Global Science Research Journals

Vol. 5 (1), pp. 158-172, December, 2018

http://www.globalscienceresearchjournals.org/

International Journal of Clinical Biochemistry

Review Article

Beta (β)-Oxidation of Fatty Acid and its associated Disorders

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Accepted 18 December, 2018

The lipids of metabolic significance in the mammalian organisms include triacylglycerols, phospholipids and steroids, together with products of their metabolism such as long-chain fatty acids, glycerol and ketone bodies. The fatty acids which are present in the triacylglycerols in the reduced form are the most abundant source of energy and provide energy twice as much as carbohydrates and proteins. Fatty acids represent an important source of energy in periods of catabolic stress related to increased muscular activity, fasting or febrile illness, where as much as 80% of the energy for the heart, skeletal muscles and liver could be derived from them. The prime pathway for the degradation of fatty acids is mitochondrial fatty acid β-oxidation (FAO). 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. Inherited defects for most of the FAO enzymes have been identified and characterized in early infancy as acute life-threatening episodes of hypoketotic, hypoglycemic coma induced by fasting or febrile illness. Therefore, this review briefly highlights mitochondrial β-oxidation of fatty acids and associated disorders with clinical manifestations.

Keywords: Carnitine, Fatty acid β-oxidation, Jamaican vomiting sickness, Stoichiometry, Sudden infant death syndrome.

INTRODUCTION

Mitochondrial β-oxidation of fatty acids plays an important role in energy production, especially during starvation, prolonged fasting or low intensity exercise. The principal sources of fatty acids for oxidation are dietary and mobilization of triacylglycerols mainly stored in adipocytes of adipose tissue (Lopaschuk et al., 1994; McGarry & Foster, 1980). The release of metabolic energy, in the form of fatty acids, is controlled by a complex series of interrelated cascades that result in the activation of hormone-sensitive lipase, which hydrolyzes fatty acids from triacylglycerols and diacylglycerols (Gibbons et al., 2000). The final fatty acid is released from monoacylglycerols through the action of monoacylglycerol lipase, an enzyme active in the absence of hormonal stimulation. Once released, these fatty acids travel through the blood to other tissues such as muscle where they are oxidized to provide energy through the mitochondrial β-oxidation pathway.

Mitochondria, as well as peroxisomes harbor all enzymes necessary for FAO. Mitochondria are the main site for the oxidation of plasma free fatty acids or lipoprotein associated triglycerides. The use of fatty acids by the liver provides energy for gluconeogenesis and ureagenesis (Liang et al; 2001). Equally important, the liver uses fatty acids to synthesize ketones, which serve as a fat derived fuel for the brain, and thus further reduce the need for glucose utilization. More than a dozen genetic defects in the fatty acid oxidation pathway are currently known. Nearly all of these defects present in early infancy as acute life-threatening episodes of hypoketotic, hypoglycemic coma induced by fasting or febrile illness (Robert MO et al., 2009). Recognition of the

fatty acid oxidation disorders is often difficult because patients can appear well until exposed to prolonged fasting, and screening tests of metabolites may not always be diagnostic. Therefore, this review briefly highlights the overall pathway of mitochondrial β-oxidation of fatty acids and its associated deficiencies with its clinical correlation.

Historical Preview of β- oxidation

Fatty acids are a key source of energy in animals. George Franz Knoop, a German biochemist in 1904 studied the biological degradation of fatty acid with his classical experiments which led him to formulate the theory of β-oxidation (Knoop, 1904). His experiments used fatty acids with phenyl residues in place of the terminal methyl groups. The phenyl residue was not metabolized which served as a reporter group and was excreted in the urine. During his experiment, Knoop fed phenyl substituted fatty acids with an odd number of carbon atoms, like phenylpropionic acid (C_6H_5-CH_2-CH_2-COOH) or phenylvaleric acid (C_6H_5-CH_2-CH_2-CH_2-CH_2-COOH) to dogs, and isolated hippuric acid (C_6H_5-CO-NH-CH_2-COOH), the conjugate of benzoic acid and glycine from their urine. In contrast, the excretory products in urine were phenyl-substituted fatty acids with an even number of carbon atoms, such as phenylbutyric acid (C_6H_5-CH_2-CH_2-CH_2-COOH), were degraded to phenylacetic acid (C_6H_5-CH_2-COOH) and excreted as phenylaceturic acid (C_6H_5-CH_2-CO-NH-CH_2-COOH). These annotations led Knoop to propose that the oxidation of fatty acids begins at carbon atom 3, the β-carbon, and that the resulting β -keto acids are cleaved between the α-carbon and β -carbon to yield fatty acids shortened by two carbon atoms. Knoop's experiment on biological degradation incited the idea that fatty acids are degraded in a stepwise method by successive β-oxidation.

Henry Drysdale Dakin followed Knoop's preliminary study and executed analogous experiments with phenylpropionic acid (Dakin, 1909). He isolated the glycine conjugates of the following β-oxidation intermediates: phenylacrylic acid (C_6H_5-CH=CH-COOH), β-phenyl-β-hydroxypropionic acid (C_6H_5-CHOH-CH_2-COOH), and benzoylacetic acid (C_6H_5-CO-CH_2-COOH) next to hippuric acid. At the same time, the unsubstituted fatty acids are degraded by β-oxidation and converted to ketone bodies in perfused livers which were verified by Embden and coworkers. As a result, by 1910 the crucial information needed for formulating the pathway of β -oxidation was available.

Due to consistent effort of researchers, after a 30-year period of little progress the oxidation of fatty acids in cell-free preparations from liver was demonstrated by Munoz and Leloir in 1943, and Lehninger in 1944. Their endeavor came true with the stage for the complete elucidation of β-oxidation. The studies and detailed investigations Lehninger with cell-free systems confirmed the need for energy as ATP to spark the oxidation of fatty acids and to be essential for the activation of fatty acids.

Wakil and Mahler, as well as by Kornberg and Pricer revealed activated fatty acids to be thioesters formed from fatty acids and coenzyme A. This progress was only made promising by prior studies of Lipmann and his collaborators who isolated and distinguished coenzyme A. The structure of active acetate is acetyl-CoA was proved by Lynen and co-workers. They also determined that the acetyl-CoA was identical with the two-carbon fragment removed from fatty acids during their degradation (Lynen, 1952-1953). Finally, the sub-cellular location of the β-oxidation system was established by Kennedy and Lehninger, who confirmed that mitochondria were the cellular components which are most active during fatty acid oxidation. The mitochondrial site of this pathway agreed with the observed coupling of fatty acid oxidation to the citric acid cycle and to oxidative phosphorylation.

Moreover, in 1950s , the laboratories of Green in Wisconsin, Lynen in Munich, and Ochoa in New York demonstrated the direct evidence for the proposed β-oxidation cycle by enzyme studies which were greatly facilitated by recently developed techniques of protein purification and by the use of spectrophotometric enzyme assays with chemically synthesized intermediates of β-oxidation as substrates (Vance & Vance, 2002). Although, several studies were carried out to confirm the steps mitochondrial β –oxidation, but the initial and conclusive remarks by Franz Knoop on β–oxidation is still considered as remarkable discovery in biochemistry. Hence, β–oxidation is also known as Knoop's pathway or β –oxidation Knoop's pathway.

β-oxidation of fatty acid

β-oxidation of fatty acid is defined as a metabolic pathway that oxidizes fatty acids, and generates fatty acyl-CoA (a thioester of fatty acid and CoA) and acetyl CoA which consists of a series of four repeated reactions, in which a molecule of acetyl CoA is generated, and an end product of the fatty acid by beta-oxidation is also acetyl CoA. Since, oxidation at the β-position of the fatty acyl-CoA was performed step wise, it was named β-oxidation. Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement (Gurr & Harwood , 1991; Schulz,1985; Schulz & Kunau,1987). The four steps are involved in β-oxidation spiral of fatty acid metabolism which is oxidation, hydration, a second oxidation, and finally thiolysis. These happens in repeating cycles through the sequential removal of 2 carbons and production of acetyl-CoA, which then enters the Krebs cycle for oxidation and ATP production. Another target of acetyl-CoA is the production of ketone bodies in the liver that are elated to tissues like the heart and brain for release of energy during starvation. Fatty acids with an odd number of carbons in

the acyl chain are left at the end with propionyl-CoA, which is converted to succinyl-CoA that then also enters the Krebs cycle. Furthermore, unsaturated fatty acids with bonds in the *cis* configuration require three separate enzymatic steps to prepare themselves for the β-oxidation pathway (Gervois et al., 2000; Thorpe & Kim,1995; Hiltunen & Qin 2000; Wanders,2001).

Activation of fatty acid

Cytosolic Fatty Acid Activation:

The transport of fatty acids between organs occurs either in the form of triacylglycerols associated with lipoproteins or as unesterified fatty acids complexed to serum albumin. The hydrolysis of triacylglycerols occurs outside of cells by lipoprotein lipase to yield free fatty acids. Even though a number of studies has been carried out with isolated cells from heart, liver, and adipose tissue but the mechanism by which free fatty acids enter cells remains poorly implicated (Kunau et al., 1995). Numerous assumed fatty acid transport proteins have been identified (Kunauet al., 1995; Abumrad N et al., 1999). However, their specific function(s) in fatty acid uptake and their molecular mechanisms remain to be clarified. For oxidation, fatty acids with carbon chains more than 14 carbons need activation before passing through the mitochondrial membrane. Free fatty acids obtained from diet or which are stored in the adipocytes are mainly 14 carbons or more in length. Fatty acids having ≤12 carbons can surpass activation and can easily pass through the mitochondrial membrane. Fatty acids with long chain once cross the plasma membrane either diffuse or are transported to mitochondria, peroxisomes, and the endoplasmic reticulum where they are activated by conversion to their CoA thioesters.

The mechanism of transfer of fatty acids between membranes is a facilitated process or occurs by simple diffusion is an unresolved issue. The identification of low-molecular-weight (14-15 kDa) fatty acid binding proteins (FABPs) in the cytosol of various animal tissues prompted the suggestion that these proteins may function as carriers of fatty acids in the cytosolic compartment (Coe & Bernlohr, 1998). FABPs may also be involved in the cellular uptake of fatty acids, their intracellular storage, or the delivery of fatty acids to sites of their utilization. The metabolism of fatty acids requires their prior activation by conversion to fatty acyl-CoA thioesters. The activating enzymes are ATP-dependent acyl-CoA synthetases/thiokinase, which catalyze the formation of acyl-CoA. Fatty acyl-CoA is formed by the formation of thioester bond between the carboxyl group of the fatty acid and the thiol group of coenzyme A. This reaction also involves use of energy, by breakdown of ATP to AMP + PP_i and which is an irreversible reaction. Fatty acyl-CoA formed in the cytosol can also be used for synthesis of phospholipids and triacylglycerols.

Transport of fatty acyl CoA to mitochondria:

Carnitine Shuttle System and Transport Mechanism

Since, the mitochondrial membrane is impermeable to acyl-CoAs, the organ liver and other tissue mitochondria is unable to oxidize fatty acids or fatty acyl-CoA's . Acyl-CoAs use the carnitine shuttle to be imported into mitochondria. Fatty acyl-CoA thioesters that are formed at the outer mitochondrial membrane cannot directly enter the mitochondrial matrix, where the enzymes of β-oxidation are located, because the inner mitochondrial membrane is impermeable to CoA and its derivatives. The reversible transfer of fatty acyl residues from CoA to carnitine is catalyzed by carnitine palmitoyltransferase I (CPT I), which is an enzyme of the outer mitochondrial membrane. There are two isoforms that are important for FAO. CPT1A (gene CPT1A), also called liver CPT1, is not only expressed in the liver, but also in the brain, kidney, lung, spleen, intestine, pancreas, ovary and fibroblasts. CPT1B (gene CPT1B) is the muscle isoform that is highly expressed in heart, skeletal muscle and testis. Both proteins are present at the outer mitochondrial membrane and are sensitive to inhibition by malonyl-CoA. Carnitine acylcarnitine translocase (CACT, SLC25A20) exchanges acylcarnitines for a free carnitine molecule from the inside. Once the acylcarnitines have entered the mitochondria, CPT2 (gene CPT2), located at the mitochondrial inner membrane, reconverts the acylcarnitines into their CoA esters, which can then undergo FAO (Ramsay et al., 2001; Bonnefont et al., 2004).

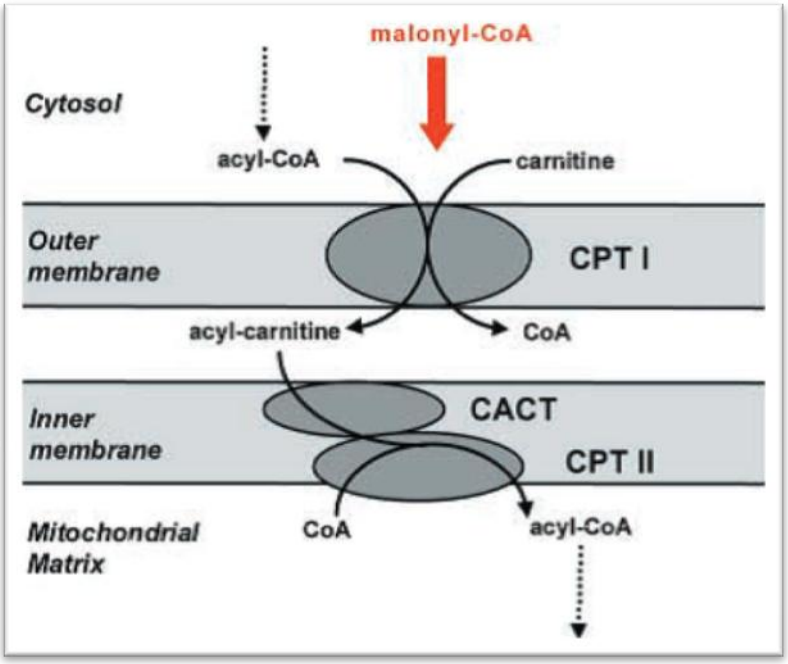

Fig 1: Carnitine shuttle system

CPT1C is a brain-specific CPT with a currently unknown function (Price, 2002). Both CPT1 and CPT2 are primarily involved in the import of (dietary) long-chain acyl-CoAs, such as palmitoyl-CoA, oleoyl-CoA, and linoleoyl-CoA. Alternatively, carnitine can be converted in the mitochondrial matrix into an acylcarnitine by the action of CPT2 or carnitine acetyl transferase (CAT, gene CRAT). These acylcarnitines can cross the mitochondrial membrane also in the opposite direction via CACT, resulting in the transport of these acylcarnitines into the cytosol. Acylcarnitines can also cross the plasma membrane, but the mechanism is currently unknown. After crossing the plasma membrane, acylcarnitines are excreted from the body via either urine or bile. This detoxification mechanism is especially important when acyl-CoAs accumulate; for example, in disorders of mitochondrial FAO.

The fatty acyl-CoA present in the mitochondrial matrix is ready for β-oxidation by the enzymes present there to yield acetyl-CoA which then enters the Kreb's cycle to give energy. This carnitine shuttle is a rate limiting step in the oxidation of fatty acids in the mitochondria and thus fatty acid oxidation can be regulated at this step. Malonyl CoA, an intermediate of fatty acid synthesis present in the cytosol is an inhibitor of carnitine acyltransferase I. This indicates that when fatty acid synthesis is in progress, oxidation of fatty acid cannot occur at the same time as the carnitine shuttle is impaired by inhibition of carnitine acyltransferase I.

Mitochondrial oxidation of fatty acids takes place in 3 stages**:**

First Stage : β- oxidation pathway

In this stage, the fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the C-16 fatty acid palmitic acid (palmitate at pH 7) undergoes 7 passes through this oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles, the last two carbons of palmitate (originally C-15 and C-16) are left as acetyl-CoA. The overall result is the conversion of 16-carbon chain of palmitate to 8 two-carbon acetyl-CoA molecules.

Second Stage: Citric acid cycle

In this stage of fatty acid oxidation, the acetyl residues of acetyl-CoA are oxidized to CO_2 *via* the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acid oxidation, thus, enters a final common pathway of oxidation along with acetyl-CoA derived from glucose *via* glycolysis and pyruvate oxidation.

Third Stage: Mitochondrial respiratory Chain

The first two stages of fatty acid oxidation produce the electron carriers, NADH and FADH2, which in the third stage donate electrons to the mitochondrial respiratory chain, through which electrons are carried to oxygen. Coupled to this flow of electrons is the phosphorylation of ADP to ATP. Thus, energy released by fatty acid oxidation is conserved as ATP.

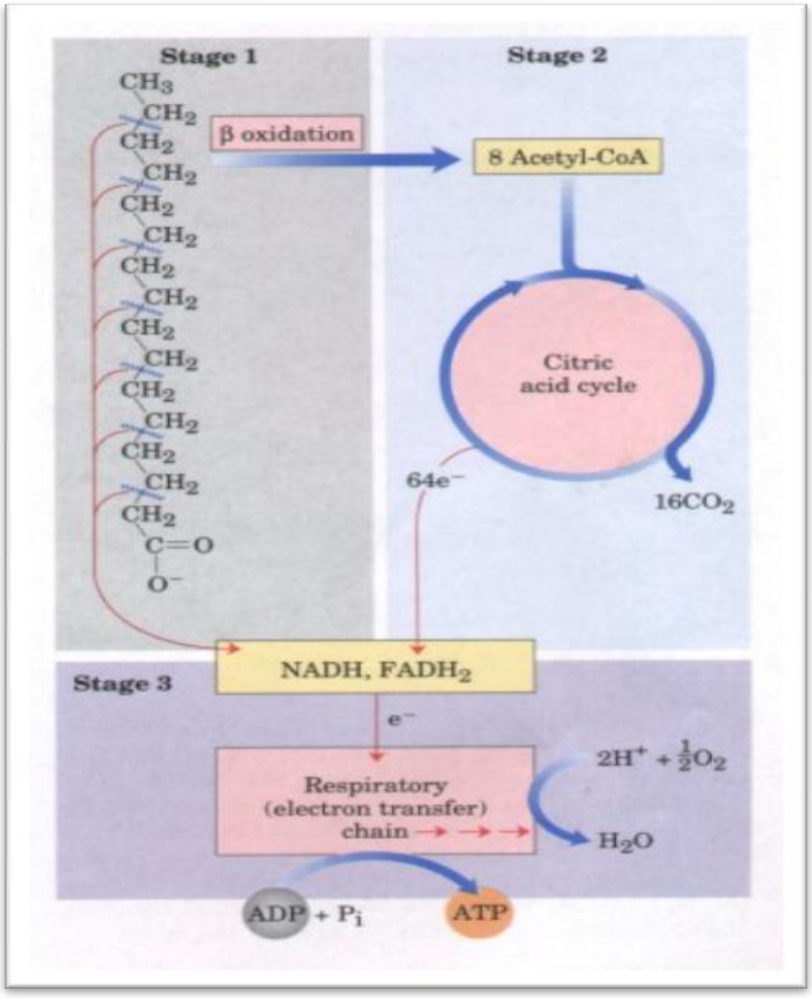

Fig 2: Stages of mitochondrial β-oxidation

Mitochondrial β-oxidation

The enzymes of β-oxidation either are associated with the inner mitochondrial membrane or are located in the mitochondrial matrix. Four acyl-CoA dehydrogenases with different but overlapping chain length specificities cooperate to assure the complete degradation of all fatty acids that can be metabolized by mitochondrial β-oxidation. The names of the four dehydrogenases, short-chain, medium-chain, long-chain, and very-long-chain acyl-CoA dehydrogenases, reflect their chain-length specificities.

Different steps

The first stage of fatty acid oxidation for the simple case of a saturated chain with an even number of carbons, and for the slightly more complicated cases of unsaturated and odd-number chains, will now be described in detail. Once the fatty acids are transported to the mitochondrial matrix via carnitine pathway, β-oxidation of fatty acyl-CoA (n carbons) occurs within the mitochondria in four steps. Each cycle of B-oxidation, liberating a two carbon unit-acetyl CoA, occurs is a sequence of four reactions:

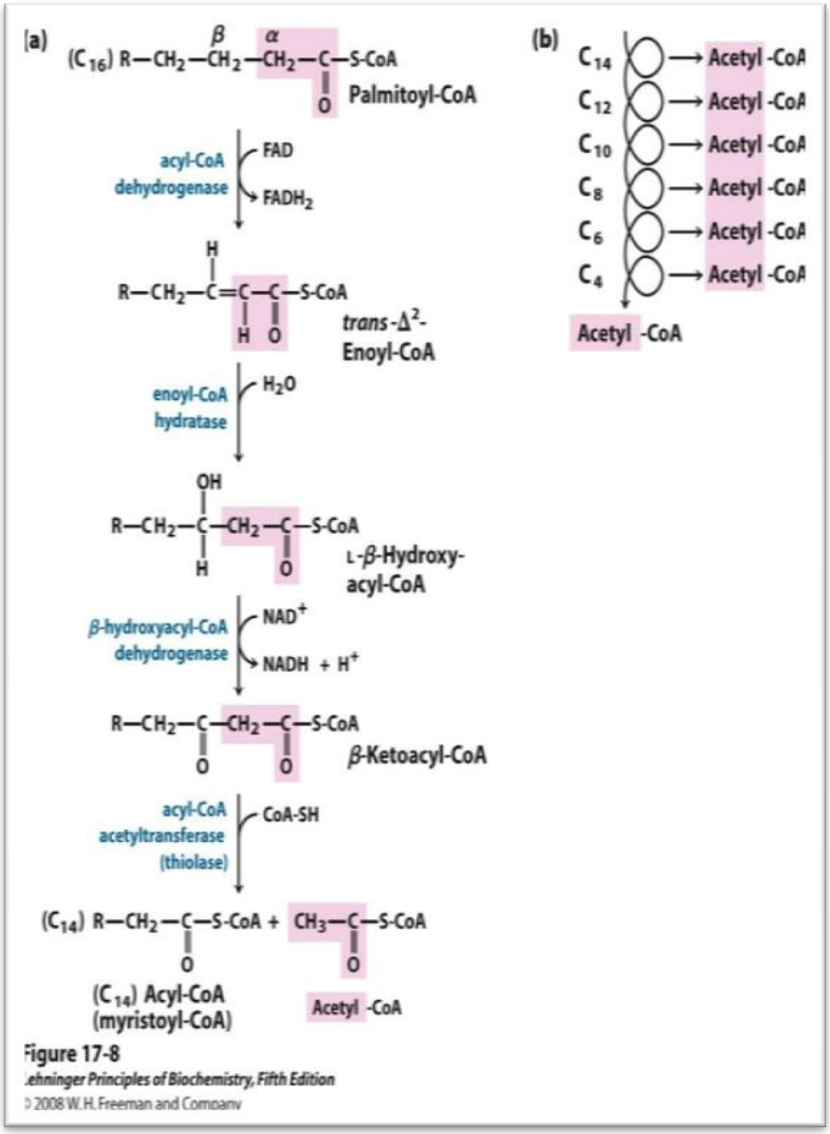

Fig 3: Reactions occurring during β-oxidation steps of fatty acid

1. Oxidation

Fatty acyl-CoA is acted upon by an enzyme *acyl-CoA dehydrogenase* which is FAD dependent enzyme. Fatty acyl-CoA undergoes dehydrogenation and forms a trans-double bond at the α and β carbons to form trans-Δ^2-enoyl-CoA. *Acyl-CoA dehydrogenase* are present as three isoenzymes each specific for a particular carbon chain length (short, intermediate and long). The electrons which were removed from the fatty acyl-CoA chain are transferred to FAD which gets reduced to $FADH_2$. This $FADH_2$ immediately via the Electron Transport System gets converted to ATP molecules.

2. Hydration

Enoyl-CoA hydratase or cronotase catalyzes this reaction where one molecule of water is added trans-Δ^2-enoyl-CoA. Hydration occurs at the double bond resulting in the formation of β-hydroxyacyl-CoA, also called as 3-hydroxyacyl-CoA.

3. Oxidation

β-hydroxyacyl-CoA undergoes dehydrogenation to form β-ketoacyl-CoA in the presence of *β-hydroxyacyl-CoA dehydrogenase*. The electrons available as a result of dehydrogenation are accepted by NAD^+ to form NADH + H^+ which immediately exchanges these electrons with oxygen in the Electron Transport System to form ATP molecules.

4. Thiolysis or Thioclastic scission

This reaction is called as thiolysis as *acyl-CoA acetyltransferase* (also known as *thiolase or* β- ketothiolase)

In the presence of CoA-SH which causes the cleavage of β-ketoacyl-CoA to form acetyl CoA and the thioester of the original fatty acid with two carbons less. This cleavage occurs as the β carbon ketone group is a good target for nucleophilic attack by the thiol (-SH) group of the coenzyme A. The shortened acyl-CoA then undergoes another cycle of oxidation, starting with the reaction catalyzed by acyl-CoA dehydrogenase. Beta-ketothiolase, hydroxyacyl dehydrogenase and enoyl- CoA hydratase all have broad specificity with respect to the length of the acyl group. Thus, by repeated turns of the cycle, a fatty acid is degraded to acetyl-CoA molecules with one being produced every turn until the last cycle, wherein two are produced. Acetyl CoA formed from the above steps now enters the Kreb's cycle to get oxidized to CO_2 and H_2O. The β-oxidation of fatty acids completes in a cyclical manner (David and Cox, 5th ed.; Jeremy 7th ed.; Reginald, 5th Edition; Satyanarayana. 3rd Edition).

Stoichiometry of β-oxidation

Each β-oxidation cycle can be represented as following:

$C_{(n)}$Acyl-CoA + CoA-SH + FAD + NAD^+ + H_2O → $C_{(n-2)}$Acyl CoA + Acetyl CoA + $FADH_2$ + NADH + H^+

Complete oxidation of Palmitoyl CoA can be represented as following:

(Equation A)

Palmitoyl CoA + 7CoA-SH + 7FAD + 7NAD^++ 7H_2O → 8Acetyl CoA + 7$FADH_2$+ 7NADH + 7H^+

Converting NADH and $FADH_2$ to their corresponding ATP equivalents from the above equation gives us:

(Equation B)

Palmitoyl CoA + 7CoA-SH + 7O_2 + 28P_i + 28ADP → 8Acetyl CoA + 28ATP + 7H_2O

And after Acetyl CoA molecules enter Kreb's cycle and Electron Transport Sytem, resulting ATP is shown below:

8Acetyl CoA + 16O_2 + 80Pi + 80ADP → 8CoA + 80ATP + 16CO_2 + 16H_2O

Thus complete energy release is shown in the following equation by combining equation A and B:

Palmitoyl CoA + 23O_2 + 108P_i + 108ADP → CoA + 108ATP + 16CO_2 + 23H_2O

Oxidation of of palmitic acid yields 7 NADH + 7 FADH2 + 8 acetyl-CoA in 7 cycles of mitochondrial beta oxidation. Every acetyl-CoA yields 3 NADH + 1 FADH2 + 1 GTP (=ATP) during Krebs cycle. Considering an average production of 2.5 ATP/NADH and 1.5 ATP/FADH2 using the respiratory chain, 108 ATP molecules are produced. However, 2 ATP molecules were consumed during the initial activation of Palmitate to Palmitoyl-CoA which is oxidized in the mitochondria. So, net energy output = (108 - 2) = 106 ATP with new concept in modern practice but ≈129 ATP with an old concept. One molecule of NADH gives 2.5 molecules of ATP and one molecule of $FADH_2$ gives 1.5 molecules of ATP with new concept but 3 molecules of ATP and one molecule of $FADH_2$ gives 2 molecules of ATP in the Electron Transport System (ETC) had been outdated and were practiced earlier.

Table 1: Energetics of Palmitic acid Oxidation

Mechanism	ATP Yield (New Concept)	ATP Yield (Old Concept)
1. β-oxidation 7 cycles 7 $FADH_2$ and 7 NADH are generated when oxidized by ETC	7$FADH_2$- 7 x 1.5 = 10.5 7 NADH- 7 x 2.5 = 17.5	7 x 2 =14 7 x 3= 21
2. From 8 acetyl CoA 1 acetyl CoA =3 NADH + 1 $FADH_2$ + 1 GTP Oxidized by citric acid cycle, each acetyl CoA provides ATP	10 x 8 = 80	12 x 8 =96
Total Energy from one mole of palmitoyl CoA	**108**	**131**
Energy utilized for activation (Formation of Palmitoyl CoA)	2	2
Net yield of oxidation of one mole of palmitate	108-2 =**106**	131-2 = **129**

Regulation of Fatty Acid Oxidation

Since mitochondrial β-oxidation functions either directly to 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 intra-mitochondrial controls. A major control on b-oxidation, and a crossover between fatty acid metabolism and carbohydrate oxidation, was elucidated by McGarry & Foster in the 1970s (McGarry &

Foster,1980). When carbohydrate is plentiful, its mitochondrial oxidation causes accumulation of citrate within the mitochondrion which may then be exported.

The carnitine shuttle is a rate limiting step in the oxidation of fatty acids in the mitochondria and thus fatty acid oxidation can be regulated at this step. Malonyl CoA**,** an intermediate of fatty acid synthesis present in the cytosol is an inhibitor of carnitine acyltransferase I. This indicates that when fatty acid synthesis is in progress, oxidation of fatty acid cannot occur at the same time as the carnitine shuttle is impaired by inhibition of carnitine acyltransferase I. Fatty acid oxidation is also regulated by the acetyl CoA to CoA ratio**:** as the ratio increases, the CoA-requiring thiolase (the enzyme participating in β-oxidation) reaction decreases. When [NADH]/[NAD+] ratio increases, the *enzyme* β-hydroxyacyl-CoA dehydrogenase is inhibited (DiMauro & DiMauro, 1973; Doege & Stahl , 2006; Kim & Simon , 2004).

Disorders of Mitochondrial Beta oxidation

Mitochondrial fatty acid β-oxidation disorders (FAODs) are a heterogeneous group of defects about 20 defects in fatty acid transport and mitochondrial β-oxidation. They are inherited as autosomal recessive disorders and have a wide range of clinical presentations. FAODs have a varied presentation, with either neonatal onset with hyperammonemia, transient hypoglycemia, metabolic acidosis, cardiomyopathy and sudden death or late onset with neuropathy, myopathy and retinopathy (Bruno & Dimauro, 2008; Shekhawat et al., 2005). Most cases with FAODs are now identified using newborn screening by mass spectrometry (MS/MS) of blood spots. Pregnancies of mothers heterozygous for FAOD have been associated with development of severe pre-eclampsia, acute fatty liver of pregnancy and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) in mothers and intrauterine growth retardation in infants (Roe & Mochel, 2006; Preece & Green , 2002).

The first inherited defects in the FAO pathway were identified in the 1970s, carnitine palmitoyl transferase 2 (CPT2) deficiency in 1973, primary carnitine deficiency in 1975 and medium chain acyl-coenzyme A (CoA) dehydrogenase (MCAD) deficiency in 1976 (Karpati et al., 1975; Gregersen et al., 1976). Most FAO enzymes were purified in the 1980s (Furuta S et al., 1981), followed by the cloning of the individual genes and the subsequent identification of disease-causing mutations in patients (Matsubara et al.,1990; Yokota et al., 1990; Kelly; 1990). For almost each enzyme involved in FAO, inherited defects have been described (Wanders et al., 1999; Rinaldo et al., 2002; Sander & Ronald, 2010). These include glutaric aciduria type 2, primary carnitine deficiency and deficiencies of CPT1, CACT, CPT2, VLCAD, MTP (including isolated LCHAD or thiolase), MCAD, M/SCHAD, SCAD and 2,4-dienoyl CoA reductase (DECR). Interestingly, CPT1b, crotonase, MCKAT and DCI deficiency have not been identified as of yet (Rinaldo et al., 2002)

In general, FAO defects have three different presentations (Wanders et al., 1999; Rinaldo et al., 2002). The first is the hepatic presentation, which is a severe, often lethal, disease in infancy or the neonatal period with hypoketotic hypoglycaemia and Reye-like syndrome. This disease is triggered by a catabolic state, for example during intercurrent infections. Most importantly, this condition can be prevented, which is the main reason for the inclusion of FAO defects in neonatal screening programmes. During infancy, patients may also present with cardiac symptoms such as dilated or hypertrophic cardiomyopathy and/or arrhythmias. Alternatively, FAO defects might present as a milder, later ('adult') onset disease. This form is characterized by exercise-induced myopathy and rhabdomyolysis (Sander & Ronald, 2010). Severely affected patients may display combinations of all three presentations. In addition, FAO defects have been associated with sudden infant death, Jamaican Vomiting Sickness that may have been caused by hypoketotic hypoglycaemia or cardiac disease.

Mitochondrial fatty acid oxidation disorders comprise 4 groups:

(1) Disorders of the entry of long-chain fatty acids into mitochondria,
(2) Intramitochondrial β-oxidation defects of long-chain fatty acids affecting membrane bound enzymes,
(3) β-oxidation defects of short- and medium- chain fatty acids affecting enzymes of the mito-chondrial matrix and
(4) Disorders of impaired electron transfer to the respiratory chain from mitochondrial β-oxidation (Blau, 2014).

Classification of FAODs

The different fatty acid oxidation disorders (The Philadelphia Guide, 2005) could be classified as follows:

(1) Disorders of plasma membrane functions
- Carnitine uptake defect
- Long-chain fatty acid transport/binding defect

(2) Disorders of fatty acid transport across the mitochondrial membranes
- CPT I deficiency
- CACT deficiency
- CPT II deficiency\

(3) Disorders of long-chain fatty acid β-oxidation
- VLCAD deficiency
- Trifunctional protein deficiency and isolated long-chain L3-hydroxyl-COA dehydrogenase deficiency

(4) Disorders of medium-chain fatty acid β-oxidation
- MCAD deficiency

- Medium- and short-chain L3-hydroxyl-COA dehydrogenase deficiency
- Medium-chain 3-ketoacyl-CoA thiolase deficiency

(5) Disorders of short-chain fatty acid β-oxidation: SCAD deficiency

Table 2: Inherited disorders of beta oxidation defect

Defect	Clinical manifestations of defect			
	Hepatic	Cardiac	Skeletal Muscle	
			Acute	Chronic
Carnitine cycle				
CTD	+	+		(+)
CPT-1	+			
Trans	+	+		+
CPT-2	+	+	(+)	+
β-Oxidation cycle				
Acyl-CoA dehydrogenases				
VLCAD	+	+	+	+
MCAD	+			
SCAD				+
3-Hydroxyacyl-CoA dehydrogenases				
LCHAD	+	+	+	
SCHAD			+	+
MCKT			+	+
DER				+

CPT, carnitine-palmitoyl transferase; *CTD*, carnitine-transporter defect; *DER*, 2,4-dienoyl-coenzyme-A reductase; *LCHAD*, long-chain 3-hydroxyacly-coenzyme-A dehydrogenase, *MCAD*, medium-chain acyl-coenzyme-A dehydrogenase; *MCKT*, medium-chain ketoacyl-CoA thiolase; *SCAD*, short-chain acyl-coenzyme-A dehydrogenase; *SCHAD*, short-chain 3-hydroxyacyl-coenzyme-A dehydrogenase; *TRANS*, carnitine/acylcarnitine translocase; *VLCAD*, very-long-chain acyl-coenzyme-A dehydrogenase

Sudden Infant Death Syndrome (SIDS)

Disorders of fatty acid oxidation play a diminutive but considerable role in high risk for metabolic diseases as the cause of unexpected death in infants and young children [(Lundemose et al., 1977). Although, a link between sudden, unexpected, infant deaths and inherited metabolic diseases was made almost 30 years ago, but there has been increased interest in this topic recently (Anonymous,1996). In particular, it has been claimed that an abnormality of fatty acid β-oxidation, medium chain acyl CoA dehydrogenase (MCAD) deficiency, could cause 3% of cases of sudden infant death syndrome (SIDS) (Howat et al., 1984) and inherited metabolic diseases could account, in total, for about 10% of these deaths (Emery et al., 1988).

SIDS is the unexpected death of an apparently well infant over one month of age, for which no cause can be found, in spite of a post-mortem examination (Beckwith,1970). The exact cause of such sudden unexplained death in infants under one year of age remains unknown in approximately 80% of cases (Hunt, 2001). Of the known causes, infections account for the highest number of “natural” causes (Platt et al., 2000; Cote et al., 1999; Sinclair-Smith et al., 1976). The diagnosis of SIDS or sudden unexplained death in infancy (SUDI), still remains the largest single cause of death in children in the industrialized world. The frequency is reported at 1:1000 live births and represents 25% of all deaths in the first year of life. Emery was the first to monitor that a broad array of metabolic disorders may present as sudden infant death syndrome (Sinclair-Smith et al., 1976). It may result from dramatic cardiac

failure, shock or cardiac arrest in many metabolic circumstances. At least 31 metabolic disorders are listed as causes of SIDS, there is some doubt as to the validity of some reports (Saudubray & Charpentier, 2000). In practice with the exception of the fatty acid oxidation defects, the majority of these disorders do not strictly present as SIDS but rather as an acute metabolic crisis with clear clinical symptoms, which precedes death by hours or even a few days.

The most likely metabolic causes of sudden unexplained death are listed below:

- Inherited defects of fatty acid oxidation and ketogenesis.
- Urea cycle disorders - most commonly ornithine transcarbamoylase (OTC) deficiency.
- Organic acidurias e.g. methylmalonic (MMA), propionic (PA) and isovaleric aciduria (IVA). Congenital lactic acidosis i.e. pyruvate dehydrogenase deficiency (PDH),
- Respiratory chain disorders,
- Biotinidase deficiency.
- Carbohydrate disorders e.g. galactosaemia, glycogen storage disease type I (GSD I), hereditary fructose intolerance, fructose 1,6-bisphosphatase deficiency.

Jamaican Vomiting Sickness (JVS)

Ackee fruit toxicity has been known since the nineteenth century and popularly called “Jamaican vomiting sickness” because of the characteristic severe bouts of vomiting (Barceloux, 2009). The term *ackee*, is derived from “anke” and “akye-fufuo,” which are used to describe the ackee apple fruit commonly found in west Africa (Grunes et al., 2012; Atolani et al., 2009). It is the national fruit in Jamaica where the toxicity is endemic (Barceloux, 2009; Emanuel & Benkeblia, 2012) 56,59]. Ackee fruit is known scientifically as *Blighia sapida* belonging to the sapindaceae family (Atolani O et al., 2009). Ackee fruit poisoning is caused by ingestion of the unripe arils of the ackee fruit, its seeds, and husks induces severe hypoglycemia presumably as a result of inhibiting fatty acid oxidation.

The study of hypoglycin, which causes Jamaican vomiting sickness in humans (Meda et al., 1999; Oludolapo et al.,2015) stimulated an interest in inhibitors of fatty acid oxidation. In animals, hypoglycin is metabolized by deamination and oxidative decarboxylation to methylenecyclopropylacetyl-CoA, which inactivates several acyl-CoA dehydrogenases and thereby inhibits β-oxidation (Joskow et al., 2006). Hassel and Reyle in 1954 first isolated the two toxic constituents, hypoglycin A and B from the arils and seeds of the unripe ackee (Hassal & Reyle, 1955) respectively which inhibits the acyl CoA dehydrogenase and thus, beta oxidation of fatty acids is blocked, leading to various complications (Satyanarayana, 3rd edn) but mainly induce severe hypoglycemia. Hypoglycin A is metabolized by the liver to methylene cyclopropyl acetic acid, a toxic metabolite that inhibits the transport of long-chain fatty acids into mitochondria, suppressing their oxidation. This impairs gluconeogenesis resulting in hypoglycemia after glycogen stores are exhausted. Hypoglycin A also inhibits the dehydrogenation of several acyl-coenzyme A, causing an accumulation of serum fatty acids (Tanaka et al., 1976). Hepatotoxicity that may occur is related to the metabolites of the toxin while CNS manifestations are attributable to direct toxic effect and hypoglycemia. The unripe ackee fruit contains hypoglycin A in a concentration 100 times higher than those in the ripe ackee fruit, whereas hypoglycin B found only in the seeds of the fruit has a less-potent hypoglycemic activity than A (Golden & Williams, 2002; Kean & Hare, 1980).

It is characterized by acute gastrointestinal illness and hypoglycemia. In severe cases, central nervous system (CNS) depression can also occur. Toxicity is dose dependent and usually manifests within 6–48 hours of ingestion with recovery usually within 1 week (Meda et al., 1999). Symptoms begin with and then subsequently more vomiting, seizures, and coma. In fatal cases, death usually occurs within 48 hours of ingestion (Meda et al., 1999; Oludolapo et al., 2015; Joskow et al., 2006).

Clinical manifestations of Jamaican vomiting sickness are: (Meda et al., 1999; Oludolapo et al., 2015; Joskow et al., 2006).

- Hypoglycemia
- Hepatic injury
- Aciduria

Clinical features of FOAD Deficiency

There may be a range of clinical presentations ranging from mild liver dysfunction, cardiomyopathy and/or skeletal myopathy to severe liver disease that may present with a recurrent Reye-like syndrome that may start in the infantile period with hepatic steatosis, unexplained hepatic failure and non-ketotic hypoglycemia (Rudolph & Rudolph, 2011). Stressors such as fasting may exacerbate the hepatic disease.

General manifestations

- Extreme sleepiness
- Behavior changes
- Irritable mood
- Enlarged heart
- Heart failure
- Fever
- Nausea/Vomiting
- Diarrhoea
- Decreased Appetite
- Hypoglycemia
- Muscle weakness

Specific Manifestations

Long Chain Fatty Acid Transport/Binding Effect

- Episodic acute liver failure

- Encephalopathy
- Hyperammonemia

Carnitine Palmitoyl transferase I (CPT-I) Deficiency (Gempel et al., 2002)

Carnitine Palmitoyl transferase II (CPT-II) Deficiency (Gempel et al., 2002)
- Classic muscle form: Fasting/Stress—Episodic Myoglobinuria
- Severe neonatal form: Hypoketotic Hypoglycemia, heart, liver,seizures/coma

Carnitine – Acyl Carnitine Translocase (CACT) Deficiency (Angelini , 1992)
- Neonatal form: Cardiac Fasting intolerance—Coma
- Mild Form: Hypoglycemia

Very Long Chain Acyl-CoA Dehydrogenase (VLCAD) deficiency
- C type: Cardiac, sudden death
- H type: Hepatic, episodic hypoketotic hypoglycemia

Long Chain Acyl-CoA Dehydrogenase (LCHAD) Deficiency
- Fasting—Episodic hypoketotic hypoglycemia
- Maternal HELLP Syndrome
- Muscle, heart, Liver and Eyes

Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency
- Fasting--- Episodic hypoketotic hypoglycemia
- Vomiting
- Acidosis
- Coma

Short Chain Acyl-CoA Dehydrogenase (SCAD) Deficiency
- Neonatal: Vomiting Acidosis, Developmental delays
- Chronic: Muscle Weakness

Diagnosis

Prenatal Diagnosis

This diagnosis is made by biochemical or molecular methods following chorionic villus sampling or amniocentesis. The most preferred technique is mutation analysis, if the molecular defect is known in the index case (Vance & Vance, 2002). With history of maternal liver disease with complicating pregnancies, prenatal diagnosis becomes compulsory (Shekhawat et al., 2005).

Diagnosis in Newborns

Acylcarnitine profiling of blood spots using tandem mass spectrometry is the screening technique for new born cases. The determination of blood acylcarnitine profiles

- Fasting Intolerance
- Hypoketotic Hypoglycemia
- Seizures
- Coma

by tandem mass spectrometry from filter paper blood spots allows detection of fatty acid oxidation disorders caused by deficiencies of MCAD, VLCAD, LCHAD/TFP, ETF/ ETF-DH, SCHAD, SCAD and HMG-CoA lyase. In CPT-1 deficiency, total carnitine levels are increased (150–200% of normal) (Stanley et al., 1992). In all of the other defects, except HMG-CoA synthase deficiency, total carnitine levels are reduced to 25-50% of normal (secondary carnitine deficiency). Thus, simple measurement of plasma total carnitine is often helpful to determine the presence of a fatty acid oxidation disorder. It should be emphasized that samples must be taken in the well-fed state with normal dietary carnitine intake because patients with disorders of fatty acid oxidation may show acute increases in the plasma total carnitine during prolonged fasting or during attacks of illness.

The following FAODs are diagnosed by newborn screening:
- CACT deficiency
- CPT II deficiency (neonatal and late onset)
- VLCAD deficiency
- MCAD deficiency
- SCAD deficiency and a few other disorders like electron transport flavoprotein-ubiquinone oxidoreductase (ETF-QO) deficiency,
- α-ETF deficiency and
- β-ETF deficiency (Sim et al., 2002)

Diagnosis in Children and Adults

Fatty acid transport studies using fibroblasts reveal possible fatty acid transporter defects. Liver biopsy may be necessary if patient present with primarily hepatic dysfunction and may reveal steatosis (Rudolph & Rudolph, 2011). The diagnosis of FAODs even postmortem may help in genetic counseling and evaluation of siblings (Blau N, 2014). Blood and urine samples collected immediately prior to treatment of an acute episode of illness can be used for this purpose, e.g. by showing elevated plasma free fatty acid but inappropriately low ketone levels at the time of hypoglycemia.

The main laboratory studies include routine labs such as:
- Complete blood count (CBC)
- Basic metabolic panel (BMP)
- Hepatic panel
- Ammonia
- Lactate
- Creatine phosphokinase (CPK)
- Acylcarnitine levels
- MS/MS analysis of organic acids,

- Plasma carnitine and
- urine acylglycine analysis, with a definitive diagnosis based on mutation analysis or measurement of specific enzyme activity (Sim et al., 2002).

Table 2: Fatty acid-oxidation disorders with distinguishing metabolic markers

Disorder	Plasma acylcarnitines	Urinary acylglycines	Urinary organic acids
VLCAD	Tetradecenoyl-		
MCAD	Octanoyl-	Hexanoyl-	
	Decenoyl-	Suberyl-	
		Phenylpropionyl-	
SCAD	Butyryl-	Butyryl-	Ethylmalonic
LCHAD	3-Hydroxy-palmitoyl-		3-Hydroxydicarboxylic
	3-Hydroxy-oleoyl-		
	3-Hydroxy-linoleoyl-		
DER	Dodecadienoyl-		
ETF and ETF-DH	Butyryl-	Isovaleryl-	Ethylmalonic
	Sovaleryl-	Hexanoyl-	Glutaric
	Glutaryl-		Isovaleric
HMG-CoA lyase	Methylglutaryl-		3-Hydroxy-3-methylglutaric

DER, 2,4-dienoyl-coenzyme A reductase; *ETF*, electron-transfer flavoprotein; *ETF-DH*, ETF dehydrogenase; *HMG-CoA*, 3-hydroxy-3-methylglutaryl-coenzyme A; *MCAD*, medium-chain acyl-coenzyme A dehydrogenase; *SCAD*, short-chain acyl-coenzyme A de hydrogenase;*VLCAD*, very-long-chain acyl-coenzyme A dehydrogenase

In vitro

Cultured skin fibroblasts or lymphoblasts from patients can also be used to demonstrate a general defect in fatty acid oxidation using 14C or 3H-labeled substrates. In addition, different chain-length fatty acid substrates can be used with these cells to localize the probable site of defect. Tandem mass spectrometry using deuterated stable isotopes fatty acids has become an important method for *in vitro* testing in cultured cells. In the hepatic presentation of any of the fatty acid oxidation disorders, a liver biopsy obtained during an acute episode of illness shows an increase in neutral fat deposits which may have either a micro- or macrovesicular appearance (Sim et al., 2002; Vishwanath, 2016).

Enzyme Assays

Cultured skin fibroblasts or cultured lymphoblasts have become the preferred material in which to measure the in vitro activities of specific steps in the fatty acid oxidation pathway. All of the known defects, except HMG-CoA synthase, are expressed in these cells and results of assays in cells from both control and affected patients have been reported. Because these assays are not widely available, they are most usefully applied to confirm a site of defect that is suggested by other clinical and laboratory data (Vishwanath, 2016)

Treatment and Management

Acute Illnesses

When patients with fatty acid oxidation disorders become ill, treatment with intravenous glucose should be given immediately. Delay may result in sudden death or permanent brain damage. The goal is to provide sufficient glucose to stimulate insulin secretion to levels that will not only suppress fatty acid oxidation in liver and muscle, but also block adipose tissue lipolysis. Solutions of 10% dextrose, rather than the usual 5%, should be used at infusion rates of 10 mg/kg per min or greater to maintain high to normal levels of plasma glucose, above 100 mg/dl (5.5 mmol/l). Resolution of coma may not be immediate, perhaps because of the toxic effects of fatty acids for a few hours in mildly ill patients or as long as 1–2 days in severely ill patients (The Philadelphia Guide, 2005)

Long-Term Therapy

The goal would be to stop fat catabolism by preventing further fatty acid oxidation. The initial steps would be the prevention of hypoglycemia in periods of catabolic stress by using frequent feeds and clinical supervision during periods of illnesses. A low fat, high carbohydrate diet is recommended. Dietary fat restriction is not indicated in MCAD deficiency and mild long-chain FAODs recently identified by newborn screening. Long chain fat, however,

needs to be restricted in severe long chain FAODs and substituted by medium-chain triglycerides [44]. Hospital admission is recommended for procedures that would require the patients to take nothing orally for >8 h, especially if less than 1 year of age. Carnitine is undisputedly effective in patients with carnitine transporter deficiency (Blau N et al., 2014). Liver transplantation may be the ultimate consideration if there is no evidence of neurological disease or other systemic involvement that may impair recovery and return to baseline function (Vishwanath, 2016; Angelini et al., 2011).

Other Therapy

Since medium-chain fatty acids bypass the carnitine cycle and enter the midportion of the mitochondrials-oxidation spiral directly, it is possible that they might be used as fuels in defects which block either the carnitine cycle or long-chain s-oxidation. For example, dietary MCT was suggested to be helpful in a patient with LCHAD deficiency. The benefits of MCT have not been thoroughly investigated, but MCT clearly must not be used in patients with MCAD, SCAD, SCHAD, ETF/ETF-DH, HMG-CoA synthase, or HMG-CoA lyase deficiencies. Some patients with mild variants of ETF/ETF-DH and SCAD deficiencies have been reported to respond to supplementation with high doses of riboflavin (100 mg/day), the cofactor for these enzymes. Triheptanoin was suggested to be of benefit I three cases of VLCAD as an anaplerotic substrate, but has not yet been confirmed by controlled studies (Roe et al., 2002).

Prognosis

Although acute episodes carry a high risk of mortality or permanent brain damage, many patients with disorders of fatty acid oxidation can be easily managed by avoidance of prolonged fasts. These patients have an excellent long-term prognosis. Patients with chronic cardiomyopathy or skeletal muscle weakness have a more guarded prognosis, since they seem to have more severe defects in fatty acid oxidation. For example, TRANS or the severe variants of CPT-2 and ETF/ETF-DH deficiencies frequently lead to death in the newborn period. On the other hand, the mild form of CPT-2 deficiency may remain silent as long as patients avoid exercise stress (Sim et al., 2002; Vishwanath, 2016)

CONCLUSION

The oxidation of fatty acids in mitochondria plays an important role in energy metabolism and genetic disorders of this pathway may cause metabolic diseases. Enzyme deficiencies can block the metabolism at defined reactions in the mitochondrion and lead to accumulation of specific substrates causing severe clinical manifestations. This review completes lucidly with the fundamentals of the pathway of mitochondrial β-oxidation, control of pathway flux, FAODS and its clinical manifestations.

ACKNOWLEDGEMENT

Author would like to thank Ms. Khushbu Yadav, Medical Microbiologist and Lecturer, Nepal Dental Hospital and Research Institute, Janakpurdham, Nepal for her valuable positive critics during preparation of this manuscript.

Conflict of Interest: None Declared

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