals can be overproduced or the natural antioxidant system defenses weakened, first resulting in oxidative stress, and then leading to oxidative injury and disease. Examples of this process include heart disease, cancer, and neurodegenerative disorders. Oxidation of human low-density lipoproteins is considered an early step in the progression and eventual development of atherosclerosis, thus leading to cardiovascular disease. Oxidative DNA damage may initiate carcinogenesis. Environmental sources of reactive oxygen species are also important in relation to oxidative stress and disease. A few examples: UV radiation, ozone, cigarette smoke, and others are significant sources of oxidative stress.

Compelling support for the involvement of free radicals in disease development originates from epidemiological studies showing that an enhanced antioxidant status is associated with reduced risk of several diseases. Vitamins C and E and prevention of cardiovascular disease are a notable example. Elevated antioxidant status is also associated with decreased incidence of cataracts, cancer, and neurodegenerative disorders. Some recent reports have suggested an inverse correlation between antioxidant status and the occurrence of rheumatoid arthritis and diabetes mellitus. Indeed, the number of indications in which antioxidants may be useful in the prevention and/or the treatment of disease is increasing.

Oxidative stress, rather than being the primary cause of disease, is more often a secondary complication in many disorders. Oxidative stress diseases include inflammatory bowel diseases, retinal ischemia, cardiovascular disease and restenosis, AIDS, adult respiratory distress syndrome, and neurodegenerative diseases such as stroke, Parkinson's disease, and Alzheimer's disease. Such indications may prove amenable to antioxidant treatment (in combination with conventional therapies) because there is a clear involvement of oxidative injury in these disorders.

In this series of books, the importance of oxidative stress and disease associated with organ systems of the body is highlighted by exploring the scientific evidence and the medical applications of this knowledge. The series also highlights the major natural antioxidant enzymes and antioxidant substances such as vitamins E, A, and C, flavonoids, polyphenols, carotenoids, lipoic acid, coenzyme $Q_{10}$, carnitine, and other micronutrients present in food and beverages. Oxidative stress is an underlying factor in health and disease. More and more evidence indicates that a proper balance between oxidants and antioxidants is involved in maintaining health and longevity and that altering this balance in favor of oxidants may result in pathophysiological responses causing functional disorders and disease. This series is intended for researchers in the basic biomedical sciences and clinicians. The potential of such knowledge for healthy aging and disease prevention warrants further knowledge about how oxidants and antioxidants modulate cell and tissue function.

**Lester Packer**

**Enrique Cadenas**

# Preface

The mitochondria serve as the powerhouses of the cells. Interest in these organelles (especially their DNA) has blossomed over the last few decades. Mitochondrial diseases have been identified. At first, it was thought that these diseases were rare and that only a few people were affected. Then, as our knowledge expanded, we began to realize that several chronic diseases were associated with malfunctioning mitochondria. Several of these malfunctions were found to be nuclear in origin. Indeed, some of the degenerative chronic diseases were found to have an adverse effect on mitochondrial number, mitogenesis, and the base sequence of the mitochondrial DNA. This idea — that disease can affect the DNA and that mutated mitochondrial DNA can result in disease — has become a puzzle. Diabetes, in particular, can be found to be associated with DNA mutation and can cause mutation.

*Mitochondria in Health and Disease* is a collection of chapters written by experts in particular areas of mitochondrial investigations. It is hoped that you, the reader, will find it of sufficient breadth and depth to meet your needs to learn more about the function of this organelle and what happens when it malfunctions. I am grateful to the authors who have taken the time to share their expertise; in

some cases, it was a difficult task, but each carried it to completion in an admirable way. Thank you.

**Carolyn D. Berdanier**

# About the Editor

**Carolyn D. Berdanier** is a professor emerita of the University of Georgia. She earned her B.S. from Pennsylvania State University and her M.S. and Ph.D. in nutrition/biochemistry from Rutgers University. After a postdoctoral year with Dr. Paul Griminger, she served as a research nutritionist with the Human Nutrition Research division of the Agricultural Research Service, USDA, and concurrently held a faculty position with the University of Maryland. After 7 years, Dr. Berdanier moved to the College of Medicine of the University of Nebraska in Omaha, where she again was a full-time researcher with some teaching assignments. Later, she moved to the University of Georgia, where she headed the Department of Nutrition for 11 years, followed by 11 years as a teacher and researcher in the genetic aspects of nutritional response in diabetes. Her research was supported by grants from NIH, USDA, several commodity groups, and USDC Sea Grant Program.

Dr. Berdanier is a member of the American Society for Nutrition Science, The American Physiology Society, The American Diabetes Association, and the Society of Experimental Biology and Medicine. She has served on a number of editorial boards, including those of the *Journal of Nutrition*, the *FASEB Journal*, *Nutrition Research*,

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# 1

# Introduction to Mitochondria

CAROLYN D. BERDANIER

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## INTRODUCTION

The mitochondria are organelles suspended in the cytoplasm that are the powerhouses or power plants of the cell. It is this organelle that traps the energy released by oxidative reactions in the high-energy bond of ATP. This trapping occurs through the process of coupling the synthesis of water via the respiratory chain to the synthesis of ATP via the $F_1F_0$ATPase in a process called oxidative phosphorylation (OXPHOS). The mitochondria have other functions as well. Fatty acid oxidation and the citric acid cycle occur here and gluconeogenesis and urea synthesis begin in this compartment. The mitochondria play a role in apoptosis and have the components for the synthesis of a few proteins of OXPHOS.

The mitochondria serve as the central integrators of intermediary metabolism. Redox and phosphorylation states are managed here. The energy "coinage" (high-energy phosphate bonds) of the cell is manufactured here and, through this manufacture, the cell is able to conserve energy and store it for use in times of need. Throughout metabolism, the flux through many of the pathways is determined by the ratio of ATP to ADP, the availability of ATP, and the ratio of reduced to oxidized metabolic intermediates. It is the purpose of this chapter to provide the framework for subsequent chapters designed to describe mitochondrial function as well as specific aspects of mitochondrial DNA in health and disease.

## ANATOMY AND PHYSIOLOGY OF THE MITOCHONDRION

### Structural Components

Morphologically, the mitochondria are remarkably similar between species in terms of structure and function (1). Cells vary in the number of mitochondria in the cytoplasm and in the shape of these organelles. The liver cell contains ~800 mitochondria, while bone cells contain fewer than 400 and erythrocytes contain none. In contrast, the ovum contains 2000–20000. Mitochondria are distributed throughout the cytoplasm in a nonrandom manner by cytoskeleton motors. High-energy dependent cells tend to have their mitochondria located close to where energy is needed. For example, muscle cells have mitochondria located in close proximity to the contracting muscle fibers. Muscle contraction requires considerable energy provided by creatine phosphate and ATP. Both of these high-energy compounds are synthesized in the mitochondrial compartment. Cells that are rapidly dividing have mitochondria close to the nucleus and to ribosomes because *de novo* protein synthesis is highly dependent on the energy provided by ATP. Nerve conduction is dependent on ATP; thus, nerve tissue has many mitochondria located close to where conduction takes place. Any shortfall in ATP production and availability will affect the function of the central and peripheral nervous systems. Sensory perception, movement, and the responses to a variety of stimuli will be affected.

The typical mitochondrion is about 0.5 μm in diameter and from 0.5 μm to several microns long. A typical mitochondrion is about the size of a typical bacterium. The shape of the mitochondrion varies with the cell type and with the metabolic activity of the cell. In the liver, the typical mitochondrion is oblong or sausage shaped; however, spindle or rod shapes can also be found in this cell type. The mitochondria in the adipose cells are generally round, but other shapes can be found as well.

**Table 1.1** Components of Mitochondria and Their Functions

| Component | Function |
|---|---|
| Outer membrane | Oxidation of neuroactive aromatic amines |
| | Cardiolipin synthesis |
| | Import of cytoplasmic synthesized proteins |
| | Ion transfer |
| Intermembrane space | Maintenance of adenine nucleotide balance |
| | Creatine kinase and adenylate kinase located here |
| | Electron transfer from complex III to complex IV of the respiratory chain |
| | Processing of proteins imported into the mitochondrion |
| Inner membrane | Oxidative phosphorylation (OXPHOS) |
| | Transport of pyridine nucleotides |
| | Calcium ion transport |
| | Transport of metabolites (pyruvate, $H_2PO_4^-$/$OH^-$, dicarboxylates, citrate/malate, carnitine/acylcarnitine) |
| Matrix | Oxidation of pyruvate to acetyl CoA (pyruvate dehydrogenase complex) |
| | Oxidation of ketone bodies |
| | Oxidation of amino acids |
| | Initiation of the urea cycle |
| | Fatty acid oxidation, citric acid cycle occurs |
| | Suppress free radical damage through Mn-SOD |
| | Process imported proteins |
| | Synthesize 13 components of OXPHOS |
| | Heme synthesis |

The mitochondrion consists of an outer membrane, an inner membrane, a space between the two membranes, and a matrix within the inner membrane. Each of these components has a specific yet different function, as listed in Table 1.1. It is possible to fractionate the cell and isolate the organelles by differential centrifugation so as to study their function. Furthermore, the mitochondria can be subfractionated so as to study the individual components and their associated functions (see Appendix 1 for methods). Enzymes typical of the matrix,

the inner membrane, the intermembrane space, or the outer membrane can be isolated and studied.

Table 1.2 lists some of the enzymes found in each of the mitochondrial components. For example, all of the enzymes of the citric acid cycle and for fatty acid oxidation can be found in the mitochondrial matrix. The intermembrane space contains several enzymes that use ATP, including creatine kinase and adenylate kinase. The $F_1F_0$ATPase has its base (the $F_0$ portion) embedded in the inner membrane and the head protrudes out into the matrix. The flux through the citric acid cycle or the fatty acid oxidation pathway or the $F_1F_0$ATPase can be studied in detail. The activity of specific enzymes as well as the changes in substrate levels of these segments of metabolism can be determined.

The matrix also contains a small genome (~16.4 kilobases in mammals) and the machinery necessary to transcribe and translate the 13 OXPHOS proteins encoded by this DNA. The size of the genome is species specific. Shown in Table 1.3 are some species and their mitochondrial DNA size. Regardless of size, the map of the genome is fairly consistent from species to species. That is, within the genome the location of each of the structural genes of the ribosomes and each of the tRNAs are found in roughly the same place. Most of the proteins of OXPHOS are encoded by the nuclear genome, synthesized in the cytoplasm, and imported into the mitochondria for insertion into their appropriate locations within the OXPHOS system. Chapter 3 of this volume describes this DNA in detail — its transcription, translation, and role in mitochondrial protein synthesis and its replication. Subsequent chapters address specific disorders associated with mitochondrial DNA mutation.

## Membranes

The composition and shape of the inner and outer membranes differ significantly. Although these membranes are composed primarily of protein and phospholipid, the proteins and phospholipids differ — particularly the composition of the fatty acids in the phospholipids. The phospholipid fatty acids of the

**Table 1.2** Enzymes in the Mitochondria

| E.C. # | Enzyme | Comment |
|---|---|---|
| | Outer Membrane | |
| 2.7.1.1 | Hexokinase | Component of the glycolytic sequence attached to the outer surface of this membrane |
| 2.7.1.11 | Phosphofructokinase | Component of the glycolytic sequence attached to the outer surface of this membrane |
| 1.2.1.12 | Glyceraldehyde-phosphate dehydrogenase | Component of the glycolytic sequence attached to the outer surface of this membrane |
| 2.7.1.40 | Pyruvate kinase | Component of the glycolytic sequence attached to the outer surface of this membrane |
| 1.1.1.27 | L-lactate dehydrogenase | Component of the glycolytic sequence attached to the outer surface of this membrane |
| 6.2.1.3 | Fatty acyl CoA synthetase (ATP) | Synthesizes long chain fatty acid CoA |
| 1.4.3.4 | Monoamine oxidase | |
| 1.14.13.9 | Kyneurenine hydroxylase (mono-oxygenase) | |
| 2.7.8.2 | Choline phosphotransferase | Enzyme in phospholipid synthesis |
| 2.3.1.15 | Glycerolphosphate acyl transferase | Enzyme in phospholipid synthesis |
| 2.7.7.41 | CDP-diglyceride pyrophosphorylase | Enzyme in phospholipid synthesis |
| 2.7.8.5 | Glycerolphosphate phosphatidyl transferase | Enzyme in phospholipid synthesis |
| 3.1.3.27 | Phosphatidylglycero-phosphatase | Enzyme in phospholipid synthesis |
| 3.1.3.4 | Phosphatidate phosphatase | Enzyme in phospholipid synthesis |
| 1.6.2.2 | Cytochrome $b_5$ reductase | |
| | Intermembrane Space | |
| 1.8.2.1 | Sulfite dehydrogenase | |
| 2.7.4.6 | Nucleoside diphosphokinase | |
| 2.7.4.3 | Adenylate kinase | |
| 4.6.1.1 | Adenylate cyclase | |
| 2.7.3.2 | Creatine kinase | |

*(continued)*

**Table 1.2 (continued)** Enzymes in the Mitochondria

| E.C. # | Enzyme | Comment |
|---|---|---|
| | Inner Membrane | |
| 2.3.1.21 | Carnitine acyltransferase | Part of fatty acid oxidation |
| 1.3.99.3 | Acyl CoA dehydrogenase (long chain) | Part of fatty acid oxidation |
| 1.3.99.2 | Acyl CoA dehydrogenase (short chain) | Part of fatty acid oxidation |
| 4.2.1.17 | Enoyl CoA hydratase | Part of fatty acid oxidation |
| 1.1.1.35 | L-Hydroxyacyl CoA dehydrogenase | Part of fatty acid oxidation |
| 2.3.1.16 | 3-Oxoacyl CoA thiolase | Part of fatty acid oxidation |
| 5.3.3.8 | *cis*-3,*trans*-2-Enoyl CoA isomerase | Part of fatty acid oxidation |
| 5.1.2.3 | 3-Hydroxyacyl CoA epimerase | Part of fatty acid oxidation |
| 1.3.99.1 | Succinate dehydrogenase | Part of the citric acid cycle |
| 1.1.1.30 | Hydroxybutyrate dehydrogenase | Important in ketone body metabolism |
| 1.6.99.3 | NADH dehydrogenase | |
| 1.6.1.1 | NAD(P) transhydrogenase | |
| 1.6.2.4 | NADPH-cytochrome reductase | |
| 1.1.99.5 | Glycerol phosphate dehydrogenase | |
| 1.5.99.1 | Sarcosine dehydrogenase | |
| 1.5.99.2 | Dimethylglycine dehydrogenase | |
| 1.3.99.2 | Acyl CoA dehydrogenase | Part of fatty acid oxidation |
| 1.1.99.1 | Choline dehydrogenase | |
| 1.1.2.3 | Lactate dehydrogenase (cytochrome $b_2$) | |
| 1.10.2.2 | Ubiquinone-cytochrome c reductase | |
| 1.9.3 | Cytochrome oxidase | |
| 3.6.1.3 | $F_1F_0$ATP synthetase | |
| 3.6.1.1 | Pyrophosphatase | |
| 2.1.2.10 | Glycine synthase | |
| 1.14.15.4 | Steroid 11β-mono-oxygenase | |
| 4.99.1.1 | Ferrochelatase | |

*(continued)*

**Table 1.2 (continued)** Enzymes in the Mitochondria

| E.C. # | Enzyme | Comment |
|---|---|---|
| | Matrix | |
| 2.7.1.99 | Pyruvate dehydrogenase kinase | Part of the citric acid cycle |
| 3.1.3.43 | Pyruvate dehydrogenase phosphatase | Part of the citric acid cycle |
| 4.1.3.7 | Citrate synthase | Part of the citric acid cycle |
| 4.2.1.3 | Aconitase | Part of the citric acid cycle |
| 1.1.1.41 | Isocitrate dehydrogenase (NAD) | Part of the citric acid cycle |
| 1.1.1.42 | Isocitrate dehydrogenase (NADP) | Part of the citric acid cycle |
| 1.2.4.2 | Oxoglutarate dehydrogenase complex | Part of the citric acid cycle |
| 2.3.1.61 | Oxoglutarate dehydrogenase complex | Part of the citric acid cycle |
| 1.6.4.3 | Oxoglutarate dehydrogenase complex | Part of the citric acid cycle |
| 6.2.1.4 | Succinate thiokinase (GTP) | Part of the citric acid cycle |
| 6.2.1.5 | Succinate thiokinase (ATP) | Part of the citric acid cycle |
| 4.2.1.2 | Fumarase (fumerate hydratase) | Part of the citric acid cycle |
| 1.1.1.37 | Malate dehydrogenase | Part of the citric acid cycle |
| 1.1.1.40 | Malic enzyme (NADP) (oxaloacetate decarboxylating) | |
| 6.4.1.1 | Pyruvate carboxylate (ATP) | |
| 4.1.1.32 | Phosphoenolpyruvate carboxykinase | Location in the matrix is species specific |
| 6.2.1.2 | Fatty acyl CoA synthetase (ATP) | Activates medium chain fatty acids |
| 6.2.1.10 | Fatty acyl CoA synthetase (GDP) | Important in fatty acid activation |
| 6.2.1.1 | Acetyl CoA synthetase | Important in fatty acid activation |
| 6.4.1.3 | Propionyl CoA carboxylase | Important to propionyl CoA metabolism |
| 5.1.99.1 | Methylmalonyl CoA racemase | Important to propionyl CoA metabolism |
| 5.4.99.2 | Methylmalonyl CoA mutase | Important to propionyl CoA metabolism |

*(continued)*

**Table 1.2 (continued)** Enzymes in the Mitochondria

| E.C. # | Enzyme | Comment |
|---|---|---|
| 2.3.1.9 | Acetoacetyl CoA thiolase | Important in ketone metabolism |
| 4.1.3.5 | Hydroxymethylglutaryl CoA synthase | Important in ketone metabolism |
| 4.1.3.4 | Hydroxymethylglutaryl CoA lyase | Important in ketone metabolism |
| 2.8.3.5 | Succinyl CoA transferase | Not in liver mitochondrial matrix |
| 1.3.1.8 | Enoyl CoA reductase | Important to fatty acid elongation |
| 1.4.1.3 | Glutamate dehydrogenase | Important to amino acid metabolism |
| 2.6.1.1 | Aspartate transaminase | |
| 3.5.1.2 | Glutaminase | |
| 6.3.4.16 | Carbamyl phosphate synthetase | First committed step in urea synthesis |
| 1.5.1.12 | Pyrroline carboxylate dehydrogenase | |
| 2.1.2.1 | Serine hydroxymethyl transferase | |
| 2.3.1.37 | δ-Aminolaevulinate synthase | Important to heme synthesis |
| 2.7.7.7 | DNA polymerase | Important to mitochondrial protein synthesis |
| 3.1.21.1 | Deoxyribonuclease | Important to mitochondrial protein synthesis |
| 2.7.7.6 | RNA polymerase | Important to mitochondrial protein synthesis |
| 1.15.1.1 | Superoxide dismutase | Important in free radical suppression |

inner membrane are highly unsaturated. Cardiolipin is abundant in the inner mitochondrial membrane and less so in the outer membrane. The inner membrane contains 19% of its phospholipids as cardiolipin and the outer membrane contains 4%. Cardiolipin is used as a marker for the identification of the mitochondrial membrane fraction when the membranes are studied. The inner membrane has very little (5.1 μg/mg protein) cholesterol. The outer membrane has some (30.1 μg/mg protein) cholesterol, but not as much as the plasma membrane.

**Table 1.3** Mitochondrial DNA in a Variety of Species

| Species | No. Bases | Remarks | Ref. |
|---|---|---|---|
| Yeast | ~50 kb | Some may be linear | 2 |
| Red alga | 25.8 kb | 51 genes | 3 |
| Amoeboid protozoan | 41.6 kb | Contains open reading frames | 4 |
| Pythuim (fungus) | 50–150 kb | Linear and circular forms | 5 |
| *Xenopus laevis* | 17.553 kb | | 6 |
| Japanese pond frog | 18.4–19.1 kb | | 7,8 |
| Honey bee | | Paternal DNA present | 9 |
| Sheep | ~16.5 kb | Wild and domestic sheep differ in control region length | 10 |
| Cows | 16.338 kb | Many polymorphisms reported | 11–13 |
| Horse | ~16.5 | Very diverse | 14,15 |
| Donkey | 16.67 kb | Length can vary | 16 |
| Rat | 16.298 | | 17 |
| Gibbon | 16.472 kb | Four genes lack complete stop codons | 18 |
| Man | 16.569 kb | Polymorphisms are common | 19,20 |

The plasma membrane has no cardiolipin but contains phosphatidylserine; the mitochondrial membranes have none of this phospholipid. Phosphatidylcholine (42% in the inner, 54% in the outer membrane) and phosphatidylethanolamine (32% in the inner and 26% in the outer membrane) are found in both mitochondrial membranes. The inner membrane contains 4% of its phospholipids as phosphatidylyinositol and the outer membrane contains 14%. The inner membrane contains ubiquinone and the outer membrane has none. Enzymes within the mitochondrial compartment play a role in the *in situ* production of some of these phospholipids (see Table 1.2). However, some of the phospholipids are synthesized outside the mitochondrion and imported (22).

The outer membrane surrounds the organelle and serves to maintain its shape (23). It is smooth and contains about 30 to 40% lipid and 60 to 70% protein. The protein in the outer membrane is rich in porin-a protein that contains many β-sheets. These sheets form large channels across the

membrane, thus explaining its relative permeability. The channels permit the free diffusion of molecules of less than 10,000 in molecular weight. The channels are voltage-dependent anion channels (VDAC) permeable to solutes up to 5 kDa. Small molecules such as ADP or ATP pass easily through the outer membrane, as do certain nonelectrolytes such as inulin. Ion movement through the outer membrane depends largely on the charge of the ion. NAD and $NADH^+$ cannot pass into the mitochondria through these membranes.

The inner membrane has many folds into the matrix that serve to increase its surface area for the metabolic activities that take place there. The wrinkles of the inner membrane form lamellar structures called cristae. On the inner membrane (facing into the matrix) are numerous small spheres on stalks, which are the structures associated with the multisubunit ATP synthetase or the $F_1F_0$ATPase. The inner membrane is permeable only to water, oxygen, and carbon dioxide and is impermeable to a wide variety of molecules and ions that, in turn, require specific transporters for entry and exit. A number of transport systems and enzymes are in the inner membrane, thus accounting for the fact that this membrane consists of ~80% by weight protein and only 20% lipid.

Located within the inner membrane are the many proteins that together comprise the respiratory chain. One of these, cytochrome c, in addition to being important to the synthesis of water via the respiratory chain, also plays an important role in apoptosis. In this role it is part of the apoptosis-inducing factor (AIF). About 90% of cytochrome c is stored in vesicles created by the in-foldings or wrinkles of the inner mitochondrial membrane. The remaining 10% is located in the intermembrane space. Cytochrome c functions in respiration and also as an electron transfer protein in many different redox reactions.

As described earlier, the inner and outer membranes have a large percentage of their structure as lipid. The lipids in these membranes are phospholipids that form lipid bilayers. The phospholipids are oriented so that their hydrophobic portions, the fatty acids at positions 1 and 2 of the phospholipid, face into the center of the membrane, thus leaving the

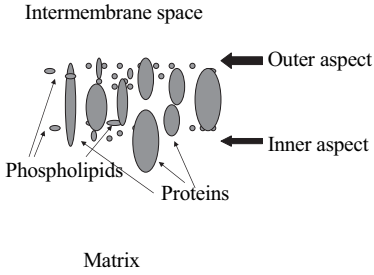


**Figure 1.1** Schematic representation of the inner mitochondrial membrane. The phospholipids are represented as having the hydrophilic head group on the outside (small circle) with the hydrophobic fatty acid tails extending in towards the center of the bilayer. Proteins are shown in several positions within the bilayer.

hydrophilic portion (the phosphorylated substituent) of their structure facing outward. Embedded in these lipid bilayers are the proteins that characterize each of the membranes.

Figure 1.1 is a theoretical representation of these membranes. Note that some of the proteins are embedded in the lipid bilayer so that they extend through it; other proteins are located to face only one side of the membrane. For example, a few of the enzymes of glycolysis are attached to the outer aspect of the outer membrane. Although glycolysis is usually thought of as a cytosolic pathway, some of the enzymes are in fact associated with the mitochondria. Another example is the enzymes of fatty acid oxidation embedded in the inner membrane but oriented so that they protrude into the matrix. The functions of these proteins can be related to their position within and on the membranes that house them.

## Diet Effects on Membrane Lipid Composition and Structure

The fatty acid composition of the membrane phospholipids can be influenced by diet (23–30) and hormonal state (31–38). In turn, these differences in composition and fluidity can affect mitochondrial function. Some examples of these influences are shown in Table 1.4 through Table 1.6. Diets rich in

**Table 1.4** Diet Effects on Selected Fatty Acids in Mitochondrial Membranes

| Treatment | Fatty Acids Mol% | | | | | Ref. |
|---|---|---|---|---|---|---|
| | 16:0 | 18:0 | 18:1 | 18:2 | 20:4 | |
| 65% sucrose–5% corn oil | 20.1 ± 0.7 | 20.9 ± 0.9 | 12.7 ± 1.6 | 20.8 ± 0.7 | 25.3 ± 0.6 | 23 |
| 65% starch–5% corn oil | 19.5 ± 0.5 | 19.9 ± 0.5 | 10.3 ± 0.8 | 24.6 ± 0.4 | 25.7 ± 0.4 | 23 |
| 65% starch–5% coconut oil | 15.4 ± 0.3 | 22.0 ± 0.4 | 12.0 ± 0.5 | 8.0 ± 0.3 | 18.0 ± 0.7 | 24 |
| Fat-free diet | 16.4 | 7.0 | 23.5 | 4.1 | 10.5 | 25 |
| 60% sucrose–9% fish oil | 30.3 ± 1 | 9.8 ± 1 | 27.8 ± 1.5 | 8.0 ± 0.7 | 2.3 ± 0.4 | Unpub. observ. |
| 60% sucrose–9% beef fat | 26.2 ± 1.0 | 9.8 ± 1.1 | 42.6 ± 1.0 | 4.7 ± 0.3 | 5.7 ± 0.8 | Unpub. observ. |

**Table 1.5** Effects of Hormones on Fatty Acid Profiles of Isolated Liver Mitochondria[a]

| Treatment | Fatty Acids (mol%) | | | | | | Ref. |
|---|---|---|---|---|---|---|---|
| | 16:0 | 18:0 | 18:1 | 18:2 | 20:4 | 22:6 | |
| 1 mg GC[a]/day | 14.6 | 22.7 | 6.6 | 28.5 | 19.0 | 3.5 | 37 |
| Control | 17.0 | 20.3 | 7.6 | 15.1 | 27.4 | 1.5 | |
| Thyroidectomy | 12.5 | 21 | 8.6 | 19.5 | 19.0 | 8.1 | 33 |
| Control | 13.0 | 20 | 9.0 | 17.4 | 15.7 | 7.0 | |
| Hypophysectomy | 27.1 | 21.5 | 13.5 | 17.0 | 14.5 | 2.0 | 38 |
| Control | 27.4 | 22 | 12.2 | 12.1 | 17.4 | 2.8 | |
| Diabetes[b] | 24.5 | 23.3 | 7.0 | 22.4 | 15.2 | 4.2 | 31 |
| Control | 16.9 | 23.2 | 9.3 | 22.7 | 22.2 | 3.1 | |

[a] GC = synthetic glucocorticoid, dexamethasone.
[b] Streptozotocin-induced diabetes.

**Table 1.6** Diet Effects on Selected Mitochondrial Functions

| Diet | Effect | Ref. |
|---|---|---|
| 5% coconut oil vs. corn oil | Increased ADP-stimulated malate–aspartate and α-glycerophosphate shuttles; increased state 4 respiration; decreased respiratory control ratio and the ADP:O ratio | 24 |
| 5% fish oil vs. corn oil | Increased state 3 respiration; decreased transition temperature (more fluid membrane) | 26 |
| 20% coconut oil vs. safflower oil | Increased desaturase activity | 28 |
| 50% fish oil vs. vegetable oil | Increased acylcarnitine transferase system and increased fatty acid oxidation; increased membrane fluidity | 28 |

polyunsaturated fatty acids (PUFAs) or saturated fatty acids influence the mitochondrial membranes so that these membranes reflect the fatty acids in the diet (Table 1.4). Diets rich in PUFAs result in membranes with more PUFAs and, as a result, these membranes are more fluid than membranes from animals fed a saturated fat diet or ones from animals fed an essential fatty acid free diet, i.e., a coconut oil diet or a fat free diet (24,25).

Feeding a high-sucrose diet to rats likewise influences these membranes: sucrose is used to support *de novo* fatty acid synthesis and these fatty acids are likely to be saturated ones. They are incorporated into the mitochondrial membranes, thus making these membranes less fluid than those in rats fed a similar amount of starch (23). Animals fed a fish oil diet have fatty acids in their membranes that reflect the fatty acids found in the fish oil (28,29). Beef tallow feeding likewise results in membranes with fatty acids reminiscent of the dietary fat (27,28). Whether the fat in the diet is high or low, the array of the fatty acids in the mitochondrial membranes will reflect the array in the diet, not the level of fat in the diet (27–31). Certain hormones also affect membrane composition and fluidity. Insulin treatment results in an increase in PUFAs (31,32), primarily because this hormone induces the activity of the desaturase enzymes (31).

Desaturation occurs in the endoplasmic reticulum and microsomes. The enzymes that catalyze this desaturation are the $\Delta 4$, $\Delta 5$, or $\Delta 6$ desaturases. Again, desaturation is species specific. Mammals, for example, lack the ability to desaturate fatty acids in the n6 or n3 position. Only plants can do this and even among plants species differences are found. Cold-water plants can desaturate at the n3 position; land plants of warmer regions cannot. The cold-water plants are consumed by cold-water creatures in a food chain that includes fish as well as sea mammals. In turn, these enter the human food supply and become sources of the n3 or omega-3 fatty acids in the marine oils.

In animals, desaturation of *de novo* synthesized fatty acids usually stops with the production of a monounsaturated fatty acid with the double bond in the 9 to 10 positions, counting from the carboxyl end of the molecule. Therefore, palmitic acid (16:0) becomes palmitoleic acid (16:1) and stearic acid (18:0) becomes oleic acid (18:1). In the absence of dietary EFA, most mammals will desaturate eicosenoic acid to produce eicosatrienoic acid. Increases in this fatty acid with unsaturations at $\omega 7$ and $\omega 9$ positions characterize the tissue lipids of EFA-deficient animals (25). They are sometimes called mixed function oxidases because two substrates (fatty

acid and NADPH) are oxidized simultaneously. These desaturases prefer substrates with a double bond in the ω6 position, but will also act on fatty acids with a double bond in the ω3 position and on saturated fatty acids.

Desaturation of *de novo* synthesized stearic acid to form oleic acid results in the formation of a double bond at the ω9 position. This is the first committed step of this desaturation/elongation reaction sequence. Oleic acid can also be formed by the desaturation and elongation of palmitic acid. Fatty acid desaturation can be followed by elongation and repeated so that a variety of mono- and polyunsaturated fatty acids can be formed; these contribute fluidity to membranes because of their lower melting point. An increase in the activity of the desaturation pathway is a characteristic response of rats fed a diet high in saturated fatty acids. The body can convert the dietary saturated fatty acids to unsaturated fatty acids, thus maintaining an optimal P:S ratio in the tissues.

Thyroidectomy results in a decrease in mitochondrial levels of linoleic and arachidonic acids (18:2 and 20:4), primarily due to a reduction in fatty acid mobilization (34–36). With a decrease in mobilization, the PUFAs have a longer residence time in the mitochondrial membranes. Treatment with glucocorticoids results in an increase in linoleic and a decrease in arachidonic acids (37). Hypophysectomy, which results in a loss in growth hormone, results in an increase in linoleic acid and a reduction in arachidonic acid (38). Each of these perturbations in membrane lipid composition also results in changes in mitochondrial function — specifically, changes in OXPHOS. Table 1.5 shows the effects of hormones on mitochondrial membrane fatty acid profiles.

Many of the proteins that are part of the OXPHOS system are embedded in the inner mitochondrial membrane and are influenced by the fluidity of that membrane (24,27,28). Their activities can be increased if the membrane is very fluid or decreased if the membrane becomes more rigid. Fluidity can be evaluated by measuring the activity of a given reaction or series of reactions such as oxygen consumption with increases in temperature (39). Careful measurements of oxygen consumption at 3° increments in the temperature of the

media surrounding the isolated mitochondria allow assessment of fluidity. By preparing an Arrhenius plot of oxygen consumption vs. temperature, the change in slope at the transition temperature can be visualized — that is, where the membrane changes from a "solid" to a "liquid." Actually, membranes are neither solid nor liquid but exist as a structure with regional differences in consistency based on the fatty acid composition of the local phospholipids and the proteins embedded in these phospholipids.

Fluidity in the outer membrane can be influenced by the ratio of the fatty acid unsaturation index to cholesterol. The fatty acid unsaturation index is defined as the sum of the unsaturated fatty acids (number of unsaturated fatty acids times the number of double bonds in these fatty acids). Because the inner membrane does not contain significant amounts of cholesterol, the fluidity of this membrane is not influenced by its cholesterol content but only by the ratio of unsaturated fatty acids to saturated fatty acids. The preparation of the Arrhenius plot that then describes membrane fluidity uses the equation:

$$d \ln k/dT = Ea/RT^2$$

where $k$ is the rate constant; $R$ is the gas constant (8.312 J/(mol•.K)); $Ea$, the activation energy; and $T$ the temperature in degrees Kelvin.

Conversion to base-10 logarithms gives the equation:

$$\log[k_2/k_1] = Ea/2.303R \bullet (T_2 - T_1/T_1\, T_2)$$

from which it can be seen that the value for $Ea$ can be obtained from the slope of the straight line when the logarithm is plotted against the reciprocal of the absolute temperature.

The transition temperature or breakpoint can be seen as the point at which the two lines intersect. This is a theoretical fluidity value. A typical Arrhenius plot is shown in Figure 1.2 and a comparison of plots using mitochondrial membranes from rats fed diets differing in fat saturation is shown in Figure 1.3. The lines shown in Figure 1.3 are from groups of rats fed a 6% hydrogenated coconut oil or a 6% menhaden oil diet for 4 weeks (25). They were killed at 56 days of age, their

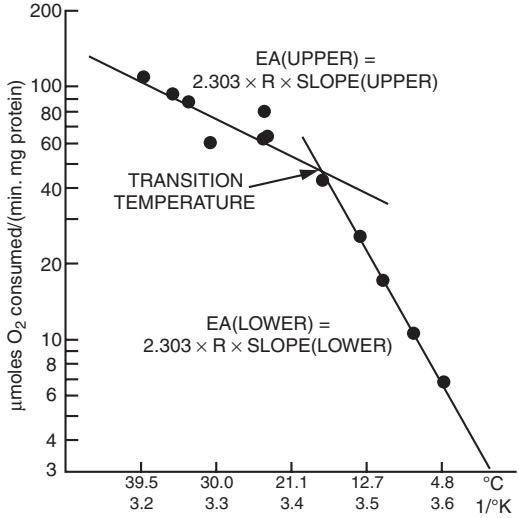


**Figure 1.2** A typical Arrhenius plot of the log of the oxygen consumption (micromoles oxygen consumed/milligrams mitochondrial protein) vs. the reciprocal of the temperature in degrees Kelvin.

mitochondria isolated, and the dependence of oxygen consumption on temperature determined.

Note in Figure 1.3 that the transition temperature for the rats fed the menhaden oil diet is lower than that for the rats fed the coconut oil diet. Shown in Table 1.6 are diet-induced effects on several other processes that depend on membrane fluidity. The shuttling of reducing equivalents is one of these, the oxidation of fatty acids is another, and gluconeogenesis is a third.

## Age Effects on Membrane Lipids

As animals age, their hormonal status changes, as does the lipid component of their membranes. With age, growth hormone production decreases, the hormones for reproduction increase and then decrease, and, as the animal ages, fat stores become larger. Larger fat cells are resistant to insulin and insulin levels may rise as a result of increased fat cell size

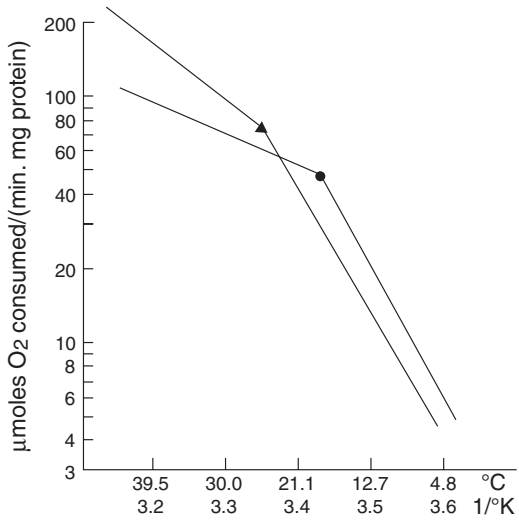


**Figure 1.3** Different Arrhenius plots from hepatic mitochondria from rats fed a 6% fish oil (-Δ-) or 6% coconut oil (-•-) diet. (Adapted from M-J.C. Kim and C.D. Berdanier. *FASEB J.* 12: 243–248, 1998.)

(insulin resistance). As mentioned in the preceding section, these hormones can affect the lipid portion of the mitochondrial membranes and thus affect mitochondrial metabolism.

With age, the degree of unsaturation of the membrane fatty acids decreases (40–44) and the number of superoxide radicals increases (45,46). This increase may be responsible for the degradation of the membrane lipids, which, in turn, might explain the age-related changes in membrane function (44). Membranes from aging animals are less fluid and have reduced transport capacities. As animals age, the hepatic mitochondrial respiratory rate declines, the respiratory ratio and the ADP/O ratio (42,47,48) decrease, and the activities of the citric acid cycle enzymes and fatty acid oxidizing enzymes decline (49). In addition, there are reports of an age-related decrease in membrane fatty acid unsaturation coupled with a decrease in membrane fluidity and a decrease in ATP synthesis (44) and a decrease in the exchange of ATP for ADP across the mitochondrial membrane (50–53). A decrease in

ATP synthesis and an amelioration of these age-related decreases in mitochondrial function by restricted feeding (energy intake reduced by 50% over the lifetime of the animals) has also been reported (54,55). Changes in mitochondrial function also take place due to free radical damage, not only to the membranes but also to the mitochondrial DNA (46). Chapter 3 and Chapter 6 in this volume address this issue.

## Membrane Function

In the previous section, the importance of diet, age, and hormonal status was described in terms of their influence on the composition of the membrane lipids. Although not emphasized, these compositional differences have important effects on metabolic regulation. This regulation consists of the control of the flux of nutrients, substrates, and/or products into, out of, and between the various compartments of the cell.

The mitochondrial membranes serve as the "gatekeepers" of the organelle. They regulate the influx and efflux of substrates and metabolic products produced or used by the compartment in the course of its metabolic activity. For example, the mitochondrial membrane through its transport of two- and four-carbon intermediates and through its exchange of ADP for ATP regulates the activity of the respiratory chain and ATP synthesis. If too little ADP enters the mitochondria because of decreased ADP transport across the mitochondrial membrane, respiratory chain activity will decrease, less ATP will be synthesized, and other mitochondrial reactions driven by ADP influx or dependent on ATP availability may decrease.

Through its export of citrate from the matrix of the mitochondria it regulates the availability of citrate to the cytosol for cleavage into oxaloacetate and acetyl CoA, the beginning of fatty acid synthesis. If more citrate is exported from the mitochondria than can be split to oxaloacetate and acetyl CoA, this citrate will feed back onto the phosphofructokinase reaction, and glycolysis will be inhibited. Thus, the activity of the mitochondrial membrane tricarboxylate transporter has a role in the control of cytosolic metabolism. Other transporters

such as the dicarboxylate transporter or the adenine nucleotide translocase have similar responsibilities vis-a-vis the control of cytosolic and mitochondrial metabolic activity. Such transporters are necessary because highly charged molecules such as ADP or ATP cannot readily traverse the mitochondrial membranes. Similarly, oxaloacetate, NAD, and NADH cannot traverse the membranes.

Closely packed proteins and lipids are in the mitochondrial membranes. The membrane-bound proteins have extensive hydrophobic regions and usually require their nearby lipids for the maintenance of their activity. Adenylate cyclase, cytochrome $b_5$, and cytochrome c oxidase have all been shown to have phospholipid affinities. Cytochrome c oxidase has tightly bound aldehyde lipid that cannot be removed without destroying its activity as the enzyme that transfers electrons to molecular oxygen in the final step of respiration.

A number of other membrane proteins have tightly bound fatty acids as part of their structures. These fatty acids are covalently bound to their proteins as a posttranslational event and act to direct, insert, and anchor the proteins in the inner and outer membranes. Some lipids are bound to membrane proteins. Some proteins are acylated with fatty acids during their passage from their site of synthesis on the rough endoplasmic reticulum to the membrane, whereas others acquire their lipid component during their placement in the membrane. β-Hydroxybutyrate dehydrogenase, for example, requires the choline head of phosphatidylcholine for its activity. If hepatocytes are caused to increase their synthesis of phosphatidyl-methylethanolamine, which substitutes for phosphatidylcholine in the mitochondrial membrane, β-hydroxybutyrate dehydrogenase activity is reduced (56).

All of these examples illustrate the importance of the mitochondrial membranes in the regulation of metabolism. They illustrate the fact that the gatekeeping property of the membrane is vested in the structure and function of the various transporters and receptors or binding proteins embedded in the bilayer lipid membrane. Whereas the genetic heritage of an individual determines the amino acid sequence of the proteins and thus their function, this function can be modified

by the lipid milieu in which they exist. Diet, hormonal state, and genetics in turn control the lipid milieu in terms of the kinds and amounts of the different lipids synthesized within the cell and incorporated into the membrane.

## METABOLIC SYSTEMS IN MITOCHONDRIA

### Fatty Acid Oxidation

Although fatty acids are important components of membranes, the main function of these molecules in the body is to provide energy to sustain life. This provision is accomplished through oxidation. Regardless of whether the fatty acids come from the diet or are mobilized from the tissue triacylglyceride store, the pathway for oxidation is the same once the triacylglyceride has been hydrolyzed. Fatty acid oxidation occurs primarily in the mitochondrial matrix.

The hydrolysis of stored lipid is catalyzed in a three-step process by one of the lipases specific to mono-, di-, or triacylglycerol. The liberated fatty acids bind to the cytosolic fatty acid binding protein and migrate through the cytoplasm to the outer mitochondrial membrane. At the outer membrane, they are activated by conversion to their CoA thioesters. This activation requires ATP and the enzyme acyl CoA synthase or thiokinase. The several thiokinases differ with respect to their specificity for the different fatty acids. The activation step depends on the release of energy from two high-energy phosphate bonds. ATP is hydrolyzed to AMP and two molecules of inorganic phosphate. Figure 1.4 shows the initial steps in the oxidation of fatty acids.

Once the fatty acid is activated, it is bound to carnitine with the release of CoA. The acyl carnitine is then translocated through both mitochondrial membranes into the mitochondrial matrix via the carnitine acylcarnitine translocase. As one molecule of acylcarnitine is passed into the matrix, one molecule of carnitine is translocated back to the cytosol and the acylcarnitine is converted back to acyl CoA. The acyl CoA can then enter the β-oxidation pathway shown in Figure 1.5.

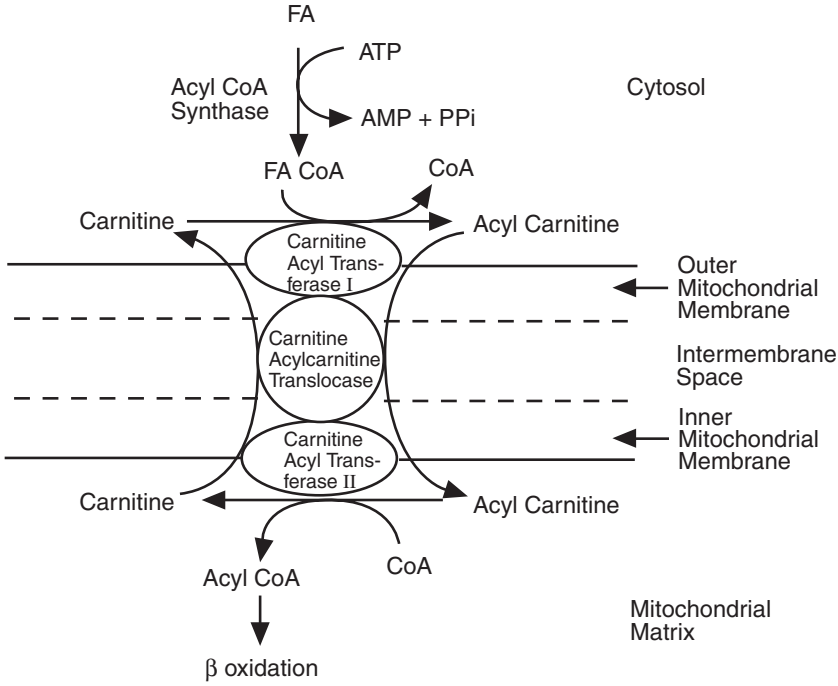


**Figure 1.4** Initial steps in the oxidation of fatty acids by the mitochondrial enzymes.

Without carnitine, the oxidation of fatty acids, especially the long chain fatty acids, cannot proceed. Acyl CoA cannot traverse the membrane into the mitochondria by itself; the translocase requires carnitine. Normally, the body synthesizes all the carnitine that it needs for fatty acid oxidation. However, in some instances, endogenous synthesis is inadequate. Premature infants, for example, may require carnitine supplementation and some children with mitochondrial disease may benefit from carnitine supplementation. In the latter case, the benefit is due to the high use of fatty acids as metabolic fuel that, in turn, increases the need for carnitine above that which can be endogenously synthesized.

The oxidation of unsaturated fatty acids follows the same pathway as the saturated fatty acids until the double bonded

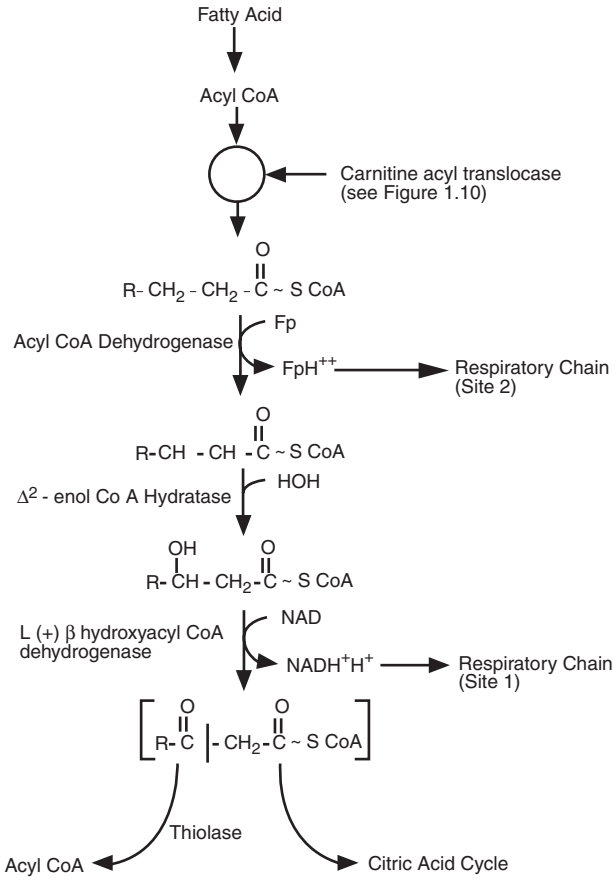


**Figure 1.5** The β-oxidation pathway.

carbons are reached. At this point, a few side steps must be taken that involve a few additional enzymes. An example of this pathway using linoleate as the fatty acid being oxidized is shown in Figure 1.6.

Linoleate has two double bonds in the *cis* configuration. β-Oxidation removes three acetyl units, leaving a CoA attached to the terminal carbon just before the first *cis* double bond. At this point, an isomerase enzyme, $\Delta^3$ *cis* $\Delta^6$ *trans* enoyl CoA isomerase, acts to convert the first *cis* bond to a *trans*

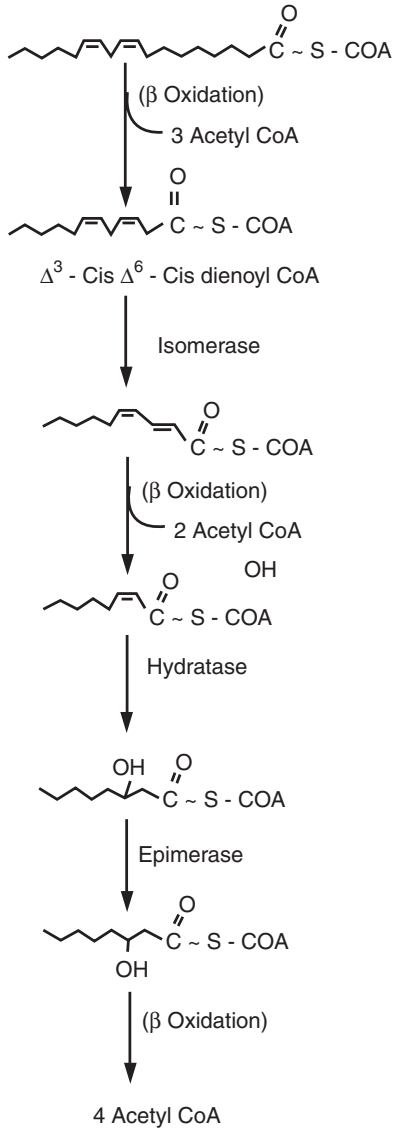


**Figure 1.6** Oxidation of linoleic acid showing the opening up of the double bond and the insertion of a hydroxyl group.

bond. The bond is opened up and a hydroxyl group is inserted. Now this part of the molecule can once again enter the β-oxidation sequence and two more acetyl CoA units are released. The second double bond is then opened and, again, a hydroxyl group is inserted. In turn, this hydroxyl group is rotated to the L position and the remaining product then reenters the β-oxidation pathway.

Other unsaturated fatty acids can be similarly oxidized. Each time the double bond is approached, the isomerization and hydroxyl group addition takes place until all of the fatty acid is oxidized. The end products of fatty acid oxidation are acetyl CoAs plus the reducing equivalents released by the dehydrogenase catalyzed reactions in the β-oxidation sequence. The reducing equivalents are sent to the respiratory chain while the acetyl CoAs enter the citric acid cycle. Thus, the fatty acids are oxidized with the production of water by the respiratory chain and the production of carbon dioxide via the citric acid cycle.

## Ketogenesis

Some of the acetyl CoA is converted to the ketones, acetoacetate, and β-hydroxybutyrate. The condensation of two molecules of acetyl CoA to acetoacetyl CoA occurs in the mitochondria via the enzyme β-ketothiolase. Acetoacetyl CoA then condenses with another acetyl CoA to form HMG CoA. This HMG CoA is cleaved into acetoacetic acid and acetyl CoA. The acetoacetic acid is reduced to β-hydroxybutyrate; this reduction depends on the ratio of $NAD^+$ to $NADH^{++}$. The enzyme for this reduction, β-hydroxybutyrate dehydrogenase, is tightly bound to the inner aspect of the mitochondrial membrane. Because of the high activity of the enzyme, the product (β-hydroxybutyrate) and substrate (acetoacetate) are in equilibrium. Measurements of these two compounds can be used to determine the redox state (ratio of oxidized to reduced NAD) of the mitochondrial compartment.

HMG CoA is also synthesized in the cytosol; however, because this compartment lacks the HMG CoA lyase, ketones are not produced here. In the cytosol, HMG CoA is

the beginning substrate for cholesterol synthesis. The ketones produced in the mitochondria can ultimately be used as fuel but may appear in the blood, liver, and other tissues at a level of less than 0.2 m*M*. In starving individuals or people consuming a high-fat diet, blood and tissue ketone levels may rise to ~3 to 5 m*M*. However, unless ketone levels greatly exceed the body's capacity to use them as fuel (as is the case in uncontrolled diabetes mellitus with levels up to 20 m*M*), a rise in ketone levels is not a cause for concern. Ketones are choice metabolic fuels for muscle and brain. Although both tissues may prefer to use glucose, the ketones can be used when glucose is in short supply. Ketones are used to spare glucose whenever possible under these conditions.

Although β-oxidation is the main pathway for the oxidation of fatty acids, some fatty acids undergo α-oxidation so as to provide the substrates for the synthesis of sphingolipids. This oxidation occurs in the endoplasmic reticulum and in the mitochondria and involves the mixed function oxidases. The oxidases require molecular oxygen, reduced NAD, and specific cytochromes. The fatty acid oxidation that occurs in organelles other than the mitochondria (i.e., peroxisomes) is an energetically wasteful reaction. It produces heat but no ATP. The peroxisomes do not have the citric acid cycle nor do they have the OXPHOS system. Peroxisomal oxidation in the kidney and liver is an important aspect of drug metabolism.

## Gluconeogenesis

The gluconeogenic pathway provides glucose to the body during starvation. It is a pathway that occurs in the cytosol but depends on the mitochondrial compartment for its starting substrate, oxaloacetate. The pathway is shown in Figure 1.7. Gluconeogenesis occurs primarily in the liver and kidney. Most tissues lack the full complement of enzymes needed to run this pathway. In particular, the enzyme phosphoenolpyruvate carboxykinase (PEPCK) is not found outside the liver and kidney. Shown in Figure 1.7 are the enzymes unique to gluconeogenesis. Some of these are found in many cell types, so some of the later steps in glucose release may be in all

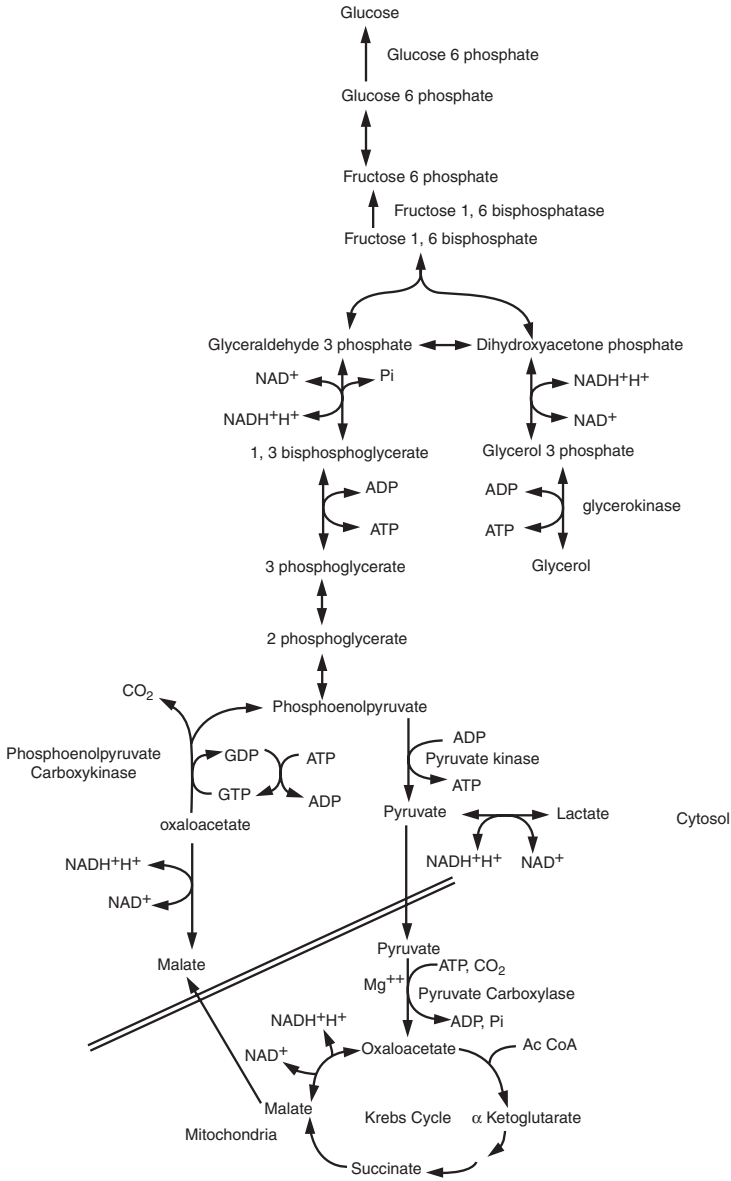


**Figure 1.7** Gluconeogenesis. Note the initial step: the malate–aspartate shuttle provides reducing equivalents to the mitochondrial compartment via metabolite exchange across the mitochondrial membrane. The key enzyme in the gluconeogenic pathway is PEPCK. In some species (e.g., the guinea pig), this enzyme is located in the mitochondria.

tissues. The other reactions shown use the same enzymes as glycolysis and do not have control properties with respect to gluconeogenesis. The rate-limiting enzymes of interest are glucose 6-phosphatase, fructose 1,6 biphosphatase, and phosphoenolpyruvate carboxykinase. Pyruvate kinase and pyruvate carboxylase are also of interest because their control is a coordinated one with respect to the regulation of PEPCK.

The control of gluconeogenesis rests in part with the mitochondrial malate–aspartate shuttle (Figure 1.8). This shuttle works to transport reducing equivalents into the mitochondria. It is stimulated by the influx of ADP in exchange for ATP. Malate is transported into the mitochondria whereupon it gives up two reducing equivalents and is transformed into oxaloacetate. Oxaloacetate cannot traverse the mitochondrial membrane, so it is converted to α-ketoglutarate in a coupled reaction that also converts glutamate to aspartate. Aspartate travels out of the mitochondria (along with ATP) and in exchange for glutamate.

Once out in the cytosol, the reactions are reversed. Aspartate is reconverted to glutamate and α-ketoglutarate reconverted to oxaloacetate. In turn, the oxaloacetate can be reduced to malate or decarboxylated to form phosphoenolpyruvate. Measurement of the activity of this shuttle has revealed that, the more active the shuttle is, the more active is gluconeogenesis (58–60) because the shuttle provides a steady supply of oxaloacetate via α-ketoglutarate in the cytosol. This oxaloacetate cannot get there any other way. As mentioned, it is generated by the Krebs cycle in the mitochondria but cannot leave this compartment. The regeneration of oxaloacetate is thus the first step in gluconeogenesis and this involves the mitochondria.

Oxaloacetate is essential to gluconeogenesis because it is the substrate for PEPCK, which catalyzes its conversion to phosphoenolpyruvate (PEP). This is an energy-dependent conversion that overcomes the irreversible final glycolytic reaction catalyzed by pyruvate kinase. The activity of PEPCK is closely coupled with that of pyruvate carboxylase. Whereas the pyruvate kinase reaction produces one ATP, the formation of PEP uses two ATPs: one in the mitochondria for the

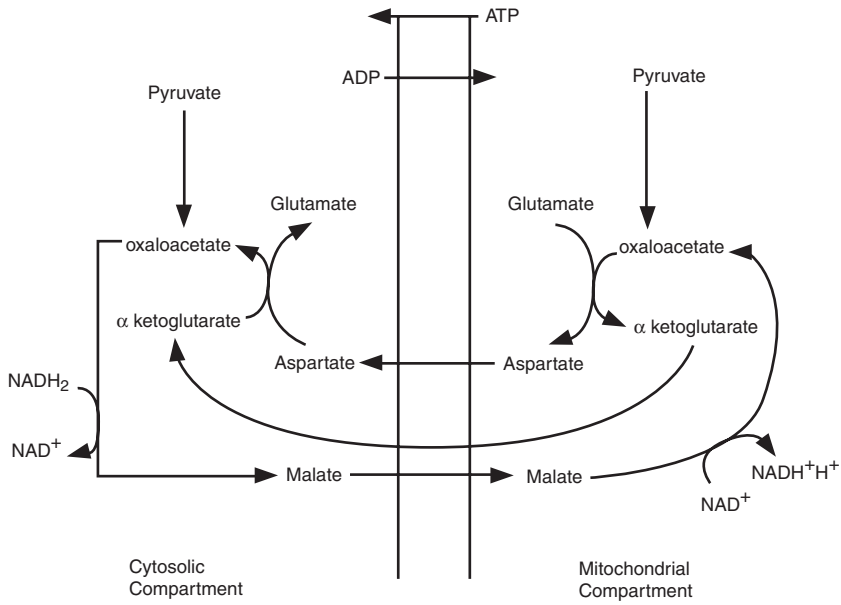


**Figure 1.8** The malate–aspartate shuttle. The shuttle is stimulated by an influx of ADP.

pyruvate carboxylase reaction and one in the cytosol for the PEPCK reaction. PEPCK requires GTP provided via the nucleoside diphosphate kinase reaction, which uses ATP. ATP transfers one high-energy bond to GDP to form ADP and GTP.

## Urea Cycle

The urea cycle consists of the synthesis of carbamyl phosphate and the synthesis of citrulline, then arginosuccinate, arginine, and finally urea (Figure 1.9). Ornithine and citrulline are shuttled back and forth as the cycle turns to get rid of the excess ammonia via urea release from arginine. The cycle functions to reduce the potentially toxic amounts of ammonia that arise when the ammonia group is removed from amino acids. Most of the ammonia released reflects the coupled action of the transaminases and L-glutamate dehydrogenase.

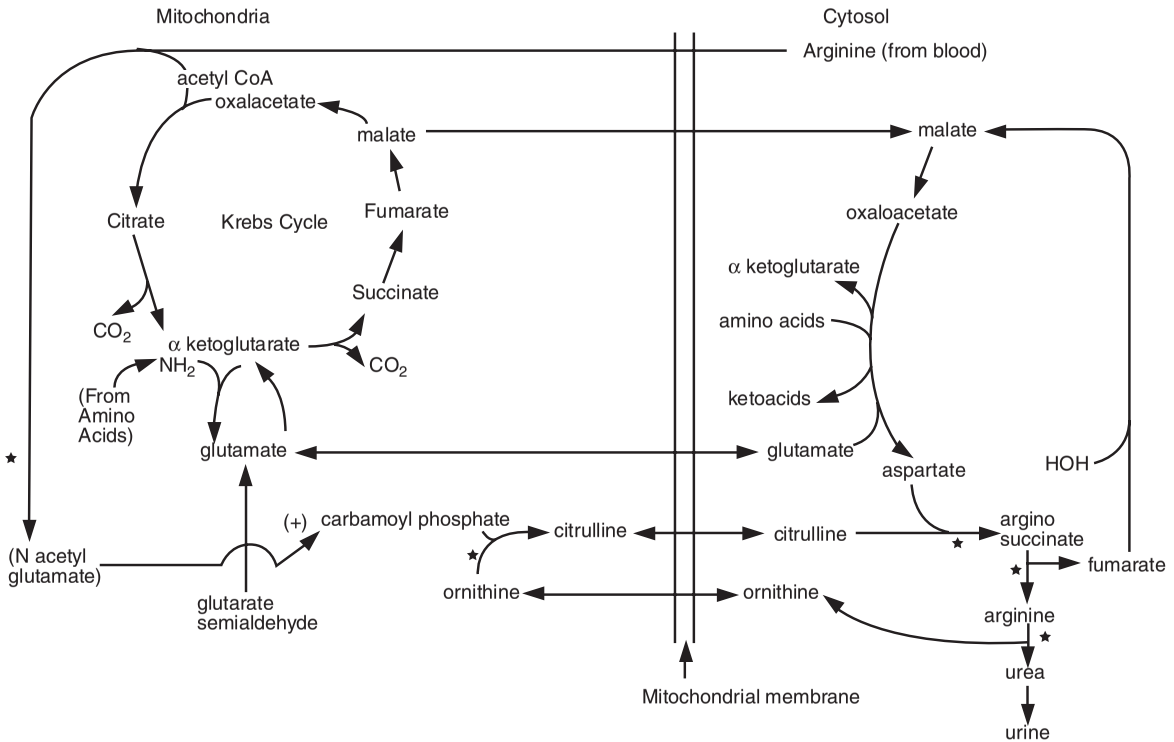


**Figure 1.9** The urea cycle. This cycle has two important steps in the mitochondrial matrix. One is the synthesis of carbamyl phosphate and the other is the synthesis of citrulline.

The glutamate dehydrogenase is a bidirectional enzyme that plays a pivotal role in nitrogen metabolism. It is present in kidney, liver, and brain. It uses $NAD^+$ or $NADP^+$ as a reducing equivalent receiver and operates close to equilibrium, using ATP, GTP, NADH, and ADP depending on the direction of the reaction. In catabolism, it channels $NH_3$ from glutamate to urea. In anabolism, it channels ammonia to α-ketoglutarate to form glutamate. In the brain, glutamate can be decarboxylated to form γ-aminobutyrate (GABA), an important neurotransmitter. The decarboxylation is catalyzed by the enzyme L-glutamate decarboxylase. Putrescine also can serve as a precursor of GABA, by deamination or via N-acetylated intermediates.

The urea cycle is, energetically speaking, a very expensive process. The synthesis of urea requires 3 mol of ATP for every mole of urea formed. This cycle is very elastic — that is, its enzymes are highly conserved, readily activated, and readily deactivated. Adaptation to a new level of activity is quickly achieved. Urea cycle activity can be high when protein-rich diets are consumed and low when low-protein diets are followed; however, the cycle never shuts down completely. Shown in Figure 1.9, the cycle is fine-tuned by the first reaction, the synthesis of carbamyl phosphate. This reaction, which occurs in the mitochondria, is catalyzed by the enzyme carbamyl phosphate synthetase. The enzyme is inactive in the absence of its allosteric activator, N-acetylglutamate, a compound synthesized from acetyl CoA and glutamate in the liver. As arginine levels increase in the liver, N-acetylglutamate synthetase is activated, which results in an increase in N-acetylglutamate.

The urea cycle is initiated in the hepatic mitochondria and finished in the cytosol. The urea is then liberated from arginine via arginase and released into the circulation whereupon it is excreted from the kidneys in the urine. Ornithine, the other product of the arginase reaction, is recyled back to the mitochondrion only to be joined once again to carbamyl phosphate to make citrulline. Rising levels of arginine turn on mitochondrial N-acetylglutamate synthetase, which

provides the N-acetylglutamate that, in turn, activates carbamyl phosphate synthetase, and the cycle goes on.

## Shuttle Systems

Changes in the dietary or hormonal status of the individual result in large changes in the cytosolic pathways for glucose and fatty acid use as well as changes in protein turnover. All of these are orchestrated by changes in oxidative phosphorylation, which, in turn, are orchestrated by changes in cytosolic activity. The coordinated decreases and increases have in common a change in compartment redox state (ratio of oxidized to reduced metabolites) and phosphorylation (ratio of ATP to ADP) state. Reducing equivalents generated in the cytosol and carried by NAD+ or NADP+ must be transferred to the mitochondria (using metabolites) for use by the respiratory chain. Through transhydrogenation, reducing equivalents generated in the cytosol through NADP-linked enzymatic reactions are transferred to NAD. In turn, because NAD cannot traverse the mitochondrial membrane, these reducing equivalents must be carried on suitable metabolites into the mitochondrial compartment.

Several shuttle systems exist (57–59); the malate–aspartate shuttle (Figure 1.8) is thought to be the most important. The shuttle requires a stoichiometric influx of malate and glutamate and efflux of aspartate and α-ketoglutarate from the mitochondria. Alterations in the rate of efflux of α-ketoglutarate can significantly alter the shuttle activity in terms of the rate of cytosolic NADH utilization. α-Ketoglutarate efflux depends on mitochondrial ATP/ADP ratios and on the concentration of cytosolic malate. Shuttle activity is controlled by cytosolic ADP levels, malate levels, and availability of NADH.

Malate can also be exchanged for citrate (Figure 1.10) or for phosphate. The malate–citrate exchange involves transport of malate into the mitochondria, where it is converted to citrate via oxaloacetate. The citrate is then transported out of the mitochondria and the reactions reversed. This exchange is thought to be particularly active in lipogenic states because

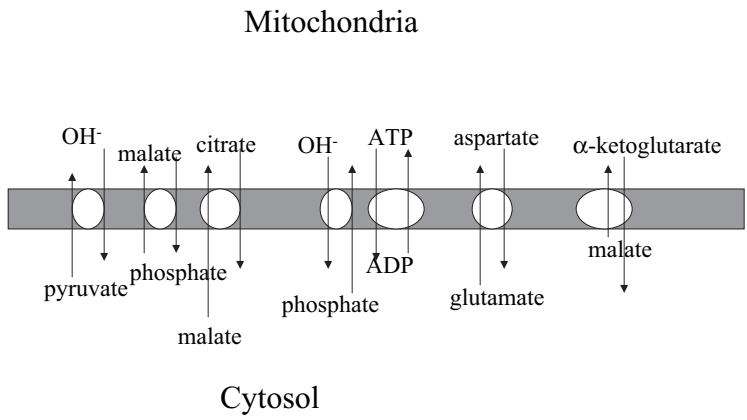


**Figure 1.10** Shuttle systems in inner mitochondrial membrane. The monocarboxylate transporter exchanges pyruvate for a hydroxyl group. The dicarboxylate transporter exchanges phosphate for malate. The tricarboxylate transporter exchanges malate for citrate. The phosphate transporter exchanges phosphate for a hydroxyl group. The adenine nucleotide transporter exchanges ADP for ATP. The aspartate–glutamate transporter exchanges glutamate for aspartate and, finally, the malate–α-ketoglutarate transporter exchanges malate for α-ketoglutarate. These shuttles are needed because several metabolic intermediates cannot cross the mitochondrial membrane without a carrier and, if no carrier is available, they must be generated within the compartment. Included in this list are oxaloacetate, NAD, NADH, ATP, and ADP.

it provides citrate to the cytosol for citrate cleavage. Because the cleavage of citrate provides the starting acetate for fatty acid synthesis, this exchange plays a key role in lipogenesis.

As shown in Figure 1.10, other exchanges also take place to varying degrees. One can recognize the components of the malate shuttle among these exchange systems as well as identify the system for the exchange of adenine nucleotides. When the activity of the malate–aspartate is increased, the redox state of the cytosol is decreased as is lipogenesis; however, gluconeogenesis is increased. All of these exchanges of metabolites are related to each other via a coordinated control

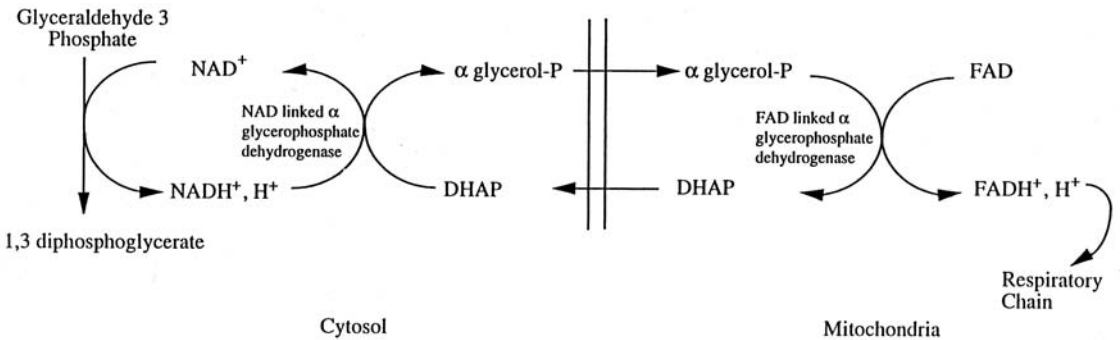


**Figure 1.11** The α-glycerophosphate shuttle. This shuttle is located on the outer side of the inner membrane. It is rate controlled by the activity of the FAD-linked mitochondrial α-glycerophosphate dehydrogenase.

exerted by the phosphorylation state, the latter in turn controlled by ADP/ATP exchange.

Of importance to the control of glycolysis is the α-glycerophosphate shuttle (Figure 1.11) located at the outer side of the mitochondrial inner membrane. Transport of α-glycerophosphate and dihydroxyacetone phosphate across the mitochondrial inner membrane is not required. The activity of the shuttle is related to the availability of α-glycerophosphate and the activity of the mitochondrial matrix enzyme, α-glycerophosphate dehydrogenase. It is particularly responsive to thyroid hormone, which is thought to act by increasing the synthesis and activity of the mitochondrial α-glycerophosphate dehydrogenase.

Adenine nucleotide translocation or the exchange of adenine nucleotides across the mitochondrial membrane (Figure 1.10) also plays a role in the regulation of oxidative phosphorylation. Although any one of the adenine nucleotides could theoretically exchange for any other one, this does not happen. AMP movement is very slow (if it occurs at all). Under certain conditions, ADP influx into the mitochondria is many times faster than ATP efflux. This occurs when the need for ATP within the mitochondria exceeds that of the cytosol. An example of this can be seen in starvation, when ATP is needed to initiate urea synthesis and fatty acid oxidation and to support gluconeogenesis.

Under normal dietary conditions, however, the exchange of ADP for ATP is very nearly equal. For every 100 molecules of ADP that enter the mitochondrial compartment, about 87 molecules of ATP exit. The number of molecules of ATP produced by the coupling of the respiratory chain to ATP synthesis depends on the point at which the reducing equivalents enter the chain. If they enter carried by NAD, they are said to enter at site I of the respiratory chain. If they enter carried by FAD, they are said to enter at site II. Site I substrates are those oxidized via NAD-linked dehydrogenases. For example, pyruvate is a site I substrate and succinate is a site II substrate. Reducing equivalents entering at site I will generate the energy for the synthesis of three molecules of ATP.

Those entering at site II will generate enough energy to result in two molecules of ATP.

Knowing how a substrate provides its reducing equivalents to the respiratory chain allows one to estimate how many ATPs will be generated by a particular reaction sequence. Because each ATP has an energy value of about 30.5 kJ or 7.3 kcal/mol, one can also estimate the amount of energy that each sequence will produce or use.

## The Citric Acid Cycle

Glucose, fatty acid, and amino acid oxidation result in the production of an activated two-carbon residue, acetyl CoA. Glucose is metabolized to pyruvate, which is converted to acetyl CoA via the pyruvate dehydrogenase complex or converted to oxaloacetate via pyruvate carboxylation (pyruvate carboxylase). Acetyl CoA is joined to oxaloacetate to form citrate and is then processed by the citric acid (also called the Krebs) cycle. This cyclic sequence of reactions is found in every cell type that contains mitochondria. The cycle is catalyzed by a series of enzymes and yields reducing equivalents ($H^+$) and two molecules of carbon dioxide.

The cycle is illustrated in Figure 1.12. Reducing equivalents and $CO_2$ are produced when α-ketoglutarate is produced from isocitrate, when ketoglutarate is decarboxylated to produce succinyl CoA. Reducing equivalents are also produced when succinate is converted to fumarate and when malate is converted to oxaloacetate. These reducing equivalents, one pair for each step, are carried to the respiratory chain by way of NAD or FAD. Reducing equivalents from succinate are carried by FAD and those produced by the oxidation of the other substrates are carried by NAD. Once oxaloacetate is formed, it can then pick up another acetate group from acetyl CoA and begin the cycle once again by forming citrate. Thus, for every turn of the cycle, 2 mol of $CO_2$ and eight pairs of reducing equivalents are produced. As long as oxaloacetate is sufficient to pick up the incoming acetate, the cycle will continue to turn and reducing equivalents will continue to be produced; in turn, these will

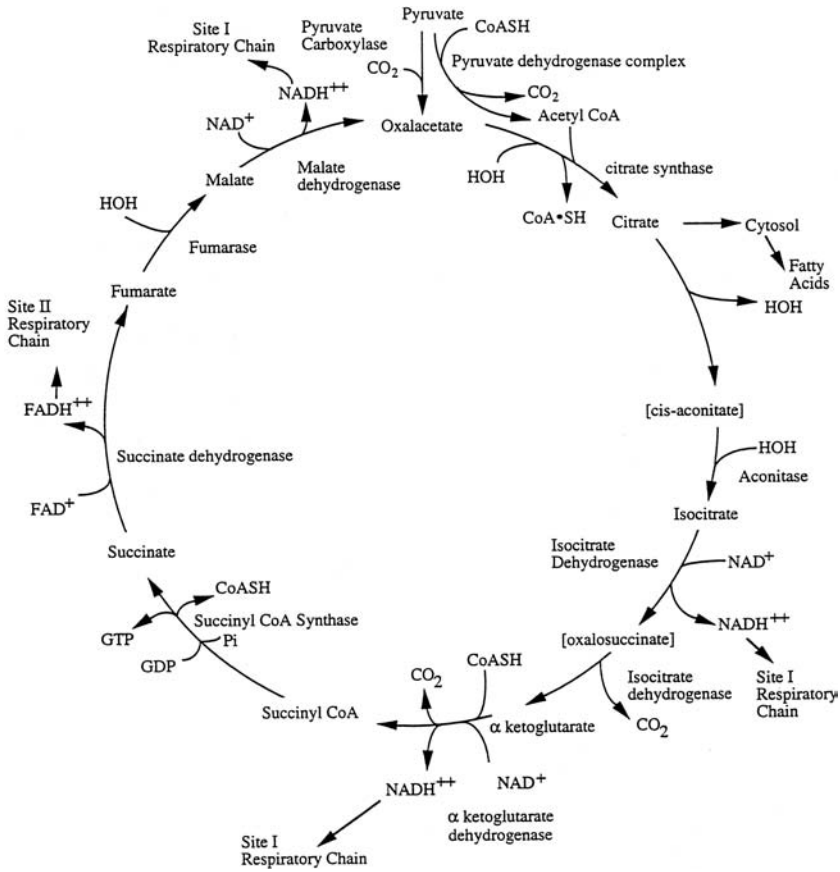


**Figure 1.12** The citric acid cycle. This cycle reduces a six-carbon intermediate to a four-carbon one, resulting in the production of two moles of carbon dioxide and reducing equivalents that are sent to the respiratory chain.

be joined with molecular oxygen to produce water, the end product (with carbon dioxide) of the catabolic process.

The preceding simplistic description of the citric acid cycle implies that it is free of controls and proceeds unhindered given adequate supplies of substrates, enzymes, and molecular oxygen. This is not true. Numerous controls regulate the cycle. Among these are the citrate synthase, isocitrate

dehydrogenase, and the α-ketoglutarate dehydrogenase reactions. These reactions have large negative ΔG values, meaning that they are essentially one-way reactions. In addition, the concentration of acetyl CoA, ATP, NAD+, and NADH exert regulatory power. Excess NADH, for example, inhibits the reactions catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The cycle is enhanced by high ADP:ATP and NAD+:NADH ratios. Lastly, excess succinyl CoA can inhibit α-ketoglutarate dehydrogenase and citrate synthase.

In summary, the citric acid cycle produces the reducing equivalents (each with an electron pair) needed by the respiratory chain and the respiratory chain must transfer these hydrogen ions and their associated electrons to the oxygen ion to produce water. In doing so, it generates the electrochemical gradient (the proton gradient) necessary for the formation of the high-energy bonds of ATP. Obviously, then, all of these metabolic processes, the citric acid cycle, the respiratory chain, and ATP synthesis are regulated coordinately.

## The Respiratory Chain

The pairs of electrons produced at the four steps described previously in the citric acid cycle as well as the electrons transferred into the mitochondria via other processes are passed down the respiratory chain to the ultimate acceptor, molecular oxygen (60). The respiratory chain enzymes embedded in the mitochondrial inner membrane are particularly complex and have a number of subunits. They catalyze a series of oxidation–reduction reactions. Each reaction is characterized by a redox potential that can be calculated using the Nernst equation:

$$E_h = E_0^1 + \frac{2.303\,RT}{nP} \log \text{n}\left(\frac{[\text{electron acceptor}]}{[\text{electron donor}]}\right)$$

where

$E_h$ = observed potential

$E_0^1$ = standard redox potential (pH = 7.0, T = 25°, 1.0-*M* concentration)
$R$ = gas constant (8.31 J $deg^{-1}$ $mol^{-1}$)
$T$ = temperature (°K)
$n$ = number of electrons transferred
$P$ = Faraday (23,062 cal $V^{-1}$ = 96, 406 J $V^{-1}$)

The more positive the potential is, the greater is the affinity of the negatively charged acceptor for the positively charged electrons. It is this potential that drives the respiratory chain reactions forward towards the formation of water. Each succeeding acceptor donor pair has a higher affinity for the electrons than the preceding pair until the point in the chain at which the product, water, is formed. By comparison to the preceding pairs, water has little tendency to give up its electrons and unless this water is immediately removed, the chain reaction will stop. Of course, water does not accumulate in the mitochondria; it leaves as quickly as it is formed and thus does not feed back to inhibit the chain. If it did accumulate, it would change the concentration of the chain components diluting them. When water accumulates, the mitochondrion swells; if in excess, the mitochondrion would burst and die. Because this does not happen, obviously, water must exit the organelle as soon as it is formed so as to maintain optimal osmotic conditions.

Although the respiratory chain usually proceeds in the forward direction (towards the formation of water) due to the exergonic nature of the reaction cascade, except for the final reaction, all of these steps are fully reversible. In order to be reversed, sufficient energy must be provided to drive the reaction in this direction. For example, the reducing equivalents derived from succinate are usually carried by FAD (as $FADH^+H^+$). These can be transferred to NAD (as $NADH^{++}H^+$) with the concomitant hydrolysis of ATP. Electron transport across the other two phosphorylation sites can also be reversed, again, only if sufficient energy is provided.

The respiratory chain consists of four major enzyme complexes (Figure 1.13) located in the inner mitochondrial membrane. The enzymes of the respiratory chain are arranged so

as to transport hydrogen ions from the matrix across the inner membrane. When this occurs, a proton gradient develops in close proximity to the $F_1F_0$ATPase (ATP synthase) complex and provides sufficient energy to drive ATP synthesis by causing a dehydration of ADP and Pi. Reducing equivalents transported into the mitochondrial compartment by the various substrate shuttle systems or generated in the compartment are passed down the respiratory chain in carefully regulated steps. Reducing equivalents enter the chain through the NAD–dehydrogenase complex (complex I) via mitochondrial shuttles or via the FAD–ubiquinone complex (complex II). With respect to the latter complex, reducing equivalents collect via three pathways:

- Succinate contributes its reducing equivalents to a flavoprotein with an iron–sulfur center.
- Glycerol 3-phosphate also uses FAD flavoprotein with an iron–sulfur center, but it is a different protein.
- The products of fatty acid oxidation (a mitochondrial process) are picked up by a FAD-flavoprotein, transferred to an electron-transferring flavoprotein, again with an iron–sulfur center, and then transferred to ubiquinone.

In mammals, complex I consists of about 42 subunits (61). Of these, seven are encoded by the mitochondrial genome and the rest by the nuclear genome synthesized on the ribosomes in the cytoplasm and imported into the mitochondrial compartment. It is the largest of the four respiratory chain complexes. Complex I is known as NADH-coenzyme Q reductase or NADH dehydrogenase. As the name implies, this complex transfers a pair of electrons from NADH to coenzyme Q, a lipid-soluble compound embedded in the inner membrane. Complex I has a molecule of flavin mononucleotide (FMN) and two binuclear iron–sulfur centers and four tetranuclear iron–sulfur centers (62). Because of its FMN, it is called a flavoprotein.

The complex catalyzes the transfer of electrons to complex III via ubiquinone and this transfer is coupled with the

vectorial transfer of protons across the mitochondrial membrane. There are two distinct species of tightly bound ubiquinones in complex I that differ in spin relaxation and redox properties. The transfer of electrons leads to the formation of a proton gradient ($\Delta\mu_{H+}$) that, in turn, drives ATP production. The stoichiometry of proton transfer for complex I is $4H^+/2e^-$. This distinguishes this complex from those that follow it in the respiratory chain. The other two $H^+$ translocating complexes (III and IV) have a stoichiometry of $2\ H^+/2e^-$.

Complex II, succinate:quinone reductase (succinate dehydrogenase), is the smallest of the respiratory chain complexes (63). None of the subunits of complex II are encoded by the mitochondrial genome. The complex consists of four subunits with several different redox prosthetic groups: a covalently bound FAD, three iron–sulfur clusters, and a cytochrome b. The head of the complex protrudes out into the matrix where its FAD can accept succinate-donated electrons from the citric acid cycle. Actually, this enzyme is a component of the respiratory chain and the citric acid cycle. It too is a flavoprotein because of its FAD content. The FAD is bound to a histidine residue (64). When succinate is converted to fumarate in the citric acid cycle, a concomitant reduction of FAD to $FADH_2$ occurs. This $FADH_2$ transfers its electrons to the iron–sulfur cluster, which, in turn, passes them on to ubiquinone. Because of insufficient energy to elicit a proton gradient, reducing equivalents and associated electrons entering the respiratory chain via complex II yield only two ATPs via OXPHOS rather than the three ATPs generated when entry occurs via complex I.

Once reducing equivalents enter the chain via site 1 or site 2 (the sites correspond to entry via complex I or II), they are passed to complex III, the ubiquinone–cytochrome $bc_1$ reductase. This complex takes the electrons passed to it from ubiquinone and then passes them to complex IV, cytochrome c oxidase. This passage uses a unique redox pathway called the Q cycle (65). Three different cytochromes (bc, b, $c_1$) are involved as well as an iron–sulfur protein. The iron is in the middle of a porphorin ring much like that of hemoglobin and

oscillates between the reduced and oxidized states (ferrous to ferric).

The Q cycle begins when a molecule of reduced ubiquinone diffuses to the $Q_p$ site on complex III near the outer face of the inner mitochondrial membrane. An electron from the reduced ubiquinone is transferred to a mobile protein called the Rieske protein. The electrons are then transferred to cytochrome $c_1$. This releases two H+ and leaves $UQ^-$, a semiquinone anion form of ubiquinone, at the $Q_p$ site. The second electron is then transferred to the $b_L$ heme, converting $UQ^-$ to ubiquinone. The Rieske protein and cytochrome $c_1$ are similar in structure; each has a globular domain and each is anchored to the inner membrane by a hydophobic segment. The segments differ: the Rieske protein has an N-terminal and the cytochrome $c_1$ has a C-terminal.

The electron on the $b_L$ heme is passed to the $b_H$ heme against a membrane potential of 0.15 V and is driven by the loss of redox potential as the electron moves from $b_L$ to $b_H$. The electron is then passed from bH to ubiquinone at the second binding site, converting the ubiquinone to $UQ^-$. The resulting $UQ^-$ remains firmly bound to the $Q_n$ site. This completes the first half of the Q cycle. The second half is similar in that a second molecule of reduced ubiquinone is oxidized at the $Q_p$ site. One electron is passed to cytochrome $c_1$ and the other is passed to heme $b_H$. The $b_H$ electron is transferred to the semiquinone anion $UQ^-$ at the $Q_n$ site. With the addition of two $H^+$, this produces $UQH_2$. The $UQH_2$ is released and returns to the coenzyme Q pool and the Q cycle is complete. Actually, the Q cycle is an unbalanced proton pump. Cytochrome c is a mobile electron carrier, as is ubiquinone. Electrons travel from c to the water-soluble $c_1$. The $c_1$ associates loosely with the inner mitochondrial membrane to acquire electrons from the iron–sulfur centers. The $c_1$ of complex III then migrates along the membrane in a reduced state so as to give these electrons to complex IV.

Complex IV, cytochrome-c oxidase, contains two heme centers (a and $a_3$) as well as two copper centers. The copper oscillates between the reduced (cuprous) and oxidized (cupric) states. Complexes III and IV elicit a proton gradient and thus

ATP is formed at each of these sites. Complex IV accepts the electrons from cytochrome c and directs them to molecular oxygen to form water. The water thus formed quickly passes out of the compartment into the cytoplasm. As was true of complex I, complexes III and IV have nuclear- and mt-encoded subunits. Complex III has 11 subunits, of which 1 is encoded by the mt genome; complex IV has 13 subunits, of which 3 are mt encoded.

## Oxidative Phosphorylation

Oxidative phosphorylation occurs in and on the inner membrane of the mitochondrial compartment and provides ATP not only to this compartment but also to the rest of the cell. The mechanism whereby this ATP synthesis is coupled to the synthesis of water was worked out many years ago. Some of the major players in this field were Mitchell (66), Lehninger (67), Boyer (68,69), Owen and Wilson (70), Brown et al. (71,72), Balaban and Heineman (73), Tager et al. (74), Hinkle and Yu (75), Brand (76), Hackenbrock (77), and Racker (78). In essence, the process links the energy released by the respiratory chain to that needed to synthesize the high-energy bond of the ATP. Not all of this energy is captured. Most of it is released as heat. The efficiency with which the energy is captured as chemical energy vs. that released as heat energy is determined by a number of factors. The composition of the diet, nutritional status, and endocrine status as well as the genetics of the individual can have major effects on this efficiency.

The components of oxidative phosphorylation are divided into five complexes. As described earlier, complexes I through IV plus ubiquinone (UQ) and cytochrome c comprise the respiratory chain. Complex V is the $F_1F_0$ ATPase shown in Figure 1.14. The electron carriers are the quinoid structures (FMN, FAD, UQ) and the transition metabolic complexes. Complexes I, III, and IV pump protons into the space between the two mitochondrial membranes. The pumping of protons creates a protomotive force that consists of a proton gradient and a membrane potential. This protomotive force is then used by