9 Oxidation of Fuels and ATP Generation: Physiological and Clinical Importance

Figure 9.1 Simple diagram of a mitochondrion showing inner and outer membranes, cristae and matrix.

Figure 9.2 Summary of reactions of the Krebs cycle. The names of the enzymes are (1) citrate synthase, (2) aconitase, (3) isocitrate dehydrogenase (there are two enzymes, one utilizes NAD\(^+\) as the cofactor, the other NADP\(^+\); it is assumed that the NAD\(^+\)-specific enzyme is that involved in the cycle), (4) oxoglutarate dehydrogenase, (5) succinyl CoA synthetase, (6) succinate dehydrogenase, (7) fumarate hydratase, (8) malate dehydrogenase.

Figure 9.3 A summary of the Physiological pathway of the Krebs cycle. The pathway starts with acetyl-CoA, since citrate synthesis is the flux-generating step. The physiological pathway includes the electron transfer chain, since there is no flux-generating step in this chain. The pathway is indicated by the broader lines. The pathway, therefore, starts with acetyl-CoA and finishes with CO\(_2\) and H\(_2\)O, which are lost to the environment. Acetyl-CoA is formed from a variety of precursors: glucose and fatty acids are presented in this figure.

Figure 9.4 Reactions of glutaminolysis: the pathway for glutamine oxidation. Reaction 1 is catalysed by glutaminase, reaction 2 by glutamate aminotransferase, and reaction 8 by aspartate aminotransferase: all other enzymes are those of the Krebs cycle (3–7). (See also Chapter 8).

Figure 9.5 A summary of pathways of the three main fuels and the positions where they enter the cycle. The figure also shows the release of hydrogen atoms/electrons and their transfer into the electron transfer chain for generation of ATP and formation of water. Glutamine is converted to glutamate by deamidation and glutamate is converted to oxoglutarate by transamination or deamination. The process of glycolysis also generates ATP as shown in the Figure.

Figure 9.6 Sequence of electron carriers in the electron transfer chain. The positions of entry into the chain from metabolism of glucose, glutamine, fatty acyl-CoA, glycerol 3-phosphate and others that are oxidised by the Krebs cycle are shown. The chain is usually considered to start with NADH and finish with cytochrome oxidase. FMN is flavin mononucleotide; FAD is flavin adenine dinucleotide.

Figure 9.7 The sequence of electron transfer complexes in the electron transfer chain. The regions enclosed by broken lines indicate the association of carriers in complexes. Their constituents are listed in Table 9.4. Note that electrons may enter at the level of ubiquinone from sources other than succinate. Complex V is F\(_{1}\)F\(_{0}\)-ATPase (Table 9.4).

Figure 9.8 Simple diagram of mitochondrial H\(^+\)-ion movement and axonal K\(^+\)-ion movement to establish membrane potentials across membranes. Note that H\(^+\) movement from the mitochondrial matrix to the outer surface of the inner membrane requires a specific proton pump that requires energy, which is transferred from electron transfer, whereas the K\(^+\)-ion movement occurs via an ion channel with energy provided from the concentration difference of K\(^+\) ions on either side of the membrane (approximately 100-fold). The movement of both the protons and K\(^+\)-ions generates a membrane potential. The potential across the membrane of the nerve axon provides the basis for nervous activity (see Chapter 14).

Figure 9.9 A diagrammatic representation of the mechanism of the proton pump that transfers protons across the inner mitochondrial membrane. The process can be divided into three parts: (1) the proton carrier (Pr) collects protons from the matrix; (2) acquisition of an electron decreases the affinity of the carrier for the proton and changes its conformation so that the binding site for the proton faces into the inter-membrane space; (3) the carrier discharges the proton into the inter-membrane space and reverts to its former conformation. The electron continues to pass along the transfer chain.

Figure 9.10 Simple diagram showing the ATPase particles attached to the cristae. The particles are the sites of ATP synthesis.

Figure 9.11 (a) Simple diagram of the two components of ATPase particle. The particle comprises two protein complexes, the F\(_{1}\) and F\(_{0}\). The latter complex (known as F\(_{1}\)-ATPase) generates ATP from ADP and P\(_i\). The F\(_{0}\) complex is present in the membrane and is responsible for movement of protons across the membrane. The arrow represents some form of communication possibly conformational, between F\(_{1}\) and F\(_{0}\). (b) Diagrammatic representation of the roles of the F\(_{1}\) and F\(_{0}\) complexes in generating ATP. The proton circuit, indicated by the dotted line, provides the energy for ATP generation. However, protons do not pass through the F\(_{1}\) protein complex as indicated in this figure. It actually rotates to transfer protons from the inter-membrane space to the F\(_{1}\) complex. Similarly, protons do not pass through the F\(_{0}\) complex, but they change its conformation, as they move into the matrix.

Figure 9.12 Diagrammatic representation of the reactions by which the F\(_{1}\) protein complex (F\(_{1}\)-ATPase) generates ATP. The enzyme (more correctly a protein component of the enzyme complex) binds ADP and P\(_i\) and forms ATP in an equilibrium reaction (i.e. no energy is transferred). The movement of protons through the F\(_{0}\)-F\(_{1}\)-complexes changes the conformation of the enzyme so that it readily discharges its ATP into the matrix, i.e. the conformation change caused by the proton motive force increases the dissociation constant for the ATP binding to the enzyme. This ‘pulls’ all the reactions towards ATP generation, so that net ATP generation results. This is illustrated by (1) the reversible arrows on the left-hand side indicating no net generation of ATP, (2) Irreversible arrows on the right-hand side indicating the effect of the conformation change on the enzyme complex. For explanation of how non-equilibrium reactions provide direction in a process, see Chapter 2.

Figure 9.13 Examples of mitochondrial transport systems for anions. (1) The antiprot transfer systems malate into but oxoglutarate out of the mitochondrion. (2) The symport system transfers both pyruvate and protons into the mitochondrion across the inner membrane. Both transport processes are electroneutral.

Figure 9.14 Simple diagram illustrating the transport of the major fuels for the Krebs cycle, and hydrogen atoms for the electron transfer chain, across the inner mitochondrial membrane.

1. Pyruvate is transported by an anion symport system (Figure 9.13).
2. Glutamine, which has no net charge, is transported by a specific glutamine transporter.
3. Fatty acyl carnitine is transported via a translocase that transports acylcarnitine into and carnitine out of the mitochondrion (Chapter 7).
4. The transport processes for hydrogen atoms are described in Figures 9.17 and 9.18. The biochemical problems in transport of hydrogen atoms are now discussed.

Figure 9.15 Fate of NADH produced in glycolysis. In hypoxic or anoxic conditions, pyruvate is converted to lactate with oxidation of NADH. In aerobic conditions, NADH is oxidised as shown in Figure 9.17 or 9.18 and pyruvate is oxidised via the Krebs cycle and the electron transfer chain.

Figure 9.16 The principle of the transfer shuttle of hydrogen atoms into the mitochondrion. A dehydrogenase in the cytosol generates XH from
NADH. XH is transported into the mitochondrion where a second dehydrogenase catalyses a reaction in which the XH reduces NAD⁺ to NADH. X then returns to the cytosol. The nature of XH is considered in Figures 9.17 and 9.18.

**Figure 9.17 The malate/aspartate shuttle.**
The shuttle involves the following reactions:
(i) In the cytosol, catalysed by cytosolic malate dehydrogenase, oxaloacetate is converted to malate.
   oxaloacetate + NADH → NAD⁺ + malate
(ii) Malate is then transported across the inner membrane, the non-equilibrium step in the shuttle.
(iii) In the mitochondrial matrix, malate is oxidised
   malate + NAD⁺ → oxaloacetate + NADH
catalysed by mitochondrial malate dehydrogenase.
(iv) The hydrogen atoms from NADH are then transferred along the electron transfer chain to be oxidised by oxygen.
(v) The oxaloacetate is then transported from mitochondrion into the cytosol but not directly, since there is no transporter for oxaloacetate in the mitochondrial membrane. This problem is solved by conversion of oxaloacetate to aspartate, by transamination, and it is the aspartate that is transported across the inner mitochondrial membrane to the cytosol, where oxaloacetate is regenerated from aspartate by a cytosolic aminotransferase enzyme.

**Figure 9.18 The glycerol phosphate shuttle.** In the cytosol, NADH is oxidised in a reaction in which dihydroxyacetone phosphate is reduced to glycerol 3-phosphate, catalysed by glycerol-3-phosphate dehydrogenase (NAD⁻ linked):

The glycerol 3-phosphate is oxidised by a second glycerol-3-phosphate dehydrogenase but this enzyme is located within the inner mitochondrial membrane. It removes electrons from glycerol 3-phosphate and transfers them directly to the electron transfer chain, at the level of ubiquinone, and the dihydroxyacetone phosphate remains in the cytosol (or at least in the intermembrane space from where it diffuses into the cytosol). The non-equilibrium reaction, which gives direction to the overall shuttle, is catalysed by the mitochondrial glycerol phosphate dehydrogenase.

There is a suggestion that this shuttle is important in brain, where glutamate and aspartate have specific roles as neurotransmitters (Chapter 14).

**Figure 9.19 Adenine nucleotide translocase and phosphate transfer into the matrix.** Phosphate is transported into the mitochondria with protons in a symport transport system. The adenine nucleotide translocase transports ATP⁻ into and ADP⁻ out of the mitochondria, i.e. it is electrogenic. The charge is neutralised by H⁺ movement into the matrix from the proton motive force which utilises about 25% of the energy in the proton motive force.

**Figure 9.20 The creatine/phosphocreatine shuttle between subsarcolemmal mitochondria and myosin ATPase in muscle.** The distance between the mitochondria that reside just below the plasma membrane (sarcolemma) and the myofibrils in which the myosin ATPase results in contraction, is long in such muscles. The advantage of the position of these mitochondria is ready access to oxygen and fuel from blood. Such mitochondria are common in endurance athletes.

**Figure 9.21 The creatine/phosphocreatine shuttle in spermatogonia.** This shuttle may not be present in all sperm: it will depend upon the distance between the mitochondria and the flagellum. Mitochondria are present in the midpiece just below the head. ATP is required for movement of the flagellum which enables the sperm to swim. Dynemin ATPase is the specific ‘motor’ ATPase, similar to myosin ATPase, that transfers energy from ATP to the flagellum. A deficiency of creatine may explain low sperm motility in some infertile men. CK – creatine kinase. Deficiencies of enzymes in the pathway for synthesis of creatine are known to occur (see Appendix 8.3).

**Figure 9.22 The relationship between ATP utilisation by myosin ATPase and ATP generation by Krebs cycle and electron transfer.** This relationship between the two major energy systems in muscle is critical. The rate of the cycle and electron transfer is controlled, in part, by ATP utilisation by muscle contraction (see below). This is equivalent to a ‘market economy’ so that the law of supply and demand applies. The greater the demand and hence the use of ATP, the greater is the rate of generation.

**Figure 9.23 Properties of the three enzymes that control the flux through the Krebs cycle.** During physical activity. The CoASH/succinyl CoA concentration ratio increases whereas that of ATP/ADP ratio decrease. These changes increase the flux through the cycle.

**Figure 9.24 Control of the oxaloacetate concentration and hence the flux through the cycle by pyruvate carboxylase.** The activity of pyruvate carboxylase is increased by an increase in its substrate, pyruvate, and its allosteric regulator, acetyl-CoA. Regulation of the activity is important to increase the concentration of oxaloacetate which increases the flux through the cycle. An increase in the rate of glycolysis increases the concentration of pyruvate, and an increase in the rate of fatty acid oxidation increases that of acetyl-CoA. Both result in an increase in the concentration of oxaloacetate and hence in the flux through the cycle, providing coordination between the rates of glycolysis, fatty acid oxidation and the cycle.

**Figure 9.25 Control of the Krebs cycle and myosin-ATPase by direct effects of Ca²⁺ ions and the resultant effects on electron transfer and oxidative phosphorylation in muscle.** The stimulation of the Krebs cycle by Ca²⁺ ions results in an increase in the NADH/NAD⁺ concentration ratio, which stimulates electron transfer. The stimulation of myosin-ATPase by Ca²⁺ lowers the ATP/ADP concentration ratio, which also stimulates electron transfer. The Ca²⁺ ions are released from the sarcoplasmic reticulum in muscle in response to nervous stimulation. In addition, generation of ADP by myosin ATPase increases the ADP concentration, which stimulates the cycle. Note that a lack of oxygen will prevent generation of ATP (Chapter 13).

**Figure 9.26 (a) Near-equilibrium and non-equilibrium reactions in the electron transfer chain.** The electron transfer chain is considered to be the latter part of the physiological Krebs cycle (see above). The non-equilibrium processes are the Krebs cycle and the terminal reaction cytochrome oxidase. All other reactions are near-equilibrium, including the ATP-generating reactions. These are not shown in the figure. (b) The similarity of electron transfer chain and glycolysis in the position of near-equilibrium/non-equilibrium reactions, in the two pathways. In both cases, non-equilibrium reactions are at the beginning and at the end of the processes (see Chapters 2 and 3 for description of these terms and the means by which such reactions can be identified).

**Figure 9.27 An overview of the mechanisms by which the rates of glycolysis, Krebs cycle, electron transfer and ATP generation are regulated by changes in the Ca²⁺ ion concentration and the ATP/ADP concentration ratio in muscle.** The symbol (-P) represents a decrease in the ATP/ADP concentration ratio. An increase in the cytosolic concentration of Ca²⁺ ions and a decrease in the ATP/ADP concentration ratio increase the activity of phosphofructokinase and hence glycogen breakdown, pyruvate dehydrogenase and hence pyruvate conversion to acetyl-CoA, and the Krebs cycle. A decrease in the ATP/ADP concentration ratio increases glycolytic flux (via effects on phosphofructokinase and pyruvate kinase). The increase in the NAD⁺/NADH concentration ratio and the decrease in the ATP/ADP concentration ratio, which are consequent upon the increase in Ca²⁺ ion concentration, increase the flux through the electron transfer chain and ATP generation. For further details on control of glycolysis and glycosylation, see Chapter 6.

**Figure 9.28 Electron micrograph of brown adipose tissue.** Kindly provided by Dr Caroline Pond of the Open University, UK.

**Figure 9.29 Control of heat production in brown adipose tissue.** Catecholamines increase cyclic AMP concentration which stimulates triacylglycerol.
Lipase which increases the long-chain fatty acid level, which increases the fluxes through β-oxidation and the Krebs cycle, and the activity of the uncoupling protein. Uncoupling decreases the ATP concentration which further increases the activity of the uncoupling.

**Figure 9.30** Flow diagram of the 'energy chain' from food to essential processes in human life. The ATP utilised by the Na⁺/K⁺ ATPase maintains the ion distribution in nerves that is essential for electrical activity and, in addition, maintains neurotransmitter synthesis, both of which provide communication in the brain and hence consciousness, learning and behaviour (Chapter 14). ATP utilisation by myosin ATPase is essential for movement and physical activity. ATP utilisation by the flagellum of sperm is essential for reproduction and ATP utilisation for synthesis of macromolecules is essential for growth.

**Figure 9.31** Mechanism by which mitochondrial damage can lead to obesity or type 2 diabetes. Damage to the proteins of the electron transfer chain or the inner mitochondrial membrane can be caused by somatic mutations, free radicals, environmental toxins or inherited genetic defects. The resultant impairment of transfer of electrons along the chain of carriers will decrease the oxidation of fatty acids by muscle and other tissues. Consequently, the fate of the fatty acids derived from fat in the diet will be storage as triacylglycerol in adipose tissue rather than oxidation in muscle and other tissues. This can lead to obesity. Furthermore, failure to oxidise fatty acyl-CoA in muscle can result in accumulation of fatty acid metabolites which can interfere with insulin signalling, resulting in insulin resistance and hence type 2 diabetes.