REVIEW ARTICLE

Mammalian mitochondrial β-oxidation

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The enzymic stages of mammalian mitochondrial β-oxidation were elucidated some 30–40 years ago. However, the discovery of a membrane-associated multifunctional enzyme of β-oxidation, a membrane-associated acyl-CoA dehydrogenase and characterization of the carnitine palmitoyl transferase system at the protein and at the genetic level has demonstrated that the enzymes of the system itself are incompletely understood. Deficiencies of many of the enzymes have been recognized as important causes of disease. In addition, the study of these disorders has led to a greater understanding of the molecular mechanism of β-oxidation and the import, processing and assembly of the β-oxidation enzymes within the mitochondrion. The tissue-specific regulation, intramitochondrial control and supramolecular organization of the pathway is becoming better understood as sensitive analytical and molecular techniques are applied. This review aims to cover enzymological and organizational aspects of mitochondrial β-oxidation together with the biochemical aspects of inherited disorders of β-oxidation and the intrinsic control of β-oxidation.

1 INTRODUCTION

β-Oxidation is the major process by which fatty acids are oxidized, thus providing a major source of energy for the heart and for skeletal muscle [1,2]. Hepatic β-oxidation serves a different role by providing ketone bodies (acetoacetate and β-hydroxybutyrate) to the peripheral circulation. Ketone bodies are an important fuel for extra-hepatic organs, especially the brain, when blood glucose levels are low. For this reason, β-oxidation is stimulated when glucose levels are low, for instance during starvation or endurance exercise, essentally as postulated in the Randle cycle [2a].

Investigation of the pathway of β-oxidation of long-chain fatty acids commenced with the celebrated early studies of Knoop [3], who fed dogs ω-phenyl odd or even carbon-number long-chain fatty acids. He inferred from the excretion of phenylacetylglycine and benzoylglycine respectively, that the metabolism of fatty acids proceeded by a process of successive removal of two-carbon fragments. These observations were subsequently confirmed by Dakin [4]; however, detailed elucidation of the pathway was not achieved until the discovery of CoA and the realization that acetyl-CoA is the product of β-oxidation [5,6]. The chemical synthesis of acyl-CoA substrates and the elucidation of the cofactor requirements of soluble extracts of mitochondria carrying out β-oxidation [7] was rapidly followed by series of papers from the laboratories of Green, Lynen and Ochoa respectively, and the discovery of the basic sequence of steps: FAD-linked dehydrogenation, hydration, NAD^+-linked dehydrogenation and thiolytic cleavage, to yield acetyl-CoA, e.g. [7a,7b]. The role of carnitine in the transport of fatty acids across the inner mitochondrial membrane (see [8]), and the function of malonyl-CoA as a regulator of transport, together with the discovery of auxiliary systems for the metabolism of polyunsaturated fatty acids (PUFA) have been more recent developments. The basic enzymology of β-oxidation is shown in Figure 1. In the present review we discuss the recent developments in the enzymology of the pathway, the discovery of a class of inherited metabolic disorders of mitochondrial β-oxidation and the organization and control of the pathway.

2 ENZYMES OF MITOCHONDRIAL β-OXIDATION

2.1 Acyl-CoA synthases

The enzymes of mitochondrial β-oxidation are summarized in Table 1. The enzymes of β-oxidation all act on CoA esters, so a preliminary to β-oxidation is the ATP-dependent formation of fatty acyl-CoA esters, catalysed by acyl-CoA synthase. Several acyl-CoA synthases are associated with mammalian mitochondria. Of these, the short-chain acyl-CoA synthases are found within the matrix and are important in ruminants [9]. Two medium-chain acyl-CoA synthases are also found in the mitochondrial matrix [10,11]. Long-chain acyl-CoA synthase activity is found in the mitochondrial outer membrane [12] and it appears to be a transmembrane protein with at least the CoA-binding domain on the cytosolic face [13].

2.2 Carnitine palmitoyltransferases (CPTs) and the acylcarnitine-carnitine translocase

Although the CPT system for the entry of acyl moieties into the mitochondrion has been known since the 1960s, until recent years the enzymology of the system has been controversial. Essentially, the controversy was over: (a) whether the outer CPT (henceforth CPT I) and the inner CPT (henceforth CPT II) were different polypeptides; and (b) whether the malonyl-CoA binding of CPT I was due to the catalytic polypeptide or a separate subunit. Now, hepatic CPT I has been purified and immunologically characterized as distinct from CPT II [14] and has been cloned and sequenced, allowing expression in yeast and demonstration of malonyl-CoA binding by the catalytic polypeptide [15]. An isoform of CPT I immunologically distinct from that in liver is present in skeletal muscle, heart and adipose tissue

Abbreviations used: CPT I, outer carnitine palmitoyl transferase; CPT II, inner carnitine palmitoyl transferase; PUFA, polyunsaturated fatty acids; SCAD, short-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase; ETF, electron transfer flavoprotein; ETF:QO, electron transfer flavoprotein:ubiquinone oxidoreductase; hsp, heat-shock protein; ACD, acyl-CoA dehydrogenase; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; SCHOAD, short-chain 3-hydroxyacyl-CoA dehydrogenase; NEFA, non-esterified fatty acids.

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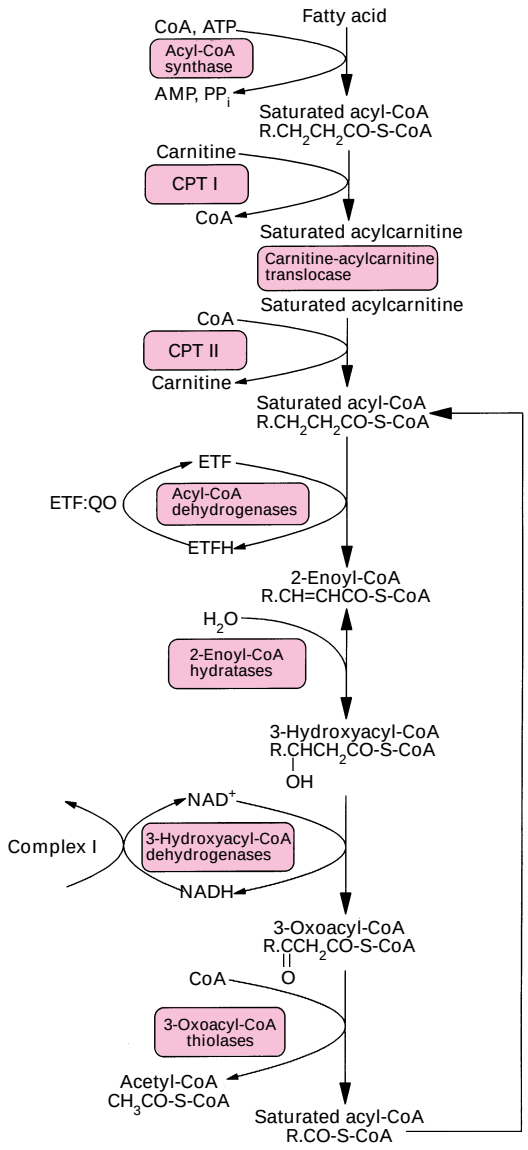

Figure 1 Enzymes of mitochondrial β-oxidation

Abbreviations: CPT, carnitine palmitoyl transferase; ETF, electron transfer flavoprotein; ETF:QO, ETF:ubiquinone oxidoreductase; ETFH, reduced ETF.

[14,16], and Weis et al. have demonstrated, on the basis of inhibitor-binding studies, that two isoforms of CPT I, the liver isoform and the skeletal muscle isoform, are simultaneously expressed in heart tissue [17]. They have subsequently shown, using the differential sensitivity of the two isoforms to DNP-etomoxir [18], that the contribution of the liver form to total heart CPT I activity decreases from 25 % in the neonatal period to 2–3 % in adult rats [19], and they hypothesize that the reason for this is that the markedly different kinetic characteristics of the two isoforms with respect to carnitine and to malonyl-CoA inhibition overcome the low perinatal carnitine levels in the heart.

The carnitine acylcarnitine translocase, which had previously only been partially purified, has been purified and found to catalyse a slow unidirectional transport of carnitine in addition to its translocase activity [20,21].

Table 1 Enzymes of mitochondrial β-oxidation

Enzyme	Structure	Molecular mass (kDa)	Reference
Acyl-CoA synthase	Unknown	78	[223]
Carnitine palmitoyl transferase I (liver)	Unknown	88	[14]
Carnitine palmitoyl transferase I (muscle)	Unknown	82	[17,18]
Carnitine acylcarnitine translocase	Unknown	32.5	[20]
Carnitine palmitoyl transferase II	Unknown	68	[224]
Very-long-chain acyl-CoA dehydrogenase	Homodimer	150	[22]
Long-chain acyl-CoA dehydrogenase	Homotetramer	180	[225]
Medium-chain acyl-CoA dehydrogenase	Homotetramer	180	[226]
Short-chain acyl-CoA dehydrogenase	Homotetramer	168	[227]
Long-chain 3-hydroxyl-CoA dehydrogenase Long-chain 2-enoyl-CoA hydratase Long-chain 3-oxoacyl-CoA thiolase (Trifunctional protein)	Heterooctomer	460	[49]
Short-chain 2-enoyl-CoA hydratase	Homohexamer	164	[32]
Short-chain 3-oxoacyl-CoA thiolase	Homotetramer	169	[228]
General 3-oxoacyl-CoA thiolase	Homotetramer	200	[48]
Electron transfer flavoprotein (ETF)	Heterodimer	57	[227]
ETF:ubiquinone oxidoreductase	Monomer	68	[30]
2,4-Dienoyl-CoA reductase	Homotetramer	124	[229]
Short-chain Δ^3,Δ^2-enoyl-CoA isomerase	Homodimer	70	[230]
Long-chain Δ^3,Δ^2-enoyl-CoA isomerase	Unknown	200	[231]
$\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase	Homotetramer	126	[232]

2.3 Acyl-CoA dehydrogenase (ACD), electron-transfer flavoprotein (ETF) and ETF:ubiquinone oxidoreductase

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 (SCAD, active with C_4 and C_6), medium-chain acyl-CoA dehydrogenase (MCAD, active with 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}). Each of these enzymes catalyses the formation of 2-enoyl-CoA from the corresponding saturated ester. SCAD, MCAD and LCAD are homotetramers located in the matrix. VLCAD, however, is a homodimer and is located in the inner mitochondrial membrane. Until recently it had been assumed that there are only three ACDs involved in mitochondrial β-oxidation; SCAD, MCAD and LCAD. However, the isolation and purification of VLCAD [22], and the demonstration that patients previously thought to have inherited deficiencies of LCAD were in fact suffering from VLCAD deficiency [23], has shown that there are in fact four enzymes. Recently a novel member of this family has been identified by expression of a cDNA with an open reading frame of 1.3 kb encoding a prercursor polypeptide of 431 amino acids, which was processed to a mature protein of 399 amino acids [24]. This enzyme had 38 % homology with SCAD and lesser homology with other members of the ACD family. The enzyme is active towards branched and straight short-chain substrates. The role of this enzyme in β-oxidation remains unclear. MCAD is the best characterized of the ACD family, and the structure of the protein at 3 Å resolution, a dimer of dimers, was reported some years ago [25]. The mechanism of action of this group of flavoproteins appears to be very similar, with the concerted removal of the pro-*R*-α-hydrogen from the acyl-CoA as a proton and elimination of the corresponding pro-*R*-β-hydrogen to the N-5 position of the flavin as a hydride equivalent [26].

Reoxidation of the FAD prosthetic group of the ACDs requires a matrix FAD-linked protein, the ETF ([27]), which in turn

passes reducing equivalents to ETF:ubiquinone oxidoreductase (ETF:QO [28]) and thence to the mitochondrial respiratory chain at the level of ubiquinone. ETF contains 1 mol of bound FAD per mol of dimer [29], and EFT:QO is a 68 kDa iron–sulphur flavoprotein of the inner membrane [28,30].

2.4 2-Enoyl-CoA hydratase

Similarly it appears that there are three 2-enoyl-CoA hydratases. One, a soluble matrix enzyme, is most active towards short-chain substrates, although it will act on substrates up to C_{16} at a much slower rate and with higher K_m values [31–33]. This was the first enzyme of mammalian mitochondrial β-oxidation to be purified (crotonase; short-chain-enoyl-CoA hydratase, EC 4.2.1.17 [34]). The long-chain enzyme (EC 4.2.1.74) is most active with C_6 to C_{10} substrates and virtually inactive with crotonyl-CoA (C_4), the preferred substrate of crotonase [35,36]. It is now apparent that the long-chain enzyme is in fact a constituent of the trifunctional enzyme described below. Studies of patients with inherited disorders suggested the existence of a third, medium-chain enzyme [37], and this activity has now been partially characterized from pig and human liver [38].

2.5 3-Hydroxyacyl-CoA dehydrogenase

The third step of the pathway, L-3-hydroxyacyl-CoA dehydrogenation, is catalysed by two enzymes with overlapping chain-length specificities. The short-chain enzyme is a soluble matrix enzyme which will act on substrates of chain-length C_4 to C_{16} although, as with crotonase, the shorter chain-length substrates are preferred [39–42]. A long-chain 3-hydroxyacyl-CoA dehydrogenase was first demonstrated by El-Fakhri and Middleton [43]. This enzyme is firmly associated with the inner mitochondrial membrane and is active with medium- and long-chain substrates, C_{16} being the preferred substrate. As with the long-chain-enoyl-CoA hydratase described above, the long-chain 3-hydroxyacyl-CoA dehydrogenase is a constituent of the trifunctional protein.

2.6 3-Oxoacyl-CoA thiolase

The final step of the pathway, thiolytic cleavage of 3-oxoacyl-CoA to yield acetyl-CoA and a chain-shortened intermediate, is catalysed by three enzymes. Two soluble activities have been identified. One is specific for acetoacetyl-CoA and 2-methylacetoacetyl-CoA (EC 2.3.1.9 [44–46]). The second thiolase, the 'general' thiolase, is active with all substrates from C_6 to C_{16} to an approximately equal extent [47,48]. The third activity is part of a newly described trifunctional enzyme which also comprises the long-chain 2-enoyl-CoA hydratase and long-chain 3-hydroxyacyl-CoA dehydrogenase activities described above.

2.7 Trifunctional protein

The trifunctional protein complex, a hetero-octomer made up of four α-units with long-chain-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities and 4 β-units with long-chain 3-oxothiolase activity, is closely associated with the inner mitochondrial membrane, and was described by Uchida et al. [49] and rapidly confirmed by others [50,51]. Evidence for the existence of such a complex was first suggested from our studies of a child with an inherited disorder of β-oxidation. Analysis of acyl-CoA and acyl-carnitine esters derived from incubations of mitochondrial fractions with [U-^{14}C]hexadecanoate revealed the presence of 3-oxo-, 3-hydroxy- and 2-enoyl- derivatives. Subsequent enzyme measurement demonstrated a total absence of long-chain 3-oxoacyl-CoA thiolase activity and markedly diminished long-chain enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities [37]. The α-unit is required for membrane binding and a mutant trifunctional protein with an absent α-unit resulted in mislocation of β-unit 3-oxoacyl-CoA thiolase activity in the matrix [52].

A complex of activities associated with CPT II has also been reported by Kerner and Bieber [53]. However, in that study the constituent activities were not characterized with any precision, and it is difficult to know if Bieber's complex also involved the trifunctional enzyme. In any event CPT II, VLCAD and the trifunctional protein are associated with the inner mitochondrial membrane together with Complex I, ETF:QO and the remainder of the respiratory chain. The implications of this arrangement are discussed below in relation to intra-mitochondrial control of β-oxidation.

2.8 Auxiliary enzymes of PUFA oxidation

PUFA are probably β-oxidized at a low rate due to the low activity of CPT towards PUFA-CoA [54]. Complete mitochondrial oxidation is important, as PUFA-CoA can act as inhibitors of β-oxidation [55]. PUFA present two main problems to the classical enzymes of β-oxidation. The first of these is that many PUFA contain *cis* double bonds at even-numbered carbon atoms. After chain shortening, a *cis*-2 double-bonded fatty acid is formed. This can be hydrated, but yields a D-3-hydroxyacyl-CoA ester which is not a substrate for the L-specific 3-hydroxyacyl-CoA dehydrogenases, and would require epimerization to the L-form before further oxidation. However, it is now generally accepted that mitochondrial oxidation of PUFA proceeds via Δ^2,Δ^4-dienoyl-CoA reduction to 3-enoyl-CoA, followed by the action of Δ^3,Δ^2-enoyl-CoA isomerase, rather than the epimerase-dependent route [56]. The second problem is the presence of *cis*-5 double bonds in PUFA, which will eventually yield 2-*trans*,5-*cis*-dienoyl-CoA. These could be oxidized by chain shortening (to 3-enoyl-CoA), followed by the action of Δ^3,Δ^2-enoyl-CoA isomerase and complete oxidation. However, a further enzyme, $\Delta^{3,5}$,$\Delta^{2,4}$-dienoyl-CoA isomerase, has been purified. This alternative route involves isomerization of 2-*trans*,5-*cis*-dienoyl-CoA to Δ^3-,Δ^5–dienoyl-CoA (by Δ^3,Δ^2-enoyl-CoA isomerase) and a further isomerization to $\Delta^{2,4}$-dienoyl-CoA by the novel enzyme, followed be the Δ^2,Δ^4-dienoyl-CoA reductase-dependent route [57,58]. This appears to be the major route operative in intact mitochondria [59].

3 INHERITED DISORDERS OF MITOCHONDRIAL β-OXIDATION

No attempt is made to review all known cases of β-oxidation disorders in the literature. The interested reader is referred to specialized reviews for a comprehensive survey of the literature [60–65].

3.1 CPT and carnitine deficiency

CPT deficiency with predominantly muscle involvement usually presents in adolescence or adulthood, and usually in males, although the inheritance is autosomal [66]. Recurrent myoglobinuria and rhabdomyolysis (muscle breakdown) induced by exercise and fasting are common presenting features. The defect is specifically of CPT II [67], and very little immunoreactive CPT II was found in the patient concerned or in the series of patients reported on by Demaugre et al. [68].

However, CPT II deficiency presenting in infancy with hypoketonaemia, hypoglycaemia and cardiomyopathy has also been described [69]. The authors postulate that in the previous cases of

CPT II deficiency, the residual activity (25 %) is sufficient for significant activity of hepatic, but not muscle, β-oxidation, whereas the profound deficiency in their patient (activity 10 % of controls) resulted in hepatic involvement. However, in other CPT II patients with profound deficiency, there was no liver involvement (e.g. [67]). In addition there are problems of interpretation, due in part to differences in assay methods [67] and in part to the occurrence of kinetic mutants [70], which makes the comparison of data from assays carried out at different substrate concentrations problematic. The human CPT II gene has been assigned to chromosome 1p32 and spans approx. 20 kb comprising five exons of 81–1305 bp. A series of studies has elucidated the molecular characteristics of this disorder, and a common S113L mutation appears to be the most frequent, but by no means the only, disease-causing mutation [71,72].

The hepatic/infantile phenotype, first described by Bougnères et al. [73], presents with fasting hypoglycaemia and hypoketonaemia in the presence of normal insulin levels. CPT I activity is absent in liver and fibroblasts, although not in muscle [74].

Only a few cases of deficiency of the carnitine/acylcarnitine translocase have been reported. The first, a boy, presented with fasting hypoketotic hypoglycaemia, muscle weakness and cardiomyopathy. Carnitine was highly esterified in both fed and fasted states [75]. A further four cases have now been described [76–78].

Some patients were originally characterized as having lipid storage myopathies associated with carnitine deficiency; however, it is now apparent that the majority of these cases are primary β-oxidation disorders with secondary carnitine deficiency. Several inherited and acquired disorders are known to cause carnitine deficiency: inborn errors of β-oxidation, of branched-chain amino acid catabolism and of the respiratory chain, alcoholic cirrhosis, valproate therapy and extended haemodialysis have all been reported to cause carnitine deficiency [79].

Primary carnitine deficiency is rare, the only well-characterized defect is of cellular carnitine uptake [80,81]. These patients present in infancy or childhood with muscle weakness and cardiomyopathy, and diagnosis is made by measurement of carnitine transport into fibroblasts. The usual lack of liver involvement could suggest a different transport mechanism in liver, or reflect increased endogenous synthesis.

3.2 VLCAD/LCAD deficiency

VLCAD/LCAD deficiency was first described by Hale et al. [82]. The defect often has a severe clinical presentation with non-ketotic hypoglycaemia, muscle weakness and hepato- and cardio-megaly; six patients died early in infancy although others had a milder clinical course [83], which taken with the biochemical evidence [84] suggests heterogeneity of the molecular defect.

Studies by Indo et al. [85] show that normal immunoreactive protein is present in most cases studied, suggesting a point mutation(s) to be the cause of the defect. However, further studies revealed normal LCAD cDNA in several patients with apparent LCAD deficiency, i.e. patients with greatly reduced activity with respect to hexadecanoyl-CoA. Immunoblot analysis of VLCAD in fibroblasts from these patients revealed absent protein, and it is now apparent that most, if not all, of the cases previously characterized as having a deficiency of LCAD, were in fact deficient in VLCAD [23,86].

3.3 MCAD deficiency

MCAD deficiency is probably the most common β-oxidation defect, with over 100 cases reported since the original patient of Kølvraa et al. [87]. Illness is often precipitated by prolonged fasting or illness, resulting in non-ketotic hypoglycaemia, elevated plasma non-esterified fatty acids (NEFA) and a spectrum of abnormal urinary and plasma metabolites, including dicarboxylic acids (DC_6–DC_{10}), suberylglycine, hexanoylglycine and medium-chain acylcarnitines (see [88,89] and the literature cited therein; [90]), with secondarily low tissue acylcarnitines. *cis*-4-Decenoic acid (a linoleic acid metabolite) has been reported to be a specific plasma metabolite present during remission [91], as have plasma octanoate [92] and plasma acyl-carnitines [93]. A number of other diagnostic procedures have been described, for example analysis of [^{14}C]acyl-CoA esters and [^{14}C]acylcarnitines generated by incubation of tissue preparations with [U-^{14}C]hexadecanoate (Figure 2), allowing the identification of several disorders of mitochondrial β-oxidation. The use of tandem MS to detect the presence of characteristic pathognomic acylcarnitines in body fluids has the advantage of being applicable to whole population screening. This is an important consideration, since MCAD deficiency is readily treated by the avoidance of fasting.

Much effort has been devoted to the study of the molecular basis of the deficiency, aided by the recognition of a common point-mutation at position 985 of the MCAD cDNA [94], which is responsible for up to 90 % of patients with MCAD deficiency. This mutation only appears in Caucasians [95], and this finding, together with the strong association of this point mutation with a particular intron haplotype [96], suggests that a mutational hot-spot at 985 is unlikely and a founder effect is more probable. Many other mutations have been identified e.g. [97,98].

The common mutation causing MCAD deficiency results in the substitution of glutamate for lysine at position 304 (K304E), and the mature protein is not detectable in tissues from affected patients. It has been suggested that, since the Lys-304 residue is located in the domain involved in dimer–dimer interaction to form the native homotetramer, the substitution by a glutamate residue in the mutant protein causes a failure of assembly and consequent instability [99–101]. However, more recent studies have suggested that the chaperonin-mediated folding pathway, which involves mitochondrial import of a 421 amino acid precursor, cleavage of a 25 amino acid leader sequence, formation of a transient complex with mitochondrial heat-shock protein 70 ($hsp70_{mit}$) followed by transfer of the polypeptide to $hsp60_{mit}$ and final assembly of the catalytically active homotetramer, is impaired [102]. Specifically, the presence of the lysine to glutamate substitution results in the formation of a K304E–$hsp60_{mit}$ complex, which is more stable than the corresponding wild-type complex, with consequent attenuation of tetramer formation. Similarly Bross et al. [103], using an *Escherichia coli* expression system, have demonstrated that chaperonin-mediated folding is affected, but also suggest that oligomer assembly and stability are involved.

The prevalence of MCAD has been estimated at 1 in 18 500 by Blakemore et al. [104] for G985 homozygotes in England, and Matsubara et al. [95] have reported an even higher figure (1 in 6400). This is of the same order as phenylketonuria and suggests that neonatal screening should be considered from Guthrie spots, either using the PCR technique (which would only detect 85–90 % of affected individuals) or by detection of specific remission metabolites, for instance by tandem MS of carnitine esters.

3.4 SCAD deficiency

SCAD deficiency is a rare defect, only having been described in a few patients since the first report by Turnbull et al. [105]; some

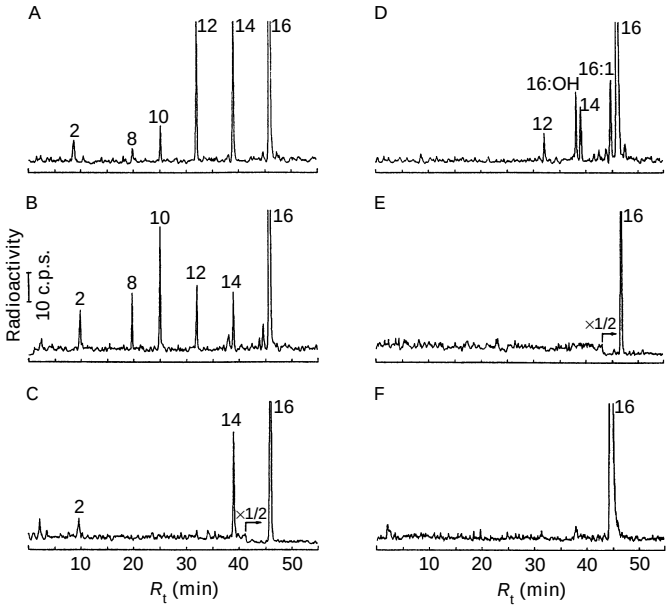

Figure 2 Radio-HPLC chromatogram of acyl-CoA esters generated from incubation of [U-^{14}C]hexadecanoate with fibroblasts from patients with inherited disorders of mitochondrial β-oxidation

(**A**) Control; (**B**) MCAD deficiency; (**C**) VLCAD deficiency; (**D**) trifunctional enzyme deficiency; (**E**) CPT II deficiency; (**F**) CPT I deficiency. Peak identification: carbon numbers are as indicated; 2,3-enoyl-CoA esters are indicated by the suffix 1; and 3-hydroxyacyl-CoA esters are indicated by the suffix OH. Taken from [221] with permission.

of the cases described may be riboflavin-responsive multiple defects. There seem to be two forms: first, a severe infantile systemic form [106,107]; and secondly, a mild, late-onset phenotype with predominantly muscle involvement [105,108]. The disorder is not expressed in the fibroblasts of patients with the muscle phenotype but is in the infantile variant [109].

The heterogeneity of the described cases has been confirmed by immunochemical studies: the first myopathic case had no immunoreactive protein in muscle [109], whereas in the three other cases, normal sized mRNA was synthesized and immunoreactive protein was present in two of the three cases [110]. In the patient with no immunodetectable protein, a heterogeneous molecular lesion was found: one allele had a point mutation at 319 bp (corresponding to a strongly conserved residue in the ACD family) and the other had a point mutation at 136 bp [111]. Recently Bhala et al. [112] have characterized the defect in fibroblasts from six patients and demonstrated marked heterogeneity in the degree of enzyme attenuation. In one patient (case 2) there was 40% residual activity with little abnormal metabolite excretion; indeed the criteria for deciding what is a deficiency and what is not can sometimes be difficult to delineate. A mouse (BALB/cByJ) model for SCAD deficiency, due to a 278 bp deletion which resulted in a mis-spliced mRNA, has been described [113].

3.5 ETF and ETF:QO deficiencies

Deficiency in either ETF or ETF:QO is termed glutaric aciduria type II [114] and was also known as multiple acyl-CoA dehydrogenation deficiency until recognition of the primary defect(s) by Christensen et al. [115] and Frerman and Goodman [116]. The activities of all dehydrogenases served by ETF and ETF:QO are impaired [117].

Defects have been recognized in three groups of patients: a fatal neonatal form associated with congenital abnormalities and cardiomyopathy [118]; an infantile or childhood form associated with episodic hypoglycaemia, metabolic acidosis and hepatomegaly [119]; and a late onset form with marked muscle involvement [120]. The molecular lesion is heterogeneous. In ETF-defective patients Ikeda et al. [121] demonstrated α-ETF to be deficient, Loehr et al. [119] have shown β-ETF to be deficient, and Yamaguchi et al. [122] have demonstrated a defect in β-ETF biosynthesis and another defect in which both α- and β-ETF were very labile. ETF:QO patients may have normal, low or absent immunoreactive protein [119].

3.6 Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (trifunctional protein deficiency)

This disorder is probably the second most common β-oxidation disorder and many cases have been described ([37,123–125] and citations therein). The age at onset of symptoms ranges from 3 days to 3 years, and clinical presentations include recurrent episodes of non-ketotic hypoglycaemia, sudden infant death and cardiomyopathy. In most cases hepatic dysfunction is a prominent feature and muscle weakness is also a common finding. The disorder was fatal in several cases although successful treatment with medium-chain triacylglycerols has been reported. The cases described by Jackson et al. [37,123] provide evidence of autosomally recessive inheritance, and in addition, long-chain enoyl-CoA hydratase and thiolase activities were diminished. It now seems certain that cases previously identified as LCHAD deficiency are in fact disorders of the trifunctional protein [37]. This only becomes apparent if enzymes are assayed with the physiologically relevant substrates or if the functional integrity of the pathway is assessed by direct measurement of the intermediates of the pathway. However, detailed studies of the molecular basis of this disorder have demonstrated the presence of a common 1528 G → C mutation, which results in a glutamate to glutamine substitution at amino acid 510 [126,127] and loss of 3-hydroxyacyl-CoA dehydrogenase activity [128]. A rather unusual finding is the association of acute fatty liver of pregnancy in mothers who are obligate heterozygotes for LCHAD deficiency [126].

3.7 Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHOAD) deficiency

SCHOAD deficiency has been described in two cases, one of them only in abstract form. The patient of Hale et al. [129] presented at the age of 9 months with a recurrent Reye-like illness and a dicarboxylic aciduria. The defect was expressed in fibroblasts. The patient reported by Tein et al. [130] presented at 16 years with hypoketotic hypoglycaemic encephalopathy, myoglobinuria and cardiomyopathy and excreted small amounts of dicarboxylic acids and a trace of hydroxydodecanedioic acid. The defect was expressed in muscle and presumably liver, but not in fibroblasts.

4 REGULATION, CONTROL AND ORGANIZATION OF MITOCHONDRIAL β-OXIDATION

4.1 Physiological regulation of mitochondrial β-oxidation

The physiological (extrinsic) regulation of mitochondrial β-oxidation depends, at least in part, on the organ in question. The

liver is capable of high rates of β-oxidation and ketogenesis, or of lipogenesis and esterification of fatty acids. The regulation of carbon flux is therefore very important so that substrate cycling does not take place. Under fed conditions, circulating glucose levels are high, NEFA levels are low, and CPT I is inhibited by high levels of malonyl-CoA. Hence, carbon flux in the liver is from glucose to *de novo* lipogenesis via citrate and malonyl-CoA. This is shown in Figure 3. During starvation, circulating levels of NEFA rise due to the action of adipose tissue triacylglycerol lipase in response to a raised [glucagon]/[insulin] ratio. Hepatic malonyl-CoA levels are lowered, due both to slower efflux of citrate from the mitochondrion and to the phosphorylation and consequent inactivation of acetyl-CoA carboxylase by AMP-dependent protein kinase, again in response to a raised [glucagon]/[insulin] ratio [131]. Hence β-oxidation and ketogenesis are activated and a rise in ketone body levels is observed. The rise in plasma NEFA levels and ketone body levels as glucose availability falls during starvation is diagnostically very useful for β-oxidation disorders; an inappropriate ketone body/NEFA ratio is suggestive of an inborn error of hepatic β-oxidation or of ketogenesis [132]. Further detailed consideration of the regulation of hepatic fatty acid metabolism is outside the scope of this review and the reader is directed to other recent reviews [133,134]. We will consider below, however, the effects that take place at the level of CPT I or intramitochondrially.

In extra-hepatic tissues in which there is no active lipogenesis, such as heart and skeletal muscle, β-oxidation serves to provide contractile energy. In these tissues, the rate of β-oxidation has been described as demand led, in that an increased work rate and ATP demand leads to faster oxidative phosphorylation and tricarboxylic acid cycle activity. NADH and acetyl-CoA levels diminish, thus increasing β-oxidation flux [135,136]. However, a role for malonyl-CoA inhibition of muscle and heart CPT I in control of β-oxidation flux has been postulated, and this is discussed below. Again the interested reader is referred to recent reviews for a more thorough discussion of the regulation of fatty-acid metabolism in the heart and skeletal muscle [137,138].

4.2 Hepatic regulation of mitochondrial β-oxidation at the level of CPT I

Much of the control and regulation of the rate of hepatic mitochondrial β-oxidation appears to reside at the level of the entry of acyl groups into the mitochondrion. Inhibition of CPT I by malonyl-CoA in the fed state was first demonstrated by McGarry and Foster [139], and has been shown to have significant control over β-oxidation flux in isolated mitochondria [140] and in intact hepatocytes under different metabolic conditions [141].

Several factors in addition to the cytosolic concentration of malonyl-CoA affect CPT I activity in the hepatocyte.

(i) The sensitivity of CPT I inhibition to malonyl-CoA itself alters [142,143] over a relatively long time-scale [144]. This may be due to a change in membrane fluidity rather than an attenuation in numbers of malonyl-CoA binding sites [145]. Phosphatidylglycerol or cardiolipin are similar in their effect to cholate extracts of mitochondria with respect to their ability to

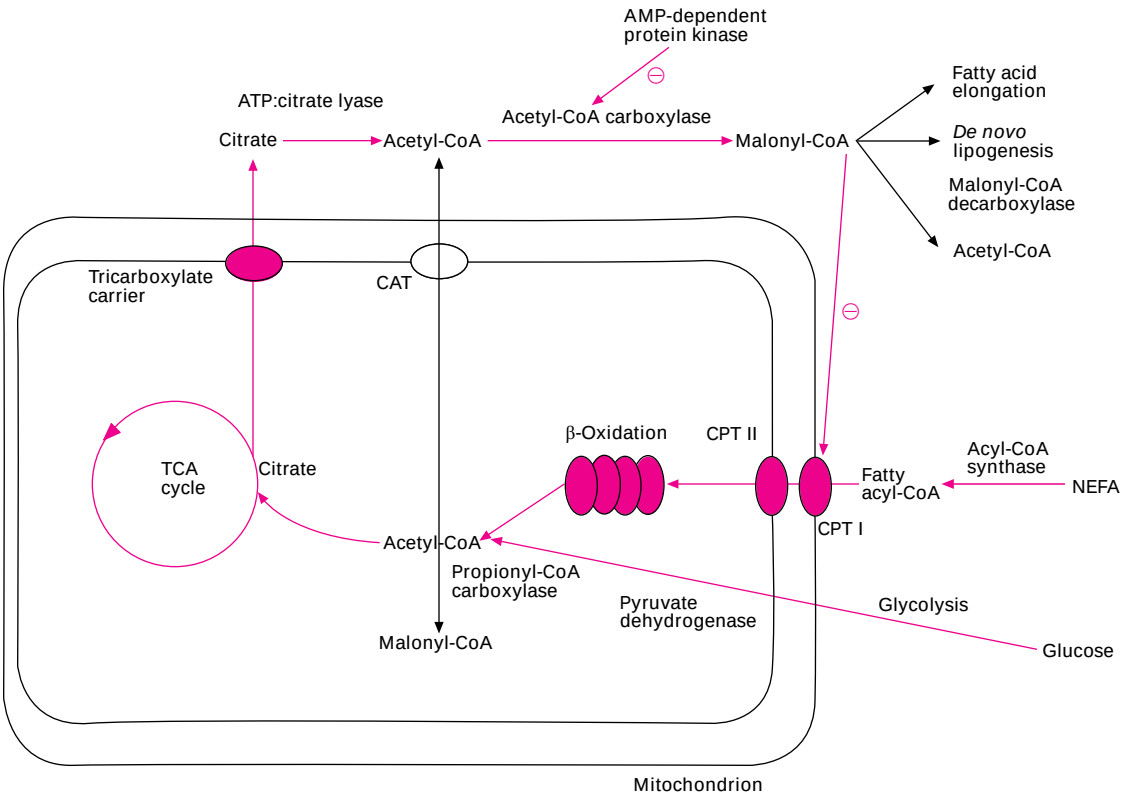

Figure 3 Modulation of CPT I activity by carbohydrates

The pathway shown in red is the established pathway for the inhibition of hepatic CPT activity by carbohydrates. The other pathways shown are alternative routes that may be operative in heart (see text). Abbreviations: CPT, carnitine palmitoyl transferase; CAT, carnitine acetyl transferase.

increase malonyl-CoA sensitivity of CPT I [146] and may account for the previous observations implying a separate malonyl-CoA binding subunit of CPT I [147].

(ii) The affinity of CPT I for its acyl-CoA substrate can alter under ketogenic conditions [148,149], although not directly in response to changes in the insulin/glucagon ratio [144].

(iii) Phosphorylation has been suggested to modulate CPT I activity. Although Harano et al. [150] showed that phosphorylation of CPT in hepatocytes in response to glucagon and forskolin increased its activity, anti-CPT II antibodies were used in this study and total CPT activity was measured. Okadaic acid, the protein phosphatase 1 and 2A inhibitor, was found to increase CPT I activity and palmitate oxidation in hepatocytes, implying phosphorylation of CPT I [151,152]. However, it has been subsequently shown that the okadaic acid-induced rise in CPT I activity is unlikely to be due to phosphorylation of CPT I, as it could not be antagonized by exogenous phosphatases 1 and 2A, was not modulated by fluoride ions and did not lead to labelling of CPT I with [^{32}P]ATP [153].

(iv) Changes in hepatocyte cell volume [154] or cytosolic pH [155] may be responsible for modulation of CPT I activity, as the effect of okadaic acid on CPT I activity is dependent on cell volume, and the sensitivity of CPT I to malonyl-CoA is strongly pH dependent [156]. All these effects may be mediated through the cytoskeleton [157], which would explain the sensitivity to the method of hepatocyte permeablization used [153].

(v) Longer term changes in CPT I activity can be brought about by changes in the expression of CPT I: insulin has been shown to inhibit transcription of CPT I mRNA [158].

4.3 Regulation of β-oxidation by modulation of CPT I activity in extrahepatic tissues

The role of malonyl-CoA in the modulation of CPT I activity in extra-mitochondrial tissues is still controversial. In the heart and skeletal muscle, β-oxidation flux is often thought of as being demand led [135,136]. However, in extrahepatic tissues, CPT I is very much more sensitive to malonyl-CoA than in the liver [159–161], and the measured levels of malonyl-CoA [161,162] would suggest that CPT I activity is almost completely inhibited in the intact tissue. However, much of the malonyl-CoA measured in heart may be intramitochondrial, due to the action of propionyl-CoA carboxylase on acetyl-CoA [163,164]. It is also possible that binding of extra-mitochondrial malonyl-CoA to mitochondrial low-affinity sites [165] or to cytosolic binding proteins, such as that described in liver [166], would also prevent malonyl-CoA from inhibiting CPT I. In order to inhibit CPT I under physiological conditions, malonyl-CoA must be formed in response to carbohydrate feeding. In liver, citrate efflux from mitochondria oxidizing carbohydrate is followed by the actions of ATP:citrate lyase and acetyl-CoA carboxylase to form malonyl-CoA, which inhibits CPT I and thus mitochondrial β-oxidation, and is itself the substrate for fatty acid synthase [139]. Muscle and heart obtain 75% of their energy requirements from fatty acids [1,2], but β-oxidation can still be suppressed to a certain extent by carbohydrate [167]. Involvement of inhibition of CPT I activity during oxidation of lactate has been inferred from the measurement of long-chain acylcarnitine levels in the perfused heart [168], and a role for regulation of β-oxidation flux by modulation of CPT I activity is suggested by the finding that AMP-dependent protein kinase phosphorylates and inhibits acetyl-CoA carboxylase during increased workload in skeletal muscle [169] and the heart [170], as it does in the liver in response to changes in glucagon/insulin ratios. However, in muscle and heart, there is only a slow efflux of citrate from mitochondria [171], although there is ATP:citrate lyase activity [172] and acetyl-CoA carboxylase activity [173,174] from a different isoform (280 kDa) than that predominant in liver (265 kDa). There is no obvious removal route for malonyl-CoA, as fatty acid synthase is absent from heart; however, Awan and Saggerson have demonstrated a fatty-acid elongation system which utilizes malonyl-CoA [172], and it has been suggested that an extra-mitochondrial malonyl-CoA decarboxylase may be responsible for disposal of malonyl-CoA [175]. Lopaschuk and co-workers suggest that a high mitochondrial efflux of acetylcarnitine during pyruvate oxidation [176] and carnitine acetyl transferase activity provide the source of extra-mitochondrial acetyl-CoA [177]. An extra-mitochondrial carnitine acetyl transferase could not, however, be detected in a variety of tissues [178], although a transient association of carnitine acetyl transferase with mitochondrial contact sites has recently been demonstrated histochemically [179].

Strong correlations between acetyl-CoA and malonyl-CoA concentrations and between malonyl-CoA concentrations and the β-oxidation rate have been found in the intact heart perfused with varying concentrations of dichloroacetate to provide a range of pyruvate dehydrogenase activities [180]. However, since the subcellular localizations of the measured acetyl-CoA and malonyl-CoA are unknown, these results could equally well fit with the model of the transfer of citrate across the membrane and the subsequent action of ATP:citrate lyase to form acetyl-CoA; a slow transfer of citrate across the mitochondrial membrane [171] could well provide enough malonyl-CoA for inhibition of CPT I activity during pyruvate oxidation. These alternative possible routes for inhibition of CPT I activity are shown in Figure 3.

4.4 Intramitochondrial control over β-oxidation

Although it appears that much of the control of mitochondrial β-oxidation flux resides in CPT I, intramitochondrial controls may be important physiologically. As mitochondrial β-oxidation consists of several enzymes of overlapping chain-length-specificities, some of which are membrane bound (the trifunctional protein and the VLCAD) and transfer reducing equivalents to the respiratory chain, the possibility of supramolecular organization of the β-oxidation enzymes and auxillary systems should be considered. The ACDs appear to have by far the lowest activity of the enzymes of β-oxidation in rat and human tissues [37,123,181,182]. However, comparison of isolated enzyme activities may be misleading, since assays of the enzymes of β-oxidation are carried out under non-physiological conditions: for example, 3-hydroxacyl-CoA dehydrogenase is measured in the reverse direction, and ACDs are measured with artificial electron acceptors with which they may have very much lower turnover numbers than with the physiological acceptor ETF. In the absence of specific inhibitors, it is difficult to determine the control strengths of the enzymes of β-oxidation, although a preliminary analysis has been performed [183]. Another method is selective overexpression of the enzymes of β-oxidation and determination of changes in flux. This has been carried out for VLCAD, in which VLCAD cDNA was introduced into three rat hepatoma cell lines. In two of the three cell lines, β-oxidation flux was increased 3-fold, although the amount of enzyme activity in the transfected cell lines was not measured [184].

4.4.1 Control by feedback inhibition and the acylation state

The ACDs have a high affinity both for their acyl-CoA substrates and for their enoyl-CoA products, resulting in product inhibition

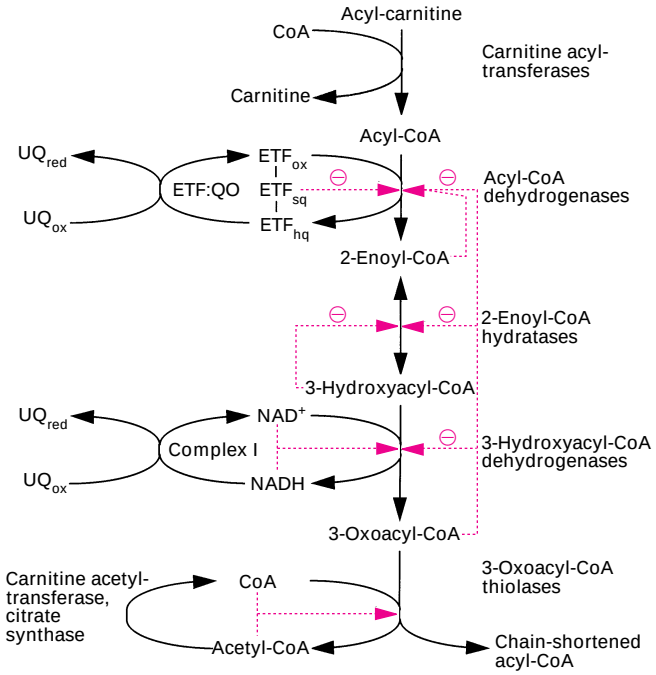

Figure 4 Mitochondrial β-oxidation showing the sites of intra-mitochondrial control in red

Abbreviations: UQ_{red}, reduced ubiquinone; Uq_{ox}, oxidized ubiquinone; ETF_{ox}, oxidized ETF; ETF_{sq}, ETF semiquinone; ETF_{hq}, reduced ETF; Complex I, NADH:ubiquinone oxidoreductase.

[185,186]. In addition, short-, medium- and long-chain ACDs are inhibited by 3-oxoacyl-CoA esters [187]. Similarly, crotonase is strongly inhibited by acetoacetyl-CoA [33], and although long-chain enoyl-CoA hydratase activity is not inhibited by acetoacetyl-CoA [36] it is not known whether it is inhibited by long-chain 3-oxoacyl-CoA esters. 3-Hydroxyacyl-CoA dehydrogenases are subject to product inhibition by 3-oxoacyl-CoA esters [42,187], and the enoyl-CoA hydratase reaction is similarly inhibited by its 3-hydroxyacyl-CoA products [188]. Hence inhibition of 3-hydroxyacyl-CoA dehydrogenase activity would lead to inhibition of 2-enoyl-CoA hydratase activity, so that accumulation of 3-oxoacyl-CoA esters within the mitochondrion would inhibit each of the individual reactions both directly and by a feedback mechanism (see Figure 4) and so be highly inhibitory to β-oxidation. The general 3-oxoacyl-CoA thiolase is inhibited by acetyl-CoA [189], so that were disposal of acetyl-CoA to ketogenesis, to the tricarboxylic acid cycle or to acetylcarnitine inhibited, feedback inhibition of β-oxidation would result. This has been suggested to be of possible regulatory importance [190], and the observation that whereas 3-oxoacyl-CoA esters are readily observed as intermediates of peroxisomal β-oxidation [191], we have never observed accumulation of 3-oxoacyl-CoA esters in mitochondrial incubations [192,193], would suggest that the accumulation of 3-oxoacyl-CoA esters is strongly prevented and that an excess of thiolase activity 'pulls' β-oxidation, as the 3-oxoacyl-CoA thiolases are not inhibited by their acyl-CoA products. It does appear that β-oxidation can be partly controlled by disposal of acetyl-CoA to ketogenesis [194], and this may be important in regulation in the immediate postnatal period in allowing ketogenesis to take place in response to raised glucagon levels, which de-succinylate and stimulate 3-hydroxymethylglutaryl-CoA synthase [195]. Connected with the inhibition of 3-oxoacyl-CoA thiolase by acetyl-CoA is the potential control of β-oxidation by the acylation state of mitochondrial CoA. As the mitochondrial CoA pool is limited, depletion of free CoA will inhibit both CPT II and the 3-oxoacyl-CoA thiolase. Other intramitochondrial enzymes dependent on unesterified CoA will also be inhibited. As these include pyruvate dehydrogenase, the branched-chain oxo-acid dehydrogenase and 2-oxoglutarate dehydrogenase, the complete acylation of the mitochondrial CoA pool would result in the breakdown of mitochondrial oxidative metabolism. Garland et al. [196] found that 95% of intramitochondrial CoA was acylated during maximal β-oxidation flux, so that only a small amount of free CoA can sustain β-oxidation. The effect, observed by Wang et al. [190], of the acetyl-CoA/CoA ratio on β-oxidation flux could be due to complete acylation. Some inhibitors of β-oxidation have been postulated to act by sequestration of intramitochondrial CoA [197,198].

4.4.2 Control by the respiratory chain

β-Oxidation is linked to the respiratory chain at two stages, that of the 3-hydroxyacyl-CoA dehydrogenase to complex I via NAD^+/NADH, and the ACDs to ubiquinone via ETF and its oxidoreductase. Inhibition of either of these stages leads to inhibition of β-oxidation [199,200]. ETF-semiquinone, which is the partially reduced form of ETF, can accumulate when the ubiquinone pool is reduced [201] and is a potent inhibitor of ACD [202]. However, ETF-semiquinone disproportionates to the fully oxidized and fully reduced forms in a reaction catalysed by ETF:QO [30], so that the levels of the various ETF species occurring in intact mitochondria are unknown. Hence the activity of the ACDs could be responsive to the redox state of the ubiquinone pool either via ETF and ETF-semiquinone or by complex I and accumulation of 3-hydroxyacyl-CoA esters (which would lead to the accumulation of 2-enoyl-CoA esters and inhibition of the ACDs). Work by Kunz [183,203] has suggested control of β-oxidation at the ETF level, and it has been suggested that the ETF reduction state is responsible for changes in β-oxidation flux with osmolality [193,204,205].

4.4.3 Organization of the enzymes of β-oxidation

The supramolecular organization of the enzymes of β-oxidation has been postulated, for which however, there is little direct evidence. Sumegi and Srere demonstrated that purified SCHOAD, crotonase and acetoacetyl-CoA thiolase bound to the inner mitochondrial membrane, although with differing dependencies on ionic strength and pH and with different binding stochiometries [206], and Kispal et al. [207] subsequently isolated a short-chain 3-hydroxyacyl-CoA binding protein of subunits 69 and 71 kDa from the inner mitochondrial membrane. The protein bound SCHOAD with a stoichiometry of 1:1 and increased SCHOAD activity. However, the authors did not examine crotonase or thiolase binding activity. Recently, Furuta and Hashimoto [208] demonstrated that rat liver mitochondrial membranes bound SCHOAD, and they subsequently isolated an SCHOAD binding protein, a homodimer of subunit mass 60 kDa. The protein bound SCHOAD and 3-oxoacyl-CoA thiolase, although it did not bind crotonase or short-, medium- or long-chain ACDs, with a stoichiometry of 1 mol of binding protein:2 mol of enzyme. However, SCHOAD activity was inhibited on binding. The relationship between these two binding proteins and their function in the intact mitochondrion remain to

be established. The β-oxidation enzyme complex isolated from rat heart mitochondria [53] included 3-hydroxyacyl-CoA dehydrogenase, crotonase and acetoacetyl-CoA thiolase activities associated with a 68 kDa protein with CPT activity. This corresponds to the mass of CPT II, but was described as having malonyl-CoA sensitivity. The chain-length dependence of the enzyme activities was not investigated, and it is possible that the complex may have been the subsequently described trifunctional protein [49,50]. Further characterization of this complex is required.

4.4.4 Intermediates of β-oxidation and the question of channelling

A method for deducing the intramitochondrial control of β-oxidation is analysis of the CoA and carnitine ester intermediates. Early studies used radio-GLC of saponified fatty acids and demonstrated that saturated esters were predominant, suggesting the ACDs to have a high control strength over β-oxidation in the intact mitochondrion [209–211]. 3-Hydroxyacyl- and 2-enoyl-moieties were only found during conditions in which the respiratory chain was inhibited, and 3-oxoacyl-CoA esters were not found. However, when radio-HPLC methods for the direct measurement of intact CoA and carnitine esters were established [212,213], 2-enoyl- and 3-hydroxyacyl-CoA esters were observed in incubations of rat skeletal muscle and liver mitochondria with [U-^{14}C]hexadecanoate under State 3 conditions [192,193], but not in human skeletal muscle or fibroblast mitochondria [37,200]. 2-Enoyl- and 3-hydroxyacylcarnitine esters also accumulated, although in lower proportions than in the CoA ester fractions, probably reflecting the lower activity of the CPT system towards 2-enoyl- and 3-hydroxyacyl-CoA esters than towards saturated CoA esters [214,215]. When the amounts of NAD^+ and NADH accumulating during oxidation of hexadecanoate were measured directly, it was found that in rat skeletal muscle mitochondria there was very little reduction of NAD(H), whereas a steady 30% reduction level was reached in rat liver mitochondria. The presence of 3-hydroxyacyl- and 2-enoyl-CoA esters in rat skeletal muscle mitochondria was implied to be either due to a small pool of rapidly turning over NAD^+/NADH [192,193] channelled between the trifunctional protein and complex I, as has been described for other dehydrogenases in contact with complex I [216], or due to high sensitivity of 3-hydroxyacyl-CoA dehydrogenase activity of the trifunctional protein to NADH [217]. The presence of saturated acyl-CoA, 2-enoyl- and 3-hydroxyacyl-CoA esters under State 3 conditions suggests that control over β-oxidation flux is shared between different steps.

The presence or absence of channelling of CoA esters in mitochondrial β-oxidation has not yet been established. It has often been assumed that because of the low concentrations of CoA esters observed by early workers, channelling of CoA esters occurred. However, the role of channelling in reducing pool size is controversial [218,219], and our more recent studies have demonstrated measurable amounts of the CoA ester intermediates of β-oxidation [192,193]. An important observation, originally made by measurement of NEFA [209,210] and subsequently by measurement of intact CoA esters [192,193], is that the amounts of the intermediates of β-oxidation are not in steady state during a pulse of β-oxidation, despite steady-state production of acetyl- units or consumption of oxygen, and do not behave as true 'intermediates'. This led to the 'leaky hosepipe' model, in which there is a small pool of rapidly turning over intermediates and the CoA esters observed are 'leaks' from the main flux of β-oxidation, which although they do not represent the true 'intermediates' must represent points at which some control over β-oxidation flux is exerted.

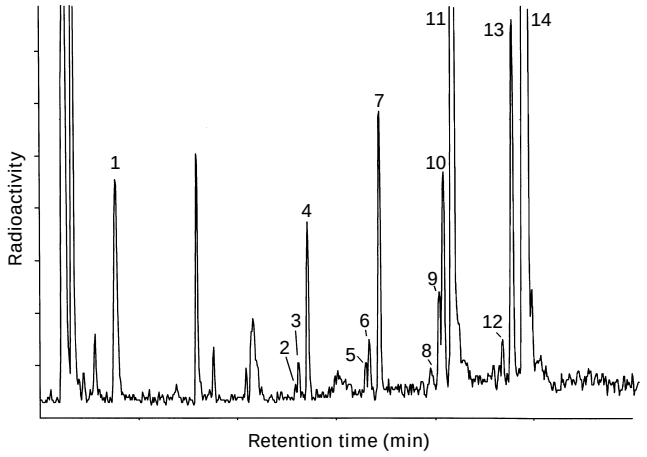

Figure 5 Radio-HPLC chromatograms showing the accumulation of CoA esters from [U-^{14}C]hexadecanoyl-CoA by rat heart mitochondria

Rat heart mitochondria were incubated with 90 μM [U-^{14}C]hexadecanoyl-CoA, and CoA esters were extracted and analysed by radio-HPLC [192]. Peak identification: 1, acetyl-; 2, dec-2-enoyl-; 3, 3-hydroxydodecanoyl-; 4, decanoyl-; 5, dodec-2-enoyl-; 6, 3-hydroxytetradecanoyl-; 7, dodecanoyl-; 8, tetradec-3-enoyl-; 9, tetradec-2-enoyl-; 10, 3-hydroxyhexadecanoyl-; 11, tetradecanoyl-; 12, hexadec-3-enoyl-; 13, hexadec-2-enoyl-; 14, hexadecanoyl-.

It has been suggested that the failure to detect long-chain intermediates of the degradation of [17,17,18,18-$^2H_4$]linoleic acid by cultured skin fibroblasts provides evidence for intermediate channelling in mitochondrial β-oxidation [220]. This hypothesis is appealing since the acyl-carnitine/carnitine translocase, carnitine palmitoyltransferase, VLCAD and trifunctional protein are bound to the inner mitochondrial membrane and would constitute a β-oxidation metabolon. Futhermore, the long-chain acyl-CoA intermediates are amphipathic in nature and it would appear advantageous to avoid having these highly surface-active agents in the mitochondrial-matrix bulk phase. There are other theoretical advantages of substrate channelling within the mitochondrial compartment, such as solvation, archipelago effects and avoidance of non-productive substrate binding, particularly in the context of β-oxidation. Nada et al. [220] carried out incubations of intact cells over a period of 96 h and, in the case of control cell lines, detected labelled butyryl-carnitine, hexanoyl-carnitine, octanoyl-carnitine and decenoyl-carnitine but no acyl-carnitines of longer chain-length. These findings are most probably due to the very prolonged incubation times employed by these workers with consequent substrate depletion or incorporation into lipid, and in our view their findings are of little relevance to the presence or otherwise of substrate channelling. We have shown in a series of papers dealing with the characteristics of β-oxidation in isolated liver, skeletal muscle, fibroblast and cardiac mitochondrial fractions from rat and man, that irrespective of whether acyl-CoA esters or acyl-carnitines are measured, long-chain intermediates are readily detected [192,193,200,221], see Figure 5. Although the detailed distribution of intermediates with respect to chain-length and type (acyl, 2,3-enoyl, 3-hydroxyacyl) is dependent upon tissue, duration of incubation, redox state and respiratory state, in no case have we ever failed to detect long-chain intermediates under conditions where there remained substrate to oxidize. These findings were true of the β-oxidation of dicarboxylates as well as monocarboxylates. Thus a limited form of channelling may exist, as exemplified by the 'leaky hosepipe' model. In the case of acyl-CoA esters it is not possible to distinguish intermediates in

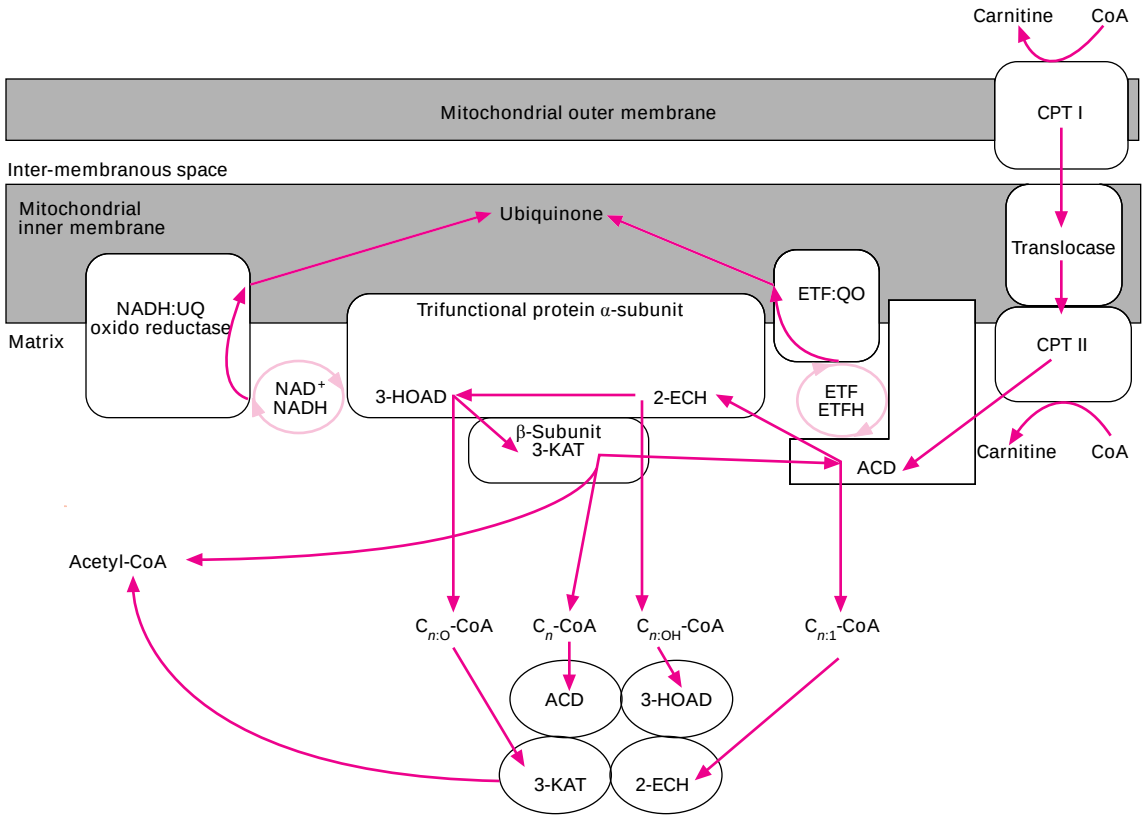

Figure 6 A model for mammalian mitochondrial β-oxidation

C_n refers to number of carbon atoms; 2,3-enoyl-CoA esters are indicated by the subscript 1; 3-hydroxyacyl-CoA esters are indicated by the subscript OH and 3-oxoacyl-CoA esters by the subscript O. Abbreviations: 2-ECH, 2-enoyl-CoA hydratase; 3-HOAD, 3-hydroxyacyl-CoA dehydrogenase, 3-KAT, 3-oxoacyl-CoA thiolase.

the matrix bulk phase from those which might be tightly bound (channelled). In the case of the acylcarnitine fraction, the intermediates seen reflect movement of acyl groups laterally from the pathway and possibly out of the mitochondrial compartment, although this will depend on the specificities of the carnitine acyltransferases and the translocase. The question of channelling in β-oxidation has recently been discussed in more detail [222].

4.5 Model for mitochondrial β-oxidation

Given the recent observations concerning the presence of membrane-bound enzymes of β-oxidation and the close relationship between β-oxidation and the respiratory chain, together with the 'leaky-hosepipe model', we propose the model of mitochondrial β-oxidation illustrated in Figure 6. In this model, long-chain acyl- moities enter the mitochondrion and are acted on by membrane-bound enzymes of β-oxidation. Reducing equivalents NAD^+ and $ETFH_2$ are channelled to complex I and ETF:QO respectively, and the concentrations of the CoA ester intermediates are kept low either by substrate channelling between the active sites of the enzymes or because of the lipophilic nature of long-chain acyl-CoA esters. The long-chain CoA esters we observe, together with chain-shortened CoA esters, are 'leaked' from this pool of rapidly turning over intermediates and are acted on by medium- and short-chain enzymes in the matrix, or are loosely associated with the inner mitochondrial membrane as suggested by Sumegi and Srere [206].

5 CONCLUSIONS

There have been significant recent advances in our knowledge of the enzymology of mitochondrial β-oxidation with the description of the VLCAD, the trifunctional protein and the medium-chain enoyl-CoA hydratase. These studies, and also the identification of inherited disorders in man, have led to the emergence of a model of the pathway that is in two parts: a complex of activities bound to the inner mitochondrial membrane and a matrix system. Paradoxically it is the longer-chain intermediates which are experimentally detectable.

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REFERENCES

1 Neely, J. R. and Morgan, H. E. (1974) Annu. Rev. Physiol. **36**, 413–459
2 Felig, P. and Wahren, J. (1975) N. Engl. J. Med. **293**, 1078–1084
2a Randle, P. J., Garland, P. B., Hales, C. N. and Newsholme, E. A. (1963) Lancet **i**, 785–789
3 Knoop, F. (1905) Beitr. Chem. Physiol. Pathol. **6**, 150–162
4 Dakin, H. D. (1909) J. Biol. Chem. **6**, 203–220
5 Lynen, F. and Reichert, E. (1951) Z. Angew. Chem. **63**, 47–48
6 Lipmann, F. (1953) Bacteriol. Rev. **17**, 1–16
7 Drysdale, G. R. and Lardy, H. A. (1953) J. Biol. Chem. **202**, 119–136
7a Wakil, S. J., Green, D. E., Mii, S. and Mahter, H. R. (1954) J. Biol. Chem. **207**, 631–638
7b Lynen, F. and Ochoa, S. (1953) Biochim. Biophys. Acta **12**, 299–314
8 Bremer, J. (1983) Physiol. Rev. **63**, 1420–1480
9 Bergman, E., Reid, R., Murray, M., Brockway, J. and Whitelaw, F. (1965) Biochem. J. **97**, 53–58

10 Mahler, H., Wakil, S. and Bock, R. (1953) J. Biol. Chem. **204**, 453–467
11 Killenberg, P. G., Davidson, E. D. and Webster, Jr., L. (1971) Mol. Pharmacol. **7**, 260–268
12 Norum, K. R., Farstad, M. and Bremer, J. (1966) Biochem. Biophys. Res. Commun. **22**, 797–804
13 Hesler, C., Olymbios, C. and Haldar, D. (1990) J. Biol. Chem. **265**, 6600–6605
14 Kolodziej, M. P., Crilly, P. J., Corstorphine, C. G. and Zammit, V. A. (1992) Biochem. J. **282**, 415–421
15 Brown, N. F., Esser, V., Foster D. W. and McGarry, J. D. (1994) J. Biol. Chem. **269**, 26438–26442
16 Esser, V., Brown, N. F., Cowan, A. T., Foster, D. W. and McGarry, J. D. (1996) J. Biol. Chem. **271**, 6872–6977
17 Weis, B. C., Esser, V., Foster, D. W. and McGarry, J. D. (1994) J. Biol. Chem. **269**, 18712–18715
18 Weis, B. C., Cowan, A. T., Brown, N., Foster, D. W. and McGarry, J. D. (1994) J. Biol. Chem. **269**, 26443–26448
19 Brown, N. F., Weis, B. C., Husti, J. E., Foster, D. W. and McGarry, J. D. (1995) J. Biol. Chem. **270**, 8952–8957
20 Indiveri, C., Tonazzi, A. and Palmieri, F. (1990) Biochim. Biophys. Acta **1020**, 81–86
21 Indiveri, C., Tonazzi, A. and Palmieri, F. (1991) Biochim. Biophys. Acta **1069**, 110–116
22 Izai, K., Uchida, Y., Orii, T., Yamamoto, S. and Hashimoto, T. (1992) J. Biol. Chem. **267**, 1027–1033
23 Yamaguchi, S., Indo, Y., Coates, P. M., Hashimoto, T. and Tanaka, K. (1993) Pediatr. Res. **34**, 111–113
24 Rozen, R., Vockley, J., Zhou, L., Milos, R., Willard, J., Fu, K. and Vicanek, C. (1994) Genomics **24**, 280–287
25 Kim, J. J. and Wu, J. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 6677–6681
26 Thorpe, C. and Kim, J. (1995) FASEB J. **9**, 718–725
27 Crane, F. and Beinert, H. (1956) J. Biol. Chem. **218**, 717–731
28 Ruzicka, F. J. and Beinert, H. (1977) J. Biol. Chem. **252**, 8440–5844
29 Furuta, S., Miyazawa, S. and Hashimoto, T. (1981) J. Biochem. (Tokyo) **90**, 1739–50
30 Beckmann, J. D. and Frerman, F. E. (1985) Biochemistry **24**, 3913–3921
31 Stern, J. R. and del Campillo, A. (1956) J. Biol. Chem. **218**, 985–1002
32 Hass, G. M. and Hill, R. L. (1969), J. Biol. Chem. **244**, 6080–6086
33 Waterson, R. M. and Hill, R. L. (1972) J. Biol. Chem. **247**, 5258–5265
34 Stern J. R., del Campillo, A. and Raw, I. (1956) J. Biol. Chem. **218**, 971–983
35 Wit-Peeters, E. M., Scholte, H. T., Van den Akker, F. and De Nie, I. (1971) Biochim. Biophys. Acta **231**, 23–31
36 Fong, J. C. and Schulz, H. (1977) J. Biol. Chem. **252**, 542–547
37 Jackson, S., Kler, R. S., Bartlett, K., Briggs, H., Bindoff, L. A., Pourfarzam, M., Gardner-Medwin, D. and Turnbull, D. M. (1992) J. Clin. Invest. **90**, 1219–1225
38 Jackson, S., Schaefer, J., Middleton, B. and Turnbull, D. (1995) Biochem. Biophys. Res. Commun. **214**, 247–253
39 Stern, J. R. (1957) Methods Enzymol. **1**, 559–567
40 Bradshaw, R. A. and Noyes, B. E. (1975) Methods Enzymol. **35**, 122–128
41 Osumi, T. and Hashimoto, T. (1980) Arch. Biochim. Biophys. **203**, 372–383
42 He, X.-Y., Yang, S. Y. and Schulz, H. (1989) Anal. Biochem. **180**, 105–109
43 El-Fakhri, M. and Middleton, B. (1982) Biochim. Biophys. Acta **713**, 270–279
44 Middleton, B. (1972) Biochem. Biophys. Res. Commun. **46**, 508–515
45 Middleton, B. (1973) Biochem. J. **132**, 717–730
46 Middleton, B. and Bartlett, K. (1983) Clin. Chim. Acta **128**, 291–305
47 Seubert, W., Lamberts, I., Kramer, R. and Ohly, B. (1968) Biochim. Biophys. Acta, **164**, 498–517
48 Staack, H., Binstock, J. F. and Schulz, H. (1978) J. Biol. Chem., **253**, 1827–1831
49 Uchida, Y., Izai, K., Orii, T. and Hashimoto, T. (1992) J. Biol. Chem. **267**, 1034–1041
50 Carpenter, K., Pollitt, R. J. and Middleton, B. (1992) Biochem. Biophys. Res. Commun. **183**, 443–448
51 Luo, M. J., He, X. Y., Sprecher, H. and Schulz, H. (1993) Arch. Biochem. Biophys. **304**, 266–271
52 Weinberger, M., Rinaldo, P., Strauss, A. and Bennett, M. (1995) Biochem. Biophys. Res. Commun. **209**, 47–52
53 Kerner, J. and Bieber, L. (1990) Biochemistry. **29**, 4326–4334
54 Gavino, G. R. and Gavino, V. C. (1991) Lipids **26**, 266–270
55 Osmundsen, H. and Bjornstad, K. (1985) Biochem. J. **230**, 329–337
56 Schulz, H. and Kunau, W.-H. (1987) Trends Biochem. Sci. **12**, 403–406
57 Smeland, T. E., Nada, M., Cuebas, D. and Schulz, H (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 6673–6677
58 Chen, L.-S., Jin, S.-J. and Tserng, K.-Y. (1994) Biochemistry **33**, 10527–10534
59 Tserng, K., Jin, S. and Chen, L. (1996) Biochem. J. **313**, 581–588
60 Vianey-Liaud, C., Divry, P., Gregersen, N. and Matthieu, M. (1987) J. Inherited Metab. Dis. **10** (suppl. 1), 159–198
61 Bartlett, K. (1993) Baillieres Clin. Endocrinol. Metab. **7**, 643–667
62 Hale, D. E. and Bennett, M. J. (1992) J. Pediatr. **121**, 1–11
63 Vockley, J. (1994) Mayo Clin. Proc. **69**, 249–257
64 Gregersen, N., Andresen, B. S., Bross, P., Bolund, L. and Kølvraa, S. (1994) in New Horizons in Neonatal Screening (Farriaux, J.-P. and Dhondt, J.-L., eds.), pp. 247–255, Elsevier, Amsterdam
65 Roe, C. R. and Coates, P. M. (1995) in The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, L., Sly, W. S. and Valle, D., eds.), pp. 889–914, McGraw-Hill Inc., New York
66 Angelini, C., Freddo, L., Battistella, P., Bresolin, N, Pierobon-Bormioli, S., Armani, M. and Vergani, L. (1981) Neurology **31**, 883–886
67 Singh, R., Shepherd, I. M., Derrick, J. P., Ramsay, R. R., Sherratt, H. S. A. and Turnbull, D. M. (1988) FEBS Lett. **241**, 126–130
68 Demaugre, F., Bonnefont, J. P., Cepanec, C., Scholte, J., Saudubray, J.-M. and Leroux, J. P. (1990) Pediatr. Res. **27**, 497–500
69 Demaugre, F., Bonnefont, J. P., Colonna, M., Cepanec, C., Leroux, J. P. and Saudubray, J.-M. (1991) J. Clin. Invest. **87**, 859–864
70 Zierz, S. and Engel, A. G. (1985) Eur. J. Biochem. **149**, 207–214
71 Taroni, F., Verderio, E., Dworzak, F., Willems, P., Cavadini, P. and DiDonato, S. (1993) Nature (Genetics) **4**, 314–319
72 Taroni, F., Verderio, E., Fiorucci, S., Cavadini, P., Finocchiaro, G. and Uziel, G. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 8429–8433
73 Bougnères, P. F., Saudubray, J. M., Marsac, C., Bernard, O., Odievre, M. and Girard, J. (1981) J. Pediatr. **98**, 742–746
74 Tein, I., Demaugre, F., Bonnefont, J. P. and Saudubray, J. M. (1989) J. Neurol. Sci. **92**, 229–245
75 Stanley, C. A., Hale, D. E., Berry, G. T., Deleeuw, S., Boxer, J. and Bonnefont, J. P. (1992) N. Engl. J. Med. **327**, 19–23
76 Pande, S., Brivet, M., Slama, A., Demaugre, F., Aufrant, C. and Saudubray, J. (1993) J. Clin. Invest. **91**, 1247–1252
77 Niezen-Koning, K., Vanspronsen, F., Ijlst, L., Wanders, R. and Brivet, M. (1995) J. Inherited Metab. Dis. **18**, 230–232
78 Brivet, M., Slama, A., Ogier, H., Boutron, A., Demaugre, F. and Saudubray, J. (1994) J. Inherited Metab. Dis. **17**, 271–274
79 Stanley, C. A. (1987) Adv. Pediatr. **34**, 59–84
80 Treem, W. R., Stanley, C. A., Finegold, D. N., Hale, D. E. and Coates, P. M. (1988) N. Engl. J. Med. **319**, 1331–1336
81 Stanley, C. A., Treem, W. R., Hale, D. E. and Coates, P. M. (1990) Progr. Clin. Biol. Res. **321**, 457–464
82 Hale, D. E., Batshaw, M..L, Coates, P. M., Frerman, F. E., Goodman, S. I., Singh, I. and Stanley, C. A. (1985) Pediatr. Res. **19**, 666–671
83 Hale, D. E., Stanley, C. A. and Coates, P. M. (1990) Progr. Clin. Biol. Res. **321**, 303–311
84 Amendt, B. A., Moon, A., Teel, L. and Rhead, W. J. (1988) Pediatr. Res. **23**, 603–605
85 Indo, Y., Coates, P. M., Hale, D. E. and Tanaka, K. (1991) Pediatr. Res. **30**, 211–215
86 Strauss, A., Powell, C., Hale, D., Anderson, M., Ahuja, A. and Brackett, J. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 10496–10500
87 Kølvraa, S., Gregersen, N., Christensen, E. and Hobolth, N. (1982) Clin. Chim. Acta **126**, 53–67
88 Millington, D. S., Norwood, D. L., Kodo, N., Roe, C. R. and Inoue, F. (1989) Anal. Biochem. **180**, 331–339
89 Millington, D. S., Kodo, N., Terada, N., Roe, D. and Chace, D. H. (1991) Int. J. Mass Spectrom. Ion Proc. **111**, 211–228
90 Bhuiyan, A. K. M. J., Watmough, N. J., Turnbull, D. M., Aynsley-Green, A. and Bartlett, K., (1987) Clin. Chim. Acta **165**, 39–44
91 Duran, M., Bruinvis, L., Ketting, D., de Klerk, J. B. C. and Wadman, S. K. (1988) Clin. Chem. **34**, 548–551
92 Pourfarzam, M., Naughten, E., Cahalane, S., Bhuiyan, A. K. M. J. and Bartlett, K. (1990) in Stable Isotopes in Paediatric Nutrition and Metabolic Research (Chapman, T. E., Berger, R., Reijngoud, D. J. and Okken, A., eds.), pp. 249–255, Intercept Press, Andover, Hampshire
93 Rinaldo, P., O'Shea, J. J., Coates, P. M., Hale, D. E., Stanley, C. A. and Tanaka, K. (1988) N. Engl. J. Med. **319**, 1308–1313
94 Matsubara, Y., Narisawa, K., Miyabayashi, S., Tada, K. and Coates, P. M. (1990) Lancet **335**, 1589
95 Matsubara, Y., Narisawa, K., Tada, K., Ikeda, H., Yao, Y. Q., Danks, D. M., Green, A. and McCabe, E. R. (1991) Lancet **338**, 552–553
96 Kolvraa, S., Gregersen, N., Blakemore, A. I., Schneidermann, A. K., Winter, V., Andresen, B. S., Curtis, D., Engel, P. C., Pricille, D. and Rhead, W. (1991) Hum. Genet. **87**, 425–428
97 Yokota, I., Tanaka, K. Coates, P. M. and Ugarte, M. (1990) Lancet **336**, 748
98 Ding, J. H., Yang, B. Z., Bao, Y., Roe, C. R., Chen, Y. T. (1992) Am. J. Hum. Genet. **50**, 229–233

99 Kelly, P. D., Whelan, A. J., Ogden, M. L., Alpers, R., Zang, Z., Bellus, G., Gregersen, N., Dorland, L. and Strauss, A. W. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 9236–9240
100 Yokota, I., Indo, Y., Coates, P. M. and Tanaka, K. (1990) J. Clin. Invest. **86**, 1000–1003
101 Gregersen, N., Andresen, B., Brosse, P., Rüdiger, N., Engst, S., Christensen, E., Kelly, D., Strauss, A. W., Kølvraa, S., Bolund, L. and Ghisla, S. (1991) Hum. Genet. **86**, 545–551
102 Saijo, T., Welch, W. J. and Tanaka, K. (1994) J. Biol. Chem. **269**, 4401–4408
103 Bross, P., Jespersen, C., Jensen T. G., Andresen, B. S., Kristensen, M. J., Winter, V., Nandy, A., Kräutle, F., Ghisla, S., Bolund, L. et al. (1995) J. Biol. Chem. **270**, 10284–10290
104 Blakemore, A. I. F., Singleton, H., Pollitt, R. J., Engel, P. C., Kølvraa, S., Gregersen, N. and Curtis, D. (1991) Lancet **337**, 298–299
105 Turnbull, D. M., Bartlett, K., Stevens, D. L., Alberti, K. G. M. M., Gibson, G. J., Johnson, M. A., McCulloch, A. J. and Sherratt, H. S. A. (1984) N. Engl. J. Med. **311**, 1232–1236
106 Amendt, B. A., Greene, C., Sweetman, L., Cloherty, J., Shih, V., Moon, A., Teel, L. and Rhead, W. J. (1987) J. Clin. Invest. **79**, 1303–1309
107 Coates, P. M., Hale, D. E., Finocchiaro, G., Tanaka, K. and Winter, S. C. (1988) J. Clin. Invest. **81**, 171–175
108 DiDonato, S., Gellera, C., Peluchetti, D., Uzieli, G., Antonelli, A., Lus, G. and Rimoldi, M. (1989) Ann. Neurol. **25**, 479–484
109 Farnsworth, L., Shepherd, I. M., Johnson, M. A., Bindoff, L. A. and Turnbull, D. M. (1990) Ann. Neurol. **28**, 717–720
110 Naito, E., Indo, Y. and Tanaka, K. (1989) J. Clin. Invest. **84**, 1671–1674
111 Naito, E., Indo, Y. and Tanaka, K. (1990) J. Clin. Invest. **85**, 1575–1582
112 Bhala, A., Willi, S., Rinaldo, P., Bennett, M., Schmidt-Sommerfeld, E. and Hale, D. (1995) J. Pediatr. **126**, 910–915
113 Hinsdale, M., Hamm, D. and Wood, P. (1996) Biochem. Mol. Med. **57**, 106–115
114 Przyrembel, H., Wendel, U., Becker, K., Bremer, H. J., Bruinvis, L., Ketting, D. and Wadman, S. (1976) Clin. Chim. Acta **66**, 227–239
115 Christensen, E., Kolvraa, S. and Gregersen, N. (1984) Pediatr. Res. **18**, 663–667
116 Frerman, F. E. and Goodman, S. I. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 4517–4520
117 Rinaldo, P., Welch, R. D., Previs, S. F., Schmidt-Sommerfeld, E., Gargus, J. J., O'Shea, J. J. and Zinn, A. B. (1991) Pediatr. Res. **30**, 216–221
118 Lehnert, W., Wendel, U., Lindenmaier, S. and Bohm, N. (1982) Eur. J. Pediatr. **139**, 56–59
119 Loehr, J. P., Goodman, S. I. and Frerman, F. E. (1990) Pediatr. Res. **27**, 311–315
120 Dusheiko, G., Kew, M., Joffe, B., Lewin, J., Mantagos, S. and Tanaka, K. (1979) N. Engl. J. Med. **301**, 1405–1409
121 Ikeda, Y., Keese, S. M. and Tanaka, K. (1986) J. Clin. Invest. **78**, 997–1002
122 Yamaguchi, S., Orii, T., Suzuki, Y., Maeda, K., Oshima, M. and Hashimoto, T. (1991) Pediatr. Res. **29**, 60–63
123 Jackson, S., Bartlett, K., Land, J., Moxon, E. R., Pollitt, R. J., Leonard, J. V. and Turnbull, D. M. (1991) Pediatr. Res. **29**, 406–411
124 Duran, M., Wanders, R. J. A., de Jeger, J. P., Dorland, L., Bruinis, L., Ketting, D., Ijlst, L. and VanSprang, F. J. (1991) Eur. J. Paediatr. **150**, 190–195
125 Rocchiccioli, F., Wanders, R. J. A. Aubourg, P., Vianey-Liaud, C., Ijlst, L., Fabre, M., Cartier, N. and Bougneres., P. F. (1990) Pediatr. Res. **28**, 657–662
126 Sims, H., Brackett, J., Powell, C., Treem, W., Hale, D. and Bennett, M. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 841–845
127 Ijlst, L., Wanders, R., Ushikubo, S., Kamijo, T. and Hashimoto, T. (1994) Biochim. Biophys. Acta **1215**, 347–350
128 Ijlst, L., Ruiter, J., Hoovers, J., Jacobs, M. and Wanders, R. J. A. (1996) J Clin Invest **98**, 1028–1033
129 Hale, D. E., Thorpe, C. and Braat, K. (1989) Pediatr. Res. **25**, 199A (abstract)
130 Tein, I., De Vivo, D. C., Hale, D. E., Clarke, J. T. R., Zinman, H., Laxer, R., Shore, A. and DiMauro, S. (1991) Ann. Neurol. **30**, 415–419
131 Hardie, D. (1992) Biochim. Biophys. Acta **1123**, 231–238
132 Bartlett, K., Aynsley-Green, A., Leonard, J. V. and Turnbull, D. M. (1991) in Inborn Errors of Metabolism (Schob, J., Van Hoof, F. and Vis, H. L., eds.), pp. 19–41, Raven Press, New York
133 Zammit, V. (1996) Biochem. J. **314**, 1–14
134 Guzman, M. and Geelen, M. (1993) Biochim. Biophys. Acta **1167**, 227–241
135 Neely, J. R., Bowman, R. H. and Morgan, H. E. (1969) Am. J. Physiol. **216**, 804–811
136 Oram, J. F., Bennetch, S. L. and Neely, J. R. (1973) J. Biol. Chem. **248**, 5299–5309
137 Vandervusse, G., Glatz, J., Stam, H. and Reneman, R. (1992) Physiol. Rev. **72**, 881–940
138 Lopaschuk, G., Belke, D., Gamble, J., Itoi, T. and Schonekess, B. (1994) Biochim. Biophys. Acta **1213**, 263–276
139 McGarry, J. D. and Foster, D. W. (1980) Annu. Rev. Biochem. **49**, 395–420
140 Quant, P. and Makins, R. (1994) Biochem. Soc. Trans. **22**, 441–446
141 Drynan, L., Quant, P. and Zammit, V. (1996) Biochem. J. **317**, 791–795
142 Cook, G. A., Otto, D. A. and Cornell, N. W. (1980) Biochem. J. **192**, 955–958
143 Cook, G. A., Stephens, T. W., and Harris, R. A. (1984) Biochem. J. **219**, 337–339
144 Grantham, B. D., and Zammit, V. A. (1988) Biochem. J. **249**, 409–414
145 Kolodziej, M. P. and Zammit, V. A. (1990) Biochem. J. **272**, 421–5
146 Mynatt, R. L., Greenhaw, J. J. and Cook, G. A. (1994) Biochem. J. **299**, 761–767
147 Ghadiminejad, I. and Saggerson, E. D. (1990) Biochem. J. **270**, 787–94
148 Brady, L. J., Silverstein, L. J., Hoppel, C. L. and Brady, P. S. (1985) Biochem. J. **232**, 445–450
149 Grantham, B. D. and Zammit, V. A. (1986) Biochem. J. **239**, 485–488
150 Harano, Y., Kashiwagi, A., Kojima, H., Suzuki, M., Hashimoto, T. and Shigeta, Y. (1985) FEBS Lett. **188**, 267–72
151 Guzman, M. and Castro, J. (1991) FEBS–Lett. **291**, 105–108
152 Guzman, M. and Geelen, M. J. H. (1992) Biochem. J. **287**, 487–492
153 Guzman, M., Kolodziej, M. P., Caldwell, A., Corstorphine, C. G. and Zammit, V. A. (1994) Biochem. J. **300**, 693–699
154 Guzman, M., Velasco, G., Castro, J. and Zammit, V. A. (1994) FEBS Lett. **344**, 239–241
155 Moir, A. M. B. and Zammit, V. A. (1995) Biochem. J. **305**, 953–958
156 Mills, S. E., Foster, D. W. and McGarry, J. D. (1984) Biochem. J. **219**, 601–608
157 Velasco, G., Sanchez, C., Geelen, M. and Guzman, M. (1996) Biochem. Biophys. Res. Commun. **224**, 754–759
158 Park, E. A., Mynatt, R. L., Cook, G. A. and Kashfi, K. (1995) Biochem. J. **310**, 853–858
159 Saggerson, E. D. and Carpenter, C. A. (1981) FEBS Lett. **129**, 229–232
160 Cook, G. A. (1984) J. Biol. Chem. **259**, 2030–2033
161 McGarry, J. D., Mills, S. E., Long, C. S. and Foster, D. W. (1983) Biochem. J. **214**, 21–28
162 Singh, B., Stakkestad, J. A., Bremer, J. and Borrebaek, B. (1984) Anal. Biochem. **138**, 107–111
163 Hulsmann, W. C. (1966) Biochim. Biophys. Acta **178**, 137–144
164 Scholte, H. R., Luyt Houwen, I. E., Dubelaar, M. L. and Hulsmann, W. C. (1986) FEBS Lett. **198**, 47–50
165 Bird, M. I. and Saggerson, E. D. (1984) Biochem. J. **222**, 639–647
166 Dugan, R. E., Osterlund, B. R., Drong, R. F. and Swenson, T. L. (1987) Biochem. Biophys. Res. Commun. **147**, 234–241
167 Taegtmeyer, H., Hems, R. and Krebs, H. (1980) Biochem. J. **186**, 701–11
168 Bielefeld, D. R., Vary, T. C. and Neely, J. R. (1985) J. Mol. Cell. Cardiol. **17**, 619–625
169 Winder, W. and Hardie, D. (1996) Am. J. Physiol. **33**, E299–E304
170 Kudo, N., Barr, A., Barr, R., Desai, S. and Lopaschuk, G. (1995) J. Biol. Chem. **270**, 17513–17520
171 England, P. J. and Robinson, B. H. (1969) Biochem. J. **112**, 8P
172 Awan, M. M. and Saggerson, E. D. (1993) Biochem. J. **295**, 61–66
173 Thampy, K. G. (1989) J. Biol. Chem. **264**, 17631–17634
174 Bianchi, A., Evans, J. L., Iverson, A. J., Nordlund, A. C., Watts, T. D. and Witters, L. A. (1990) J. Biol. Chem. **265**, 1502–1509
175 Kudo, N., Barr, A. J., Barr, R. L., Desai, S. and Lopaschuk, G. D. (1995) J. Biol. Chem. **270**, 17513–17520
176 Lysiak, W., Toth, P. P., Suelter, C. H. and Bieber, L. L. (1986) J. Biol. Chem. **261**, 3698–3703
177 Lopaschuk, G. D., Belke, D. D., Gamble, J., Itoi, T. and Schonekess, B. O. (1994) Biochim. Biophys. Acta **1213**, 263–276
178 Edwards, Y. H., Chase, J. F., Edwards, M. R. and Tubbs, P. K. (1974) Eur. J. Biochem. **46**, 209–215
179 Bakker, A., Biermans, W., Vanbelle, H., Debie, M., Bernaert, I. and Jacob, W. (1994) Biochim. Biophys. Acta **1185**, 97–102
180 Saddik, M., Gamble, J., Witters, L. A. and Lopaschuk, G. D. (1993) J. Biol. Chem. **268**, 25836–25845
181 Reichmann, H. and DeVivo, D. C. (1991) Comp. Biochem. Physiol. **98B**, 327–331
182 Melde, K., Jackson, S., Bartlett, K., Sherratt, H. S. A. and Ghisla, S. (1991) Biochem. J. **274**, 395–400
183 Kunz, W. S. (1991) Biomed. Biochim. Acta. **50**, 1143–1157
184 Aoyama, T., Ueno, I., Kamijo, T. and Hashimoto, T. (1994) J. Biol. Chem. **269**, 19088–19094
185 Davidson, B. and Schulz, H. (1982) Arch. Biochem. Biophys. **213**, 155–162
186 Powell, P. J., Lau, S. M., Killian, D. and Thorpe, C. (1987) Biochemistry **26**, 3704–3710
187 Schifferdecker, J. and Schulz, H. (1974) Life Sci. **14**, 1487–1492
188 He, X.-Y., Yang, S. Y. and Schulz, H. (1992) Arch. Biochem. Biophys. **298**, 527–531
189 Olowe, Y. and Schulz, H. (1980) Eur. J. Biochem. **109**, 425–429

190 Wang, H. Y., Baxter, Jr., C. F. and Schulz, H. (1991) Arch. Biochem. Biophys. **289**, 274–280
191 Sleboda, J., Pourfarzam, M., Bartlett, K. and Osmundsen, H. (1995) Biochim. Biophys. Acta **1258**, 309–318
192 Eaton, S., Bhuiyan, A. K. M. J., Kler, R. S., Turnbull, D. M. and Bartlett, K. (1993) Biochem. J. **289**, 161–168
193 Eaton, S., Turnbull, D. M. and Bartlett, K. (1994) Eur. J. Biochem. **220**, 671–681
194 Quant, P. A., Robin, D., Robin, P., Girard, J. and Brand, M. D. (1993) Biochim. Biophys. Acta **1156**, 135–143
195 Quant, P. A., Robin, D., Robin, P., Ferre, P., Brand, M. D. and Girard, J. (1991) Eur. J. Biochem. **195**, 449–454
196 Garland, P. B., Shepherd, D. and Yates, D. W. (1965) Biochem. J. **97**, 587–594.
197 Pacanis, A., Strzelecki, T. and Rogulski, J. (1981) J. Biol. Chem. **256**, 3035–3038
198 Turnbull, D. M., Bone, A. J., Bartlett, K., Koundakjian, P. P. and Sherratt, H. S. A. (1983) Biochem. Pharmacol. **32**, 1887–1892
199 Bremer, J. and Wojtczak, A. B. (1972) Biochim. Biophys. Acta. **280**, 515–530
200 Singh Kler, R., Jackson, S., Bartlett, K., Bindoff, L. A., Eaton, S., Pourfarzam, M., Frerman, F. E., Watmough, N. J. and Turnbull, D.M (1991) J. Biol. Chem. **266**, 22932–22938
201 Frerman, F. E. (1987) Biochim. Biophys. Acta. **893**, 161–169
202 Beckmann, J. D., Frerman, F. E. and McKean, M. C. (1981) Biochem. Biophys. Res. Commun. **102**, 1290–1294
203 Kunz, W. S. (1988) Biochim. Biophys. Acta. **932**, 8–16
204 Halestrap, A. P. and Dunlop, J. L. (1986) Biochem. J. **239**, 559–565
205 Halestrap, A. P. (1987) Biochem. J. **244**, 159–164
206 Sumegi, B. and Srere, P. A. (1984) J. Biol. Chem. **259**, 8748–8752
207 Kispal, G., Sumegi, B. and Alkonyi, I. (1986) J. Biol. Chem. **261**, 14209–14213
208 Furuta, S. and Hashimoto, T. (1995) J. Biochem. (Tokyo) **118**, 810–818
209 Stanley, K. K. and Tubbs, P. K. (1974) FEBS Lett. **39**, 325–328
210 Stanley, K. K. and Tubbs, P. K. (1975) Biochem. J. **150**, 77–88
211 Lopes-Cardozo, M., Klazinga, W. and Bergh, S. G. (1978) Eur. J. Biochem. **83**, 629–634
212 Watmough, N. J., Turnbull, D. M., Sherratt, H. S. A. and Bartlett, K. (1989) Biochem. J. **262**, 261–269
213 Bhuiyan, A. K. M. J., Jackson, S., Turnbull, D. M., Aynsley-Green, A., Leonard, J. V. and Bartlett, K. (1992) Clin. Chim. Acta. **207**, 185–204
214 Al-Arif, A. and Blecher, M. (1971) Biochim. Biophys. Acta. **248**, 406–415
215 Mahadevan, S., Malaiyandi, M., Erfle, J. D. and Sauer, F. (1970) J. Biol. Chem. **245**, 4585–4595
216 Fukushima, T., Decker, R. V., Anderson, W. M. and Spivey, H. O. (1989) J. Biol. Chem. **264**, 16483–16488
217 Middleton, B. (1994) Biochem. Soc. Trans. **22**, 427–431
218 Cornish-Bowden, A. and Cardenas, M. (1993) Eur. J. Biochem. **213**, 87–92
219 Mendes, P., Kell, D. and Westerhoff, H. (1996) Biochim. Biophys. Acta **1289**, 175–186
220 Nada, M. A., Rhead, W. J., Sprecher, H., Schulz, H. and Roe, C. R. (1995) J. Biol. Chem. **270**, 530–535
221 Pourfarzam, M., Schaefer, J., Turnbull, D. M. and Bartlett, K. (1994) Clin. Chem. **40**, 2267–2275
222 Osmundsen, H., Bartlett, K., Pourfarzam, M., Eaton, S. and Sleboda, J. (1996) in Channelling in Intermediary Metabolism (Agius L. and Sherratt H. S. A., eds.) Portland Press, London, in the press
223 Suzuki, H., Kawarabayasi, Y., Kondo, J., Abe, T., Nishikawa, K. and Kimura, S. (1990) J. Biol. Chem. **265**, 8681–8685
224 Clarke, P. R. and Bieber, L. L. (1981) J. Biol. Chem. **256**, 9861–9868
225 Ikeda, Y., Dabrowski, C. and Tanaka, K. (1985) J. Biol. Chem. **258,** 1066–1076
226 Finocchiario, G., Ito, K. and Tanaka, K. (1987) J. Biol. Chem. **262**, 7982–7989
227 Furuta, S., Miyazawa, S. and Hashimoto, T (1981) J. Biochem. (Tokyo) **90,** 1739–1750
228 Gehring, U. and Repertinger, C. (1968) Eur. J. Biochem., **6**, 281–292
229 Dommes, V. and Kunau, W. H. (1984) J. Biol. Chem. **259**, 1789–1798
230 Palossari, P. M., Kilponnen, J. M., Sormunen, R. T., Hassinen, I. E. and Hiltunen, J. K. (1990) J. Biol. Chem. **265**, 3347–3353
231 Kilponen, J. M., Palosaari, P. M. and Hiltunen, J. K. (1990) Biochem. J. **269**, 223–226
232 Luo, M. J., Smeland, T. E., Shoukry, K. and Shulz, H. (1994) J. Biol. Chem. **269**, 2384–2388