1. Introduction

Despite the abundant literature dealing with the metabolism of fatty acid in the heart, there is a limited understanding (and to the best of our knowledge no comprehensive review) concerning the role that cardiac lipid and fatty acid metabolism plays in the genesis and progression of cardiac failure.

What is presently known is:

(a) Fatty acids and associated lipids play an important role in cardiomyocytes structure and function. There is considerable evidence that in the post-natal and adult mammalian heart, fatty acid β oxidation is the preferred pathway for the energy that is required for efficient cardiac pumping.

(b) Specific defects (either inherited or acquired) in mitochondrial fatty acid metabolism may cause cardiomyopathy and arrhythmias that can lead to cardiac failure.

In this review, we discuss the information available concerning the molecular and cellular basis of fatty acid and lipid metabolic perturbations which can lead to cardiac failure. In this context, we will focus on the molecular and biochemical players as well as the events that occur in both the genetic abnormalities in fatty acid metabolism that lead to cardiomyopathy and cardiac failure, as well as in cardiac hypertrophy and apoptosis. The term cardiac failure is broadly used as a pathophysiologic state where the heart is unable to meet the metabolic requirements of the body. Most of this review is directed to our understanding of the mechanisms, diagnosis and treatment of fatty acid defects occurring in human cardiac failure. Experimental findings of defects in fatty acid metabolism are also discussed with respect to current and future use of animal models.

Since the literature is abundant (including numerous reviews) on the subject of acquired and inherited lipid disorders in the development of coronary artery disease and stroke (e.g., cholesterol, the apolipoproteins ... LDL), we have omitted these subjects from our review.

2. Role of fatty acids and their metabolism in the normal cardiomyocyte

2.1. Structural and regulatory roles in cardiac cell membranes

Fatty acids play an integral role in determining the...
structural and functional nature of the cardiac plasma and mitochondrial membranes. Their influence on the fluidity and stability of membrane structure markedly impacts on membrane functions such as transport of the ions and substrates, and the electrophysiology which is intrinsic to cardiac function and cardiac excitability. In addition to the structural and functional roles played within the cardiac membrane, fatty acids and associated lipids are also recognized as regulatory molecules with roles in cell signaling, second messengers in transduction, as effectors in apoptosis (cell death) and in responses to oxidative and ischemic damage.

2.2. Transporters and carriers

Entry of fatty acids into the myocardial cell while not fully delineated is presently thought to be mediated by several proteins including fatty acid binding proteins (FABPs) and a myocardial-specific integral membrane transporter (fatty acid translocase or FAT). The non-enzymatic FABP also serves as a facilitator of intracellular transport of relatively insoluble long-chain fatty acids to sites of metabolic utilization (e.g., mitochondria). In mammals, the FABP content in skeletal and cardiac muscle is related to the fatty acid oxidation capacity of the tissue [1].

Prior to transport into the mitochondria, fatty acids must be activated in the cytoplasm. The net result of this activation process is the consumption of ATP, and requires CoA-SH. Activation is catalyzed by fatty acyl-CoA synthetases (at least three different acyl-CoA synthetase enzymes have been described whose specificities depend on fatty acid chain length) associated with either the endoplasmic reticulum or the outer membrane of the mitochondria.

For the β oxidation pathway to function, the fatty acyl-CoA has to be transported across the inner mitochondrial membrane. Long-chain fatty acyl-CoA molecules cannot pass directly across the inner mitochondrial membrane and need to be transported as carnitine esters whereas short-chain and medium-chain fatty acids can be easily transported without the assistance of carnitine. The transport of long-chain fatty acyl-CoA into the mitochondria depicted in Fig. 1 is accomplished via an acyl-carnitine intermediate, which itself is generated by the action of carnitine palmitoyltransferase I (CPT-I), an enzyme residing in the inner face of the outer mitochondrial membrane. The resulting acyl-carnitine molecule is subsequently transported into the mitochondria by the carnitine translocase, a transmembrane protein residing in the inner membrane which delivers acyl-carnitine in exchange for free carnitine from the mitochondrial matrix. CPT-II located within the inner mitochondrial membrane catalyzes the regeneration of the fatty acyl-CoA molecule, with the acyl group transferred back to CoA from carnitine. Once inside the mitochondrion the fatty acid-CoA is a substrate for the β oxidation machinery.

A key regulatory event in the uptake of fatty acids into myocardial mitochondria involves changes in the levels of the metabolite malonyl CoA which functions as a potent allosteric inhibitor of CPT-I. Malonyl CoA is synthesized by the enzyme acetyl coA carboxylase (ACC) from cytoplasmic acetyl CoA. Levels of malonyl CoA are altered by changed levels of the acetyl CoA or by modulation of the ACC activity. Levels of the cytoplasmic acetyl CoA increase either as a function of decreased TCA cycle activity reflecting lowered metabolic demand or as a result of increased pyruvate dehydrogenase activity. Therefore malonyl CoA production linked to altered metabolic demand or utilization of carbohydrate resources can in turn impact on either the down or up-regulation of fatty acid import into mitochondria and myocardial fatty acid oxidation. ACC activity is allosterically regulated by citrate and by kinase-mediated phosphorylation.

2.3. Bioenergetics of fatty acid oxidation

The mitochondrial fatty acid β oxidation pathway contains four reaction steps including acyl-CoA dehydrogenases (short-chain, SCAD, medium-chain, MCAD, long-chain, LCAD and very-long-chain, VLCAD), short-chain enoyl-CoA hydratase, β-hydroxyacyl CoA dehydrogenase and β-ketoacyl CoA thiolase as shown in Fig. 2.

In the initial acyl-CoA dehydrogenase reaction, VLCAD and LCAD are responsible for the enzymatic dehydrogenation of long-chain C8–C22 fatty acids (e.g., palmitate and linoleic acids) with VLCAD having greater activity with the longer-chain substrates (e.g., C22 and C24 acyl-CoA esters), MCAD is active with the C4–C12 straight-chain fatty acids (e.g., decanoic acid) and SCAD primarily is active with C2–C4 fatty acids (e.g., butyryl coA).

The remaining enzymatic reactions of β oxidation are performed by a highly organized single enzymatic complex known as the mitochondrial trifunctional protein (MTP) associated with the mitochondrial inner membrane. Recent studies have indicated that an entirely different set of enzymes responsible for the β oxidation of medium and short-chain fatty acids is present in the mitochondrial matrix [2,3].

The process of fatty acid oxidation is termed β oxidation since it occurs through the sequential removal of 2-carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule. Each round of β oxidation produces NADH, FADH₂, and acetyl-CoA. The acetyl-CoA, the end product of each round of β oxidation, enters the TCA cycle, where it is further oxidized to CO₂ with the concomitant generation of NADH, FADH₂ and ATP. The NADH and FADH₂ generated during the fat oxidation and acetyl-CoA oxidation in the TCA cycle will subsequently enter the respiratory pathway for the production of ATP. Consequently, the oxidation of fatty acids yields more energy per carbon atom than does the oxidation of
Fig. 1. Fatty acid and carnitine entry into the myocyte and transport into the mitochondria for oxidation. FABP, Fatty acid binding protein; FAT, fatty acid translocase; CPT-I, carnitine palmitoyltransferase I; CPT-II, carnitine palmitoyltransferase II; MCAD, medium-chain acyl CoA dehydrogenase; SCAD, short-chain acyl CoA dehydrogenase; LCAD, long-chain acyl CoA dehydrogenase.

2.4. Cellular location

Both peroxisomes and mitochondria have multiple enzymes involved in fatty acid β oxidation. The peroxisomal enzymes include palmitoyl CoA oxidase, 1-functional carbohydrates. However, while fatty acids produce more ATP during complete aerobic oxidation than glucose, this occurs at the expense of a higher rate of oxygen consumption. The supply of oxygen can be an important determinant of myocardial fuel utilization.
Fig. 2. Intersection of three mitochondrial bioenergetic pathways: fatty acid β-oxidation, electron transport chain (OXPHOS) and TCA cycle. ETF, Electron transfer flavoprotein; cytc, cytochrome c; MTP, mitochondrial trifunctional protein; MCAD, medium-chain acyl CoA dehydrogenase; LCAD, long-chain acyl CoA dehydrogenase; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; TCA cycle, tricarboxylic acid or Krebs cycle; OAA, oxaloacetate; M, malate; C, citrate; F, fumarate; IC, isocitrate; KG, ketoglutarate; S, succinate; S-CoA, succinyl CoA.

protein and 3-ketoacyl oxidase which are all inducible enzymes acting on straight-chain substrates; in addition, peroxisomes contain branched-chain acyl CoA oxidase, d-functional protein and sterol-carrier protein X, which are non-inducible and primarily use branched-chain substrates. The inducible enzymes increase in response to the peroxisomal proliferating activating receptor (PPAR) resulting in increased peroxisomal biogenesis (see below).

It is important to note that while specific deficiencies in the mitochondrial-located enzymes involved in fatty acid β-oxidation may result in cardiomyopathy (as discussed more fully below), defects in the peroxisomal fatty acid β-oxidation enzymes primarily result in neurological complications including seizures, hypotonia and psychomotor retardation. Interestingly, cardiac abnormalities have been rarely described in peroxisomal deficiencies. This is also true of diseases involving general peroxisomal biogenesis abnormalities such as Zellweger syndrome and neonatal adrenoleukodystrophy where there is little or no cardiac involvement. Recent evidence (discussed below) demonstrates a pivotal role of the PPAR regulation in both mitochondrial fatty acid oxidation and mitochondrial biogenesis, not only in normal cardiac growth and development but also in cardiac failure. This suggests an important inter-relationship between the two cellular compartments and further underscores the mitochondrial compartment as a critical effector of cardiac homeostasis. The commonality of biogenesis and potential feedback between these two
cellular organelles needs further elucidation in both normal growth and development and in cardiac disease (both fatty acid and mitochondrial OXPHOS disorders).

3. Changes occur in fatty acid regulation during cardiac growth and development

3.1. Fetal to post-natal transition

In the fetal heart which functions in a relatively hypoxic environment, glucose and lactate are the predominant fuel substrates utilized by glycolysis and lactate oxidation, respectively. Postnatally, a switch occurs so that fatty acids become the chief energy substrate thereafter in the adult mammalian heart [4]. Genes encoding the fatty acid enzymes are expressed at low levels in the fetal and neonatal rat heart and are significantly and coordinately upregulated (≥70%) in adult rat hearts compared to the fetal expression patterns. This switch operates at both transcriptional and post-transcriptional levels [4]. For example, MCAD mRNA levels in rats increased 2–3-fold at birth followed by a decline during the first postnatal week in heart and liver. The pattern of accumulation of MCAD mRNAs in rat heart during the weaning and early adult periods was similar [5].

3.2. Post-natal to adult

The expression patterns of mitochondrial carnitine palmitoyltransferase (CPT) enzymes examined in the developing rat heart show a marked change during this period. The specific activity of CPT-II increases approximately threefold during the first month of life as does myocardial carnitine levels. Two kinetically different tissue-specific isoforms of CPT-I, CPT-I α and CPT-I β, have been identified which are immunologically distinct proteins [6]. The CPT-I α or L form is expressed in highest abundance in the liver, pancreas and heart; the β gene or M form is expressed predominantly in skeletal muscle, adipose tissue, heart and testis. Cardiac ventricular myocytes are the only cells known to express both CPT-I isoforms. Developmentally, expression of CPT-I α gene is very high in the fetal heart and declines following birth. CPT-I β, is also highly expressed in fetal myocytes and remains so throughout development. Therefore, in the adult heart CPT-I α represents a very minor constituent, while its contribution is much greater in the newborn. CPT-I α is also subject to hormonal regulation, increasing during fasting and diabetes, and decreasing upon weaning with a high carbohydrate diet; CPT-I β gene is not subject to the same developmental or hormonal controls imposed on CPT-I α [6,7].

As previously noted, specific membrane-associated and cytoplasmic proteins are involved in the uptake of long-chain fatty acids by cardiac and skeletal muscle cells [8]. This is supported by data demonstrating the co-occurrence of these proteins in muscle and their co-ordinate regulation during development in both cardiac and muscle tissue [9]. Transcripts for the integral membrane fatty acid transport protein (FATP), fatty acid translocase (FAT), the plasma membrane-associated fatty acid-binding protein (FABPpm) and the cytoplasmic heart-type fatty acid-binding protein (H-FABPc) showed high expression levels in heart, somewhat lower in red skeletal muscle and significantly lower in white skeletal muscle. However, FATP, FAT and H-FABPc displayed three- to fivefold increases in mRNA expression during cardiac growth, while the FABPpm expression remained relatively constant [9].

3.3. Aging and senescence

Many changes have been noted in cardiac muscle with advancing age related to modifications in membrane fatty acid composition, decreasing levels of polyunsaturated fatty acids and increasing levels of saturated fatty acids. Several studies have also reported significant reduction in heart mitochondrial cardiolipin content with aging [10,11]. The unique anionic phospholipid cardiolipin is the principle polyglycerophospholipid (carrying four acyl groups and two negative charges) found in the heart, the most unsaturated cellular phospholipid and localizes predominantly in mitochondria. The age-related reduction in cardiolipin is considered to have major impact on cardiac mitochondrial membrane transport function, fluidity and stability. Studies with cultured adult cardiomyocytes isolated from rat hearts of broad age range also exhibited changes in the fatty acid profile related to alterations in the mechanism of desaturation and elongation of essential fatty acids. The ability of heart cells to metabolize linoleic acid to higher and more unsaturated metabolites decreased with age. With aging, the pattern of fatty acids of the cultured cardiomyocytes showed a gradual but significant shift, similar to that reported in the whole heart [12].

Presently, data on cardiac carnitine levels in healthy aging people is sparse. However, a marked reduction of carnitine and its derivatives in muscle, and of long-chain acylcarnitine has been reported in hearts of older mice and rats, when compared to younger animals. Analysis of muscle samples of healthy humans showed drastic reduction of carnitine and acetyl carnitine in older subjects with strong reverse correlation between age and carnitine levels [13,14].

4. Disorders of fatty acid metabolism affect cardiac structure/function

In cardiac failure following cardiac hypertrophy, there is a major switch in myocardial bioenergetic substrate used, from fatty acid to glucose. A key component and marker of the switch is the co-ordinate down-regulation of fatty acid
β oxidation enzymes and mRNA levels (>40%) in the human left ventricle [15].

This switch is thought to represent a reversal to a fetal energy substrate preference pattern in the heart. During the development of cardiac hypertrophy, a fetal metabolic gene program is initiated via the complicity of transcription factors which bind to regulatory elements reducing fatty acid oxidation gene expression (e.g., MCAD and CPT-I β). Although the molecular mechanism(s) mediating this down-regulation is not fully understood, the participation of several nuclear receptors, intermediate metabolites and transcription factors (e.g., SP1 and PPAR) has been implicated in this programmatic change in gene expression (see further discussion in Section 5) [16].

4.1. Specific heritable (inborn) deficiencies in fatty acid metabolism are associated with cardiomyopathy and cardiac failure

Table 1 presents a list of disorders affecting fatty acid metabolism which can result in cardiomyopathy and/or cardiac failure, with their characterized genetic loci.

Heritable defects in mitochondrial acyl-CoA dehydrogenase have been described in cardiomyopathy and cardiac failure [17]. In general, defects in the oxidation of long-chain fatty acids are more likely to cause cardiomyopathy than defects in medium-chain or short-chain fatty acids. Specific defects in enzymes involved in long-chain and very long-fatty-acid-chains have been identified [18–20] and the genetic defects will be described further in the molecular section below. Moreover, although severe cardiomyopathy is unusual in patients with MCAD deficiency, sudden death in children is a common outcome, and its pathogenetic mechanism is presently undetermined. Similarly, a recent study indicates that cardiomyopathy can be part of the clinical phenotype of SCAD deficiency [21].

Carnitine deficiency has been frequently associated with severe cardiomyopathy. Mutations in proteins that participate in carnitine transport and metabolism may cause dilated cardiomyopathy as a recessive trait [22,23].

One of the loci effected in carnitine-associated cardiac involvement includes the plasma-membrane localized carrier which transports carnitine into the cell; its deficiency has been described as primary carnitine deficiency [24]. This transport deficiency is due to specific defects in the gene (OCTN2) encoding the plasma-membrane localized organic cation/carnitine transporter [25]. Defects in a second locus, the mitochondrial membrane localized carnitine translocase also lead to carnitine deficiency resulting in cardiomyopathy and cardiac failure [26].

Although generally not found associated with cardiomyopathy/cardiac failure, new evidence suggests that CPT-I deficiency can result in cardiac involvement [27]. In contrast, there is general consensus that deficiencies in CPT-II (specifically infantile CPT-II deficiency), an autosomal recessive disorder, is associated with cardiac damage and sudden death [28].

4.2. Secondary effects on mitochondrial fatty acid β oxidation: relationship to mitochondrial respiration and OXPHOS

The utilization of fatty acids as an energy source requires the functional operation of the mitochondrial electron chain and OXPHOS; the NADH feeds into the electron transport chain at complex I and electrons are transferred from acyl CoA dehydrogenases via the electron-transfer flavoprotein (ETF), ETF dehydrogenase and ubiquinone (or coenzyme Q) to complex III as depicted in Fig. 2 [29]. Affected individuals with deficiencies in the ETF pathway display impaired fatty acid oxidation and abnormal intra-mitochondrial accumulation of fatty acids and glutaric acid and may develop a fatal cardiomyopathy [29,30]. Similarly, patients with defects in respiratory complexes (e.g., complexes I and IV) will frequently develop cardiomyopathy and cardiac failure largely as a result of impaired energy production [31] However, the extent of the effects on the cardiac fatty acid β oxidation and on lipid accumulation in patients with defined respiratory activity defects and with defective coenzyme Q levels has not yet been fully assessed.

4.3. Fatty acid metabolism defects can be associated with either HCM or DCM

With the exception of defects in the MTP that affect long-chain L-3 hydroxylacyl-CoA activity and are associated with DCM [32], most of the disturbances in fatty acid metabolism are found in patients with hypertrophic cardiomyopathy (HCM) and many of the reported mutations in fatty acid β oxidation pathway result in HCM rather than dilated cardiomyopathy (DCM) [33].

Barth syndrome, an X-linked disorder, is characterized by a triad of dilated cardiomyopathy, neutropenia and
increased levels of 3-methylglutaconic aciduria with onset often occurring in infancy. Arrhythmias and heart failure are frequently present. The protein tafazzin (encoded by the G4.5 gene) is mutated and responsible for Barth syndrome with associated cardiomyopathy [34,35]. While the biochemical function of the tafazzin protein has not yet been determined, structural analysis suggests that tafazzin belongs to a family of acyltransferases involved in phospholipid synthesis [36].

A recent report reveals that the levels of cardiolipin are markedly reduced in cultured fibroblasts from patients with the G4.5 mutation. This may be the first report of a human disorder with a defect in cardiolipin metabolism [37], and it may prove to be useful to assess the cardiac levels of cardiolipin in patients with Barth syndrome.

4.4. Abnormalities in fatty acid oxidation lead to cardiac arrhythmias and conduction defects

Conduction defects and cardiac arrhythmias have been shown to be frequently present in patients with certain defects in fatty acid oxidation [38]. Specifically, these cardiac defects were present in patients with deficiencies in CPT-II deficiency, carnitine translocase and MTP deficiency. Cardiac arrhythmias were notably absent in patients with: deficiencies in CPT-I, the primary carnitine carrier and MCAD.

These findings strongly suggest that the accumulation of arrhythmogenic intermediary metabolites of fatty acids (e.g., long-chain acylcarnitines) may be responsible for arrhythmias and potentially contributory to cardiac failure and sudden death. This is consistent with the findings that long-chain acylcarnitines accumulate with the defects in CPT-II, carnitine translocase and MPT whereas MCAD, CPT-I and carnitine carrier do not result in accumulation of these intermediates.

Amphiphilic long-chain acylcarnitines have detergent-like properties, can extensively modify membrane proteins and lipids, and have a variety of toxic effects on the electrophysiological function of cardiac membranes including ion transport (Na⁺, Ca²⁺), and impaired gap junction activity. This is further supported by the demonstration that patients with cardiomyopathy due to inborn defects in carnitine translocase have an increased onset of cardiac arrhythmias [39]. Moreover, the accumulation of long-chain fatty acid intermediates (e.g., acylcarnitine) has been implicated in the genesis of ventricular rhythm disorders during myocardial ischemia [40]. Selective blocking of CPT-I activity prevents the accumulation of potentially toxic long-chain esters during hypoxia/ischemia, thereby reducing the risk of electrophysiologic disturbance and membrane disruption [41].

Studies to define the precise site of action of these toxic long-chain intermediates within the cardiomyocytes e.g., at the level of the plasma membrane, mitochondrial matrix or membrane(s) may prove to be of great significance in the development of new therapies.

4.5. Fatty acids and cardiac apoptosis

Apoptosis (programmed cell death) plays a prominent role in the myocyte loss that occurs in human cardiac failure [42,43]. It also plays a major role in the extensive cardiac remodeling that encompasses the transition from cardiac hypertrophy to heart failure (in models such as the spontaneously hypertensive rat) [44].

One of the hallmarks of apoptosis and an early regulatory event is the release of cytochrome c from mitochondria into the cytosol. The release of cytochrome c has been shown to be necessary for the activation of a cascade of downstream cysteine–aspartate proteases (caspases) and nuclear endonucleases. The mechanism of cytochrome c release from the inner mitochondrial membrane has not been fully elucidated. In addition, mitochondrial membrane permeabilization is a critical early step of apoptosis preceding the caspase cascade. The permeabilization is accompanied by an early dissipation of the mitochondrial transmembrane potential [45]. Opening the mitochondrial permeability transition pore is accompanied by the depolarization of the mitochondrial membrane. A component of the inner membrane which is associated with the mitochondrial pore as well as with cytochrome c is the phospholipid cardiolipin. Cardiolipin also mediates the targeting of the pro-apoptotic protein, tBid to mitochondria implicating cardiolipin in the pathway for cytochrome c release [46]. It is also thought to play a role in membrane permeability and proton conductance as well as functioning of cytochrome c oxidase.

During ischemia, oxidation of the saturated fatty acid palmitate is associated with diminished myocyte function [47]. Saturated long-chain fatty acid substrates such as palmitate (but not mono-unsaturated fatty acids) readily induce apoptosis in rat neonatal cardiomyocytes [48,49].

As an early feature of palmitate-induced cardiomyocyte apoptosis, palmitate diminishes the content of the mitochondrial cardiolipin by causing a marked reduction of cardiolipin synthesis. Decreased levels of cardiolipin synthesis and cytochrome c release have been recently reported to be directly temporally correlated. This suggests that cardiolipin modulates the association of cytochrome c with the mitochondrial inner membrane [50].

Palmitate also decreases the oxidative metabolism of fatty acids and causes increases in the intracellular second messenger ceramide [47] paralleling a decrease in complex III activity. The decrease in fatty acid metabolism (e.g., CPT-I activity declines) and complex III activities and ceramide accumulation have been shown to be downstream events occurring well after cytochrome c release (and changes in the permeability transition pore) [49–51].
5. Molecular players and events in fatty acid related cardiac diseases; genes and modulation of gene expression

5.1. MCAD

MCAD deficiency is autosomally recessive and associated with sudden death. Over 90% of cases of MCAD deficiency are associated with a homozygous mutation at base pair (bp) 985 (A985G). This mutation directs a glutamate replacement of lysine at residue 304 in the mature MCAD subunit causing impairment of tetramer assembly and increased protein instability [52]. MCAD deficiency is the most frequent inborn metabolic disorder in populations of Northwestern European origin [53].

At the gene level, three of the seven reported non A985G mutations found in MCAD deficiency localize to exon 11. At the protein level, the mutant residues cluster in helix H of the MCAD protein and are proposed to have their primary effect on the correct folding and assembly of the tetrameric MCAD enzyme structure. The amino acid residues effected are: M301T, S311R and K304E [54].

5.2. VLCAD

Pediatric cardiomyopathy is the most common clinical phenotype of VLCAD deficiency. A severe form of infantile cardiomyopathy is found in over 67% of cases, often resulting in sudden death [20]. VLCAD deficiency is characterized by a marked reduction of VLCAD mRNA and decreased levels and/or absence of VLCAD enzyme activity. Mutation analysis of the VLCAD gene revealed a large number of different mutant loci (21 in 19 patients) with few repeated mutations [55]. Distinguishing between truly pathogenic mutations and polymorphic variations remains to be done.

5.3. CPT-II

The infantile form of CPT-II deficiency has frequent cardiac involvement and is associated with specific CPT-II mutations. This is in contrast to the adult form of CPT-II deficiency which does not present with cardiac involvement; hence mutations involved in the adult CPT-II phenotype (although residing within the same gene) are not further discussed in this review.

The infantile CPT-II deficiency has been associated with several mutations including a homozygous mutation at A2399C causing a Tyr→Ser substitution at residue 628. This mutation produces a marked decrease in CPT-II activity in patient fibroblasts [56]. Another mutation has been reported at C1992T predicting an Arg→Cys substitution at residue 631 which is associated with drastic reduction of CPT-II catalytic activity [57].

5.4. MTP

MTP, an enzyme of β oxidation of long-chain fatty acids, is a multi-enzyme complex composed of four molecules of the α-subunit (encoded by HADHA) which contains both the enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase domains, and four molecules of the β-subunit (encoded by HADHB) containing the 3-ketoacyl-CoA thiolase domain [58].

MTP deficiency is classified into two different biochemical phenotypes: (1) both α and β subunits are present and only the 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity is effected; (2) the absence of both subunits, and the complete lack of all three enzymatic activities of MTP. Although there is some overlap between the clinical features found in each molecular/biochemical phenotype, patients with neonatal cardiomyopathy have the second biochemical phenotype only. The most common mutation associated with MTP deficiency (G1528C) is associated with the first phenotype and not with the second. Mutations have been localized to the 5′ donor splicing site of the α subunit gene which can result in the entire loss of an exon in the mRNA (exon 3) [32] and are associated with the second phenotype. Both DNA and enzymatic testing can be performed in fetal screening of this often devastating disease [59].

5.5. PPAR

A family of nuclear gene receptors, peroxisomal proliferating activating receptors (PPARs) has been identified as playing a key role in the transcriptional regulation of genes involved in intra-cellular lipid and energy metabolism including fatty acid oxidation enzymes [60]. These receptors are enriched in tissues that are dependent on lipid utilization for energy metabolism (e.g., heart, liver, brown adipose tissue) playing a major role in the rapid mobilization of bioenergetic stores in response to physiological stresses.

PPAR is a transcription factor which activates expression of a constellation of genes encoding enzymes involved in both peroxisome and mitochondrial fatty acid oxidation (e.g., mitochondrial MCAD, CPT-I and peroxisomal acyl CoA oxidase). PPAR activity is dependent on the presence of a variety of activating ligands (e.g., prostaglandins, eicosanoids, long-chain unsaturated fatty acids, etc.) and interacting proteins (i.e., co-activators and co-repressors). Activated PPAR–ligand complex binds to a DNA response element in the promoter region of specific genes activating transcription [61]. Cardiac metabolic gene expression is activated by PPARγ regulation during postnatal development, during short-term starvation and in response to exercise training. One marker of PPAR activation is up-regulated MCAD expression.

Conversely, pressure-overload hypertrophy results in the
de-activation of PPARα with lower fatty acid oxidation expression, abnormal cardiac lipid homoeostasis and reduced energy production [62]. The negative regulation of PPARα is mediated at several levels with PPAR gene expression being reduced during ventricular overload (in mice). In addition, PPAR activity is altered at the post-transcriptional level via the extracellular signal-regulated MAP kinase pathway. Ventricular overload therefore results in hypertrophied myocytes with intracellular fat accumulation (in response to oleate loading). At this time, the role of PPAR in the activation of the fetal gene program occurring during hypertrophy and cardiac failure has not yet been fully delineated.

There is also evidence that the PPAR interacting co-activators affect gene regulation by modulation of the chromatin structure surrounding the DNA (by changing the extent of acetylation of histone residues).

Recently, it has been shown that PPAR plays a pivotal role in mediating the effect of hypoxia on mitochondrial fatty acid oxidation in cardiac myocytes resulting in diminished CPT-I β mRNA levels. This is accomplished via PPAR transcriptional regulation (due to reduced binding of PPARα) and its obligate partner, retinoid X receptor α (RXRα) to a DNA response element residing within the CPT-I β promoter [63]. Immunoblot analysis has shown that during hypoxia, nuclear and cytoplasmic levels of RXRα are reduced whereas there is no change in PPAR levels.

5.6. Peroxisome proliferator-activated receptor gamma co-activator (PGC-1)

PGC-1 has been identified as a regulator of mitochondrial respiratory function in tissues specialized for thermogenesis, e.g., brown adipose tissue and skeletal muscle [64]. PGC-1 gene expression is induced in the mouse heart after birth and in response to short-term fasting, conditions known to increase cardiac mitochondrial energy production.

Expression of PGC-1 in cardiac myocytes has been reported to induce nuclear and mitochondrial gene expression involved in multiple mitochondrial energy-transduction production pathways, increased mitochondrial number, and increased respiration. Cardiac-specific overexpression of PGC-1 in transgenic mice resulted in uncontrolled mitochondrial proliferation in cardiac myocytes leading to loss of sarcomeric structure and dilated cardiomyopathy. These results identify PGC-1 as a critical regulatory molecule in controlling mitochondrial number and function in response to energy demands [65].

PGC-1 also has been implicated in both the increased expression of mitochondrial transcription factor A (mtTFA), which is involved in control of both mitochondrial DNA transcription and replication, and levels of the NRF-1 transcription factor which mediates the expression of a number of nuclear genes involved in mitochondrial OXPHOS including subunits of cytochrome c oxidase and ATP synthase [66].

6. Animal models of defective fatty acid metabolism and cardiac failure

A number of animal models, utilizing some of the molecular findings described in the previous section have been informative in our understanding of the initiation, severity and progression of the cardiac phenotypes associated with specific disturbances in fatty acid metabolism. Mice lacking PPARα have a cardiac phenotype of increased myocyte lipid accumulation [67]. Mice lacking MTP α and β subunits alleles show necrosis and acute degradation of the cardiac myocytes. They also accumulate long-chain fatty acid metabolites, have low birth weight and develop neonatal hypoglycemia with sudden death between 6 and 36 h after birth [68].

To test the hypothesis that disturbance in myocardial fatty acid uptake and utilization leads to the accumulation of cardiotoxic lipid species, and to establish a mouse model of metabolic cardiomyopathy, transgenic mouse lines that overexpress long-chain acyl-CoA synthetase in the heart were generated. These mice demonstrate cardiac-restricted expression of the transgene and marked cardiac myocyte triglyceride accumulation. Lipid accumulation was associated with initial cardiac hypertrophy, followed by the development of left-ventricular dysfunction and premature death [69].

The role of RXRα in cardiac failure has been further probed in the transgenic mouse as well. RXRα null mutant mice display ocular and cardiac malformations, liver developmental delay, and die from cardiac failure around embryonic day (E) 14.5. To dissect the molecular basis of the RXRα-associated cardiomyopathy, subtractive hybridization experiments to identify putative downstream target genes that were selectively lacking in the mutant embryos, showed 50% of the subtracted clones (61/115) encoded proteins that were involved in fatty acid metabolism and electron transport, suggesting an energy deficiency in the null RXRα embryos. ATP content and MCAD mRNA were lower in RXRα mutant hearts compared to wild-type mice. These findings suggest that defects in intermediary metabolism may be a causative factor of the RXRα −/− phenotype, an embryonic form of DCM [70].

7. Advances in diagnostics and treatment of fatty acid/cardiac disease

At the outset, a number of treatment recommendations can be suggested from the foregoing discussion. It is
critical that information concerning the site of defect within the fatty acid/carnitine metabolic pathway should be evaluated before treatment is undertaken. Diagnostic evaluation of fatty acid defects and carnitine levels at the biochemical level is easily performed. Early and correct diagnosis (including newborn screening using a non-invasive highly-sensitive methodology profiling acylcarnitines via tandem mass spectrometry on a blood spot collected on a Guthrie card) is important since dramatic recovery and/or prevention of arrhythmias and cardiac failure has been demonstrated in some of these disorders (e.g., VLCAD deficiency) [71]. Genetic analysis is also increasingly available, although the presence of non-repeating mutations makes this analysis more problematic.

Treatment of CPT-II deficiency includes the avoidance of fasting and/or exercise, a low fat diet enriched with medium-chain triglycerides and carnitine supplementation [72]. Acute cardiomyopathy associated with VLCAD deficiency which can be evaluated diagnostically by acylcarnitine analysis even in the neonatal period, can be treated with dietary therapy including medium-chain triglycerides [73].

Patients with cardiomyopathy secondary to primary carnitine deficiency can be treated with oral l-carnitine. Often, there is resolution of the cardiomyopathy and its recurrence may be prevented for more than 5 years [74].

Treatment of disorders of mitochondrial long-chain fatty acid oxidation is based on the avoidance of fasting and the replacement of normal dietary fat by medium-chain triglyceride. Diagnosis of these disorders is based on direct assay of the enzyme involved; however, preliminary indicators may come from determination of carnitine and intermediate metabolites in plasma, urinary organic acid profiling, and radioisotopic screening assays with lymphocytes or cultured fibroblasts [75,76]. Knowledge of the precise site of the biochemical/molecular defect is critical in the therapeutic modality to be used. For instance, deficiencies in CPT-II, carnitine acylcarnitine translocase or MTP can be treated with targeted drugs which enhance glucose use and pyruvate oxidation energy, at the expense of fatty acid oxidation, in order to prevent the accumulation of long-chain acylcarnitines that can result in increased cardiac conduction defects and arrhythmias. Long-chain fatty acid accumulation and their consequences can also be effectively reversed by inhibition of CPT-I activity with perhexiline and amiodarone treatment. The treatment of patients with congestive heart failure using carvedilol, a β-adrenoceptor blocker, has recently been shown to lead to marked improvement in myocardial energy efficiency by employing a shift of myocardial oxidative substrates from fatty acid to glucose [77].

In addition, gene therapy for some of the fatty acid disturbances of cardiac function and structure, to decrease long-chain fatty acid intermediates and redirect metabolic programs, holds great promise.

References


