Review

Cardiac fatty acid uptake and transport in health and disease

Ger J. van der Vusse*, Marc van Bilsen, Jan F.C. Glatz

Department of Physiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

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Abstract

Fatty acids are important energy donors for the healthy heart. These substrates are supplied to the myocardium bound to albumin to overcome their low solubility in aqueous solutions such as blood plasma. Transport from the microvascular compartment to the mitochondria inside the cardiomyocytes is most likely a combination of passive and protein-mediated diffusion. Alterations in tissue content of fatty acid-transport proteins may contribute to myocardial diseases such as the diabetic heart, and cardiac hypertrophy and failure. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Under normal physiological conditions long-chain fatty acids are important oxidizable substrates for the heart, serve as building blocks of cellular membranes after their esterification into phospholipids and are involved in signal transducing pathways [1,2]. Because of their low solubility in water, fatty acids are supplied to the heart either attached to plasma albumin or covalently bound in the triacylglycerol core of circulating lipoproteins. After dissociation of the albumin–fatty acid complex or hydrolysis of the triacylglycerols, fatty acids are transferred from the capillary lumen through the capillary endothelium and interstitial compartment to the cardiac muscle cell [3,4]. Subsequently, fatty acids cross the sarcolemma and cytoplasm to be converted into fatty acyl-CoA at the mitochondrial outer membrane or the sarcoplasmic reticulum. In a carnitine mediated process the bulk of these fatty acid derivatives pass the mitochondrial inner membrane and are degraded in the β-oxidation pathway and citric acid cycle. The remaining part of the acyl moieties of fatty acyl-CoA is incorporated into the esterified lipid pool, mainly consisting of phospholipids and triacylglycerols [1].

Recent findings strongly suggest that in addition to passive diffusion, a variety of proteins are involved in the transfer of fatty acids from the cardiac microvascular compartment to the intracellular sites of conversion [5–9]. In particular, plasmalemmal fatty acid-binding protein (FABPpm), fatty acid translocase (FAT/CD36), and/or fatty acid transport protein (FATP) may play dominant roles in transmembrane trafficking of fatty acids. A cytoplasmic fatty acid binding protein (heart-type FABP) is thought to be instrumental in maintaining sufficient flux levels of fatty acids from the sarcolemma to the mitochondrial or sarcoplasmic membrane.

The notion that proteins are most likely involved in the uptake and intracardiac transport of fatty acids has prompted research exploring the regulation of the expression of genes encoding for these fatty acid handling proteins. In particular, the role of fatty acids themselves and their derivatives in gene expression in the heart is becoming subject of intensive studies [2,10]. Moreover, if cardiac fatty acid uptake and transport are mediated by specific proteins, it is conceivable that various inherited or acquired cardiac myopathies are causally related to defects in the synthesis of these proteins, occurring either at the transcriptional or translational level. Recent observations indicate that some cardiomypathies are indeed associated with impaired expression of proteins putatively involved in the intracardiac fatty acid transfer process [11,12].

*Corresponding author. Tel.: +31-43-388-1086; fax: +31-43-388-4166.
E-mail address: vandervusse@fys.unimaas.nl (G.J. van der Vusse)

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In this survey we will, in a concise manner, reiterate the significance of fatty acids for cardiac energy conversion, and their possible route(s) of transport from the microvascular compartment into the interior of the cardiac myocyte. Further, the mechanisms underlying intracardiac fatty acid transport and the potential regulatory role of fatty acid handling proteins and the regulation of their expression by fatty acids themselves will be reviewed. Finally, the consequences of alterations in the expression of proteins putatively involved in fatty acid uptake and transport, as seen in selected pathophysiological states, will be briefly discussed.

2. Cardiac need of fatty acids

Since the pioneering studies of Bing et al. [13] it has been fully appreciated that fatty acids are important substrates for myocardial energy conversion. Under normal physiological conditions about 60–70% of the total amount of ATP required for adequate contraction of the heart is generated by mitochondrial oxidation of fatty acids [1]. The remaining part is mainly accounted for by oxidation of carbohydrates, such as glucose and lactate [14–16].

Myocardial tissue contains substantial amounts of fatty acyl moieties. The bulk is incorporated in the membrane phospholipid pool and, hence, should be considered as structural fatty acids. On the average about 40 μmol fatty acyl moieties per gram wet weight of tissue is present in this distinct lipid pool. Although exchange of phospholipid-incorporated fatty acids with the cellular unesterified fatty acid pool readily occurs, the phospholipid-fatty acyl pool is quantitatively not important as storage form of oxidizable fatty acids since a substantial decline or major fluctuations of the size of the membrane phospholipid pool is most likely not compatible with life [1]. Myocardial triacylglycerol contains about 4 μmol fatty acyl moieties per gram of tissue, stored in lipid droplets in the cytoplasm of the cardiac muscle cells in the close vicinity of the mitochondrial outer membrane [17]. Under steady state conditions no major alterations in the absolute amount of triacylglycerol fatty acids take place. The level of fatty acids in the unesterified form in cardiac tissue is extremely low, i.e., on the order of 30 to 60 nmol fatty acids per gram of tissue [18]. These considerations lead to the conclusion that in normal physiological circumstances, cardiac muscle cells rely heavily on an adequate and unimpeded supply of blood-borne fatty acids to fulfil their energy requirements.

In addition to supplying energy, fatty acids may take part in intracellular signal transduction [10,19]. Recent experimental findings indicate that several cardiac genes are under the control of fatty acids, while fatty acids are also involved in post-translational modification of proteins through protein acylation and activation of protein kinases [10,20,21]. Moreover, fatty acids such as arachidonic acid serve as precursor of biologically active eicosanoids, produced in cardiac endothelial cells and to a minor extent also in cardiac muscle cells [22–24].

A variety of studies on cardiac energy metabolism has shown that at physiological workloads the amount of fatty acids oxidized is on the order of 50–100 nmol/min per gram wet weight [1]. Thus, as discussed above, in steady-state conditions a similar amount of fatty acyl moieties must be transferred from the microvascular compartment to the cardiac parenchymal cells, i.e., the cardiomyocytes. Blood-borne fatty acids are delivered to the heart basically in two different forms (Fig. 1). The first source is plasma albumin carrying fatty acids, which are originally released from fat cells. Second, circulating lipoproteins, such as very low density lipoproteins (VLDL) and chylomicrons, originating from liver and gut, respectively, contain appreciable amounts of triacylglycerols, the fatty acyl moieties of which can be set free by lipoprotein lipase [25,26]. This enzyme is produced in the cardiac muscle cell and transported to the endothelium, where it becomes attached to the luminal side of the endothelial cell (Fig. 1). Due to technical limitations, the quantitative contribution of circulating triacylglycerols to cardiac fatty acid utilization in situ has not yet been precisely determined, but is considered to be moderate [27]. Recently, Wang et al. [28] estimated the contribution of lipoprotein triacylglycerol fatty acids to total fatty acid utilization in the isolated rat heart to be 20–25% at maximum. Studies in which arterio-

![Fig. 1. (A) simplified schematic representation of fatty acid uptake and transport in the heart, highlighting potential membrane barriers such as the endothelium and sarcolemma. Aqueous compartments such as the interstitial compartment and cardiac cytoplasm are also considered. LPL, lipoprotein lipase; TG, circulating triacylglycerols (being part of chylomicrons or very low-density lipoproteins); FA, (unesterified) long-chain fatty acids; alb, albumin; FABP, cytoplasmic fatty acid-binding protein; ACS, acylCoA synthetase.](image-url)
local venous differences of albumin-bound fatty acids were measured, indicate that the latter source of plasma-borne fatty acyl moieties significantly contributes to overall myocardial lipid oxidation [1]. Therefore, in the present overview we will mainly focus on cardiac uptake and transport of albumin-derived fatty acids.

3. Intracardiac transport routes of fatty acids

The notion that fatty acids are supplied to cardiac muscle cells from the capillary lumen implies that on their way from this compartment to the intracellular site of conversion potential barriers have to be crossed by these hydrophobic compounds. The first constraint encountered by fatty acids translocating from the microvascular compartment to the cardiac parenchymal cells is the endothelium (Fig. 1). Theoretically, fatty acids can cross the endothelial barrier either complexed to the fatty acid carrier albumin or as free, non-protein-bound fatty acyl moieties. The first option is rather unlikely since the transendothelial transfer rate of albumin, either by transcytosis (Fig. 2, route 1) or diffusion through the interendothelial clefts (Fig. 2, route 2), is far too low to account for a transendothelial influx of fatty acids of about 50–100 nmol/min per gram [3,4], although some albumin will escape from the intravascular compartment to compensate for loss of interstitial albumin via lymphatic flow. The movement of albumin–fatty acid complexes through the endothelial clefts is particularly hampered by the size of the albumin molecule and by tethering of the glycocalyx lining the endothelial cells [3,4]. The corollary to this notion is that fatty acids should be released from albumin prior to transfer across the endothelial barrier. Non-protein-bound fatty acids might pass the endothelial layer either via the interendothelial clefts or through the endothelial cells composing the microvascular wall. Experimental evidence and theoretical considerations indicate, however, that diffusion of fatty acids through interendothelial clefts is inconsequential for bulk transport to the cardiac muscle cells and, hence, that fatty acids traffic through the microvascular endothelial cells [3,4,29].

Fig. 2. Alternative transport routes of long-chain fatty acids from the microvascular compartment to the main site of metabolic conversion, i.e., the interior of the cardiac myocyte. (1) Transport of the albumin–fatty acid complex through the interendothelial clefts; (2, 3.1, 3.2 and 4) various transport routes of the albumin–fatty acid complex or of the dissociated fatty acids through the endothelial cells. Alb, albumin; FABP, cytoplasmic fatty acid-binding protein; FA, long-chain fatty acids; p, membrane-associated (complex of) protein(s). See text for detailed explanation.
Fatty acids might cross the endothelial cells by periendothelial diffusion [30]. This would be accomplished by dissolution of fatty acids in the endothelial luminal surface membrane followed by movement of the fatty acids by diffusion within the plasmalemma down a concentration gradient to the abluminal surface membrane (Fig. 2, route 3.1). This hypothetical route has been dismissed by Bassingthwaighte et al. [3] on the basis of calculations rather than experimental findings. Their calculations predict that peri-endothelial transfer of fatty acids would require a very high concentration gradient within the endothelial cell membrane. To accomplish such a gradient the fatty acid content in the luminal surface membrane should surpass the phospholipid content. This condition was thought not to be compatible with the normal chemical composition and physiological function of a biological membrane [3]. Therefore, fatty acids most likely pass the endothelium by transport across the luminal membrane, through the cytoplasm, and then across the abluminal membrane of the endothelial cell (Fig. 2, route 3.2 and 4). After reaching the second barrier, the interstitial compartment separating endothelial and cardiac muscle cells, the fatty acid molecules bind to interstitial albumin and are transferred to the cardiomyocytes by this carrier protein. The third barrier is represented by the sarcolemma, enclosing the cardiac muscle cell. Subsequently, the bulk of fatty acids taken up by the cardiomyocytes crosses the fourth barrier, the cytoplasm, to reach the sarcoplasmic reticulum or mitochondrial outer membrane.

4. General aspects of fatty acid transport through aqueous compartments and phospholipid bilayers

As can be inferred from the above, potential barriers for intra-organ transport of fatty acids are aqueous compartments and biological membranes. Although the diffusion rate of fatty acids in an aqueous environment is relatively high, their low solubility in aqueous solutions implies that only minute amounts of fatty acids are translocated in this manner. Therefore, carrier proteins are required to transport physiologically relevant amounts of hydrophobic fatty acids in an aqueous environment. In the vascular and interstitial compartments this requirement is met by plasma albumin, present in a relatively high concentration (on the order of 0.6 and 0.3 mM in the vascular and interstitial compartments, respectively). The same holds for the cytoplasm of the majority of parenchymal cells, where the abundant presence of low-molecular-mass FABPs is supposed to guarantee physiologically relevant flux levels of fatty acids from the plasmalemma to intracellular sites of conversion [31].

Although general agreement exists on the transport mechanism of fatty acids in aqueous compartments, the precise mechanism underlying transmembrane movement of the lipophilic fatty acids is a matter of continuous debate [8,32–34]. Two opposing views emerge from experimental studies performed in the last decade. On the one hand, it is proposed that trafficking of fatty acids across a biological membrane is accomplished by simple diffusion, governed by the physicochemical properties of water, the phospholipid bilayer of the membrane, and fatty acids [32,33,35]. On the other hand, fatty acids are assumed to traverse a biological membrane by facilitated mechanisms involving specific membrane-associated fatty acid-binding and carrying proteins [8,36,37].

Proponents of the physicochemical mechanism base their view on findings mainly obtained in preparations of artificial membranes [38,39]. These experiments show that absorption of fatty acids from an aqueous environment to the outer leaflet of a vesicle, composed of pure phospholipids, is fast. Translocation of fatty acids from the outer leaflet to the inner leaflet of the vesicles via a flip-flop mechanism takes a few milliseconds. The desorption of the fatty acyl moieties from the outer leaflet into the second aqueous compartment is also reported to be a fast process [32]. Recent findings strongly suggest that fatty acids traverse the vesicular phospholipid bilayer mainly in the unionized form, indicating that transmembrane diffusion of fatty acids is proton-mediated [33]. Due to the physicochemical properties of the anionic phospholipids in the membrane the pKₐ value of fatty acids dissolved in the phospholipid bilayer is about 7.5, a value close to the physiological pH of blood and interstitial fluid. This notion implies that approximately 50% of fatty acids dissolved in the outer leaflet of a biological membrane is present in the protonated form, which substantially facilitates bulk diffusion of fatty acids from the outer to the inner membrane leaflet. Moreover, recent observations of Trigatti and Gerber [40] in isolated 3T3-L1 adipocytes indicated that acidification of the cytoplasm significantly reduced the transfer of fatty acids from the surrounding medium into the cell interior. Since the latter effect could not be related to alterations in the cellular rate of fatty acid metabolism, the authors concluded that their findings are in line with the notion that transmembrane movement of fatty acids occurs by simple diffusion of protonated fatty acids across a lipid bilayer.

Some uncertainty exists, however, with respect to the rate of delivery of fatty acids to a (biological) membrane. Since it is generally acknowledged that fatty acids are supplied to a plasma membrane complexed to albumin, the rate of dissociation of fatty acid–albumin should be taken into account when considering the overall fatty acid flux. Zakim [32] suggested that in the liver the relative slowness of the rate of dissociation of fatty acids from the carrier albumin (k = 0.036 s⁻¹) does not limit the rate of uptake by the hepatocytes. It is, however, uncertain whether this conclusion does also hold for cardiac tissue.

Proponents of the protein-mediated transmembrane transport mechanism base their theory on the discovery of membrane-associated proteins able to bind fatty acids or
fatty acid analogues. At present, a set of at least three distinct proteins putatively involved in transmembrane transport has been identified in various biological membranes (Table 1). Stremmel et al. were the first to identify in the hepatocyte plasma membrane a 40–43 kDa protein that displays a high affinity for fatty acids [41]. This peripherally bound protein, designated plasmalemmal fatty acid-binding protein (FABP<sub>pm</sub>), is found in several other tissues including cardiac endothelium and cardiac muscle, and appears closely related, if not identical to mitochondrial aspartate aminotransferase (mAAT) [42]. Functional evidence for the involvement of FABP<sub>pm</sub> in cellular fatty acid uptake stems from studies showing that antibodies against this protein inhibit cellular fatty acid uptake [43] and that transfection of 3T3-L1 fibroblasts with a cDNA for FABP<sub>pm</sub> confers a saturable and 10-fold increased rate of fatty acid uptake into these cells [44].

By labeling with fatty acid analogues, Abumrad et al. [45] found in rat adipocytes an 88 kDa integral membrane protein, called fatty acid translocase (FAT), which most likely is a species homologue of the human leukocyte differentiation antigen CD36. The highly glycosylated FAT/CD36 presumably has one or two membrane spanning regions [46] and is expressed in a number of tissues, including the heart, but absent in liver [45,47]. FAT/CD36 mRNA increases during differentiation of preadipocytes into adipocytes and this induction is paralleled by an increase in fatty acid transport into the cell [45,48]. Moreover, transfection of FAT/CD36 cDNA into fibroblasts lacking the protein led to induction of a saturable, high-affinity component of oleate uptake [49].

More recently, Schaffer and Lodish [50] discovered another candidate protein for facilitating the transmembrane transport of fatty acids. This candidate protein was identified via expression cloning of an adipocyte cDNA library in COS7 cells and subsequent screening for cells exhibiting elevated uptake of a fluorescent fatty acid analogue. This putative transporter, named fatty acid transport protein (FATP), has a molecular mass of 63 kDa and is an integral membrane protein with multiple transmembrane sequences [50,51]. FATP is found in most mammalian tissues investigated [47,50]. Interestingly, in 1987 Fujii et al. [52] reported the purification of a 60 kDa high affinity fatty acid receptor from heart and kidney membranes. This protein has not been further characterized, but on the basis of molecular size and in vitro properties, may be identical to the later identified FATP.

5. Mechanisms of intracardiac fatty acid transport

5.1. Transendothelial transport of fatty acids

Since diffusion of fatty acids through the interendothelial clefts or through the plasmalemmal membrane of the endothelial cells each are quantitatively inconsequential [3] (Fig. 2, routes 2 and 3.1), fatty acids first must cross the luminal membrane of the cardiac endothelial cell. The mechanism underlying cardiac transendothelial transfer of fatty acids has been extensively studied in isolated rabbit hearts with the use of radiolabeled albumin and palmitate [3,4]. The experimental findings indicate that the influx of fatty acids, delivered to the membrane in a non-protein bound form, from the microvascular compartment into the cardiac endothelium is on the order of 50 pmol/min per gram [3,4], taking into account that the non-protein-bound concentration of fatty acids in plasma is ≈5 nM [53]. This influx is three orders of magnitude lower than the amount of fatty acids extracted by the heart under normal physiological circumstances, i.e., 50–100 nmol/min per gram [1]. This conclusion entails that instead of the non-protein bound fatty acid fraction in plasma, albumin-bound fatty

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular mass (kDa)</th>
<th>Current designation</th>
<th>Occurrence</th>
<th>References</th>
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<td>Albumin</td>
<td>68</td>
<td>Albumin</td>
<td>Blood plasma, interstitial fluid</td>
<td>Spector et al., 1969 [135]</td>
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<td><strong>Membrane associated</strong></td>
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<td>Albumin binding protein</td>
<td>18, 31</td>
<td>ABP (Alb, BP)</td>
<td>Endothelial and parenchymal cells in various organs</td>
<td>Popov et al., 1992 [56]</td>
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<td>Plasmamembrane FABP</td>
<td>40–43</td>
<td>FABP&lt;sub&gt;pm&lt;/sub&gt;</td>
<td>Endothelium, cardiomyocyte (Peripheral protein)</td>
<td>Sorrentino et al., 1988 [43]</td>
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<td>FA transport protein</td>
<td>63</td>
<td>FATP</td>
<td>Endothelium, cardiomyocyte (Transmembrane protein)</td>
<td>Schaffer and Lodish, 1994, 1995 [7,50]</td>
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<tr>
<td>FA translocase</td>
<td>88</td>
<td>FAT/CD36</td>
<td>Endothelium, cardiomyocyte (Transmembrane protein)</td>
<td>Abumrad et al., 1993 [45]</td>
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<td><strong>Intracellular</strong></td>
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<td>Epidermal-type FABP</td>
<td>15</td>
<td>E-FABP</td>
<td>Endothelium</td>
<td>Massouye et al., 1997 [64]</td>
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<td>Heart-type FABP</td>
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<td>H-FABP</td>
<td>Endothelium, cardiomyocyte</td>
<td>Ockner et al., 1972 [136]</td>
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<td>ACBP</td>
<td>10</td>
<td>ACBP</td>
<td>Cardiomyocyte</td>
<td>Antohe et al., 1998 [65]</td>
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*Abbreviations: FA, long-chain fatty acid; FABP, fatty acid-binding protein; ACBP, acyl CoA-binding protein.*
acids are of physiological relevance as driving force for transendothelial transport.

Exploring this issue in more detail, it was found that the transcapillary transfer of fatty acids is saturable with increasing albumin concentrations. However, the capacity to transport fatty acids across the capillary wall at a fixed albumin concentration was found to be linearly related to the total amount of fatty acids complexed to albumin, without any sign of saturation up to 2.0 mM fatty acid [4].

Although the experimental findings suggest that plasma fatty acids are directly translocated from the plasma fatty acid carrier, albumin, to the luminal membrane of the capillary endothelium, it is incompletely understood how under physiological conditions the transfer of fatty acids to cardiac endothelial cell membrane is accomplished. Direct transfer of fatty acids from albumin to the luminal endothelial membrane implies physical contact between the albumin molecule and the membrane (Fig. 2, route 4). The observation that unidirectional influx of fatty acids becomes saturated at elevated albumin concentrations suggests that a limited number of interaction sites for albumin with the luminal endothelial membrane is available. These findings do not allow for discrimination between interaction of albumin with either (domains of) phospholipids of the outer membrane leaflet or with specific proteins attached to or localized within the phospholipid bilayer. If proteins are involved, likely candidates are a family of recently identified membrane proteins, capable of binding albumin with a relatively high affinity [54–56]. At present, the physiological function of these proteins is supposed to be the trapping of plasma albumin prior to endothelial transcytosis of the albumin molecule. However, a potential role of albumin binding proteins in the transfer of fatty acids from the fatty acid carrier albumin into the luminal membrane of the cardiac endothelial cells is worth to consider.

Irrespective of the identity of the membrane components involved in albumin–membrane interaction, the physical contact between the carrier protein and the cardiac endothelial membrane should result in a substantial acceleration of the release rate of fatty acids from their albumin binding sites. Scheider [57] and Svenson et al. [58] previously reported dissociation rates on the order of 15 to 20 s, and Zakim [32] over 25 s, which are far too low to accommodate physiological flux levels of fatty acids across the cardiac endothelium, considering an average dwell time of blood in the cardiac microvascular compartment of 1 to 2 s [3] and a single pass extraction of fatty acids of 40–60% [1]. This situation is therefore basically different from that in liver, where the extraction ratio of plasma fatty acids is generally lower [32].

Although passive diffusion as possible mechanism of fatty acid transport across the luminal endothelial membrane cannot be ruled out entirely [32,33], recent findings suggest that membrane-associated proteins are implicated in transendothelial trafficking of fatty acids in the heart. Goeresky et al. [59] observed that in the isolated rat heart a specific, peripheral membrane fatty acid binding protein (FABP_{pm}) is required for transition of fatty acids from the microvascular compartment into the luminal endothelial membrane, since antibodies directed against this protein substantially decreased the unidirectional influx of fatty acids. In addition to the 40 kDa FABP_{pm}, other membrane proteins putatively involved in transport of fatty acids across the endothelial barrier, such as FAT/CD36 and FATP, have been shown to be present in cardiac endothelial cells [47,60]. The exact function of these proteins and their contribution to overall transendothelial fatty acid transport remain, however, to be established.

After desorption from the inner leaflet of the luminal membrane fatty acids should permeate the aqueous cytoplasm of the endothelial cell to reach the abluminal membrane [3]. Earlier reports from our laboratory indicated that the transfer of the poorly water soluble fatty acids across the endothelial cytoplasm does not require a carrier protein [1]. This conclusion was based on the notion that, due to the short diffusion distance between the endothelial luminal and abluminal membrane, bulk transport of fatty acids might occur by passive diffusion in spite of their low solubility in water (Fig. 2, route 3.2). This idea was further supported by the failure to identify significant quantities of a soluble fatty acid-binding protein in homogenates of stable endothelial cell lines, derived from the adult rat heart [61,62]. However, recent computer simulations and histochemical analysis of intact rat and mouse hearts have shed new light on this issue. The model studies predicted the need of a protein with a high affinity for fatty acids in the endothelial cytoplasm required to facilitate desorption of the fatty acid molecules from the inner leaflet of the luminal membrane [63]. Massouyé et al. [64] provided histochemical evidence for the presence of small quantities of an epithelial-type fatty acid binding protein in human cardiac endothelial cells, making this protein a likely candidate for transcytotic transfer of fatty acids in the cardiac endothelium. Recently, the presence of heart-type FABP was immunohistochemically established in the microvascular endothelium of the intact mouse heart [65]. Obviously, more detailed experiments are required to solve the question as to whether and, if so, to what extent soluble fatty acid binding proteins are required for transendothelial fatty acid transport.

Movement of fatty acids from the cytoplasm across the abluminal endothelial membrane into the interstitial compartment may take place in a mirror-like fashion as described above for the luminal membrane. After albumin-facilitated diffusion from the abluminal membrane of the endothelium to the cardiomyocytes, fatty acids again have to be released from the albumin carrier prior to transsarcolemmal trafficking (Fig. 2).

5.2. Sarcolemmal transport of fatty acids

The last membrane barrier to be taken by fatty acids on their way to intracellular conversion into acyl-CoA is the
plasmalemma of the cardiac muscle cell. The mechanisms by which fatty acids are translocated across the sarcolemma are most likely identical to those for endothelial membranes. Also in this case, the two extremes are a simple physicochemical process based on passive diffusion of the fatty acid molecule and the passage through the membrane barrier by means of (a set of) membrane-associated proteins (Table 1).

The possible involvement of sarcolemmal proteins in the uptake of fatty acids by cardiac myocytes was recently explored by Luiken et al. [66]. They observed in isolated adult rat cardiomyocytes saturation of palmitate uptake when the concentration of palmitate (complexed to albumin with a constant ratio) in the incubation medium was raised from 10 to 300 μM. Interpretation of these findings is, however, not unambiguous. It might indicate either saturation of intracellular consumption of fatty acids or saturation of membrane-associated fatty acid transfer proteins. Interestingly, mild pre-treatment of isolated cardiomyocytes with low concentrations of trypsin, a well-known proteolytic enzyme, significantly depressed the rate of cardiac palmitate uptake, while leaving the cellular integrity intact (Fig. 3). Moreover, palmitate uptake by isolated rat cardiac myocytes was found to be substantially blocked by treatment of the cells with either phloretin, an universal inhibitor of membrane-associated transport proteins, or sulfo-N-succinimidyl oleate (SSO), a ligand of the fatty acid transporter, FAT/CD36 [66,67], suggesting the involvement of membrane proteins in fatty acid uptake by cardiac muscle cells.

The studies of Luiken et al. [66] also confirm that fatty acid uptake by cardiac myocytes is most likely driven by their rapid intracellular metabolic conversion, and that this close link between uptake and metabolism hampers the elucidation of the precise cellular uptake mechanism. However, in subsequent studies, Luiken et al. [68] used giant sarcolemmal vesicles prepared from rat heart to study fatty acid uptake in the absence of metabolism. These vesicles, devoid of fatty acid metabolizing enzymes, contain cytoplasmic FABP which acts as intravesicular sink for fatty acids. Similar to the studies with cardiac myocytes, palmitate uptake by sarcolemmal vesicles derived from these cells could be inhibited for up to 80% in the presence of protein-modifying agents (trypsin, phloretin), of antibodies against FABPpm or of highly reactive fatty acid derivatives such as SSO [68]. The observation that none of these agents, either alone or in combination, can fully inhibit palmitate transfer through the isolated sarcolemma suggests that for cardiac myocytes there is a non-inhibitable component contributing to about 20% of cellular fatty acid uptake. The latter uptake is likely due to passive diffusion.

Interestingly, the inhibitory actions of anti-FABPpm antibodies and SSO were non-additive [68], suggesting that FABPpm and FAT/CD36 (and/or FATP) may cooperate to translocate fatty acids across the sarcolemma. This raises the attractive hypothesis that the peripheral membrane protein FABPpm located at the extracellular leaflet could act as a trapping protein for fatty acids in order to facilitate fatty acid adsorption while the integral membrane protein(s) FAT/CD36 (and/or FATP) would function as a fatty acid translocase. 

In other studies, Bonen et al. [69] observed that sarcolemmal vesicles prepared from skeletal muscles that are electrically stimulated for 25 min display a markedly higher rate of fatty acid uptake than vesicles from non-stimulated control muscles, and that this increase was paralleled by a higher sarcolemmal content of FAT/CD36, but not FABPpm, in vesicles prepared from stimulated muscles. Since the duration of the period of muscle contraction is too short for de novo protein synthesis, this increase in sarcolemmal FAT/CD36 most likely is due to protein translocation. Such a mechanism may show analogy to the short-term regulation of glucose uptake into muscle by insulin, which is due to translocation of GLUT4 from intracellular stores to the plasma membrane. However, it remains to be proven whether and, if so, to what extent fatty acid uptake by cardiac myocytes is regulated by subcellular redistribution of membrane-associated fatty acid-binding proteins.

The chain length and degree of acyl chain unsaturation of the (long-chain) fatty acid may also influence their rate

Fig. 3. Effect of selected inhibitors on fatty acid uptake by cardiac muscle cells. Isolated adult rat cardiomyocytes were incubated in the presence of 90 μM palmitic acid and 300 μM albumin for 3 min. 14C-palmitate was added to monitor the initial uptake rate of fatty acids. Interventions included (pre)treatment of the cells with trypsin (0.25%), phloretin (0.4 mM) or sulfo-N-succinimidyl oleate (SSO) (0.4 mM). Asterisk indicates significantly different from control cells. See for details Luiken et al. [66].
of uptake by cardiac myocytes. The degree of acyl chain unsaturation is an important parameter determining the aqueous solubility of the fatty acid, and was found to play a significant role in the equilibrium between fatty acid association with specific proteins [53,70] and with biological membranes [33]. Despite these differences, the affinities of the various proteins and cell membranes for fatty acids are such that, under normal conditions, the unbound fatty acid concentrations are extremely low [53] and will hardly elicit a possible selective uptake of fatty acid types. Possible differences in binding specificities of the sarcolemmal proteins (putatively) involved in fatty acid uptake, to our knowledge, have only been explored by Baillie et al. [71], who studied the in vitro properties of FAT/CD36 isolated from rat adipocytes to find comparable binding affinities for palmitate, oleate, stearate, linoleate and arachidonate. It remains to be established whether the other membrane proteins would favor the uptake of selected fatty acid types.

Collectively, these recent observations point to the involvement of sarcolemmal proteins in fatty acid handling of cardiac muscle cells. To cross the bridge between the two extreme views of either non-protein-mediated or protein-mediated fatty acid transport, we like to suggest that both processes occur in parallel with their relative contribution to total fatty acid uptake depending on the physiological condition. Transport of protonated fatty acyl moieties may occur by rapid flip-flop across the phospholipid bilayer, whereas proteins are involved in the transport of ionized fatty acyl moieties through the membrane barrier, enhancing the total capacity of the cell to utilize fatty acids supplied from the extracellular environment. The protein-mediated mechanism might be of great importance during elevated metabolic activity (high workload) when the content of protonated fatty acids inside the cell most likely increases due to enhanced generation of protons by glycolytic and mitochondrial activity. The intracellular increase of protonated fatty acids may counteract the diffusion of protonated fatty acids from the interstitium to the cytoplasmic compartment. This adverse effect may be compensated for by protein-mediated transport of ionized fatty acids from the interstitial compartment into the cardiac muscle cell interior.

5.3. Transsarcoplasmic transport of fatty acids

Mathematical models as well as in vitro studies have indicated that intracellular fatty acid carriers would be indispensable for adequate transport of fatty acids from the sarcolemma to the mitochondria interspersed between the myofibrils [72,73]. In this respect, it is generally accepted that cytoplasmic heart-type FABP (H-FABP) functions as intracellular fatty acid carrier inside the myocytes. H-FABP belongs to a family of 14–15 kDa lipid binding proteins, currently consisting of 13 members and also including cellular retinoid-binding proteins, and shows one high-affinity fatty acid-binding site per protein molecule [9,74,75]. H-FABP is abundantly expressed in the cytoplasm of cardiac myocytes, and in rat heart H-FABP accounts for approximately 3% of the cytoplasmic proteins (50 nmol/g wet weight) [76]. Immuno-electronmicroscopic studies revealed its presence primarily in the soluble cytoplasm [9], although minute amounts of H-FABP are found inside the nucleus and in the mitochondrial matrix [77]. H-FABP is also expressed in skeletal muscles, and a positive relation is found between the H-FABP content and the oxidative capacities of muscles tissues (heart>red muscles>white muscles) [76,78], suggesting a functional involvement of H-FABP in muscle lipid metabolism. An acyl-CoA binding protein (ACBP) of 10 kDa, which most likely binds the fatty acid after activation to its acyl-CoA thioester has also been detected in the cardiomyocyte [79] (Table 1).

The physiological significance of cytoplasmic H-FABP in cardiac fatty acid handling was recently firmly established by Binas et al. [80] and Schaap et al. [81]. They studied fatty acid uptake by the heart of intact mice, carrying a targeted disruption of the gene coding for H-FABP, and utilization of fatty acids by cardiomyocytes isolated from these animals, respectively. Cardiac mRNA levels of several genes involved in plasmalemmal fatty acid transport and intracellular metabolism were comparable in H-FABP knock-out and wild-type mice. Moreover, no compensatory expression of other FABP types was detected in heart tissue. Cardiomyocytes isolated from H-FABP knock-out mice showed a markedly lowered uptake rate of palmitate (≈ −50%), while the uptake of octanoate was not affected. In addition, palmitate oxidation by isolated cardiac myocytes from knock-out mice was also significantly depressed despite the fact that the capacity for fatty acid oxidation, as measured in heart homogenates, was similar in H-FABP knock-out and wild-type mice [81]. These observations give the first conclusive evidence for a role of H-FABP in fatty acid uptake and oxidation in cardiac muscle cells.

As pointed out earlier FABP most likely facilitates bulk diffusion of fatty acids in the sarcoplasm by enhancing substantially the solubility of fatty acids in this aqueous environment. Despite the fact that the diffusion rate of the fatty acid–FABP complex is slower than that of non-protein bound fatty acids, the overall diffusion capacity is enhanced approximately 20-fold [73]. Moreover, cytoplasmic FABP may interact with the membrane-bound FAT/CD36 to facilitate the transfer of fatty acids from the sarcolemma into the cell interior [82]. This finding implies that a continuum of proteins and protein interactions might be involved in guiding fatty acids from the vascular compartment to the intracellular site of metabolic conversion. This notion is supported by the fact that computer models predict that FABP is required for desorption of fatty acids from the inner leaflet of the sarcolemma into the cytoplasm [63]. Direct delivery of the fatty acid molecule
to the active centre of acyl-CoA synthetase may be also facilitated by the fatty acid carrier FABP.

Studies by Richieri et al. [70] revealed that in vitro H-FABP will bind various fatty acid types (palmitate, oleate, stearate, linoleate, and arachidonate) with comparable affinity, suggesting that there is no selective intracellular trafficking of fatty acid types by H-FABP.

6. Fatty acid handling proteins and cardiac abnormalities

The discussion as to whether cardiac uptake and transport of fatty acids are protein-mediated or not might have important clinical ramifications, since proteins are subject to alterations in production rates due to up- or downregulation of transcriptional and translational activity, and post-translational modifications such as phosphorylation. As a consequence, altered levels and/or activities of fatty acid handling proteins may give rise to cardiac abnormalities. The reverse may also be true, namely that pathological changes in myocardial performance will result in alterations in level and/or activity of fatty acid handling proteins in cardiac cells. During the past decade information is becoming available suggesting that cardiac pathophysiologic conditions such as diabetes, cardiac hypertrophy and failure, and other types of cardiomyopathies, are associated with chronic alterations in cardiac energy metabolism in general and fatty acid utilization in particular [2,83–87]. Although detailed biochemical investigations have shown that some types of cardiomyopathies are related to malfunctioning of enzymes involved in cardiac fatty acid metabolism [88–91], recent findings suggest that proteins putatively involved in fatty acid uptake and transport may also play a role in cardiac abnormalities. Below, two pathological conditions, i.e., diabetes and cardiac hypertrophy, will be discussed.

6.1. Diabetic heart

In the diabetic heart fuel selection is appreciably changed, but the direction and magnitude of changes in glucose and fatty acid utilization appear to differ among various species and to be experimental model dependent. It is of interest to note that similar model dependency is also reported for the sensitivity of the diabetic heart towards ischemic injury [92]. In rats rendered diabetic by streptozotocin-treatment glucose oxidation is severely depressed and fatty acid oxidation enhanced [85,93]. This condition is found to be associated with increased levels of cytoplasmic FABP [94–96] and mRNA and protein levels of FAT/CD36 [97,98]. Heyliger et al. [99] provided circumstantial evidence that in the diabetic rat heart protein-mediated binding of fatty acids to the sarcolemma is elevated. Studies conducted on diabetic mice revealed substantially increased FAT/CD36 levels in cardiac endothelium [60]. The functional consequences of elevated FAT/CD36 in this animal species are, however, less clear, since uptake of the radiolabeled fatty acid analogue \(^{123}\)I-BMIPP in the murine diabetic heart was found to be depressed [100]. Contrasting data were also reported in human diabetes. Both in non-insulin dependent (NIDDM) and insulin dependent (IDDM) patients cardiac oxidation of \(^{123}\)I-heptadecanoic acid was not significantly different from healthy control subjects [101]. Strikingly, in subjects with impaired glucose tolerance, without overt signs of NIDDM or IDDM, the myocardial \(^{123}\)I-heptadecanoic acid oxidation rate was significantly decreased [101]. Another remarkable difference between diabetic rats and diabetic humans may concern the concentration of circulating fatty acids. In the streptozotocin-treated rat plasma fatty acid levels are consistently increased [99]. In diabetic patients, however, some investigators failed to disclose a significant difference in plasma fatty acid levels between patients and healthy controls [102].

Collectively, the present findings indicate that the direction of changes in cardiac fatty acid utilization in the diabetic heart, if any, is species and/or experimental model dependent. Moreover, it remains to be explored whether in diabetes mellitus alterations in myocardial fatty acid utilization and in the tissue content of proteins (putatively) involved in fatty acid uptake and transport, if any, are causally related to the diseased state of the heart.  

6.2. Cardiac hypertrophy

During the past decade experimental and clinical evidence has been provided that fuel selection is considerably altered in the hypertrophying heart [2,84]. The striking decline in myocardial fatty acid oxidation with a concomitant enhancement of glucose utilization strongly suggests that energy metabolism in the hypertrophied heart switches to the fetal programme [103]. The decline in fatty acid oxidation was found to be due to the hypertrophic condition per se rather than alterations in blood pressure and/or plasma concentration of fatty acids [104,105]. The observation that the oxidation rate of the medium-chain fatty acid octanoate did not differ between hypertrophied and control hearts indicates a crucial role of carnitine-dependent processes in the decline of fatty acid utilization in the hypertrophied heart [106]. This finding may also point to the importance of protein-mediated transport processes since the solubility of octanoate is appreciably higher than that of long-chain fatty acids.

The mechanism responsible for the switch from fatty acid to glucose metabolism in the hypertrophied heart might be pluriform. Alterations in, for instance, the cellular concentration of cofactors or the degree of phosphorylation of enzymes have to be taken into consideration. In this respect the reported decline in myocardial carnitine content in animal models of cardiac hypertrophy and failure [107,108] and in patients with end-stage heart failure
observed in the failing human heart [111]. Animal studies showed that in the hypertrophied heart of spontaneously hypertensive rats the cytoplasmic FABP content is slightly, but significantly decreased [112]. Unfortunately, no information is yet available on the tissue contents of membrane fatty acid-binding proteins such as FAT, FATP or FABP prostate in the compensated or failing hypertrophied heart.

It is of interest to note that in addition to pressure overload direct interventions in cardiac fatty acid handling eventually entail myocardial hypertrophy. Chronic inhibition of carnitine acyl transferase I by POCA resulted in a significantly increased left ventricular weight of the adult rat [113]. More recently, Kusaka et al. [114] reported that prolonged treatment of laboratory rats with sulfo-N-succinimidyl palmitate, an effective blocker of FAT/CD36 [67], gives rise to a 12% increase in heart weight, compared to untreated controls. Moreover, spontaneously hypertensive rats (SHR), the hearts of which are significantly enlarged, appear to be defective in the FAT/CD36 gene [115]. Studies by Binas et al. [80] on H-FABP knock out mice indicated the absence of this protein to elicit the development of cardiac hypertrophy at the older age (>1 year). These findings are in favor of the notion that in small rodents defects in carrier-mediated fatty acid uptake lead to impaired fatty acid utilization and alterations of the cardiac muscle cell phenotype.

Clinical studies [11,12] revealed in the Japanese population a high prevalence of a point mutation in the FAT/CD36 gene (48T→48C) resulting in FAT/CD36 deficiency. Patients, being homozygous for the 48T→48C substitution developed hypertrophic cardiomyopathy. These patients showed a complete lack of myocardial extraction of the radiolabeled fatty acid analogue 123I-BMIPP from the vascular compartment. These findings are the first clinical evidence of a possible causal relationship between disruption of a gene encoding for a putative fatty acid transport protein and impaired cardiac function. Very recently, however, the notion of a causal relationship between FAT/CD36 deficiency and cardiac abnormalities in man has been challenged by Nakamura et al. [116]. They failed to disclose a significant difference in scintigraphic findings, echocardiographic data, and hemodynamic parameters between patients with hypertrophic cardiomyopathy showing normal FAT/CD36 expression and those with FAT/CD36 deficiency.

Finally, a recent report of Hwang et al. [117] describing various members of a Japanese family with virtually no cardiac uptake of 123I-BMIPP associated with QT prolongation, but normal cardiac perfusion, suggests the involvement of a hereditary myocardial metabolic abnormality. It remains to be established, however, whether this abnormality is caused by disruption of genes involved in cardiac fatty acid uptake and transport.

7. Regulation of genes involved in cardiac fatty acid handling

As described above various lines of evidence suggest that chronic alterations in the cardiac utilization of fatty acids can be attributed to adjustments of the expression of genes involved in uptake, transport and metabolic conversion of fatty acids. So far the mechanisms involved remain to be elucidated. On the basis of experimental observations in parenchymal cells, such as adipocytes and hepatocytes, it can be concluded that substrates for cellular energy conversion themselves are capable of controlling transcriptional activity of metabolic genes [118–121]. Both oxygen, and glucose and fatty acids or their derivatives are supposed to influence the intracellular level of proteins involved in fuel selection and intermediary metabolism [2]. Recent findings in cultured neonatal cardiomyocytes [10] support the notion that exogenous fatty acids act also as modulators of cardiac transcriptional activity. When rat neonatal cardiomyocytes are exposed to physiological levels of fatty acids in the surrounding medium, the RNA transcripts of a set of cellular proteins, involved in fatty acid uptake and mitochondrial catabolism, are appreciably increased compared to cells chronically exposed to glucose in the absence of fatty acids (Fig. 4). mRNA levels of FAT/CD36, FABP, fatty acyl-CoA synthetase and long-chain acyl-CoA dehydrogenase all showed a 3-fold increase [10,122]. If one assumes that enhanced mRNAs are

![Fig. 4. H-FABP mRNA content of isolated rat neonatal cardiomyocytes as a function of type of substrates present in the culture medium. Cells were cultured for 48 h in medium containing either glucose (10 mM, left bar), or a combination of palmitate and oleate (each 0.25 mM, middle bar) or a combination of glucose (10 mM) and these fatty acids (each 0.25 mM, right bar). Values of the glucose group were arbitrarily set at 1.0. Values of the other groups were normalized on the glucose group. Asterisk indicates significantly different from the glucose group; for details see Van Bilsen et al. [10].](image-url)
associated with elevated protein levels, fatty acids or their derivatives can control their own rate of utilization through fine-tuning of gene expression (in other words, fatty acids beget fatty acid utilization). Indeed, the capacity to oxidize exogenous fatty acids is significantly increased in cultured neonatal cardiomyocytes previously exposed to fatty acids in the incubation medium [122]. In this respect it is also of interest to note that during maturation a significant increase of cardiac fatty acid oxidation is observed, starting a couple of days after birth [84,123,124], most likely due to hormonal influences, although the effect of enhanced circulating levels of fatty acids cannot be excluded. The maturational increase in cardiac fatty acid oxidation was found to be associated with appreciably increased tissue contents of putative fatty acid transport proteins, such as FAT/CD36, FATP, and of cytoplasmic FABP [47,125].

The observation that chronic administration of fatty acids to cultured neonatal cardiomyocytes results in transcriptional upregulation of a set of fatty acid handling genes, implies that the cells are capable of sensing changes in the supply of fatty acids (or fatty acid derivatives). Although precise information about the mechanism underlying fatty acid sensing in cardiac cells is lacking, it is tempting to speculate that intracellular proteins that bind fatty acids or fatty acyl derivatives are involved. Both FABP and acylCoA-binding protein have been postulated to confer fatty acids to the fatty acid sensing machinery [9]. Evidence for a crucial role of the former protein in fatty acid modulated gene expression in the heart is, however, poor, since a recently generated heart-type FABP knock-out mouse did not show major phenotypic alterations up to 14 months after birth, apart from a 50% reduction in cardiac fatty acid oxidation, suggesting that this protein does not play a crucial role in fatty acid mediated signal transduction [80].

It is of interest to note that within the promoter region of lipoprotein lipase, FATP as well as FAT/CD36 cis-regulatory elements are present which contain binding sites for specific hormone receptors [2,103]. Sophisticated molecular biological techniques have revealed that proteins, such as the transcription factors COUP/TF, PPAR, RXR, HFN-4 and oestrogen-related receptor-α, interact with these cis-regulatory elements [103,126]. In particular, PPAR is a likely candidate for cellular fatty acid sensing, as evidence is accumulating that fatty acids may act as natural ligands of the PPARs [127–129]. The PPARs are well-equipped to act as general sensors of the lipid status, since they demonstrate limited specificity towards individual fatty acid species. Although subtle differences are apparent between the different PPAR isoforms, both saturated and polyunsaturated fatty acid species with chain lengths longer than 14 carbon atoms bind to and activate PPARs [130].

By now three isoforms of PPAR have been described, PPARα, PPARβ (also referred to as PPARδ, FAAR and NUC1), and PPARγ. These isoforms are encoded by different genes, the level of expression being cell type dependent [131–134]. PPARα is expressed in brain, kidney, liver, skeletal muscle and heart. PPARβ shows an ubiquitous expression pattern, while PPARγ appears to be more or less confined to fat cells [2]. The presence of both PPARα and PPARβ in cardiac muscle cells opens the intriguing possibility that the interaction between these transcription factors and their potential ligands, i.e., long-chain fatty acids or their derivatives, helps to control the rate of gene transcription of proteins involved in fatty acid transport in this cell type. Further experiments are required to obtain a complete view on this important aspect of cardiac fatty acid uptake and metabolism.

8. Summary and future directions

Circumstantial evidence is accumulating that proteins are involved in the uptake and intracellular transport of long-chain fatty acids. In addition to cytoplasmic fatty acid-binding protein (heart-type FABP), a set of membrane-associated proteins (FABPpm, FAT/CD36, FATP) has been identified that might facilitate transfer of fatty acids across both endothelial and muscular cell membranes, as additional mechanism to passive diffusion.

Recent findings indicate that expression of fatty acid handling proteins in cardiac cells is controlled (at least in part) by fatty acids themselves. Further experiments are required to fully understand the molecular mechanisms underlying cardiac fatty acid-sensing and transcriptional control of genes encoding for fatty acid-binding and/or transporting proteins.

Involvement of proteins in fatty acid handling by the heart might have clinical ramifications. Alterations in the cellular content or intracellular localization of proteins (putatively) involved in fatty acid uptake and transport may compromise cardiac function. Conversely, cardiac diseases may lead to alterations in the expression and/or localization of these fatty acid-handling proteins. Elucidation of the precise relationship between cardiac function under normal and pathological conditions, such as diabetes, hypertrophy and pump failure on the one hand, and proteins involved in fatty acid uptake and transport on the other, may open new avenues for sophisticated and targeted interventions aiming at mitigating, preventing or restoring these cardiac abnormalities.

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