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High fat diet induced diabetic cardiomyopathy

Ellen Dirkx*, Robert W. Schwenk, Jan F.C. Glatz, Joost J.F.P. Luiken, Guillaume J.J.M. van Eys

Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands

A B S T R A C T

In response to a chronic high plasma concentration of long-chain fatty acids (FAs), the heart is forced to increase the uptake of FA at the cost of glucose. This switch in metabolic substrate uptake is accompanied by an increased presence of the FA transporter CD36 at the cardiac plasma membrane and over time results in the development of cardiac insulin resistance and ultimately diabetic cardiomyopathy. FA can interact with peroxisome proliferator-activated receptors (PPARs), which induce upregulation of the expression of enzymes necessary for their disposal through mitochondrial β-oxidation, but also stimulate FA uptake. This then leads to a further increase in FA concentration in the cytoplasm of cardiomyocytes. These metabolic changes are supposed to play an important role in the development of cardiomyopathy. Although the onset of this pathology is an increased FA utilization by the heart, the subsequent lipid overload results in an increased production of reactive oxygen species (ROS) and accumulation of lipid intermediates such as diacylglycerols (DAG) and ceramide. These compounds have a profound impact on signaling pathways, in particular insulin signaling. Over time the metabolic changes will introduce structural changes that affect cardiac contractile characteristics. The present mini-review will focus on the lipid-induced changes that link metabolic perturbation, characteristic for type 2 diabetes, with cardiac remodeling and dysfunction.

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1. Introduction

Myocardial metabolic remodeling is central to the pathogenesis of cardiac diseases such as ventricular hypertrophy and diabetic cardiomyopathy. To produce energy, the heart utilizes mainly long-chain fatty acids (FAs) and glucose, and minor quantities of lactate and ketone bodies [1]. Under normal conditions, the majority of the acetyl-CoA that enters the Krebs cycle is generated by β-oxidation of FA, while about a third is derived from oxidation of pyruvate, which is the product of glycolysis [2]. The shift in oxidation towards FA, at the cost of glucose, that occurs in type 2 diabetes is well documented [3]. Less studied is the mechanism by which diabetes affects morphology and structure of the heart. However, there are a number of reports that link type 2 diabetes to a disproportionate increase in left ventricular wall mass [4,5]. At present, metabolic disturbances due to lipid overload are thought to be the underlying cause of cardiac hypertrophy in type 2 diabetes.

2. Fatty acid uptake and hyperlypidemia

In the heart FAs are the substrate for β-oxidation, and they cover 60–70% of the energy needed for the generation of ATP. Since the heart must respond to continuously changing energy demands but has no large capacity for storage of either FA or glucose, substrate uptake must match energy demands. FA can be taken up by passive diffusion (20%) although most of the transport is protein-mediated (80%) [6]. This protein-mediated transport of FA depends largely on the plasma membrane transporter CD36 and the plasmalemmal fatty acid-binding protein (FABPpm) [7].

The FA transporter CD36 is a 472-amino acid (88 kDa) protein that has a hairpin membrane topology with two transmembrane spanning regions, with both the NH_2 and the COOH termini as short segments in the cytoplasm [8]. The current knowledge on its structure has been extensively reviewed by Glatz et al. [9]. In cardiac myocytes of CD36 knockout (CD36KO) mice, insulin stimulation of FA uptake was markedly impaired (+21%) compared with wild type mice (+60%) [10]. These reductions in FA uptake also contributed to altered rates of FA metabolism. For example, in working hearts, FA oxidation was 40–60% lower in CD36KO than in WT mice [11,12]. In both heart and skeletal muscle FA uptake rates have been found to run in parallel with the expression level of CD36 [9]. Thus, CD36 overexpression results in elevated rates of FA uptake [13] whereas CD36KO or knockdown impairs the transport of FA across the plasma membrane [14]. It is remarkable that Asian and African individuals relatively frequently have a genetic CD36 deficiency. CD36 deficiency showed some features of the 'metabolic syndrome'. Middle aged patients had significantly higher plasma triacylglycerol

* Corresponding author. Tel.: +31 433881697; fax: +31 433884574.
E-mail address: ellen.dirkx@gen.unimaas.nl (E. Dirkx).

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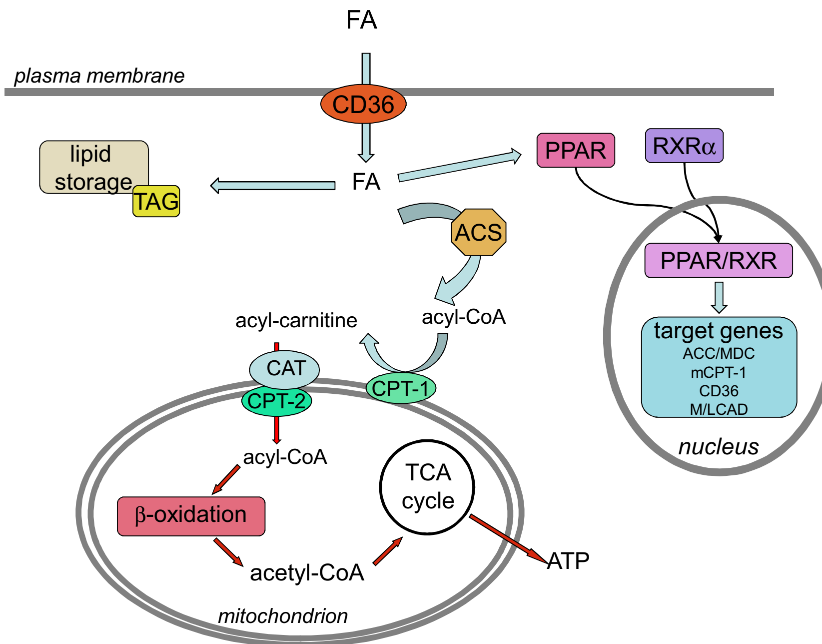

Fig. 1. Regulation of long-chain fatty acid (FA) uptake and oxidation in cardiomyocytes. FAs are imported from the blood facilitated by CD36 or by passive diffusion. FAs then are activated by ACS to acyl-CoA. Acyl-CoA is converted into acyl-carnitine by carnitine palmitoyl transferase I (CPT-1). Acyl-carnitine is translocated into the mitochondria by carnitine acyl translocase (CAT). In the mitochondrial matrix, CPT-II regenerates acyl-CoA, which enters β-oxidation and is further processed to ATP. Besides oxidation, cytoplasmic FA can be stored or can interact with peroxisome proliferator-activated receptors (PPARs) to stimulate the expression of genes coding for lipid metabolic enzymes and transporters.

concentrations, lower high-density lipoprotein cholesterol levels, higher plasma glucose levels and higher blood pressure than aged-matched control subjects. The cardiovascular pathophysiology of these individuals underscores the importance of CD36 for transport of FA into cardiomyocytes [15].

During increased contraction or insulin stimulation, CD36 translocates from endosomal pools to the plasma membrane to facilitate FA uptake [16]. In the cytoplasm FA bind to cytosolic fatty acid-binding protein ($FABP_c$) after which they are transported through the cytoplasm to the mitochondria. Then, fatty acyl-CoA synthetase (ACS) present at the outer mitochondrial membrane will esterify FA into acyl-CoA (Fig. 1) [17]. At the outer membrane of the mitochondria, acyl-CoA is converted into acyl-carnitine by carnitine palmitoyl transferase I (CPT-I) [18,19]. Translocation of acyl-CoA across the inner mitochondrial membrane is facilitated by carnitine acyl translocase (CAT). In the mitochondrial matrix, carnitine palmitoyl transferase II (CPT-II) regenerates acyl-CoA, which then undergoes β-oxidation (Fig. 1) [18,19]. These transferase reactions are rate-limiting steps in oxidation. Furthermore, FA oxidation rates are directly dependent on myocardial substrate availability, which is regulated primarily by levels of malonyl-CoA, a physiological inhibitor of CPT-I [20].

2.1. Hyperlipidemia and diabetes

The diabetic state is characterized by hyperinsulinemia, late-onset hyperglycemia and hyperlipidemia; the latter as a result of increased levels of triacylglyceroles and (non-esterified) FA [21]. In advanced diabetes, the combined effects of high levels of circulating FA and of insulin resistance drive cardiomyocytes towards an almost exclusive use of FA to generate ATP.

Diabetic cardiomyopathy is associated with an increase in FA uptake and oxidation, and an increase in cytoplasmic FA concentration [22]. Cardiomyocytes respond to increased FA concentration by upregulating the expression of the enzymes necessary for their utilization through mitochondrial β-oxidation [23]. These enzymes are under transcriptional control of PPARα and PPARβ/δ (see below). In addition, diabetic FA levels inhibit pyruvate dehydrogenase, which impairs myocardial energy production and leads to accumulation of both glycolytic intermediates and intracellular lipids, because FA oxidation then exceeds the mitochondrial capacity [24].

2.2. The role of PPARs

Cardiac FA utilization is largely controlled by metabolic gene programs that are under the control of nuclear receptors that bind FA or its derivatives [25,26]. Peroxisome proliferator-activated receptors (PPARs) are members of a nuclear receptor superfamily and some of them function as FA sensors and as transcriptional regulators of FA uptake and oxidation [27]. Upon activation PPARs form heterodimers with retinoid X receptors (RXRs) and then bind to the so-called PPAR responsive elements (PPREs) located in 5′ upstream regions of a number of genes encoding metabolic enzymes (Fig. 1) [28]. The binding to PPREs is enhanced by peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1). Once bound to the PPRE, the PPAR/RXR/PGC-1 complex increases the rate of transcription of, amongst others, genes involved in FA transport and oxidation [29]. For instance, PPARα upregulates the expression of FA transporters such as fatty acid-transport protein (FATP). CD36, however, has no PPRE in the upstream promoter region, but still is activated by PPAR ligands, probably in an indirect manner [30]. PPARα also induces the synthesis of metabolic enzymes of the β-oxidation pathway, such as CYP4A, and CPT-I [23,31,32]. In the promoter region of CPT-I and CYP4A functional PPREs have been identified. On the one hand, under standard conditions, PPARα stimulates FA oxidation, on the other hand, under diabetic conditions, PPARα agonists, such as fibrates, down-regulate FA oxidation and upregulate glucose oxidation. Such a shift towards glucose utilization may be due to normalization of circulating triacylglyceroles and FA concentrations, as observed in db/db mice and in diabetic Zucker rats [33,34]. The importance of the whole body effects seems to be underscored by results in transgenic mice with cardiac specific PPARα overexpression. In the hearts of these mice, an increase in

FA and a decrease in glucose utilization was found [35], an effect that was exacerbated by a high fat diet [36]. In line with this, PPARαKO mice have been generated and found to display no severe phenotypic defects. However, the hearts of these mice exhibit serious metabolic abnormalities. When challenged by a high cytoplasmic FA concentration, these mice are unable to upregulate cardiac transporters and enzymes crucial for FA metabolism. The mice accumulate myocardial lipids, due to a lower FA oxidation rate but they are resistant to high fat-induced insulin resistance [37,38]. Interestingly, the absence of PPARα was found to result in a decreased expression of malonyl-CoA carboxylase, leading to increased malonyl-CoA concentrations, which strongly inhibit FA oxidation [39]. The complex picture that arises from the available data establishes the role of PPARα in cardiac metabolic homeostasis, but at the same time demands further study. Such studies have to include the other PPAR types, in particular PPARβ/δ, since these are also highly expressed in cardiac myocytes and increasing evidence points towards a significant role of other PPAR types in myocardial lipid homeostasis [40].

2.3. CD36 relocation

Exposure to a high fat diet induces cardiac contractile dysfunction, which is associated with a permanent relocation of CD36 to the plasma membrane [41]. Furthermore, relocation of CD36 appears to be a general phenomenon in insulin resistant hearts [42], and precedes cardiac contractile dysfunction [41]. Also, in skeletal muscle biopsies from type 2 diabetic subjects, CD36 was relocated to the sarcolemma, and this increased sarcolemmal localization of CD36 is closely correlated with muscular triacylglycerol accumulation [43].

This raises the question what mechanism underlies the continuous presence of CD36 at the cardiac plasma membrane in case of a high fat diet. Cardiac CD36 is stored in endosomal storage pools that are regulated by AMP-activated kinase (AMPK) and PI3-kinase (PI3K)-Akt, respectively. Activation of AMPK is critical for contraction induced CD36 translocation to the plasma membrane, and activation of PI3K-Akt is critical for insulin-induced CD36 translocation (reviewed in [9]). In rats on a high fat diet, cardiac basal Akt phosphorylation was elevated, whereas insulin-stimulated Akt phosphorylation, CD36 translocation, and FA uptake each were decreased [41]. However, AMPK activity was not affected by this diet. These observations suggest that increased basal Akt activity may contribute to the continuous presence of CD36 at the plasma membrane in hearts during high fat diet feeding (Fig. 2). Another potential mechanism to stimulate relocation of CD36 to the plasma membrane is via PPARα and PPARβ/δ activation (Fig. 2). It has been shown that these PPARs are able to upregulate FA uptake and oxidation in the heart by inducing the translocation of CD36 to the plasma membrane. Importantly, observed effects of continuous PPAR activation were not related to AMPK activation or plasma FA concentrations [44]. Furthermore, oral treatment with the PPARδ agonist GW501516 was associated with large increases in total muscle CD36 protein content, but not diacylglycerol or ceramide contents [45]. Together, this indicates that PPAR activation can induce both CD36 expression and its relocation to the plasma membrane.

3. Insulin signaling and hyperlipidemia

Insulin-stimulated glucose and FA uptake is initiated by binding of insulin to the α-subunit of the insulin receptor at the sarcolemma [46]. This induces a conformational change resulting in the autophosphorylation of a number of tyrosine residues present in the β-subunits [47]. Tyrosine autophosphorylation leads to activation of the receptor's intrinsic tyrosine kinase activity, which results in phosphorylation of the two insulin receptor substrates IRS-1 and IRS-2. Phosphorylated IRS1/2 then binds and activates the regulatory subunit of phosphotidylinositol 3-kinase (PI3K) to produce phosphatidylinositol 3,4,5-triphosphate, and activate phosphoinositide-dependent protein kinases, kinase B/Akt, 3-phosphoinositide-dependent kinase-1 (PDK1), as well as the atypical protein kinase C isoforms ζ and λ [46,48,49]. PDK1 and PKC ζ/λ are necessary for both GLUT4 [50,51] and CD36 [52] translocation, likely via activation of vesicle-activated membrane protein-2 (VAMP2), to ensure that both transporters are specifically delivered to the sarcolemma, and not to other subcellular membrane systems [53]. Additionally, activated Akt phosphorylates and inhibits its 160 kDa substrate (AS160), thereby inducing GLUT4 translocation from the intracellular pools to the sarcolemma [50]. In addition to the effect of insulin on

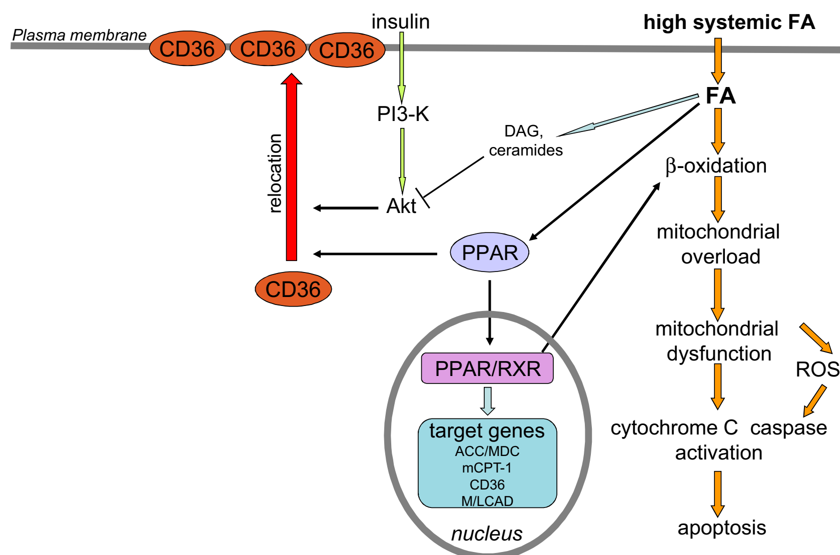

Fig. 2. Schematic depiction of consequences of increased FA uptake by cardiomyocytes (metabolic remodeling). High plasma FA concentrations lead to an increase in cytoplasmic FA levels. This stimulates β-oxidation (directly or indirectly via PPAR) and also stimulates CD36 relocation to the plasma membrane (via PPAR or via increased basal Akt phosphorylation), which leads to an even further increase in cellular FA uptake. The increased intracellular FA concentration causes mitochondrial overload, leading to dysfunction and oxygen radical production. Eventually, this results in cytochrome c leakage and in caspase activation, which induces apoptosis.

GLUT4, insulin receptor signaling also affects CD36-mediated FA uptake [54,55]. Whether AS160 also plays a role in insulin-stimulated CD36 translocation is not yet investigated. However, pharmacological experiments and studies in Akt2KO mice suggest that in the heart the CD36 translocation pathway largely parallels the regulation of GLUT4 translocation [9].

The role of several proteins of the insulin-GLUT4/CD36 cascade has been tested in transgenic mouse models. Cardiomyocyte-specific insulin receptor knockout mice (CIRKO) displayed decreased glucose and FA oxidation and mitochondrial dysfunction [56]. Deletion of the Akt2 gene resulted in insulin resistance and type 2 diabetes [57]. Finally, conditional mouse models have been generated that resulted in an up- or down-regulation of PI3K and its downstream effector PDK1, linking the metabolic state to changes in heart morphology [58,59]. These mouse models confirm the complexity and intrinsic fine-tuning of the insulin-initiated pathway that leads to cardiac substrate uptake. However, the precise nature of the crosstalk between the pathways directing glucose and FA uptake still has to be elucidated.

3.1. Insulin resistance

Insulin resistance is a risk factor of left ventricular dysfunction and heart failure [60], and is one of the hallmarks of type 2 diabetes [61,62]. It is associated with hyperinsulinemia and hyperglycemia. However, despite whole body hyperinsulinemia and hyperglycemia, the diabetic heart relies almost entirely on FA utilization at the expense of glucose [3]. This change in substrate utilization has been described in rodent models as well as in humans and can be largely attributed to nutrition of excessive fat containing food. Rodents can be made diabetic by prolonged periods of exposure to a high fat diet. Alterations in FA metabolism, that occur long before the diabetic state has been reached, will lead to a permanent relocation of CD36 to the plasma membrane and a reduced insulin-stimulated GLUT4-mediated glucose uptake (see above) [63]. Furthermore, the increase in cytoplasmic FA results in an increased ROS production (as a by-product of increased FA oxidation) and accumulation of lipid intermediates such as diacylglycerols (DAG) and ceramide (as a result of a mismatch between FA uptake and FA oxidation). These latter compounds have a profound impact on insulin signaling [64]. Kinases, notably JNK and IKK, will be activated by ROS, and PKC will be activated by DAG. Together they will down-regulate insulin action through serine phosphorylation of IRS-1 [65]. In contrast to tyrosine phosphorylation, serine phosphorylation leads to the inhibition of IRS-1. Furthermore, ceramides neutralize insulin action by inhibiting Akt [66]. Thus, in the diabetic heart intracellular accumulation of lipid metabolites, most notably ceramides and DAG, and generation of ROS promote a permanent CD36 relocation and a decreased insulin-stimulated GLUT4 translocation. The latter decreases the ability of the heart to utilize glucose.

4. Diabetic cardiac remodeling

In a healthy individual, cardiac hypertrophy is the answer to an increased work load. It is a beneficial compensatory process as it decreases wall stress and increases cardiac output and stroke volume. However, hypertrophic growth in the context of disease is in the end maladaptive because it will progress to decompensation, contractile dysfunction and ultimately heart failure [67]. Physiologically and biochemically, hypertrophic remodeling is a delicate process, which balances between adaptation and disease, and includes participation of a number of pathways that direct growth and differentiation. When challenged, as in diabetes, only a small percentage of the cells in the heart (myoblasts) can divide and proliferate. A recent paper by Efe et al. [68] describes genetic reprogramming of cardiac fibroblasts into cardiomyocytes. This opens new avenues for cardiac remodeling. Still, cardiac enlargement and capacity increase occurs primarily as a result of growth of resident myocytes [69]. Myocyte enlargement may follow two patterns: on the one hand concentric hypertrophy is achieved by the addition of sarcomeres in parallel, increasing myocyte width; on the other hand eccentric hypertrophy is the result of the addition of sarcomere series, causing the myocyte to elongate [70].

The cause of concentric hypertrophy is increased stress on the ventricle walls, which adversely affects the cardiac output. The increased pressure is balanced by myocyte hypertrophy and by increased wall thickness, which together eventually equalize the wall stress [71]. This results in a concentric form of hypertrophy in which the ventricle walls increase in thickness while the chamber volume is unaffected. The increase in wall thickness is proportional to the increase in systolic pressure, and wall stress is normalized. However, there is a greater oxygen demand of the heart due to the increased cardiac mass. In eccentric hypertrophy, chamber volume is increased with little or no effect on wall thickness. Ventricular dilation allows for increased pressure necessary for ejection, but wall stress is not normalized. Interestingly, prolonged aortic stenosis also leads to a drop in cardiac output, elevated end-diastolic pressure and dilation of the left ventricle. This suggests that concentric hypertrophy may in time deteriorate into a dilated eccentric growth [72].

4.1. The role of FA

Diabetic cardiomyopathy is characterized by ventricular dysfunction occurring independently of a recognized cause such as coronary atherosclerosis or hypertension [73]. Several studies have shown that diabetes results in structural, mostly hypertrophic, and functional cardiac changes and subsequently heart failure [4,74,75]. The underlying pathological mechanisms of diabetic cardiomyopathy are still poorly understood, although there is accumulating evidence that this cardiomyopathy is associated with an altered metabolism. Increased FA uptake and lipid accumulation in cardiomyocytes are associated with insulin resistance, type 2 diabetes, hypertrophy and eventually heart failure [3,76]. In this respect, several rodent models have been studied, including rats on a high fat diet [41], leptin-deficient animals [77] and cardiac PPARα overexpression mice [35]. Interestingly, the absence of CD36 in cardiac PPARα overexpression mice prevented myocyte triacylglycerol accumulation and cardiac dysfunction both under basal conditions and following administration of a high fat diet. The rescue of the cardiac PPARα overexpression phenotype by CD36 ablation was associated with increased glucose uptake and oxidation rather than changes in FA utilization [78]. All these data indicate that intramyocardial lipid accumulation underlies diabetic cardiac dysfunction. In this respect a recent paper by Glenn et al. [79] is of interest. These investigators generated a transgenic mouse model with cardiomyocyte-specific overexpression of diacylglycerol acyl-transferase-1 (DGAT1). DGAT1 is involved in synthesizing triacylglyceroles. As expected, the DGAT1 transgenic mice displayed cardiac lipid accumulation. In addition, the mice also exhibited cardiac fibrosis and contractile dysfunction. As a result, this report strongly supports the concept that lipid accumulation in the heart may be directly responsible for metabolic cardiomyopathy.

4.2. The role of Akt signaling

Akt signaling is an important regulator of cardiac growth, and its overexpression leads to enhanced contractility, cell survival

and pathological cardiac hypertrophy [80,81]. High fat diet feeding did not change the cardiac expression of Akt and Foxo3a but basal phosphorylation levels of Akt were increased, which leads to inhibition of Foxo3a [82]. In these hearts, insulin-stimulated phosphorylation of Akt and Foxo3a was blunted, leading to reduced insulin sensitivity. These data also favor a role for Akt and its downstream signal Foxo3a in cardiac hypertrophy during lipid overload (Fig. 3). Specifically, increased basal phosphorylation of Akt and decreased activation of Foxo3a, as occurring after high fat diet feeding, promote cardiac hypertrophy and suppress atrophy-specific gene transcription involving atrogin-1 and MuRF-1 [82]. In addition, a reduced insulin action hampers Foxo3a phosphorylation and apoptosis, which was supported by elevated caspase activities in cardiomyocytes after high fat diet intake [82]. The observation of enhanced basal Foxo3a phosphorylation and suppressed atrophy-specific gene suppression coincides with cardiac hypertrophy during lipid overload. Taken together, during a high fat diet, basal Akt phosphorylation levels increase and this will cause both metabolic and hypertrophic cardiac remodeling.

4.3. The role of ROS

In diabetic patients, as in several animal models, ventricular wall remodeling is often compromised by the development of fibrosis, changes in the extracellular matrix and even apoptosis [83–85]. Such changes are the result of a genetic reprogramming often started by oxidative stress. ROS has been reported as a product of excessive FA oxidation in diabetic cardiomyocytes (Fig. 3). As second messenger, ROS can mediate hypertrophic signals by regulating various intracellular signal transduction cascades and the activity of various transcription factors, such as NF-κB and activator protein-1 [86], and by activating mitogen-activated protein kinases (MAPKs) [87,88]. In addition, ROS has been shown to activate matrix metalloproteinases, in particular MMP-2 [89]. MMPs are well known for their role in extracellular matrix remodeling, but they can also cleave sarcomeric proteins such as troponin-I and MLC-1, which leads to contractile dysfunction and may contribute to apoptosis [90]. Both intracellular and extracellular matrix actions of MMP may affect structure and function of the heart. The standing body of literature on the ROS–MMP–hypertrophy axis in ischemia-reperfusion is rather extensive, but in insulin resistance/diabetes this aspect has been neglected so far, since focus has been aimed at the contractile performance. Although mitochondria are a major source of ROS, the organelles themselves can be damaged by ROS. Mitochondrial injury is reflected by mtDNA damage as well as by a decline in the mtRNA transcripts, protein synthesis and mitochondrial function [91]. Eventually, this can lead to apoptosis due to the release of proapoptotic proteins by the mitochondria [92]. In the diabetic heart, mitochondrial oxidative stress induces apoptosis by release of cytochrome c and upregulation of caspase-3 and caspase-9 [93,94]. Thus, in earlier stages of insulin resistance, mitochondrial FA oxidation increases, while in advanced stages of insulin resistance, ROS production leads to mitochondrial injury, which results in a decreased FA oxidation.

5. Concluding remarks

Our current understanding of the mechanism by which systemic hyperlipidemia leads to cardiac remodeling and dysfunction can be summarized as follows. Initially, high fat diet-induced hyperlipidemia will lead to an increased facilitated diffusion of FA over the plasma membrane into the cytoplasm. The higher intracellular concentration of FA will lead to an activation of PPAR signaling pathways. This not only enhances mitochondrial β-oxidation but also CD36 translocation, which will speed-up FA import and further boost PPAR stimulation. The resulting vicious circle of increased uptake of FA and FA-induced uptake stimulation, eventually leads to mitochondrial FA overload. FA becomes the preferred substrate for mitochondrial β-oxidation at the expense of glucose. Excessive β-oxidation results in massive ROS production. Over time this may contribute to cardiac remodeling by inducing the expression of genes such as MMP-2. ROS production also can cause

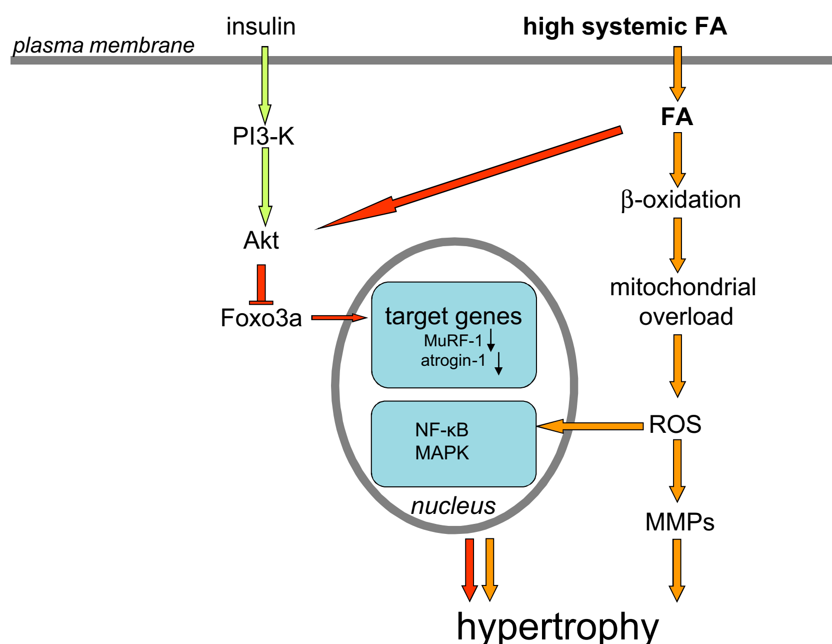

Fig. 3. Schematic depiction of the consequences of increased FA uptake by cardiomyocytes (hypertrophic remodeling). Increased cytoplasmic FA concentrations will induce hypertrophic remodeling by at least two mechanisms. Firstly, high cytoplasmic FA levels increase basal Akt phosphorylation, which leads to the inactivation of Foxo3a. Inactivation of Foxo3a leads to the inhibition of several atrophy-related genes, which leads to hypertrophic remodeling. Secondly, high cytoplasmic FA levels cause an increase in mitochondrial ROS generation. This will activate MMPs, which induce extracellular matrix remodeling and cleave sarcomeric proteins leading to cardiac dysfunction and remodeling. In addition, ROS will (indirectly) affect the expression of genes involved in inflammation and growth.

mitochondrial dysfunction, which leads to cardiomyocyte apoptosis. Finally, FA overload affects the complex signaling patterns of insulin action and glucose utilization directly (via Akt), and indirectly (via ROS). Thus, whole body lipid overload causes an increased uptake of FA into the heart, which is facilitated by CD36, which hampers glucose oxidation. In the end this is detrimental to the cardiomyocyte. Although the regulation of FA uptake and the effects of FA on ongoing cellular processes are not yet completely understood, the increasing insight in this complex diabetic pathology may already offer opportunities for therapeutic intervention.

Conflict of interest

The authors declare that there are no conflicts of interest.

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