Eur. J. Biochem. **271**, 462–469 (2004) © FEBS 2004 doi:10.1046/j.1432-1033.2003.03947.x

MINIREVIEW

Mitochondrial β-oxidation

Kim Bartlett[1] and Simon Eaton[2]

[1]*Department of Child Health, Sir James Spence Institute of Child Health, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne;* [2]*Surgery Unit and Biochemistry, Endocrinology and Metabolism Unit, Institute of Child Health, University College London, UK*

Mitochondrial β-oxidation is a complex pathway involving, in the case of saturated straight chain fatty acids of even carbon number, at least 16 proteins which are organized into two functional subdomains; one associated with the inner face of the inner mitochondrial membrane and the other in the matrix. Overall, the pathway is subject to intramitochondrial control at multiple sites. However, at least in the liver, carnitine palmitoyl transferase I exerts approximately 80% of control over pathway flux under normal conditions. Clearly, when one or more enzyme activities are attenuated because of a mutation, the major site of flux control will change.

Introduction

The β-oxidation of long-chain fatty acids is central to the provision of energy for the organism and is of particular importance for cardiac and skeletal muscle. However, a number of other tissues, primarily the liver, but also the kidney, small intestine and white adipose tissue, can utilize the products of β-oxidation for the formation of ketone bodies which can, in turn, be utilized for energy by other tissues. The relationship of fat oxidation with the utilization of carbohydrate as a source of energy is complex and depends upon tissue, nutritional state, exercise, development and a variety of other influences such as infection and other pathological states. A full description of the regulatory mechanisms involved is beyond the scope of the present review and the interested reader is referred to recent treatments of the subject [1–5]. In the present review we concentrate on; the response to stress and fasting at the level of the whole body, the principal differences between tissues and organs, the enzymology and regulation of the pathway at the level of the mitochondrion. Although long chain fatty acids are also β-oxidized by a peroxisomal pathway, this pathway is quantitatively minor, and, although inherited disorders of the peroxisomal system result in devastating disease, is not considered further. Similarly, the auxiliary systems required for the metabolism of polyunsaturated and branched-chain long-chain fatty acids are not discussed and the interested reader is referred to recent reviews. This review is the first of several dealing with various aspects of mitochondrial β-oxidation and its disorders.

The basic pathway of mitochondrial β-oxidation (Fig. 1) was one of the first biochemical pathways to be investigated, and the concept of the progressive removal of acetate arose from the studies of Knoop and was confirmed by Dakin ([6] and literature cited therein). It was some years later with the discovery of coenzyme A (CoA), that the role of acetyl-CoA as the product of β-oxidation was appreciated and the well-known sequence of FAD-linked dehydrogenation, hydration, NAD^+-linked dehydrogenation and thiolytic cleavage, to yield acetyl-CoA, was elucidated. In the present review we include the transport of fatty acyl moieties into the mitochondrial matrix as a functional component of the pathway. The role of carnitine in this process is of particular relevance to the control of β-oxidation flux and there have been significant recent advances in this area.

Whole body response to stress and fasting – regulation and control

Under fasting conditions, the insulin : glucagon ratio is low which results in the stimulation of lipolysis. Triacylglycerol stores in fat depot are hydrolysed to free fatty acids that are then released into the circulation and subsequently taken up and oxidized by most tissues apart from the CNS and erythrocytes. In the liver, under these conditions, fatty acids are broken down to acetyl-CoA, most of which is used for the formation of ketone bodies (acetoacetate and 3-hydroxybutyrate). Ketone bodies are, in turn, exported for oxidation by extra-hepatic tissues. Simultaneously, glycogenolysis occurs, and in the liver, and to a lesser extent the kidney, glucose is mobilized for extra-hepatic utilization. Skeletal muscle also has substantial glycogen reserves, but

Correspondence to K. Bartlett, Department of Child Health, Sir James Spence Institute of Child Health, University of Newcastle upon Tyne, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK.
Fax: + 44 1912 023 041, Tel.: + 44 1912 023 040,
E-mail: kim.bartlett@ncl.ac.uk

Abbreviations: lCPTI, carnitine palmitoyl transferase I (liver); mCPTI, carnitine palmitoyl transferase I (muscle); CPTII, carnitine palmitoyl transferase II; MCAD, medium-chain acyl-CoA dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase; ETF, electron transfering flavoprotein; ETFD, ETF-ubiquinone oxidoreductase; NEFA, nonesterified fatty acids; AMPK, AMP-activated protein kinase.

Enzyme: trimethylamine dehydrogenase from *Methylophilus methylotrophus* (EC 1.5.99.7).

(Received 26 August 2003, revised 12 November 2003, accepted 1 December 2003)

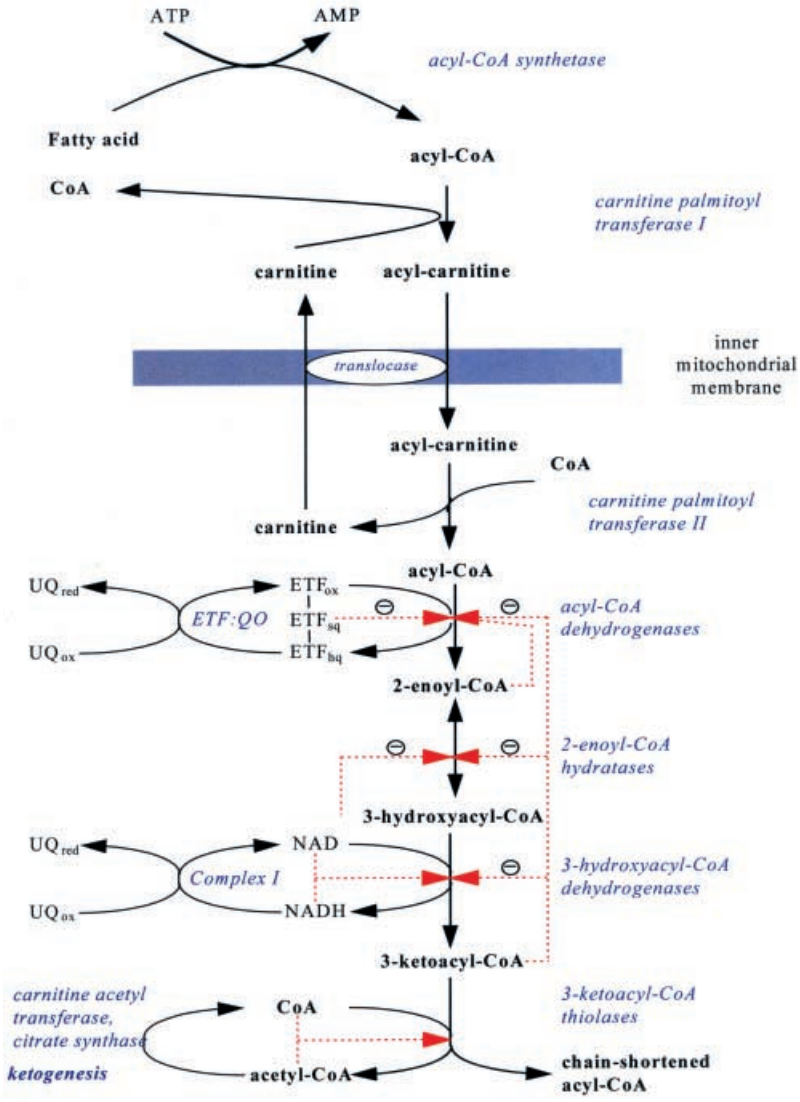

Fig. 1. The pathway of mitochondrial β-oxidation. ETF, electron transfer flavoprotein; UQ, ubiquinone; ETF:QO, electron transfer flavoprotein-ubiquinone oxidoreductase, CoA, coenzyme A. The dotted red lines indicate points of feedback control.

these are utilized endogenously particularly during exercise. Thus the net affect of fasting or indeed any stress leading to counter-regulation of insulin, is a switch from a fuel economy based on carbohydrate to one in which a greater proportion of energy is derived from the oxidation of lipid (Fig. 2). The resultant sparing of glucose allows the movement of glucose in the direction of those tissues with an obligatory requirement, such as CNS. This, in brief, is the conventional view of the whole body response to fasting and is mediated by regulatory mechanisms which will not be discussed further here. It is clear from the above that impaired activity of any of the enzymes of β-oxidation or of the auxiliary systems concerned with fatty acid transport, with disposal of reducing equivalents, with disposal of acetyl-CoA, or with the degradation of polyunsaturated fatty acids, is likely to have a major impact on glucose-sparing during periods of counter-regulation. Furthermore, gluconeogenesis may well be attenuated due to lowered availability of reducing equivalents. This is particularly apparent in patients with disorders of the long- and medium-chain specific enzymes. However, in patients with the short-chain disorders, milder variants and in older patients, in whom exercise intolerance and muscle and heart involvement are the predominant presenting features, hypoglycaemia and an inappropriate ketotic response to fasting may not be present.

The concentrations of intermediary metabolites from patients with medium-chain acyl-CoA dehydrogenase deficiency and from patients with other causes of hypoketotic hypoglycaemia and hyperinsulinism, are shown in Table 1. Whether or not hypoglycaemia is accompanied by an appropriate ketonaemia is clearly of importance. In order to distinguish an appropriate ketotic response to hypoglycaemia, particularly in the context of impaired β-oxidation, it is helpful to relate log ([acetoacetate] + [3-hydroxybutyrate]) to the concentration of nonesterified fatty acids (NEFA) [7]. Most patients with disorders of β-oxidation have high concentrations of free fatty acids but inappropriately low concentrations of ketone bodies for that degree of lipolysis. Figure 3 (dashed lines) shows the changes, with time, in the relationship between the blood concentrations of free fatty acids and of ketone bodies during the progression of the

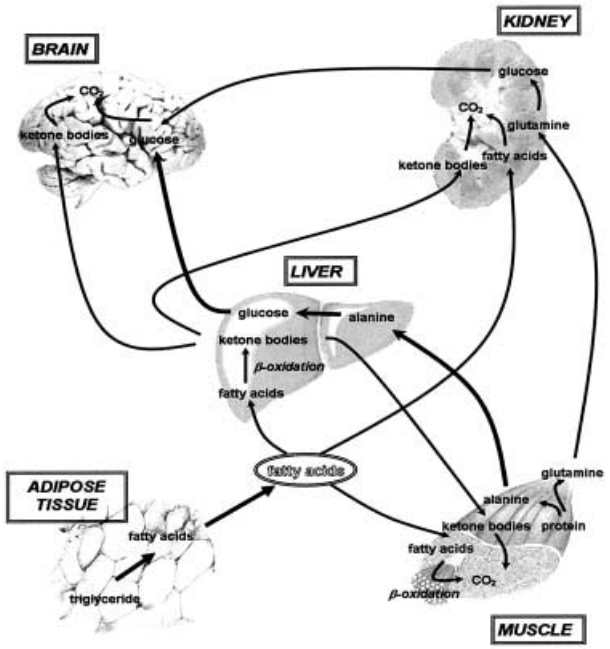

Fig. 2. Relationship of organs with respect to fuel utilization in the fasted state.

starvation provocation test in three children with medium-chain acyl-CoA dehydrogenase deficiency. It is clear that whilst the relationship is normal at the onset of the starvation test, the relationship rapidly becomes abnormal with increased starvation-induced stress. The sequential changes in children in whom there was no evidence of metabolic disease (Fig. 3, continuous lines) are also shown, and it is apparent that these data points stay within the 95% confidence limits derived from cross-sectional data. It is informative to compare these children with hyperinsulinaemic children who have a relationship which falls within the 95% confidence limits [7]. Thus, although hyperinsulinaemic children had an inappropriately low concentration of ketone bodies relative to the degree of glycaemia, the relationship with free fatty acids was appropriate. It is evident that the hypoketonaemia arose from decreased free fatty acid release as a result of the antilipolytic effect of insulin on adipose cells. However, it appears that some hyperinsulinaemic children have an inborn error of short-chain 3-hydroxyacyl-CoA dehydrogenase (see below) such that ketogenesis itself may be impaired [8].

Overview of enzymology

After entry into the cell, fatty acids are activated to acyl-CoA esters by acyl-CoA synthetases and can be targeted to esterification or to mitochondrial β-oxidation (reviewed in [9]). Mitochondrial β-oxidation can be conceptually divided into two: (a) the process of getting acyl groups into the mitochondrion for oxidation and (b) intramitochondrial chain shortening by oxidative removal of two-carbon (acetyl) units. The enzymes involved in these processes are summarized in Table 2.

Carnitine palmitoyl transferases and the carnitine-acylcarnitine translocase

Acyl-CoA esters cannot directly cross the mitochondrial inner membrane, and their entry to the mitochondrion is a major point for control and regulation of the β-oxidation flux ([9]; see below). After entry, the acyl moiety can be considered as committed to complete oxidation. Transfer across the mitochondrial membrane is achieved by transference of the acyl group from CoA to carnitine, transfer across the inner membrane, and reconversion to acyl-CoA ester intramitochondrially. This is accomplished by carnitine palmitoyl transferase I (CPTI) on the outer mitochondrial membrane, carnitine acylcarnitine translocase in the inner membrane, and carnitine palmitoyl transferase II (CPTII) on the inner face of the inner membrane (Fig. 4). The carnitine acyl-carnitine translocase exchanges acylcarnitine for carnitine, so that the cytosol does not become carnitine depleted relative to the mitochondrion.

Chain shortening

Mitochondrial chain shortening takes place via a series of four repeated enzyme steps (Fig. 1): (a) acyl-CoA dehydrogenase, producing *trans*-2,3-enoyl-CoA (b) 2-enoyl-CoA hydratase, producing L-3-hydroxyacyl-CoA (c) L-3-hydroxyacyl-CoA dehydrogenase (NAD^+-linked), producing

Table 1. Concentrations of intermediary metabolites in the blood of normal subjects, patients with medium chain acyl-CoA dehydrogenase deficiency and patients with hyperinsulinism. Controls were fasted for 24 h. Modified from [7] with permission. –, SDs for < 1.0 cannot be calculated.

		Analyte								
Subject		Lactate ($mmol \cdot L^{-1}$)	Pyruvate ($mmol \cdot L^{-1}$)	Alanine ($mmol \cdot L^{-1}$)	3OHbutyrate ($mmol \cdot L^{-1}$)	Acetoacetate ($mmol \cdot L^{-1}$)	Glucose ($mmol \cdot L^{-1}$)	NEFA ($mmol \cdot L^{-1}$)	Glycerol ($mmol \cdot L^{-1}$)	Insulin (mU)
Controls [n = 19]	Mean	1.26	0.11	0.20	1.98	0.74	3.6	1.58	0.16	<1.0
	SD	0.56	0.07	0.06	1.38	0.56	0.60	0.39	0.04	–
Hyperinsulinemics [n = 13]	Mean	1.07	0.09	0.24	0.29	0.14	2.5	0.58	0.74	9.8
	SD	0.53	0.04	0.08	0.47	0.17	0.9	0.45	0.11	7.6
MCAD deficiency	Mean	1.24	0.09	0.15	0.40	0.23	2.54	2.28	0.28	<1.0
	SD	0.39	0.04	0.01	0.20	0.24	0.65	0.42	0.17	–
	n	8	3	3	8	3	8	7	3	5

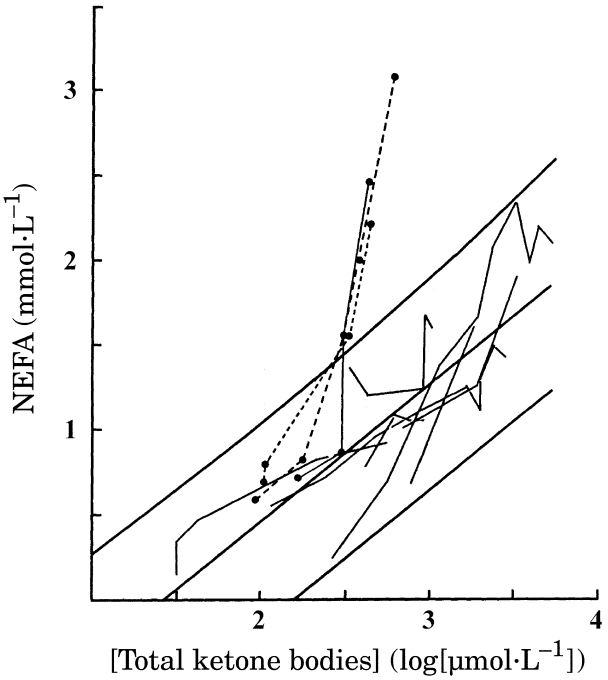

Fig. 3. The relationship of plasma NEFA concentrations to (log) blood ketone body concentrations. The line of best fit and the 95% confidence limits are derived from cross-sectional data from 46 control subjects. Also shown are data from patients with medium-chain acyl-CoA dehydrogenase deficiency (--●) and normal children (—) during the course of a fasting provocation stress test. Modified from [7] with permission.

3-oxoacyl-CoA and (d) 3-oxoacyl-CoA thiolase, producing saturated acyl-CoA shortened by 2 carbons, plus an acetyl-CoA. The first dehydrogenation step is linked to the respiratory chain via electron transfer flavoprotein (ETF) and ETF-ubiquinone oxidoreductase (ETFD), and the second dehydrogenation is linked to complex I of the respiratory chain via NADH. Hence, ATP production from β-oxidation comes both from direct production of reduced cofactors, and from subsequent oxidation of acetyl-CoA.

There are multiple enzymes for each of the constituent steps of the pathway, which vary in their chain-length specificity. In the case of acyl-CoA dehydrogenation there are four enzymes: short-chain acyl-CoA dehydrogenase (active with C_4 and C_6), medium-chain acyl-CoA dehydrogenase (MCAD, C_4 to C_{12}), long-chain acyl-CoA dehydrogenase (LCAD, active with C_8 to C_{20}) and very-long-chain acyl-CoA dehydrogenase (VLCAD, active with C_{12} to C_{24}). LCAD may be relatively unimportant in the β-oxidation of saturated fatty acids and more important for the oxidation of unsaturated [10] and 2-methyl-branched chain fatty acids [11], although comparison of mice with disrupted LCAD and VLCAD supports a role for LCAD in β-oxidation of saturated fatty acids [12]. Recently, a further, ninth, member of the acyl-CoA dehydrogenase family has been documented (ACAD-9) [13], which appears to have properties and tissue distribution (based on mRNA) very similar to VLCAD, except that, unusually for the acyl-CoA dehydrogenases, there are substantial levels in brain. This novel enzyme has optimal activity with palmitoyl-CoA and very little activity with octanoyl-CoA and branched-chain substrates. The role of ACAD-9 in mitochondrial β-oxidation remains to be established.

There are two 2-enoyl-CoA hydratase activities; the short-chain 2-enoyl-CoA hydratase ('crotonase') which has a broad substrate specificity, and the long-chain 2-enoyl-CoA hydratase. Similarly, there are short- and long-chain L-3-hydroxyacyl-CoA dehydrogenases, and short-, medium- ('general') and long-chain 3-oxoacyl-CoA thiolases, although the short-chain enzyme is more important for ketogenesis and branched-chain amino acid oxidation than β-oxidation. The long-chain activities of

Table 2. Enzymes of mitochondrial β-oxidation. Refer to [6] for relevant primary literature.

Enzyme	Abbreviation	Structure	MW (kDa)
Carnitine palmitoyl transferase I (liver)	lCPTI	unknown	88
Carnitine palmitoyl transferase I (muscle)	mCPTI	unknown	82
Carnitine acyl-carnitine translocase	CACT	unknown	32.5
Carnitine palmitoyl transferase II	CPTII	unknown	68
Very-long-chain acyl-CoA dehydrogenase	VLCAD	homodimer	150
ACAD-9	ACAD-9	homodimer	140
Long-chain acyl-CoA dehydrogenase	LCAD	homotetramer	180
Medium-chain acyl-CoA dehydrogenase	MCAD	homotetramer	180
Short-chain acyl-CoA dehydrogenase	SCAD	homotetramer	168
Trifunctional protein	TFP	heterooctomer	460
Long-chain 3-hydroxyacyl-CoA dehydrogenase	LCHAD		
Long-chain 2-enoyl-CoA hydratase			
Long-chain 3-oxoacyl-CoA thiolase			
Short-chain 2-enoyl-CoA hydratase (crotonase)	SCEH	homohexamer	164
Short-chain 3-oxoacyl-CoA thiolase	SCOT	homotetramer	169
Short-chain 3-hydroxyacyl-CoA dehydrogenase	SCHAD	homodimer	68
General (medium-chain) 3-oxoacyl-CoA thiolase	GOT	homotetramer	200
Electron transfering flavoprotein	ETF	heterodimer	57
ETF-ubiquinone oxidoreductase	ETFD	monomer	68
Carnitine acetyltransferase	CAT	monomer	60

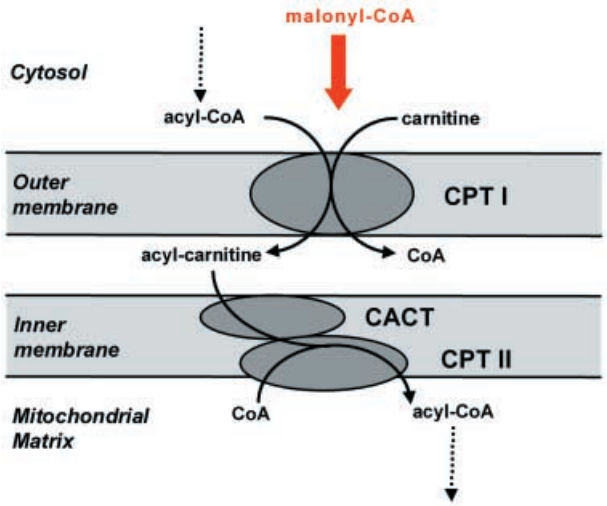

Fig. 4. Transfer of acyl-CoA ester into the mitochondrion by the combined activities of: carnitine palmitoyl transferase I (CPTI), carnitine acylcarnitine translocase (CACT) and carnitine palmitoyl transferase II (CPTII).

2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-oxoacyl-CoA thiolase are constituents of a single protein of the inner mitochondrial membrane, the trifunctional protein [6].

The acetyl-CoA produced by chain-shortening has different fates, depending on the tissue: in ketogenic tissues (e.g. liver) most of the acetyl-CoA is used to form ketone bodies (acetoacetate and β-hydroxybutyrate) for export and peripheral oxidation, whereas in most tissues (e.g. heart, skeletal muscle), acetyl-CoA enters the Krebs cycle and is used in ATP generation. However, the rate of β-oxidation may exceed the rate of acetyl-CoA oxidation in the Krebs cycle, so acetyl-CoA can be exported from the mitochondrion by the action of the auxiliary enzyme carnitine acetyl transferase and the carnitine acyl-carnitine translocase. The resultant cytosolic acetyl-carnitine acts as an acetyl buffer, re-entering the mitochondrion and being oxidized when intramitochondrial acetyl-CoA levels are lower. In addition to the enzymes discussed above, there are various other auxiliary enzymes which act to enable a wide range of polyunsaturated and xenobiotic fatty acids to be completely oxidized; for a discussion of these enzymes, the reader is referred to other recent reviews [14].

Mitochondrial control/integration of systems

As mitochondrial β-oxidation functions either to directly produce ATP, or to produce ketone bodies for ATP generation by peripheral tissues, the rate of β-oxidation flux is integrated with the oxidation of other substrates, particularly glucose. This is achieved by control both at the level of entry of fatty acids into the mitochondrion, and by further intramitochondrial controls.

A major control on β-oxidation, and a crossover between fatty acid metabolism and carbohydrate oxidation, was elucidated by McGarry & Foster in the 1970s [4]. When carbohydrate is plentiful, its mitochondrial oxidation causes accumulation of citrate within the mitochondrion which may then be exported. The resultant cytosolic citrate is cleaved by ATP-citrate lyase to malate and acetyl-CoA. The acetyl-CoA forms malonyl-CoA by the action of acetyl-CoA carboxylase, which is then activated by the presence of high citrate concentrations. Malonyl-CoA is the substrate for fatty acid synthesis and has been called the 'signal of plenty'. In order that fatty acid oxidation does not occur simultaneously with synthesis, malonyl-CoA is a physiological inhibitor of CPTI, and thus of fatty acid entry to the mitochondria for β-oxidation. CPTI has been shown to be rate-controlling for β-oxidation flux (i.e. it has a high flux control coefficient), but not necessarily rate-limiting under most conditions ([9]; however, tissue-specific differences are discussed below). In addition to the feedback control mediated by malonyl-CoA, it has been demonstrated that the AMP-activated protein kinase (AMPK) stimulates CPTI [15]. Thus, as well as inactivating acetyl-CoA carboxylase and thereby lowering malonyl-CoA concentrations and releasing the inhibition of CPTI, AMPK increases CPTI activity, and mediates a concerted response to metabolic stress by stimulating fatty acid oxidation. The mechanism of this effect of AMPK on CPTI appears to involve the phosphorylation of the cytoskeletal components, cytokeratins 8 and 18.

Intramitochondrial controls on β-oxidation flux can be considered mostly in terms of recycling of cofactors, which are at a limited concentration within the mitochondrion. There is clearly a requirement for NAD^+ by the 3-hydroxyacyl-CoA dehydrogenases, a requirement for oxidized ETF by the acyl-CoA dehydrogenases, and a requirement for unesterified CoA both by CPTII and by the 3-oxoacyl-CoA thiolases. If re-oxidation of NADH (by complex I) or reduced-ETF (by ETFD and the coenzyme Q pool) is impaired, then β-oxidation itself will be inhibited at the levels of the 3-hydroxyacyl-CoA dehydrogenase and acyl-CoA dehydrogenase, respectively. Accumulation of 3-hydroxyacyl-CoA esters subsequent to inhibition of the 3-hydroxyacyl-CoA dehydrogenases will cause feedback inhibition of the 2-enoyl-CoA hydratases [16] and then the acyl-CoA dehydrogenases [17,18]. Redox control of β-oxidation has been shown where the respiratory chain is impaired, either by enzyme deficiency [19], enzyme inhibition [20,21], high ATP/ADP ratio [22], anoxia [23] or in normal respiring liver mitochondria [24]. The mitochondrial CoA pool is of limited size, and so intramitochondrial accumulation of acyl-CoA esters or unrestricted entry of acyl groups to the mitochondrion would lead to lack of free CoA for esterification. This would inhibit β-oxidation at the 3-oxoacyl-CoA thiolase step and lead to accumulation of 3-oxoacyl-CoA esters, which inhibit the 3-hydroxyacyl-CoA dehydrogenases [16,25], the 2-enoyl-CoA hydratases [26] and the acyl-CoA dehydrogenases [18] and are thus potential potent feedback inhibitors of β-oxidation. As acetyl-CoA is an inhibitor of 3-oxoacyl-CoA thiolases [27,28], an increase in the acetyl-CoA/CoA ratio would lead to inhibition of 3-oxoacyl-CoA thiolase activity, accumulation of 3-oxoacyl-CoA esters and inhibition of β-oxidation. This has been proposed to be important in the control of β-oxidation flux [27,29], however, we have never detected 3-oxoacyl-CoA esters in mitochondria under any condition, suggesting that their intramitochondrial concentration

may never rise high enough to be important physiological regulators of β-oxidation flux [20,24,30]. In addition, CPTII would become inhibited by lack of unesterified CoA before 3-oxoacyl-CoA thiolase, thus preventing entry of further acyl groups to the mitochondrion; the kinetic characteristics of CPTII and the carnitine acylcarnitine translocase would favour export of acyl groups under these circumstances [9].

The pathway of long-chain fatty acid oxidation to acetyl-CoA is one of the longest unbranched pathways in metabolism, and it has long been suggested that the enzymes of β-oxidation are organized into a multienzyme complex. This was initially based on the detection of low concentrations of intermediates [31] and later the observation that the intermediates of β-oxidation that did accumulate behaved more like products than intermediates [20,21,24,30,32–34]. This led to the 'leaky hosepipe' model for the control of β-oxidation flux [21,32,33] in which the channelling of a small, quick turnover pool of intermediates is implied. In addition, the measured concentrations of acyl-CoA esters are close to the concentrations of the enzymes of β-oxidation themselves [35]. As carnitine acyl-carnitine translocase, CPTII, VLCAD, the trifunctional protein, ETFD and complex I are bound to the inner membrane and could be associated with CPTI and acyl-CoA synthetase in contact sites, all the enzymes required for β-oxidation of long-chain acyl-CoA esters, serviced by NAD and ETF (which is present at substrate levels in mitochondria) could be associated in a metabolon [6,36]. However, direct evidence is lacking, both for such a complex and for channelling within such a metabolon. The only direct evidence for channelling of long-chain acyl-CoA esters is between the alpha- and beta-subunits of the trifunctional protein [28] and it cannot be assumed that simply because it would appear to make sense that β-oxidation is a channelled process undertaken by a metabolon that it does take place in that way [37]. Another explanation for the low concentrations of intermediates observed could simply be that most of the control within the pathway of β-oxidation is exerted at or before the level of CPTI. In addition, as pointed out by Srere & Sumegi, the teleological argument that channelling of β-oxidation would have evolved because it is more efficient, does not hold because evolution appears to have developed from a multifunctional system in prokaryotes to multiple enzymes, rather than the reverse [35]. In addition, it is possible that the observed 'free' enzymes are an artefact of mitochondrial disruption and subsequent dilution.

Although direct evidence for a β-oxidation metabolon is lacking, there is much evidence for associations of various types between β-oxidation enzymes and between β-oxidation enzymes and their associated proteins. Complex I binds several dehydrogenases, including SHOAD [38] and NADH can be channelled between them [39,40]; we have also suggested that there is a pool of NAD/NADH that is channelled between the trifunctional protein and complex I [30,34]. General 3-ketoacyl-CoA thiolase binds to citrate synthase [41], and there is at least one SHOAD binding protein in the inner mitochondrial membrane [42,43] which could provide an anchor for the binding of soluble enzymes of β-oxidation. The finding that gently sonicated mitochondria oxidized short-chain substrates more rapidly than disrupted mitochondria was also interpreted as providing evidence for association of the soluble enzymes of β-oxidation with their redox partners (i.e. ETF, ETF-QO and complex I) [44]. Recently, Parker & Engel showed that functional assemblies consisting of MCAD or sarcosine dehydrogenase (an enzyme of one-carbon metabolism which is also dehydrogenated by ETF) together with ETF, ETFD, coenzyme Q (ubiquinone) and complex III could be isolated from sonicated porcine liver mitochondria [45]. Similarly, Jones *et al.* [46] have characterized electron transfer and conformational changes in a complex of trimethylamine dehydrogenase (EC 1.5.99.7, derived from the methylotroph *Methylophilus methylotrophus*) and ETF, and demonstrate that electron transfer occurs during metastable states of the complex. Further, they show that ETF undergoes a stable conformational change when it interacts with TAMDH which they term 'structural imprinting' and that this form of semiquinone ETF has an increased rate of electron transfer to the artificial electron acceptor ferricenium. However, these effects were not observed with the human MCAD and human ETF_{ox}. These authors conclude that *in vitro* studies of interprotein electron transfer reactions must be interpreted with caution, particularly with regard to extrapolation to the *in vivo* situation. Hence, evidence for a true β-oxidation metabolon appears tantalizingly close, but has not been conclusively demonstrated.

Tissue/organ differences

The intramitochondrial enzymes of the β-oxidation spiral are not known to have any tissue-specific isoforms and are thought to be expressed in all tissues that are active in β-oxidation. However, there are two isoforms of CPTI which vary in their expression between tissues and in their regulatory properties. The liver isoform of CPTI (lCPTI) is expressed in the liver, kidney, pancreatic islets, intestine and brain, whereas the muscle isoform (mCPTI) is found in skeletal and cardiac muscle, in the testis and in brown adipocytes [47]. The liver and muscle CPTI isoforms have markedly different kinetic characteristics: lCPTI has a low K_m for carnitine whereas mCPTI is very much more sensitive to malonyl-CoA than the lCPTI [48]. Cardiac muscle expresses both m and l CPTI isoforms, but the relative proportions of the isoforms change during development [49] so that the overall sensitivity to malonyl-CoA and affinity for carnitine, alters.

Although there are no tissue-specific isoforms of the enzymes of the β-oxidation spiral per se, there do appear to be differences in relative amounts between tissues. Thus, the low rate of β-oxidation in brain has been hypothesized to be due to the very low activity of 3-oxoacyl-CoA thiolase relative to the other β-oxidation enzymes [50]. Moreover, within tissues there may be differences in the distribution of β-oxidation activity, for example, there is good evidence for zonation within the liver acinus. In rats that have been fed, periportal (afferent) hepatocytes have higher rates of β-oxidation than perivenous (efferent) hepatocytes [51]. Moreover, CPTI activities are higher in periportal hepatocytes. Also in the rat, the ratio of β-oxidation flux between these two zones varies with the physiological state: the periportal/perivenous ratios are 1.5, 2.0, 1.0 and 0.4 for fed, starved, re-fed and cold-exposed animals, respectively [52]. Similar variation in zonation is observed in CPTI, and in the activity of the key regulatory

enzyme of ketogenesis, 3-hydroxy-3-methylglutaryl-CoA synthase [52].

Conclusions

Whilst the fundamentals of the pathway of mitochondrial β-oxidation are now well established and our understanding of the control of pathway flux, both at the point of fatty acid uptake and within the mitochondrial matrix, is reasonably complete, there remain to be answered questions relating to the topology of the system. Similarly, inherited disorders of the pathway have been known for almost 20 years but there remains uncertainty regarding the relationship between known mutations and the observed clinical phenotype. Recent progress in this area and related biochemistry are described in the other reviews in this series.

References

1. Gibbons, G.F., Islam, K. & Pease, R.J. (2000) Mobilisation of triacylglycerol stores. *Biochim. Biophys. Acta* **1483**, 37–57.
2. Gervois, P., Torra, I.P., Fruchart, J.C. & Staels, B. (2000) Regulation of lipid and lipoprotein metabolism by PPAR activators. *Clin. Chem. Lab. Med.* **38**, 3–11.
3. Lopaschuk, G.D., Belke, D.D., Gamble, J., Itoi, T. & Schonekess, B.O. (1994) Regulation of fatty-acid oxidation in the mammalian heart in health and disease. *Biochim. Biophys. Acta* **1213**, 263–276.
4. McGarry, J.D. & Foster, D.W. (1980) Regulation of hepatic fatty acid oxidation and ketone body production. *Ann. Rev. Biochem.* **49**, 395–420.
5. Reidy, S.P. & Weber, J.M. (2000) Leptin: an essential regulator of lipid metabolism. *Comp. Biochem. Physiol., Part A Mol. Integr. Physiol.* **125**, 285–297.
6. Eaton, S., Bartlett, K. & Pourfarzam, M. (1996) Mammalian mitochondrial beta-oxidation. *Biochem. J.* **320**, 345–357.
7. Bartlett, K. (1993) Methods for the investigation of hypoglycemia with particular reference to inherited disorders of mitochondrial beta-oxidation. *Baillieres Clin. Endocrinol. Metab.* **7**, 643–667.
8. Clayton, P.T., Eaton, S., Aynsley-Green, A., Edginton, M., Hussain, K., Krywawych, S., Datta, V., Malingre, H.E.M., Berger, R. & van den Berg, I.E.T. (2001) Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. *J. Clin. Invest.* **108**, 457–465.
9. Eaton, S. (2002) Control of mitochondrial β-oxidation flux. *Prog. Lipid. Res.* **41**, 197–239.
10. Le, W.P., Abbas, A.S., Sprecher, H., Vockley, J. & Schulz, H. (2000) Long-chain acyl-CoA dehydrogenase is a key enzyme in the mitochondrial beta-oxidation of unsaturated fatty acids. *Biochim. Biophys. Acta* **1485**, 121–128.
11. Wanders, R.J.A., Denis, S., Ruiter, J.P.N., IJlst, L. & Dacremont, G. (1998) 2,6-dimethylheptanoyl-CoA is a specific substrate for long-chain acyl-CoA dehydrogenase (LCAD): evidence for a major role of LCAD in branched-chain fatty acid oxidation. *Biochim. Biophys. Acta* **1393**, 35–40.
12. Cox, K.B., Hamm, D.A., Millington, D.S., Matern, D., Vockley, J., Rinaldo, P., Pinkert, C.A., Rhead, W.J., Lindsey, J.R. & Wood, P.A. (2001) Gestational, pathologic and biochemical differences between very long-chain acyl-CoA dehydrogenase deficiency and long-chain acyl-CoA dehydrogenase deficiency in the mouse. *Hum. Mol. Genet.* **10**, 2069–2077.
13. Zhang, J., Zhang, W., Zou, D., Chen, G., Wan, T., Zhang, M. & Cao, X. (2002) Cloning and functional characterization of ACAD-9, a novel member of human acyl-CoA dehydrogenase family. *Biochem. Biophys. Res. Comms* **297**, 1033–1042.
14. Hiltunen, J.K. & Qin, Y.M. (2000) β-Oxidation – strategies for the metabolism of a wide variety of acyl-CoA esters. *Biochim. Biophys. Acta* **1484**, 117–128.
15. Velasco, G., Gomez, d. P., Carling, D. & Guzman, M. (1998) Evidence that the AMP-activated protein kinase stimulates rat liver carnitine palmitoyltransferase I by phosphorylating cytoskeletal components. *FEBS Lett.* **439**, 317–320.
16. Schifferdecker, J. & Schulz, H. (1974) The inhibition of L-3-hydroxyacyl-CoA dehydrogenase by acetoacetyl-CoA and the possible effect of this inhibitor on fatty acid oxidation. *Life Sci.* **14**, 1487–1492.
17. Powell, P.J., Lau, S.M., Killian, D. & Thorpe, C. (1987) Interaction of acyl coenzyme A substrates and analogues with pig kidney medium-chain acyl-coA dehydrogenase. *Biochemistry* **26**, 3704–3710.
18. Davidson, B. & Schulz, H. (1982) Separation, properties, and regulation of acyl coenzyme A dehydrogenases from bovine heart and liver. *Arch. Biochem. Biophys.* **213**, 155–162.
19. Watmough, N., Bindoff, L., Birch-Machin, M., Jackson, S., Bartlett, K., Ragan, C., Poulton, J., Gardiner, R., Sherratt, H.S.A. & Turnbull, D.M. (1990) Impaired mitochondrial beta-oxidation in a patient with an abnormality of the respiratory chain. Studies in skeletal muscle mitochondria. *J. Clin. Invest* **85**, 177–184.
20. Eaton, S., Pourfarzam, M. & Bartlett, K. (1996) The effect of respiratory chain impairment on beta-oxidation in rat heart mitochondria. *Biochem. J.* **319**, 633–640.
21. Stanley, K.K. & Tubbs, P.K. (1975) The role of intermediates in mitochondrial fatty acid oxidation. *Biochem. J.* **150**, 77–88.
22. Lopez-Cardozo, M., Klazinga, W. & van den Bergh, S.G. (1978) Accumulation of carnitine esters of beta-oxidation intermediates during palmitate oxidation in rat liver mitochondria. *Eur. J. Biochem.* **83**, 629–634.
23. Moore, K.H., Radloff, J.F., Hull, F.E. & Sweely, C.C. (1980) Incomplete fatty acid oxidation by ischaemic heart: beta-hydroxy fatty acid production. *Am. J. Physiol.* **239**, H257–H265.
24. Eaton, S., Turnbull, D.M. & Bartlett, K. (1994) Redox control of beta-oxidation in rat liver mitochondria. *Eur. J. Biochem.* **220**, 671–681.
25. He, X.-Y., Yang, S. & Schulz, H. (1989) Assay of 1-3-hydroxyacyl-coenzyme-a dehydrogenase with substrates of different chain lengths. *Anal. Biochem.* **180**, 105–109.
26. Waterson, R. & Hill, R. (1972) Enoyl coenzyme A hydratase (crotonase). Catalytic properties of crotonase and its possible regulatory role in fattyacid oxidation. *J. Biol. Chem.* **247**, 5258–5265.
27. Wang, H.Y., Baxter, C.F. Jr & Schulz, H. (1991) Regulation of fatty acid beta-oxidation in rat heart mitochondria. *Arch. Biochem. Biophys.* **289**, 274–280.
28. Yao, K.W. & Schulz, H. (1996) Intermediate channeling on the trifunctional beta-oxidation complex from pig-heart mitochondria. *J. Biol. Chem.* **271**, 17816–17820.
29. Oram, J.F., Bennetch, S.L. & Neely, J.R. (1973) Regulation of fatty acid utilization in isolated perfused rat hearts. *J. Biol. Chem.* **248**, 5299–5309.
30. Eaton, S., Bhuiyan, A.K.M.J., Kler, R.S., Turnbull, D.M. & Bartlett, K. (1993) Intramitochondrial control of the oxidation of hexadecanoate in skeletal muscle. A study of the acyl-CoA esters which accumulate during rat skeletal-muscle mitochondrial beta-oxidation of [U-14C]hexadecanoate and [U-14C]hexadecanoylcarnitine. *Biochem. J.* **289**, 161–168.
31. Garland, P.B., Shepherd, D. & Yates, D.W. (1965) Steady-state concentrations of CoA acetyl-CoA and long-chain fatty acyl-CoA in rat liver mitochondria. *Biochem. J.* **97**, 587–594.
32. Stanley, K.K. & Tubbs, P.K. (1974) The occurrence of intermediates in mitochondrial fatty acid oxidation. *FEBS Lett.* **39**, 325–328.

33. Stewart, H.B., Tubbs, P.K. & Stanley, K.K. (1973) Intermediates in fatty acid oxidation. *Biochem. J.* **132**, 61–76.
34. Eaton, S., Bartlett, K. & Pourfarzam, M. (1999) Intermediates of myocardial mitochondrial beta-oxidation: possible channelling of NADH and of CoA esters. *Biochim. Biophys. Acta* **1437**, 402–408.
35. Srere, P.A. & Sumegi, B. (1994) Processivity and fatty-acid oxidation. *Biochem. Soc. Trans.* **22**, 446–450.
36. Eaton, S., Bursby, T., Middleton, B., Pourfarzam, M., Mills, K., Johnson, A.W. & Bartlett, K. (2000) The mitochondrial trifunctional protein: centre of a beta-oxidation metabolon? *Biochem. Soc. Trans.* **28**, 177–182.
37. Cornish-Bowden, A. (1997) Kinetic consequences of channelling. In: *Channelling in Intermediary Metabolism* (Agius, L. & Sherratt, H.S.A., eds), pp. 53–70. Portland Press, London.
38. Sumegi, B. & Srere, P.A. (1984) Complex I binds several mitochondrial NAD-coupled dehydrogenases. *J. Biol. Chem.* 259, 15040–15045.
39. Fukushima, T., Decker, R.V., Anderson, W.M. & Spivey, H.O. (1989) Substrate channeling of NADH and binding of dehydrogenases to complex I. *J. Biol. Chem.* **264**, 16483–16488.
40. Ushiroyama, T., Fukushima, T., Styre, J.D. & Spivey, H.O. (1992) Substrate channeling of NADH in mitochondrial redox processes. *Curr. Top. Cell. Regul.* **33**, 291–307.
41. Sumegi, B., Gilbert, H.F. & Srere, P.A. (1985) Interaction between citrate synthase and thiolase. *J. Biol. Chem.* **260**, 188–190.
42. Kispal, G., Sumegi, B. & Alkonyi, I. (1986) Isolation and characterization of 3-hydroxyacyl coenzyme A dehydrogenase-binding protein from pig heart inner mitochondrial membrane. *J. Biol. Chem.* **261**, 14209–14213.
43. Furuta, S. & Hashimoto, T. (1995) Purification and properties of 3-hydroxyacyl coenzyme-a dehydrogenase-binding protein from rat-liver mitochondria. *J. Biochem.* **118**, 810–818.
44. Sumegi, B., Porpaczy, Z. & Alkonyi, I. (1991) Kinetic advantage of the interaction between the fatty acid beta-oxidation enzymes and the complexes of the respiratory chain. *Biochim. Biophys. Acta* **1081**, 121–128.
45. Parker, A. & Engel, P.C. (2000) Preliminary evidence for the existence of specific functional assemblies between enzymes of the beta-oxidation pathway and the respiratory chain. *Biochem. J.* **345**, 429–435.
46. Jones, M., Talfournier, F., Bobrov, A., Grossmann, J.G., Vekshin, N., Sutcliffe, M.J. & Scrutton, N.S. (2002) Electron transfer and conformational change in complexes of trimethylamine dehydrogenase and electron transferring flavoprotein. *J. Biol. Chem.* **277**, 8457–8465.
47. Brown, N.F., Hill, J., Esser, V., Kirkland, J., Corkey, B. & Foster, D.W. (1997) Mouse white adipocytes and 3t3-l1 cells display an anomalous pattern of carnitine palmitoyltransferase (CPT) I isoform expression during differentiation – inter-tissue and interspecies expression of CPT I and CPT II enzymes. *Biochem. J.* **327**, 225–231.
48. Saggerson, E.D. & Carpenter, C.A. (1981) Carnitine palmitoyltransferase and carnitine octanoyltransferase activities in liver, kidney cortex, adipocyte, lactating mammary-gland, skeletal-muscle and heart. *FEBS Lett.* **129**, 229–232.
49. Brown, N.F., Weis, B.C., Husti, J., Foster, D.W. & McGarry, J.D. (1995) Mitochondrial carnitine palmitoyltransferase-I isoform switching in the developing rat-heart. *J. Biol. Chem.* **270**, 8952–8957.
50. Yang, S.Y., He, X.-Y. & Schulz, H. (1987) Fatty acid oxidation in rat brain is limited by the low activity of 3-ketoacyl-coenzyme A thiolase. *J. Biol. Chem.* **262**, 13027–13032.
51. Tosh, D., Alberti, K.G.M.M. & Agius, L. (1988) Glucagon regulation of gluconeogenesis and ketogenesis in periportal and perivenous rat hepatocytes – heterogeneity of hormone action and of the mitochondrial redox state. *Biochem. J.* **256**, 197–204.
52. Guzman, M., Bijleveld, C. & Geelen, M.J.H. (1995) Flexibility of zonation of fatty-acid oxidation in rat-liver. *Biochem. J.* **311**, 853–860.