Molecular and genetic aspects of cardiac fatty acid homeostasis in health and disease

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Introduction

This review will present evidence of the versatile nature and many homeostatic functions of fatty acids, one of our more common dietary substrates. Many still tend to think of fatty acids as molecules that play an obscure role in energy metabolism or associate these compounds with their role in the development of atherogenesis. We intend to demonstrate the diverse and essential role of fatty acids and, hopefully, will ignite broader clinical interest in this area of metabolism. Especially for the cardiologist, it must be exciting to realize that the myocardium is almost completely dependent upon fatty acid for the generation of energy for its indefatigable task during fasting and exercise (section 1). The balance between health and disease is tilted the wrong way when genetic changes lead to the disruption of this continuous flow of energy (section 2). There exists a balance between glucose and fatty acid utilization for energy metabolism, and utilizing one substrate to the exclusion of the other appears to favour the pathogenesis of cardiac disease (section 3). Fatty acids can further act as a double-edged sword in their capacity to either induce or prevent myocardial electrical remodelling, depending upon the dietary fatty acid species supplied to the heart (section 4). And finally, the gene expression profile of a cardiomyocyte may well be influenced by the supply of fatty acids provided on a day-to-day basis. Thus the expression 'you are what you eat' can take on new significance (section 5). The goal of this review is to distil complexities of fatty acid metabolism described in the literature and discuss the potential this complexity offers us for novel approaches in the daily treatment and management of myocardial disease.

Fatty acid energy metabolism

Fatty acids are simple molecules belonging to the lipid family and their basic biochemical structure consists of a hydrophilic (or water-soluble) group attached to one end of a hydrophobic (water-insoluble) hydrocarbon chain. Figure 1(a) depicts an example of palmitic acid, a common dietary fatty acid consisting of 16 carbon (C) atoms with all the carbons saturated with hydrogen (H) atoms. Palmitic acid is therefore referred to as a saturated fatty acid. Many important, naturally occurring fatty acids are unsaturated such as palmitoleic acid, meaning that the carbon atom chain contains one or more double bonds (Fig. 1(b)). These unsaturated bonds have important effects on the molecular structure of fatty acids, for each double bond inserts a bend in the hydrocarbon chain of the molecule, which will influence the melting point of the fatty acid. Ultimately, the fluidity of the membrane structure strongly depends upon the relative content of unsaturated to saturated fatty acid species in the membrane (Fig. 1(c) and section 4, Structural function of fatty acids).

When used to generate ATP, fatty acids are catalytically broken down in mitochondria and peroxisomes by the ω-oxidation reaction. To this end, the fatty acyl-CoA
Esters are transported into the mitochondrial matrix via a carnitine-dependent shuttle mechanism (Fig. 2). The translocation of activated fatty acids across the mitochondrial membrane involves the combined action of three enzymes: carnitine palmitoyltransferase I (CPT I) at the outer mitochondrial membrane; carnitine:acylcar- nitine translocase (CT) and carnitine palmitoyltransferase II (CPT II), which are both located at the inner mitochondrial membrane (Fig. 2). Once inside the mito-

chondrial matrix, the fatty acyl-CoA esters enter the β-oxidation, four sequential reactions that result in the cleavage of two carbon atoms from the amino terminal end of the fatty acyl-CoA molecule, generating one molecule of acetyl CoA, a fatty acyl-CoA two carbons shorter, and reducing equivalents after each turn of the cycle (Fig. 2). The shorter fatty acyl-CoA is subsequently returned to the β-oxidation spiral. The other product, acetyl CoA, can enter the citric cycle where it is

### Figure 1 Structure of fatty acids. (a) The basic chemical structure of a fatty acid exemplified by the chemical structure of a common dietary saturated fatty acid, palmitic acid. A fatty acid consists of a long hydrophobic (water insoluble) hydrocarbon chain (CH₂ or CH₃) linked to a hydrophilic head group (COOH). This fatty acid species is called saturated because it lacks a double bond in the hydrocarbon chain. (b) Chemical structure of an unsaturated fatty acid, palmitoleic acid, which only differs from its saturated counterpart palmitic acid by one double bond in the hydrocarbon chain between carbon atoms at positions 9 and 10 counted from the COOH group. Double bonds have a major impact on the molecular structure of fatty acids as they introduce a bend into the hydrocarbon chain, which ultimately influences the melting point of the fatty acid species. (c) List of most common saturated and unsaturated fatty acid species present in the human myocardium with their respective chemical abbreviation, structure formula and melting point. Note that the relative melting point of fatty acid species lowers as the number of double bonds increases.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Abbreviation</th>
<th>Structure</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>16:0</td>
<td>CH₃(CH₂)₁₄COOH</td>
<td>63.1</td>
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<tr>
<td>Stearic acid</td>
<td>18:0</td>
<td>CH₃(CH₂)₁₆COOH</td>
<td>69.6</td>
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<tr>
<td>Oleic acid</td>
<td>18:1Δ9</td>
<td>CH₃(CH₂)₁₆CH=CH(CH₂)₇COOH</td>
<td>16</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18:2Δ9,12</td>
<td>CH₃(CH₂)₁₄CH=CHCH₂CH=CH(CH₂)₇COOH</td>
<td>5</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>20:4Δ5,8,11,14</td>
<td>CH₃(CH₂)₁₈CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₇COOH</td>
<td>−50</td>
</tr>
</tbody>
</table>
Figure 2  Mitochondrial fatty acid transport and β-oxidation cycle. Fatty acids are imported across the mitochondria membranes into the mitochondrial matrix by the cooperative effort of specialized shuttle enzymes CPT-I, CT and CPT-II. The β-oxidation cycle itself is catalyzed by a series of four enzymes in the mitochondrial matrix. At the same relative position of MCAD within the β-oxidation cycle, SCAD, LCAD and VLCAD are also positioned depending on the length of the chain of the fatty acid that is being oxidized. Each turn of the cycle shortens the fatty acid chain by two carbon atoms (shown in bold) and generates one molecule of acetyl CoA and one molecule each of NADH and FADH2. The electrons carried by these coenzymes will be subsequently transferred to the electron transfer flavoproteins (ETF) and ETF-dehydrogenase (ETF-QO) which shuttle electrons to coenzyme Q in the respiratory chain in the mitochondrial inner membrane, to generate energy in the form of ATP. ACS=acyl-CoA synthase; CPT-I=carnitine palmitoyl transferase I; CT=carnitine:carnitine translocase; CPT-II=carnitine palmitoyltransferase II; MCAD=medium chain acyl-CoA dehydrogenase; SCAD=short-chain acyl-CoA dehydrogenase; LCAD=long chain acyl-CoA dehydrogenase; VLCAD=very long chain acyl-CoA dehydrogenase; ECH=enoyl-CoA hydratase; HACD=hydroxyacyl-CoA dehydrogenase; OAT=keto-acyl thiolase.

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oxidized further to generate NADH and FADH₂ as endproducts. The latter two compounds are used to generate ATP via oxidative phosphorylation (Fig. 2)[8].

**Genetic deficiencies in fatty acid metabolism**

To date, a number of specific mutations in the human genes directly involved in mitochondrial metabolism have been identified. The clinical features and severity of the pathology of these disorders vary according to the particular enzyme defect, with the most severe effects involving genes in mitochondrial β-oxidation, the process aimed at converting fatty acids into energy. In general, cardiac involvement is manifested as hypertrophic or dilated cardiomyopathy, which is occasionally accompanied by lethal ventricular arrhythmias and sudden cardiac death. The genetic defects in human cardiac fatty acid energy metabolism can be subdivided in mutations in enzymes involved in (1) fatty acid uptake, (2) fatty acid β-oxidation and (3) electron transfer to the respiratory chain.

**Mutations in genes involved in mitochondrial fatty acid uptake**

Mutations in the human genes encoding the transporter of carnitine across the sarcolemma and those encoding the mitochondrial transporters carnitine:acylcarnitine translocase (CT) and carnitine palmityltransferase II (CPT II) have been reported. Defects in these transporters would severely affect the ability of the cardiac muscle to take up fatty acids into the mitochondria for energy production. The most common human disorder of an enzyme that is involved in fatty acid transport is CPT-II deficiency, although this genetic disorder is rarely associated with muscular or cardiac disorders (see Fig. 2 for location of CPT-II)[5–8]. Primary carnitine deficiency (CD), an autosomal recessive inherited disorder, is caused by a defect in proteins essential for carnitine transport across the sarcolemma[9]. The main clinical cardiac feature is dilated cardiomyopathy and skeletal myopathy[9]. Although lethal if left untreated, CD patients respond rapidly to carnitine supplementation. Finally, mutations in carnitine:acylcarnitine translocase (CT) lead to cardiomyopathy, muscle weakness and hypoketotic hypoglycaemia and is associated with low plasma free carnitine and high levels of long-chain acylcarnitines[10], but is manageable by carnitine supplementation (see Fig. 2 for location of CT).

**Mutations in genes involved in mitochondrial β-oxidation**

Other cardiac disorders have been linked to mutations in genes from patients encoding enzymes involved in the actual β-oxidation process by which fatty acids are broken down to acetyl CoA for direct energy production (see Fig. 2 for circular process starting with MCAD and ending with OAT). The most common β-oxidational genetic defect in humans is medium chain acyl-CoA dehydrogenase (MCAD) deficiency[9]. Typically, hypoglycaemia, increased excretion of medium-chain carboxylic acids and low plasma and tissue carnitine levels have been reported in MCAD deficient patients, without obvious signs of cardiac and skeletal muscle involvement[9]. Deficiencies in the β-oxidation enzymes, very long chain acyl-CoA dehydrogenase (VLCAD) and long chain acyl-CoA dehydrogenase (LCAD) are less common, but have a more severe clinical presentation than MCAD deficiency and affect the liver, heart and muscles[9–15]. The most severe cardiac phenotypes in primary defects of β-oxidation (hypertrophic/dilated cardiomyopathy and presence of arrhythmias) are found in patients with genetic defects in very long chain acyl-CoA dehydrogenase. The reason for cardiac involvement during the progression of the disease in VLCAD deficient patients may be the formation of deleterious long chain fatty acid oxidation intermediates, which have powerful arrhythmical effects and which are not observed with the medium chain fatty acid metabolites formed in MCAD deficient patients. Clinical features of VLCAD deficiency vary from hypoketotic hypoglycaemia and dicarboxylic aciduria to lethal hypertrophic cardiomyopathy and recurrent metabolic crises[9–15].

The discovery of VLCAD and a long-chain mitochondrial trifunctional protein complex (MTP), both located in the mitochondrial membrane, suggest the existence of a distinct pathway responsible for oxidation of long-chain β-oxidation[15]. In contrast, the pathway responsible for oxidation of medium-chain and short-chain acyl-CoA is located in the mitochondrial matrix. MTP includes the activities of long-chain enoyl-CoA hydratase (ECH), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and long-chain keto-acyl thiolase (OAT). Patients with mutations in LCHAD, which affect the whole MTP enzyme complex, or with mutations in other parts of the MTP complex can develop myopathy, cardiomyopathy, heart failure and myoglobinuria[16–19].

**Mutations in genes involved in electron transfer to the mitochondrial respiratory chain**

Finally, a select number of patients with metabolic cardiomyopathy have mutations in genes encoding the mitochondrial flavoproteins, electron transfer flavoprotein (ETF) and electron transfer flavoprotein dehydrogenase (ETF-QO), which transfer electrons to coenzyme Q, that serves as a shuttle molecule in the respiratory chain. The respiratory chain serves as a molecular ‘motor’ to generate energy for the cardiac muscle cell in the form of ATP (see bottom of Fig. 2 for location of
ETF, ETF-QO and respiratory chain). Glutaric aciduria type II is a cardiac disorder that is caused by mutations in ETF or ETF-QO and is associated with massive urinary excretion of organic acids\cite{20–24}. Affected infants present with hypoglycaemia, metabolic acidosis, hypotonia, hepatomegaly and multiple congenital anomalies, leading to premature death. Infants that survive longer develop cardiomyopathy\cite{20–23}.

Animal models of mitochondrial cardiomyopathy

On a more fundamental level, molecular biological strategies have been implemented for the development of animal models displaying human metabolic cardiomyopathy, like the murine short-chain acyl-CoA dehydrogenase (SCAD)-deficiency model\cite{24–28}. Recently, the murine genes coding for long-chain and very long-chain acyl-CoA dehydrogenase (LCAD and VLCAD), the enzymes involved in the first step of mitochondrial $\beta$-oxidation of fatty acids with carbon-lengths of 10 or greater, were disrupted\cite{29}. LCAD deficient mice display many biochemical and clinical features of VLCAD deficiency in human patients. When fasted, the LCAD deficient mice become less active, maintain a hunched posture and develop a Reye-like syndrome marked by hypoketotic hypoglycaemia and accumulation of metabolites in blood, tissues and urine. Also, a subset of male LCAD null animals develop a cardiomyopathy and suffer sudden death\cite{29}, which could be aggravated by maintaining the animals on soybean and alfalfa free food (Unpublished observations, Keith Cox). These data demonstrate that the outcome of metabolic cardiomyopathy might be influenced by age, genotype, gender or environmental factors such as diets, an observation in line with the phenotypical variability of patients with metabolic cardiomyopathy. In general, VLCAD deficient mice display a phenotype reminiscent of the LCAD deficient phenotype, but less severe\cite{29}.

Collectively, these genetic models do not only confirm the causative nature of the specific mutation in certain $\beta$-oxidation genes and/or mitochondrial function, but also provide a valuable resource of animal models for testing candidate drugs or therapies to manage metabolic cardiomyopathy in patients. Interestingly, these animal models also confirm the existence of an intrinsic myocardial mechanism that senses disruption of fatty acid energy metabolism. These signals are translated into morphological changes (cardiac hypertrophy and dilatation), which in turn have negative effects on whole body physiology. As such, future research efforts should be directed towards understanding the molecular mechanisms and intracellular hypertrophic signalling pathways underlying the pathogenic cardiac responses in the genetic models of metabolic cardiomyopathy.

Genetic-based deficiencies with possible cardiac involvement can be diagnosed by molecular biological analytical methods, using a number of techniques such as polymerase chain reaction (PCR), genetic linkage analysis and DNA sequencing\cite{30,31}. With the sequencing of the human genome completed\cite{32,33}, the future undoubtedly will uncover novel human mutations related to mitochondrial disease. Efforts in basic research may demonstrate the feasibility of somatic gene therapy, aimed at replacing the mutated genetic locus by a functional one in genetically altered animal models of human inborn deficiencies of fatty acid metabolism as the ultimate treatment of inborn errors of fatty acid energy metabolism in patients.

Shift in fatty acid energy metabolism and cardiac disease

Fatty acid energy metabolism in the diabetic heart

Recent studies indicate that disrupted cardiac physiology arises not only from primary defects in energy metabolism, but that fatty acid metabolism contributes secondarily to the pathogenesis of more common disorders such as diabetic cardiomyopathy. It is well established that patients with insulin dependent diabetes mellitus (IDDM) are particularly susceptible for heart failure\cite{34,35}. Experimental evidence suggests that the chronic cardiac metabolic changes in the IDDM patient largely contribute to the deteriorated state of the heart, independent from the various vascular effects associated with the disease\cite{36}. Diabetic patients have increased adipose tissue lipolysis and subsequently have higher circulating fatty acid levels. The rate of lipolysis within the diabetic heart is also increased, in concert with an expanded myocardial triacylglycerol pool. These factors, in addition to the reduced uptake of glucose across the sarcolemma, will lead to an almost complete reliance on fatty acids of both endogenous and exogenous sources for cardiac ATP generation and has been correlated to a direct inhibitory effect on glucose oxidation\cite{37–43}.

One explanation for the impaired glucose utilization by fatty acids in type II diabetes is through a reduction of glucose transport capacity, in particularly GLUT-4 activity\cite{39,40}, through increased glycogen deposition\cite{41} or through indirect activation of the pyruvate dehydrogenase complex\cite{42}. Additionally, concomitant intracellular accumulation of potentially toxic intermediates of fatty acid oxidation has been demonstrated to modify ion channel activity and properties\cite{43–46}, interfere with adenine nucleotide pathways and reduce myocardial ATP levels. These detrimental effects will ultimately lead to cardiac dysfunction through a disturbance in intracellular calcium ($Ca^{2+}$) handling, intracellular $Ca^{2+}$ overload and activation of $Ca^{2+}$ dependent proteases and toxic $Ca^{2+}$ deposition in mitochondria and depletion of ATP stores\cite{47,48}.

Studies performed in streptozotin-induced diabetic animals indicate that activation of the pyruvate dehydrogenase complex by dichloroacetate administration
resulted in a switch from a reliance on fatty acids to glucose oxidation for energy production. An improvement in cardiac performance was observed, at least in the early stages of chronic diabetes[49]. Pharmacological intervention with other drugs such as etomoxir, which specifically block CPT-I (and indirectly fatty acid oxidation), have also demonstrated an improvement in cardiac function in a number of experimental studies using diabetic animal models[50,51].

Collectively, these promising results in animal models have provided greater insight into IDDM-related cardiac dysfunction. Furthermore, from a clinical point of view, the future development and testing of drugs that stimulate cardiac glucose utilization at the expense of fatty acids might provide a new avenue for a more favourable cardiac energy metabolism and improved myocardial performance in patients with diabetic cardiomyopathy.

**Fatty acid metabolism in ischaemic heart disease**

During oxygen deprivation as occurs in acute myocardial infarction (AMI) or during cardiac bypass surgery, both glucose and fatty acid oxidation are interrupted as a result of hypoxia[52,53]. During periods of hypoxia, affected regions of the heart are completely dependent on glycolysis (or anaerobic metabolism) for ATP production. As a result ATP levels fall rapidly as the energy yield of 1 mole glucose converted to lactate is only 5% of that yielded from the completed oxidation of 1 mole palmitate in the mitochondrial β-oxidation. However, fatty acid activation (creation of fatty acyl CoA esters) continues to take place in the cytosol and, as a consequence, the level of fatty acyl esters of CoA and acylcarnitine species rise and are detectable 2 min after the onset of experimental global ischaemia[54].

The reinstitution of coronary blood flow (reperfusion) e.g. by thrombolytics or percutaneous intervention after AMI or after release of the cross-clamp during bypass surgery, will lead to a rapid return of both oxygen consumption and overall respiration chain activity to pre-ischaemic levels. Paradoxically, despite the return of oxidative metabolism and oxygen consumption during reperfusion, which is an absolute requirement for ultimate survival of the ventricular tissue, severe post-ischaemic depression of contractile function is commonly observed. Because intracellular Ca²⁺ concentrations rise during ischaemia and further increases after reperfusion, activation of Ca²⁺ transport processes, aimed at normalizing the intracellular Ca²⁺ homeostasis, may contribute to the rise in energy expenditure[55,56]. Evidence consistent with this hypothesis was demonstrated in isolated rat hearts rendered globally ischaemic, which demonstrated improved contractile function following addition of NiCl₂ or ruthenium red during reperfusion to inhibit trans-sarcolemmal Ca²⁺ transport and Ca²⁺ transport at the inner mitochondrial membrane[57–62]. These results indicate that the oxidative rate during reperfusion might arise from processes aimed at normalizing the intracellular ion homeostasis.

Although the accumulation of fatty acid intermediates during ischaemia and changes in energy expenditure during reperfusion clearly contribute to the deterioration in mechanical function, it appears that the rise in fatty acid catabolism in the post-ischaemic period has detrimental effects on the myocardium[63,64]. It has been observed that fatty acid oxidation accounts for most of cellular oxidative metabolism during reperfusion, while glucose oxidation remains lower as compared to pre-ischaemic levels. This metabolic alteration may be due to the higher plasma fatty acid levels, which are generally observed following AMI or cardiac surgery[65]. An alternative explanation for the higher post-ischaemic fatty acid oxidation, however, relates to the increase in cardiac AMP to ATP conversion due to limited ATP generation during ischaemia. This accumulation of AMP results in phosphorylation and activation of 5'-AMP-activated protein kinase (AMPK). Activated AMPK phosphorylates serine residues in acetyl-CoA carboxylase (ACC) during reperfusion resulting in the inhibition of ACC[66–68]. Inactivation of ACC results in decreased malonyl-CoA levels and release of the malonyl-CoA inhibition of CPT-I. Elevated CPT-I activity in turn results in increased mitochondrial fatty acid uptake and oxidation, leading to inhibition of the pyruvate dehydrogenase complex and glucose oxidation. The concomitant decrease in glucose oxidation causes impaired coupling of glycolysis to mitochondrial glucose oxidation and increased proton production from glycolytically derived ATP. Indeed, recent studies indicate that elevated proton production contributes to decreased functional recovery of hearts rendered experimentally ischaemic[69,70].

Efforts to pharmacologically stimulate glucose oxidation over fatty acid oxidation during reperfusion have emphasized the detrimental effects of loss of glucose oxidation capacity of the post-ischaemic myocardium. Stimulation of the pyruvate dehydrogenase complex by administration of dichloroacetate or by using perfusion solutions with high concentrations of glucose (which increases intracellular pyruvate), have been shown to improve post-ischaemic haemodynamic recovery[71–75]. Similar improvement was observed by inhibition of fatty acid oxidation by administering compounds as etomoxir (inhibitor of CPT-I), ranolazine and l-carnitine during reperfusion, all of which inhibit fatty acid β-oxidation and favour glucose oxidation[75,76–79]. Beneficial effects were also observed in clinical studies using a reperfusion solution containing high glucose, insulin and potassium[73,76–81], although these effects have not always been reproduced[82]. Clearly, the beneficial effects of enhancing glucose oxidation during reperfusion at the expense of fatty acid oxidation merits further investigation, both in animal studies and in clinical trials. The ultimate goal would be to achieve an adjuvant therapy that would improve clinical mortality and morbidity in patients experiencing AMI by pharmaceutical
stimulation of cardiac glucose oxidation over fatty acid oxidation e.g. during the acute reperfusion phase.

**Structural function of fatty acids**

*Fatty acids in control of cardiac muscle cell survival*

Fatty acids and fatty acid-derived compounds play a critical role in cellular viability and manipulation of fatty acid homeostasis may open novel therapeutic avenues to enhance myocardial viability. In addition to serving as a source for energy production, fatty acids play a crucial role as structural components of cardiomyocyte cell membranes. The cellular membranes enclose all cells and cellular organelles and function as semi-permeable barriers that are essential for the maintenance of specific intracellular and intraorganellar environments to maintain cellular homeostasis. The major components of cell membranes are phospholipids. Phospholipids are complex molecules consisting of a hydrophilic alcohol headgroup connected via a phosphate group to the sn-3 carbon atom of the glycerol backbone and two fatty acid residues connected to the two other carbon atoms (sn-1 and sn-2 carbon atom) of glycerol (Fig. 3). As such, phospholipids are amphiphilic molecules: the hydrophobic (water insoluble) fatty acid tails will not readily mix with the extra- and intracellular aqueous environments and are

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*Figure 3* Chemical structure of phospholipids, fatty acids and lysophospholipids. Upper part of the figure indicates the chemical structure of 1-palmitoyl, 2-arachidonoyl, phosphatidylethanolamine, a common phospholipid species present in the sarcolemma of cardiac muscle cells. The two fatty acyl chains from palmitic acid and arachidonic acid are indicated by the two shaded boxes on the left, the diacylglycerol backbone by the shaded box in the centre and the alcoholic head group ethanolamine by the shaded box on the right. The position of the cleavage site of phospholipases A$_2$ (PLA$_2$) and the resultant degradation products, a fatty acid and a lysophospholipid, are indicated in the lower part.
pointed towards the inside of the double membrane layers. The hydrophilic (water soluble) polar head groups, however, have a high affinity for aqueous environments. Because of their shape and chemical nature, phospholipids readily form bilayers in aqueous environments\(^8\). The hydrophilic headgroup of the sarcolemmal phospholipids of the inner leaflet, for instance, point towards the intracellular space, while the head groups of the outer sarcolemmal leaflet are directed towards the extracellular space. The fatty acyl residues of both leaflets are buried in the inner part of the membrane (Fig. 3)\[^84\]. Different classes of phospholipids exist, which differ in the composition of the alcoholic headgroup and/or the type of fatty acid tails esterified to the glycerol backbone. In fact, cardiac membrane phospholipids are subject to a continuous turnover of both the fatty acids and the hydrophilic head groups. The cardiac muscle cell is equipped with a complement of enzymes to catalyze hydrolysis and resynthesis of membrane components\[^3\]. Regulation of cell membrane turnover allows the cardiomyocyte to respond appropriately to changes in the extracellular environment by altering the physico-chemical properties of its membranes.

Under normoxic conditions the resynthesis processes are in strict balance with the hydrolytic processes, so that the amount of intracellular non-esterified fatty acids in the normoxic cardiac tissue are extremely small\[^85,86\].

In contrast, many experimental and clinical studies have provided evidence that under pathological circumstances such as myocardial ischaemia, cardiac phospholipid homeostasis is severely disrupted, resulting in a net degradation of membrane phospholipids\[^83,87–90\], and an accompanying increase in phospholipid degradation products, fatty acids and lysophospholipids\[^85,86\]. Although restoration of flow is crucial to alleviate the impact of ischaemia and for the survival of the compromised tissue, reperfusion results in a dramatic rise in already elevated tissue levels of fatty acids and lysophospholipids. Experimental studies have demonstrated that phospholipid degradation is a critical event in the development of cardiomyocyte necrosis\[^86,91–94\]. It is therefore hypothesized that degradation of membrane phospholipids during ischaemia and reperfusion will destabilize crucial cardiac membranes such as the sarcolemma, sarcoplasmatic reticulum and mitochondrial membranes, and result in a loss of barrier function, as evidenced by the release of intracellular macromolecules, such as creatine kinase and tropon I into the extracellular environment\[^83\]. Indeed, the extent of post-ischaemic cardiac damage is clinically diagnosed by measuring plasma levels of such macromolecules. In this manner, the destabilization of muscle cell membranes contributes to necrotic cell death, and results in a reduction of functional contractile units and, ultimately, haemodynamic dysfunction.

The mechanism underlying ischaemia and reperfusion-induced phospholipid degradation is incompletely understood, but is most probably multifactorial. The observation that fatty acids, including arachidonic acid, that under normoxic conditions are predominantly esterified in membrane phospholipids (Fig. 3), readily accumulate in flow-deprived cardiac tissue, points toward an imbalance between hydrolysis and re-esterification of phospholipids. Indeed, several studies using pharmacological inhibition of phospholipase A\(_2\) support the notion that the degradation of phospholipids results directly from the activation of myocardial phospholipases A\(_2\), enzymes that degrade phospholipids to lysophospholipids and fatty acids (Fig. 3)\[^83,87–90\]. To unequivocally prove that myocardial hydrolytic enzymes are critically involved in post-ischaemic membrane phospholipid degradation, irreversible cell death and haemodynamic dysfunction, gene targeted approaches in animal models lacking phospholipase A\(_2\) enzymes are warranted. This issue is further complicated by the fact that multiple phospholipase A\(_2\) enzyme classes and isoforms exist in the mammalian myocardium, and these appear to provide functional redundancy through overlapping enzymatic activities. Accordingly, we have recently found that following acute ischaemia and reperfusion the extent of membrane damage, cellular death or post-ischaemic functional recovery was not attenuated in hearts from mice that are genetically deficient for one subtype of cardiac phospholipase A\(_2\)\[^95\].

Phospholipids and fatty acids not only play a role in necrotic cell death. During apoptosis it has been demonstrated that the normal asymmetric localization of different phospholipid species is distributed and various phospholipids become symmetrically distributed in the two leaflets because of increased activity of the enzyme scramblase\[^96–99\]. During this very early phenotypic phase of apoptosis the architecture of the sarcolemma is changed such that the cardiac muscle cell exposes large amounts of phosphatidylserine at its outer membrane leaflet. Annexins are members of a family of proteins with phospholipid-binding properties, and have strong affinity for phosphatidylserine and bind outer cell membranes of cells with an activated cell death programme\[^97,100–102\]. This feature of phospholipids is currently being used for diagnostic purposes to quantify the extent of cardiac programmed cell death in both experimental studies\[^103\] and in patients following acute myocardial infarction\[^104\]. Other potential therapeutic options based upon the characteristics of cardiomyocyte phospholipid homeostasis would be to prevent membrane damage and necrotic cell death by inhibition of cardiac phospholipase species in the setting of acute ischaemic heart disease to improve post-ischaemic functional recovery.

**Fatty acids and arrhythmogenesis**

Another phospholipid degradation product which accumulates in flow-deprived cardiac tissue in addition to fatty acids, is lysophospholipid\[^85\]. Lysophospholipids, amphiphilic metabolites that can be produced by both cardiac phospholipases A\(_1\) and A\(_2\) (Fig. 3), are recognized for their arrhythmogenic potential\[^89\]. Because lysophospholipids are metabolites derived from cardiac
membranes, the physical and chemical properties of membranes change after an increase in lysophospholipid concentrations in the membrane by inducing alterations in membrane fluidity\textsuperscript{105–107}. Some evidence points toward the ability of lysophospholipids to induce intracellular Ca\textsuperscript{2+} overload in myocytes\textsuperscript{108,109}.

Lysophospholipids have been demonstrated to inhibit sarcolemmal sodium/potassium ATPase (Na\textsuperscript{+}/K\textsuperscript{+}-ATPase) and cause bursts of sodium (Na\textsuperscript{+}) influx by prolonging the time channels remain closed\textsuperscript{110,111}. Alternatively, lysophospholipids may modify the voltage-dependent Na\textsuperscript{+} channel leading to increased Na\textsuperscript{+} permeability\textsuperscript{111}. Secondary to the above mechanisms, intracellular Ca\textsuperscript{2+} overload would then be caused by sodium- calcium (Na\textsuperscript{+}/Ca\textsuperscript{2+}) exchanger activity. Lysophospholipids have also been suggested to increase non-specific sarcolemmal permeability to Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} ions, possibly by making micro-pores in the sarcolemma through which ions non-selectively pass\textsuperscript{112}.

In animal studies it has been observed that certain polyunsaturated fatty acids (PUFAs), a special class of fatty acids with multiple unsaturated bonds, prevent fatal ischaemia-induced cardiac arrhythmias. The mechanism of the antiarrhythmic action of PUFAs has been studied in spontaneously contracting cultured cardiac myocytes of neonatal rats. Adding arrhythmogenic agents to the myocyte cultures caused tachycardia and contracture of the cultured myocytes. Adding PUFAs to the culture medium before adding the arrhythmogenic agents prevented tachyarrhythmias. Addition of PUFAs to the medium subsequent to induction of arrhythmias terminated the arrhythmias\textsuperscript{113–115}. This antiarrhythmic action was specific to dietary n-3 and n-6 PUFAs, specific fatty acid species with the unsaturated bond at position 3 or 6 within the hydrocarbon chain (see Fig. 1) which are highly enriched in fish oil. Saturated or monounsaturated fatty acids failed to attenuate arrhythmias. In fact, PUFAs are able to slightly hyperpolarize the resting membrane potential and raised the voltage threshold for opening the fast sodium channel\textsuperscript{116}. These effects demonstrate that the antiarrhythmic mode of action of PUFAs may be related to the fact that they are able to electrically stabilize myocyte membranes. In a clinical study it was inadvertently found that subjects that consume relative high amounts of n-3 fatty acids are less vulnerable to sudden death\textsuperscript{117,118} and these findings were supported by other clinical studies\textsuperscript{119–122}.

Collectively, these examples clearly demonstrate the versatile and even opposing actions of fatty acids and their derivatives on the electrophysiological characteristics of the myocardium, with fatty acyl esters and lysophospholipids exerting arrhythmogenic effects, while certain dietary polyunsaturated fatty acids may prevent life-threatening arrhythmias. From a clinical point of view, the notion that certain dietary components can help to prevent lethal arrhythmias creates an option as becoming part of antiarrhythmic treatment for patients with selected rhythm disorders.

**Fatty acids as regulators of gene expression**

As mentioned above, a number of pathological conditions of the myocardium are accompanied by a reduction in fatty acid metabolism. In recent years, evidence has accumulated that this decrease in fatty acid utilization is due to, at least in part, a downregulation of genes that are involved in the catabolism of cardiac fatty acids. It was soon realized that the expression patterns of these genes result from the influence of fatty acid metabolites directly on gene expression patterns. In fact, feeding rats a diet enriched with PUFAs increased CPT-I expression and activity in cardiac and skeletal muscle\textsuperscript{123}. Moreover, primary cardiomyocyte cultures incubated with certain fatty acid species at physiological levels displayed specific upregulation of mRNA levels of a transsarcolemmal fatty acid transporter (FAT), heart-type fatty acid binding protein, ACS and LCAD\textsuperscript{124,125}. One example of how fatty acids are thought to influence transcriptional activity of certain target genes is described below.

The peroxisome proliferator activated receptors (PPARs) are transcription factors in the family of steroid hormone receptors. PPARs have been demonstrated to bind to and be activated by fatty acids as well as a number of biological active fatty acid derivatives\textsuperscript{126–128}. Activated PPARs heterodimerize with the retinoic acid receptor X protein and bind to canonical peroxisome proliferator response elements in the promoter of target genes\textsuperscript{127,128}. Peroxisomes are essential organelles that have as one role the processing and oxidation of very-long chain fatty acids. Analysis of the cis-regulatory elements in genes responsive to PPAR activation revealed a consensus DNA site consisting of a direct repeat of two hexameric half-sites (AGG(A/T)CA), separated by one nucleotide. In addition to genes involved in peroxisome function, these peroxisome proliferator-cis-regulatory DNA elements or PPREs have been identified in other genes directly involved in fatty acid metabolism or transport, such as acyl-CoA oxidase, ACS, medium-chain acyl-CoA dehydrogenase, the liver-type fatty acid binding protein and apolipoprotein A-II\textsuperscript{127,128}. PPARs do not act alone. For their activation they need to dimerize with retinoic-X-receptors (RXR), which are activated by the vitamin A derivative 9-cis-retinoic acid\textsuperscript{129,130}. Consistent with this view, 9-cis-retinoic acid and fatty acid ligands can act together to synergistically activate the transcription of the peroxisomal gene acyl-CoA oxidase. Currently, multiple isoforms of PPARs with differing ligand selectivity and tissue distribution have been identified\textsuperscript{131,132} and are currently under investigation as to the role of fatty acid-PPAR-RXR mediated influence on gene expression in the heart.

Conclusively, the notion that common dietary components may directly regulate the gene expression profile is a relatively new and exciting cardiovascular research field. Clearly, additional research is clearly needed to unravel the significance of fatty acids on the gene
**Table 1  Explanatory list of molecular genetic terms**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Acetyl</td>
<td>Chemical group derived from acetic acid. Acetyl groups are important in energy metabolism and often added as a covalent modification of proteins.</td>
</tr>
<tr>
<td>Acetyl CoA</td>
<td>Small water-soluble molecule that carries acetyl groups in cells. Comprises an acetyl group linked to coenzyme A (CoA).</td>
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<tr>
<td>Apoptosis</td>
<td>Programmed cell death</td>
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<tr>
<td>ATPase</td>
<td>One of a large class of enzymes that catalyzes a process that involves hydrolysis of ATP</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Contents of a cell that is contained within the plasma membrane (sarcolemma) but outside of the nucleus</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>Lipid produced by cleavage of phospholipids. Composed of two fatty acid chains linked to glycerol</td>
</tr>
<tr>
<td>Ester</td>
<td>Molecule formed by the condensation reaction of an alcohol group with an acidic group</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>Compound that has a carboxylic acid attached to a long hydrocarbon chain. Used as a major source of energy during metabolism and as a starting point for the synthesis of phospholipids</td>
</tr>
<tr>
<td>Gene</td>
<td>Region of DNA that controls a discrete hereditary characteristic usually corresponding to a single protein or RNA</td>
</tr>
<tr>
<td>Genetic linkage analysis</td>
<td>Procedure to study coinheritance of a specific human disease by analysing the frequency of coinheritance of genetic markers located closely to the (unknown) disease gene</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>Ubiquitous metabolic pathway in the cytosol in which sugars are incompletely degraded with production of ATP (energy)</td>
</tr>
<tr>
<td>Membrane</td>
<td>Double layer of lipid molecules and associated proteins that encloses all cells</td>
</tr>
<tr>
<td>Mutation</td>
<td>Heritable change in the nucleotide sequence of a chromosome</td>
</tr>
<tr>
<td>NAD+/NADH</td>
<td>Nicotinic adenine nucleotide. Coenzyme that participates in an oxidation reaction by accepting an ion from a donor molecule. The NADH formed is an important carrier of electrons for oxidative phosphorylation</td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>Process in bacteria and mitochondria in which ATP formation is driven by the transfer of electrons from food molecules to molecular oxygen</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction. Technique for amplifying specific regions of DNA by multiple cycles of DNA polymerase, each followed by a brief heat treatment to separate complementary chains</td>
</tr>
<tr>
<td>Promoter</td>
<td>Regulatory nucleotide sequence in DNA to which RNA polymerase (the enzyme that produces RNA) bind to begin transcription (process of generating RNA from DNA)</td>
</tr>
<tr>
<td>RNA</td>
<td>Polymer formed from covalently linked ribo nucleotide monomers which, in case of messenger RNA (mRNA), carries genetic information for one single protein</td>
</tr>
</tbody>
</table>

expression pattern and whether this phenomenon constitutes a passive or active role in the pathogenesis of acquired forms of metabolic heart diseases. When established, this particular characteristic of dietary constituents like fatty acids may be utilized in the future to change the genetic profile of the cardiac muscle cell by simply adjusting the diet of patients to aid in the attenuation or reversal of common acquired forms of heart disease.

**Conclusions**

Fatty acids are an essential energy source for the healthy human heart. In the present overview, evidence was presented that the role of fatty acids in the heart is not limited to the generation of energy. In fact, fatty acids are the major components of crucial structural molecules as phospholipids, essential building blocks of cellular membranes. Moreover, fatty acids can act as signalling molecules that have the capacity to affect nuclear gene expression. In recent years it has become clear that under various pathological circumstances such as ischaemia/reperfusion, hypertrophy, diabetes or heart failure, cardiac fatty acid energy metabolism and phospholipid homeostasis is severely disrupted and this phenomenon may play a role in disease progression. In light of this, it seems feasible to speculate that manipulation of cardiac fatty acid homeostasis may become of importance for future treatment of certain forms of heart disease.

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**References**

Aoyama T, Souri M, Ushikubo S
Hale DE, Batshaw ML, Coates PM


[78] van Bilsen M, van der Vusse GJ, Willemsen PH, Coumans WA, Roemen TH, Reneman RS. Effects of L-NAME and mepergine on fatty acid accumulation and myocardial

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