The Review of DIABETIC STUDIES

REVIEW

Fatty Heart, Cardiac Damage, and Inflammation

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■ Abstract

Type 2 diabetes and obesity are associated with systemic inflammation, generalized enlargement of fat depots, and uncontrolled release of fatty acids (FA) into the circulation. These features support the occurrence of cardiac adiposity, which is characterized by an increase in intramyocardial triglyceride content and an enlargement of the volume of fat surrounding the heart and vessels. Both events may initially serve as protective mechanisms to portion energy, but their excessive expansion can lead to myocardial damage and heart disease. FA overload promotes FA oxidation and the accumulation of triglycerides and metabolic intermediates, which can impair calcium signaling, β-oxidation, and glucose utilization. This leads to damaged mitochondrial function and increased production of reactive oxygen species, pro-apoptotic, and inflammatory molecules, and finally to myocardial inflammation and dysfunction. Triglyceride accumulation is associated with left ventricular hypertrophy and dysfunction. The enlargement of epicardial fat in patients with metabolic disorders, and coronary artery disease, is associated with the release of proinflammatory and proatherogenic cytokines to the subtending tissues. In this review, we examine the evidence supporting a causal relationship linking FA overload and cardiac dysfunction. Also, we disentangle the separate roles of FA oxidation and triglyceride accumulation in causing cardiac damage. Finally, we focus on the mechanisms of inflammation development in the fatty heart, before summarizing the available evidence in humans. Current literature confirms the dual (protective and detrimental) role of cardiac fat, and suggests prospective studies to establish the pathogenetic (when and how) and possible prognostic value of this potential biomarker in humans.

Keywords: type 2 diabetes · obesity · cardiovascular disease · imaging · ectopic fat · epicardial fat · lipid · lipotoxicity

Introduction

Cardiac complications are the leading cause of mortality in patients suffering from type 2 diabetes (T2D) or obesity. In these patients, an increased supply of fatty acids is correlated with augmented cardiac adiposity and cardiac dysfunction. Indeed, the increased prevalence of metabolic and cardiovascular diseases has directed attention to the action of myocardial metabolic regulation mechanisms. Recent advances in imaging technology have allowed to explore, and partly support, the association between cardiac adiposity and systemic inflammation in patients with obesity, diabetes, and heart disease. However, correlative human studies have not yet established any causal mechanisms. Animal models of obesity and diabetes, and genetically modified (molecularly targeted) models, have been developed and used to characterize the relationship between intramyocardial lipid accumulation and ventricular dysfunction.

Cardiac adiposity refers to the presence of a large number of lipid droplets inside the myocytes that emerges because of excessive fat accumulation in the cardiac muscle. Intramyocardial triglyceride depots, which are increased in obesity and type 2 diabetes, are associated with greater

Abbreviations:

ACS - acyl-CoA synthetase
Acyl-CoA - acyl-coenzym A
AGPAT - acylglycerolphosphate acyltransferase
AMP - adenosine 5'-monophosphate
AMPKα - AMP-activated protein kinase-alpha
ANP - atrial natriuretic factor
ATGL - adipose tissue triglyceride lipase
ATP - adenosine triphosphate
BMI - body mass index
BNP - brain natriuretic peptide
CAD - coronary artery disease
CCL2 - chemokine (C-C motif) ligand 2
CPT1 - carnitine palitoyl transferase 1
CTE-1 - cytosolic thioesterase-1
DAG - diacyglycerol
DGAT1- diacyglycerolacyltransferases isoform 1
eNOS - endothelial nitrix oxide sinthase
ER - endoplasmic reticulum
FA - fatty acids
FABPpm - FA binding protein
FAT - fatty acid translocase (also called CD36)
FATP1/6 - FA transport protein 1 (and 6)
GPAT - glycerolphosphate acyltransferase
GLUT4 - glucose transporter 4
GPAT - glycerol-3-phosphate acyltranspherase
HFD - high fat diet
HSL - hormone sensitive lipase
IKK - I kappaB kinase
IL - interleukine
JNK - C-Jun N-terminal kinase
KO - knock-out
LFD - low-fat diet
LPA - lysophosphatidic acid
LpL - lipoprotein lipase
LV - left ventricular
MAPK - mitogen-activated protein kinase
MCP1 - monocyte chemoattractant proein-1
MHC-a/b - myosin heavy chain isoform a/b
MMP - matrix metalloproteinases
MRS - magnetic resonance spectroscopy
MTE-1 - mitochondrial thioesterase-1
MTP - microsomal triglyceride transfer protein
MVO2 - myocardial oxygen consumption
NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells
PA - phosphatidic acid
PDH - pyruvate dehydrogenase
PDK-4 - pyruvate dehydrogenase kinase-4
PET - positron emission tomography
PKB/C - protein kinase B/C
PPARα - peroxisome proliferator activated receptor alpha
ROS - reactive oxygen species
SOD - superoxide dismutase
SPT - serine palmitolyltransferase
SR - sarcoplasmic reticulum
STZ - streptozoticin
T2D - type 2 diabetes
TG - triglyceride
TNFα - tumor necrosis factor alpha
Tpl2 - tumor progression locus 2
UCP-3 - uncoupling protein-3
WD - western diet

left ventricular (LV) mass, impaired diastolic function, higher cardiac work, and oxygen consumption [1, 2]. In this context, cardiac dysfunction correlates with myocyte accumulation of lipotoxic intermediates and apoptosis. Another definition of cardiac adiposity is increased thickness or volume of adipose tissue layers surrounding the heart and vessels. These fat layers are described as epicardial (between myocardium and visceral pericardium), or pericardial (between visceral and parietal pericardium, or on the external surface of the pericardium).

Both fat depots inside and around the heart may initially serve as a fatty acid (FA) deposits and exert a protective role in energy portioning. However, their excessive expansion has been associated with inflammation, various kinds of myocardial damage, and heart disease. Increased FA availability is being considered as one of the major players in the development of myocardial damage and chronic inflammation. In fact, increased lipolysis leading to a higher FA supply to non-adipose tissues is a common feature in diabetes, insulin resistance, and obesity (once the adipocyte buffering capacity saturates), and may be associated with the adrenergic activation that correlates with heart failure [3]. In the healthy heart, FAs represent the main fuel, being responsible for 70% of the oxidative ATP production [4]. Intracellular FAs activate the transcription factor peroxisome proliferator activated receptor alpha (PPARα), which regulates several genes involved in FA metabolism [5], including those involved in FA uptake, esterification, transport into mitochondria, and β-oxidation. As cardiac FA influx increases, PPARα is activated in a feed-forward fashion. Thereby, in response to FA overload, both FA oxidation and esterification are increased. Eventually, TG accumulation occurs, and is further promoted by the exhaustion of oxidative capacity [6].

Increased FA oxidation is associated with production of reactive oxygen species (ROS) which leads to myocardial dysfunction and cardiomyopathy [7-9]. Moreover, FAs might be diverted to non-oxidative pathways, including the formation of lipotoxic intermediates and pro-apoptotic species. FA overload also decreases glucose oxidation by inhibiting pyruvate dehydrogenase (PDH) activity through a PPARα-regulated mechanism, and reduces the expression of sarcolemma glucose transporter 4 (GLUT4), thus deteriorating insulin sensitivity [8, 10].

In this review, we examine the evidence supporting a causal relationship linking FA overload

with cardiac dysfunction. Also, we disentangle the separate roles of FA oxidation, and TG accumulation, in causing cardiac damage, and we focus on the mechanisms underlying the development of inflammation in the fatty heart. Finally, we summarize the available evidence for the fatty heart in humans.

Fatty acid overload and cardiac function

In lipotoxic heart disease, increased plasma FA availability is a first and likely causative event correlated with cardiac hypertrophy and dysfunction. FA cellular uptake is mediated by sar-

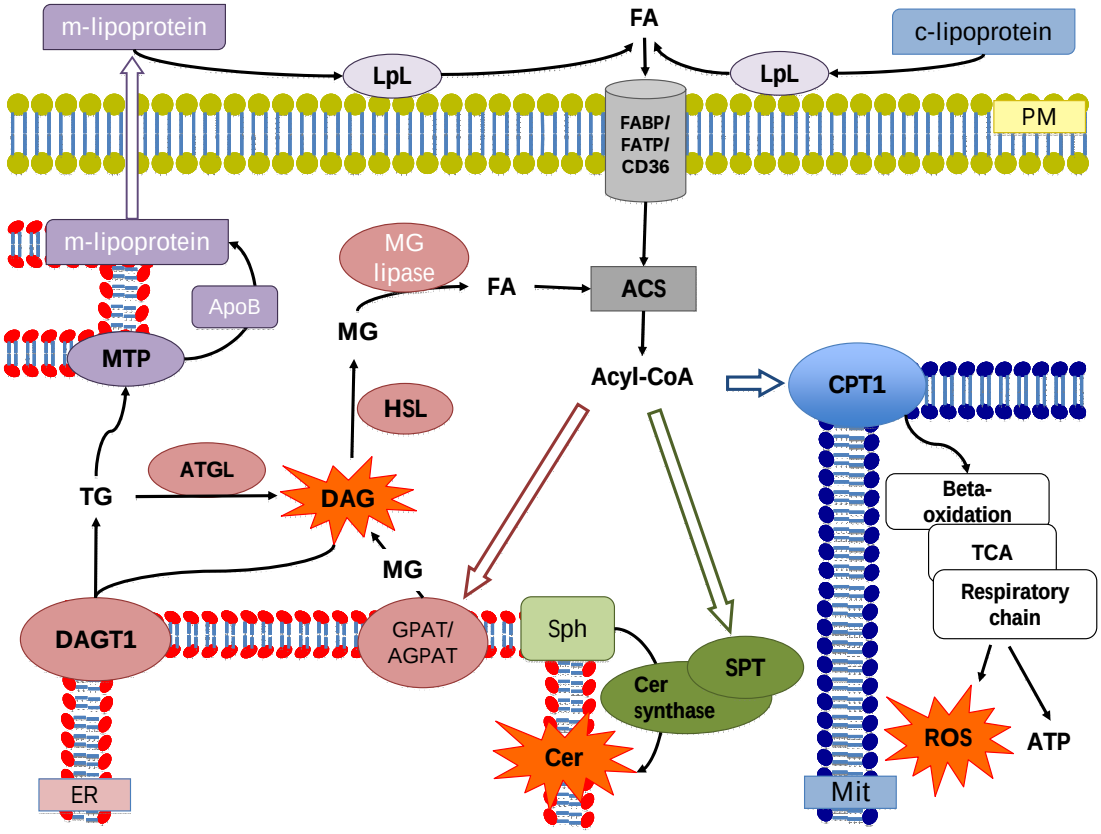

Figure 1. Enzymatic pathways regulating fatty acid (FA) metabolism in the heart. FAs enter the cardiomyocyte through specific transporters, and they are converted to acyl-CoA by acyl-CoA synthethase (ACS). Acyl-CoAs might be used for β-oxidation (blue pathway), or they can be diverted to non-oxidative pathways, including esterification and TG synthesis (pink pathway) and formation of lipotoxic intermediates, namely ceramides (Cer) (bottom, green pathway). Reactive oxygen species (ROS) and diacylglycerol (DAG), resulting from oxidation or esterification, are also toxic intermediates. Intracellular TG may accumulate in lipoproteins (top left, purple pathway) to be eventually released in the circulation, or be hydrolyzed by adipose tissue triglyceride lipase (ATGL), leading to intracellular release of FA. Intracellular FAs can follow one of the pathways mentioned above, with further accumulation of cytotoxic molecules. Potentially toxic products are shown in orange. **Abbreviations**: Acyl-CoA: acyl coenzyme A. ACS: acyl-CoA synthetase. AGPAT: acyl glycerolphosphate acyltransferase. ApoB: apolipoprotein B. ATGL: adipose tissue triglyceride lipase. Cer: ceramide. CPT1: carnitine palmitoyl transferase-1. DAG: diacyloglycerol. DGAT1: diacylglycerol-acyltransferase 1. ER: endoplasmic reticulum. FA: fatty acid. FABP: fatty acid binding protein. FATP: fatty acid transport protein. GPAT: glycerolphosphate acyltransferase. HSL: hormone sensitive lipase. LpL: lipoproein lipase. m-/c-lipoprotein: cardiomyocyte released / circulating lipoprotein. MG: monoacylglycerol. Mit: mitochondrion. MTP: microsomal triglyceride transfer protein. PM: plasma membrane. ROS: reactive oxygen species. Sph: sphingosine. SPT: serine palmitoyl-transferase. TCA: tricarboxylic acid cycle. TG: triglyceride.

colemma proteins CD36, plasma membrane FA-binding protein (FABPpm), and FA transport protein 1 and 6 (FATP1/6). Also, intracellular proteins may independently enhance FA uptake by binding and modifying the intracellular FA pool, thereby creating an inward FA gradient. Among them, acyl-CoA synthetase (ACS) represents the first step in FA intracellular metabolism, since it converts FAs to acyl-CoA derivates, which may eventually be oxidized, or incorporated into lipids (Figure 1).

Studies have been carried out in wild type and genetically modified rodent models, undergoing high-fat or control diet feeding, to elucidate the effect of an FA overload on cardiac function. Mutations in leptin, and leptin receptor, genes are responsible for augmented food intake observed in db/db, and ob/ob, mice models, in which diabetes and reduced cardiac power are observed at 5 and 15 weeks of age, respectively. Hearts of diabetic db/db mice develop a 2-3-fold increase in myocardial TG accumulation, which is associated with LV contractile dysfunction [8]. Isolated hearts of insulin-resistant ob/ob mice showed augmented FA oxidation and myocardial oxygen consumption (MVO2) at the expense of glucose utilization, in response to perfusion with increasing palmitate concentrations. This finding was independent of insulin administration. It indicates that ob/ob mice are unable to modulate substrate utilization [2].

In obese compared to lean Zucker rats, the higher number of lipid droplets found in cardiomyocytes is due to enhanced FA transport through FABPpm and CD36, which is not accompanied by an increase in the FA oxidation rate [11]. Moreover, Burgmaier *et al*. observed that obese Zucker rats fed a western diet (WD, with excess calories and lipids) showed a significant decrease in cardiac power, increased TG synthesis and accumulation after only 7 days of WD feeding [12]. These changes were not seen in animals fed a control-diet, or in lean rats, in which FA oxidation was upregulated after 7 days of high-calorie diet. The latter effect counteracted the initial FA overload. This response was observed with a delay of 4 weeks from the start of WD in the obese group, in which the mismatch between FA uptake and FA oxidation led to a massive myocardial TG accumulation and LV dysfunction at 7 days of WD. The subsequent (though late) activation of oxidation prevented a further impairment in cardiac function. Metabolic findings correlated with gene expression in the obese animal group, in which the levels of glycerol-3-phosphate acyltranspherase (GPAT), promoting TG synthesis, were increased after 7 days of WD. Whereas, the levels of oxidative enzymes (pyruvate dehydrogenase kinase-4 PDK-4, uncoupling protein-3 UCP-3, mitochondrial and cytosolic thioesterase-1 MTE-1 and CTE-1 respectively) did not increase until the fourth week of diet. Ouwens *et al*. reported that rats fed a high-fat diet (HFD) for 8 weeks exhibited increased TG accumulation myocyte hypertrophy, and a decreased LV fractional shortening and ejection fraction [13]. Also, they showed impaired systemic glucose tolerance, relative to rats fed an isocaloric low-fat diet (LFD).

A HFD induces cardiac hypertrophy, and alters the subcellular localization of CD36. Current evidence suggests that the sarcolemma relocalization of CD36 is induced by FA overload itself (through PPARα activation). It precedes the onset of cardiac dysfunction, and it is related to the phosphorylation and activation of protein kinase B (PKB/Akt) signaling, which regulates insulin sensitivity [8, 14]. In fact, CD36 deficiency prevents myocardial TG accumulation, and counteracts LV dysfunction in rats overexpressing cardiac PPARα. CD36 deficiency does not affect the FA oxidative pathway, but it enhances glucose utilization [5, 6]. Accordingly, in isolated working hearts of FAT/CD36-null mice perfused with either low or high concentrations of palmitate, the rate of FA oxidation is significantly lower than in hearts of wild type mice. Notably, this animal model displayed a compensatory increase in myocardial glucose utilization, and an improvement in cardiac output and coronary blood flow [15].

Although CD36 is responsible for 40-60% of FA uptake, there are other membrane-associated transporters that provide the heart with this substrate. For example, cardiac overexpression of FATP1 results in higher FA uptake, which leads to a 2-fold increase in FA oxidation, and a 50% decrease in glucose metabolism, as measured by radioactive tracers (^{11}C-palmitate, ^{14}C-glucose, ^{3}H-palmitate) [16]. Enhanced FATP1-mediated FA uptake, similar to CD36 membrane translocation, is associated with greater LV mass and internal diameter, a reduction in heart rate, and an impairment in diastolic function [16]. Thus, FAs are specifically implicated in the pathogenesis of diastolic dysfunction, which is a typical finding in obesity and diabetes, and a recognized early predictor of systolic dysfunction and heart failure.

In addition to membrane transporter proteins, FA uptake is regulated by the activity of ACS,

Table 1. Summary of genetic and pharmacological animal models investigating the relation between fatty heart and cardiac function

Target gene or pathway	Genetic or pharmacological manipulation	Myocardial metabolic changes	Cardiac damage/ dysfunction	Reference
FA uptake				
PPARα	Overexpression (mice)	↑ FA oxidation ↑ TG ↓ Glucose usage	LV hypertrophy and dysfunction	Finck BN, *et al.* 2002[5]
PPARα + CD36	Overexpression + knock out (mice)	Prevents ↑ TG ↓ Glucose use	CD36 deficiency rescues the PPARα phenotype and the associated LV dysfunction	Finck BN, *et al.* 2002[5]
CD36	Null (isolated mice hearts)	↓ FA oxidation ↑ Glucose usage	↑ Cardiac perfusion, output	Kuang M, *et al.* 2004 [15]
FATP1	Overexpression (mice)	↑ FA oxidation ↓ Glucose usage	↑ LV mass, diameter ↓ Heart rate, diastolic function	Chiu HC, *et al.* 2005 [16]
LpL	Overexpression (mice)	↑ TG ↑ Ceramides ↑ PPARα ↑ ANP, BNP, caspase-3 ↓ GLUT4	LV hypertrophy, dilated cardiomyopathy	Yagyu H, *et al.* 2003 [20]
ACS	Overexpression (mice)	↑ FA oxidation ↑ TG ↑ Ceramides ↓ Antioxidant enzymes	LV hypertrophy ↓ Fractional shortening, systolic function Myocyte enlargement, myofiber disorganization, fibrosis, apoptosis	Lee Y, *et al.* 2006 [17] Lee Y, *et al.* 2004 [18] Chiu HC, *et al.* 2001 [19]
FA storage				
DGAT1	Overexpression (mice)	↑ TG ↓ Ceramides and DAG ↑ FA usage genes	Neutral	Liu L, *et al.* 2009 [23]
DGAT1 + ACS	Double overexpression (mice)	↑ TG Prevents ↓ AE	Coexpression improves ACS phenotype (cardiac function and apoptosis)	Chiu HC, *et al.* 2001 [19]
ATGL	Knock-out (mice)	↑↑ TG	Massive LV dilatation and hypertrophy, severe fibrosis, heart failure	Haemmerle G, *et al.* 2006 [24]
HSL	Overexpression (mice)	↓ TG ↓ DAG ↑ FA oxidation genes	Prevents steatosis and fibrosis	Suzuki J, *et al.* 2001 [27]
MTP-A	Knock-out (mice)	↑ TG	Not defined	Bartels ED, *et al.* 2009 [28]
Apo-B	Overexpression (mice)	↓ TG ↓ CD36, FATP1, UCP3	Prevents cardiac dysfunction in fat-fed obese mice	Bartels ED, *et al.* 2009 [28]
LpL + Apo-B	Double-overexpression (mice)	↓ TG ↓ ANP, BNP, caspase-3 ↑ GLUT4	↑ LV function Coexpression improves the LpL phenotype	Yokoyama M, *et al.* 2004 [29]
FA oxidation				
Anti-diabetic drug	Troglitazone (ZDF rat and ob/ob mice)	↓ TG ↓ Ceramide ↑ AMPK activation ↑ FA oxidation ?	↑ Cardiac function	Wang MY, *et al.* 20095[43]
CPT1-inhibitor	Oxfenicine/etomoxir (rat)	↓ FA oxidation ↑ Glucose oxidation ↑ MHC-a and -b	↑ Post-ischemic function recovery	Lopaschuck GD, *et al.* 1990 [44]

Legend: AE: antioxidant enzymes. AMPK: AMP-activated protein kinase. ANP: atrial natriuretic peptide. Apo-B: apolipoprotein B. ATGL: adipose tissue triglyceride lipase. BNP: brain natriuretic peptide. CD36: cluster of differentiation 36, also called fatty acid translocase (FAT). CPT1: carnitine palitoyl transferase 1. DAG: diacyglycerol. DGAT1: diacyglycerolacyltransferases isoform 1. HSL: hormone sensitive lipase. FA: fatty acid. FATP1: FA transport protein 1. GLUT4: glucose transporter 4. LpL: lipoprotein lipase. LV: left ventricle. MHC: myosin heavy chain. MTP-A: microsomal triglyceride transfer protein A. PPARα: peroxisome proliferator-activated receptor alpha. TG: triglyceride. UCP: uncoupling protein.

which converts FA to acyl-CoA, and thus creates an intracellular deposit for FA. Lee *et al*. showed that in ACS transgenic mice, an augmented FA import caused heart hypertrophy, increased the LV anterior and posterior wall thicknesses, and compromised fractional shortening and systolic function [17, 18]. This cardiac dysfunction was associated with enlarged myocytes, accumulation of lipid droplets (FA uptake was increased by 59%, against a 16% increase in β-oxidation), myofiber disorganization, interstitial fibrosis, and a 3.3-fold rise in ceramide levels and apoptosis. [19].

FA overload resulting from overexpression of the membrane anchored lipoprotein lipase (LpL) in cardiomyocytes also induces lipid accumulation, involving TGs, ceramides, and cholesterol. LpL transgenic mice exhibit LV hypertrophy, increased PPARα expression, and mortality from dilated cardiomyopathy [20].

Molecular mechanisms underlying cardiac dysfunction caused by FA overload include alterations in cardiomyocyte excitation-contraction coupling and Ca^{2+} handling [13]. According to Haim *et al*., exposure to palmitate decreases contractility by reducing Ca^{2+} transients through an effect on sarcolemma membrane excitability in isolated ventricular myocytes from rodents [21]. Specifically, palmitate exposure increases the voltage-dependent K^{+} outward current resulting in potential action shortening, reduction in voltage-dependent Ca^{2+} influx, and Ca^{2+}-dependent release of Ca^{2+} from intracellular stores. These effects reduce cell contractility. By comparing wild type with diabetic ob/ob mice, Fauconnier *et al*. suggested that in wild type mice, palmitate dissipates the membrane potential and increases mitochondrial ROS production, which are responsible for impaired Ca^{2+} handling [22]. According to this study, these effects are not observed in ob/ob mouse cardiomyocytes exposed to palmitate, suggesting that a prolonged exposure to high FA concentrations eventually results in cardiomyocyte adaptation.

Consequently, the available evidence indicates that FA overload exerts toxic effects on cardiac function, since it enhances metabolic pathways that alter intracellular metabolites concentrations and signaling molecules. Either FA oxidation, or FA accumulation in TG, are amplified in FA overload states, and it is difficult to quantify their individual contributions to cardiac damage. The following paragraphs address the evidence implicating each of these two pathways. Table 1 summarizes these studies.

Triglyceride accumulation and cardiac function

Increased FA levels result in TG accumulation in cardiomyocytes. In fact, as FA oxidation becomes saturated, FA storage within TG depots provides a protective buffer mechanism, which prevents the incorporation of excessive FA (especially the long-chain saturated FAs like palmitate) into lipotoxic species, namely ceramides and diacyglycerols (DAGs). Nevertheless, massive intracellular TG accumulation correlates with ventricular hypertrophy, cardiac dysfunction, insulin resistance, and inflammatory markers. Esterification of intracellular FAs is catalyzed by acylglycerolphosphate acyltransferases (GPAT and AGPAT), which leads to formation of DAG. This is converted to TG by diacyglycerolacyltransferases, which is mostly represented by isoform 1 (DGAT1) in the heart. Intracellular TG lipolysis is catalyzed by adipose tissue triglyceride lipase (ATGL) and hormone sensitive lipase (HSL). Moreover, the microsomal triglyceride transfer protein (MTP), which is located in the endoplasmatic reticulum (ER), transfers neutral lipids onto the Apo-B polypeptide, contributing to triglyceride packaging and secretion in lipoproteins. Overall, the cardiac triglyceride pool is not inert, but rather depends on, and responds dynamically to, the intracellular fluxes of fatty acids. Figure 1 summarizes the pathways described above.

Cardiac overexpression of DGAT1 in mice increases TG accumulation, while decreasing ceramides, DAG, and FA by 35%, 26%, and 20% respectively, without altering cardiac function [23]. Interestingly, in mice which overexpress ACS and exhibit enlarged TG depots because of an imbalance between FA uptake and oxidation, overexpression of DGAT1 partially compensates for the impairment in fractional shortening, LV dysfunction, endoplasmic reticulum (ER) stress, and apoptosis [19]. When ACS and DGAT1 are increased simultaneously, gene expression patterns suggest that TG storage and FA oxidation are both enhanced, resulting in a protective metabolic milieu. DGAT1 overexpression induces expression of ATGL and carnitine palitoyl transferase-1 (CPT1), and partially reverts the downregulation of antioxidant genes catalase and glutathione peroxidase observed in ACS transgenic mice [23].

In the abovementioned model, TG accumulation due to DGAT1 overexpression appears to be a beneficial metabolic adaptation. Contrary to this, knock-out of the TG lipolytic enzyme ATGL re-

sults in heart enlargement (20-fold), and a generalized increase in all tissue fat depots. Haemmerle *et al*. showed that ATGL-KO mice have severe cardiac fibrosis, increased posterior LV wall thickness, and ventricular dilatation of both ventricles [24]. Thereby, mice die from cardiac insufficiency caused by a mechanical contraction defect which is due to massive lipid accumulation. This effect is consistent with the described disarrangement in contractile protein composition observed in obese Zucker rats [25, 26]. In the latter, FA overload and TG accumulation are associated with upregulation of isoform b of the myosin heavy chain (MHC-b), but not MHC-a, which leads to a depressed cardiac power. Thus, excessive intracellular lipid accumulation might represent a physical hindrance, which mechanically disturbs protein contraction and contributes to cell dysfunction. Whereas, in control and streptozoticin (STZ)-induced diabetic mice, cardiac-specific overexpression of the lipolytic enzyme HSL prevents TG accumulation under fasting conditions, increases expression of FA oxidative genes, and prevents interstitial fibrosis and mortality [27]. TG and DAG HSL-mediated hydrolysis contributes to intracellular FA production and upregulation of several genes involved in lipid metabolism.

Lipoprotein secretion also plays a role in modulating intra-cardiac TG accumulation. Fasting and fat-feeding conditions induce the expression of cardiac MTP and formation of Apo-B-containing lipoparticles, which promote TG release. Transgenic mice exhibit an expansion in TG content upon both fasting and fat feeding, when the cardiac-specific MTP-A isoform is deleted [28]. In contrast, cardiac Apo-B overexpression prevents TG accumulation and the expression of genes involved in FA uptake, cytosolic release, and mitochondrial oxidation uncoupling such as CD36, FATP1, UCP3. The latter molecules are implicated in the lipotoxic effect. As a result, cardiac function is more preserved in Apo-B transgenic mice than control animals during high fat-feeding [28]. Moreover, the double overexpression of Apo-B and LpL in mice reduces the severe lipotoxic cardiomyopathy of the LpL transgenic model by decreasing cardiac TG and FA content, atrial natriuretic factor (ANP) gene expression, and brain natriuretic peptide (BNP) gene expression, while increasing GLUT4 [29].

All considered, these results suggest that it remains unclear whether, and when, cardiac TG synthesis and accumulation are protective or detrimental. The available studies suggest that cardiac TG storage can be beneficial to cardiomyocyte function and survival, at least to some extend. A modest increase in TG may not be detrimental, and can be seen as a preferential pathway preventing FA from ceramide and toxic intermediate formation in situations of FA overload. Saturation of this storage capacity results in the formation of intermediate species. As the intracellular lipid pool grows, it may undergo peroxidation, and become a source of oxidative damage. Once it becomes excessively large, mechanical decoupling and disruption may occur.

Fatty acid oxidation and cardiac function

β-oxidation *per se* is a protective pathway. It provides energy for cell contraction and survival, and it deters FA from excessive TG accumulation and formation of toxic species. However, an increase in FA oxidation lowers myocardial glucose utilization and enhances myocardial oxygen consumption (MVO2). Moreover, increased FA oxidation—especially that of long-chain saturated FA, e.g. palmitate—can induce apoptotic signaling through dissipation of the mitochondrial membrane potential and overproduction of ROS [30, 31]. Most likely, this occurs at the level of complexes I and III of the mitochondrial respiratory chain.

ROS overproduction damages mitochondrial structure, which results in the release of pro-apoptotic mitochondrial proteins, citochrome c leakage, and activation of caspase 3 and 9 [32, 33]. Besides programmed cell death, ROS activate kinase pathways, which are associated with cardiac hypertrophy and loss of function. For instance, induction of BNP promoter depends on NF-κB, which can be activated by ROS and plays a role in cardiac hypertrophy [34]. ROS may also activate the mitogen-activated protein kinase (MAPK) signaling pathway [35] and matrix metalloproteinases (MMP), which may in turn contribute to cardiac growth and hypertrophic response. MMP-2 in particular is emerging as an important signaling protease implicated in the proteolytic regulation of intracellular proteins in myocardial oxidative stress injury [36]. ROS can damage excitation-contraction coupling, which impairs Ca^{2+} homeostasis in isolated cadiomyocytes treated with palmitate. In fact, increased ROS production can decrease L-type Ca^{2+} flow amplitude. This increases the open probability of sarcoplasmic reticulum (SR) Ca^{2+} release channels, and slows down SR Ca^{2+} uptake. At the same time it increases sarcolemma Na^{+}/Ca^{2+} exchange activity,

Table 2. Relationship between intramyocardial or epicardial fat and obesity, T2D, cardiac metabolism, and cardiac function in humans

Patient type	Study type	Cardiac / metabolic features	Cardiac function	Reference
Studies measuring intramyocardial fat (TG content)				
Obesity (BMI ≥ 30)	Cross-sectional comparison	↑ FA uptake and oxidation, ↑ TG content	Not defined	Peterson LR, *et al.* 2004[51]
T2D	Cross-sectional comparison	↑ TG content	↓ LV diastolic function	Rijzewijk LJ, *et al.* 2008[53]
T2D	Short-term caloric restriction	↑ Plasma FA ↑ TG content	↓↓ LV diastolic function	Hammer S, *et al.* 2008 [54]
T2D	Short-term caloric restriction + acipimox	= Plasma FA = TG content	= LV diastolic function	Hammer S, *et al.* 2008 [54]
Obesity or T2D	Long-term caloric restriction (↓ BMI)	↓ TG content	↓ LV mass ↑ LV diastolic function ↓ Blood pressure	Hammer S, *et al.* 2008 [55]
T2D	Pioglitazone	= TG content	↑ LV diastolic function	van der Meer RW, *et al.* 2009 [69]
T2D	Metformin	= TG content	↓ Cardiac work	van der Meer RW, *et al.* 2009 [69]
T2D	Insulin + pioglitazone	↓ TG content	= Cardiac function = Blood pressure	Zib I, *et al.* 2007 [68]
Obesity (BMI ≥ 30)	Trimetazedine	↓ FA oxidation ↑ Glucose metabolism = TG content	= Cardiac work ↑ Cardiac efficiency	Bucci M, *et al.* 2011 [47]
Studies measuring epicardial fat (volume or thickness)				
Obesity (BMI = 28-33)	Cross-sectional comparison	↑ FA levels ↑ TG content ↑ Epicardial fat	↓ Cardiac index ↑ LV mass ↑ Peripheral vascular resistances	Kankaanpaa M, *et al.* 2006 [57]
Obesity (BMI ≥ 30, ↑ abdominal visceral fat)	Cross-sectional comparison	↑ Epicardial fat ↑ Peri-coronary fat thickness	Not defined	Gorter PM, *et al.* 2008 [62]
Metabolic syndrome (≥ 3 metabolic abnormalities)	Cross-sectional comparison	↑ Epicardial fat ↑ Peri-coronary fat thickness	Not defined	Gorter PM, *et al.* 2008 [62]
T2D	Cross-sectional comparison	↑ Epicardial fat ↑ Peri-coronary fat thickness	Not defined	Gorter PM, *et al.* 2008 [62]
Obesity (BMI = 30.7±3.3)	Low-calorie diet and exercise (↓ BMI and waist)	↓↓ Epicardial fat	↓ LV mass ↑ LV diastolic function	Kim MK, *et al.* 2009 [65]
T2D	Pioglitazone	↑ Pericardial fat	↑ LV diastolic function	Jonker JT, *et al.* 2010 [70]

Legend: =: unchanged. BMI: body mass index. FA: fatty acid. LV: left ventricle. T2D: type 2 diabetes. TG: triglyceride.

which eventually leads to reduced SR Ca^{2+} content and depressed myocyte shortening [22].

Increased FA oxidation is associated with a reduced energetic efficiency in ATP production. Compromised ATP production suggests an FA-mediated activation of uncoupling proteins (UCP). Again, ROS are also responsible for the oxidation of lipids and proteins to produce highly reactive species, which may activate UCPs [37, 38]. UCPs represent a mechanism by which protons can re-enter the mitochondrial matrix, which lowers the membrane potential and bypasses ATP synthesis. UCP-2 and UCP-3 are expressed in the heart. Their content is elevated in db/db mice [39], but it

is reduced in hypoxic hearts, which use glucose as main energy source [40]. Upregulation in UCP3 expression has not been uniformly found by all authors. This suggests that mitochondrial uncoupling may reflect the allosteric activation of uncoupling mechanisms independent of changes in gene expression. The occurrence of uncoupling seems to be in agreement with observations in hearts of obese ob/ob mice. Despite an increase in mitochondrial mass, these hearts manifest an impaired expression in complexes I, III, and IV of the electron transport chain, and a defect in oxidative phosphorylation in response to increased FA uptake and oxidation [8, 41].

Whether modulating β-oxidation has a beneficial effect on ventricular function is still controversial. Treatment with the anti-diabetic drug troglitazone lowers cardiac TG and ceramide accumulation, and improves cardiac function [42]. The mechanism mediating these effects of troglitazone has been identified in the activation of AMP-activated protein kinase alpha (AMPKα) in ZDF rats or ob/ob mice [43].

On the other hand, the pharmacological inhibition of FA oxidation has been widely studied as a therapeutic strategy against ischemic cardiomyopathy to prevent the superoxide-related consequences. CPT1 inhibitors, such as oxfenicine and etomoxir, have been shown to benefit post-ischemic cardiac function recovery. In the reperfused ischemic myocardium, FAs are the preferred substrate, providing up to 90% of the required ATP. In this model, etomoxir administration can enhance glucose oxidation and increase the expression of both the a and the b isoform of MHC, which improves heart functional recovery [44]. Conversely, etomoxir was recently reported to fail in reversing heart failure in a rat model with mechanical-induced hypertrophy [45]. This suggests that only the ischemic, i.e. poorly oxygenated heart, may benefit from the metabolic shift from FA oxidation to the utilization of glucose, which is an oxygen-sparing substrate.

Trimetazidine is another anti-ischemic drug acting as a metabolic modulator, which shifts cardiac energy metabolism from FA to glucose oxidation [46]. Recently, a positron emission tomography (PET) study showed that trimetazedine administration preferentially reduces the oxidation of TG-released FA, while activating glucose metabolism in the myocardium of obese, otherwise healthy human subjects [47]. This drug has been shown to improve LV ejection fraction and cardiac function in patients with heart failure, with and without diabetes [48-50].

Genetic and pharmacological interventions affect lipid uptake and accumulation. Therefore, it has been difficult to isolate the effect of modulation of FA oxidation. Generally, the beneficial outcome of FA oxidation seems to be counterbalanced by the proportional rate of ROS production, which contributes to tissue damage and oxygen consumption. Therefore, the suppression of FA oxidation, leading to the utilization of glucose as energy source by the myocardium, seems to be beneficial in conditions of oxygen deficiency such as ischemia.

Cardiac adiposity and dysfunction in humans

Cardiac complications represent the major cause of mortality in diabetic and severely obese patients. These conditions are characterized by an elevated release of FA from adipose tissue, likely representing the major contributor to the development of heart adiposity. Both intramyocardial stores and epicardial fat depots are augmented in these patients compared to respective control populations. Table 2 summarizes the results from studies carried out in diabetic and obese patients, which are designed to clarify the relationship between cardiac fat and function.

Intramyocardial fat

Consistent with animal studies, in non-diabetic obese [51] or type 2 diabetic patients, myocardial FA uptake and oxidation (as measured with PET) and TG content (determined by MRS) are increased compared to those in lean individuals [52]. In type 2 diabetic patients, myocardial TG content is associated with impaired left ventricular diastolic function, independent of age, body mass index (BMI), heart rate, visceral fat, and diastolic blood pressure [53]. In both type 2 diabetic and healthy individuals, a short-term caloric restriction leads to an elevation in FA levels, myocardial TG accumulation, and deterioration of LV function, unless FA overload is prevented, e.g. by the pharmacologic suppression of lipolysis [54]. However, when obese or type 2 diabetic patients undergo a very low calories diet for a longer term (6-16 weeks), a reduction in myocardial TG content and LV mass combined with a significant improvement in cardiac diastolic function beyond BMI and blood pressure are observed [55]. These results suggest that intracellular TG content is highly flexible, and rapidly adapts to the changing metabolic milieu.

Epicardial fat

In addition to cardiac steatosis, diabetes and obesity have been positively correlated with the amount of fat surrounding heart and vessels [56]. Consistently, a positive correlation between epicardial fat volume and intramyocardial lipid content has been found [57]. Epicardial fat accounts for about 20% of the total ventricular mass, and is significantly greater in the hypertrophied heart. It is characterized by smaller adipocyte size, different fatty acid composition, lower glucose utilization rates, and higher fatty acid synthesis and metabolism compared to other fat depots [56]. Because of its close anatomical relationship to the heart, and the absence of physical fascial boundaries, epicardial fat may modulate vessels and heart metabolism via paracrine, or local direct secretion. Several protective roles have been ascribed to epicardial fat. Firstly, it serves as local energy supplier and buffer against excessive FA. Secondly, it is a source of protective cytokines and adipokines, such as adiponectin and anti-inflammatory interleukine-10 (IL-10). Finally, it mechanically attenuates vascular tension. Conversely, under pathological circumstances, such as obesity and diabetes, it may contribute to local inflammatory cell infiltration [56] and secretion of pro-inflammatory cytokines [58, 59]. These events are associated with a deterioration in coronary artery disease (CAD).

Epicardial fat thickness [60] and volume [61] are considered to be independent predictors of metabolic syndrome and pro-inflammatory citokine level. Its expansion is associated with lipotoxic heart disease. Epicardial adipose tissue volume correlates with abdominal visceral fat and BMI, and increases with the number of metabolic syndrome components [62]. This means that epicardial adipose tissue progressively increases in patient classes, from lean to obese individuals with normal glucose tolerance to those having impaired glucose tolerance and type 2 diabetes [63]. Also, its thickness is positively correlated with several circulating proatherogenic and proinflammatory adipokine levels [64], while it is inversely related to adiponectin concentrations. Kankaanpaa *et al*. have shown that in moderately obese men, epicardial fat is positively related to peripheral vascular resistances, and negatively associated with the cardiac index [57]. Low calorie diet and exercise have been shown to decrease epicardial fat thickness in obese men, resulting in changes in LV mass and diastolic function [65]. This is consistent with data suggesting that there is a relationship between epicardial fat mass and LV or RV mass [66].

Imaging studies have documented that patients with CAD have larger depots of pericardial fat compared to healthy individuals. Also, these studies have shown that epicardial fat thickness, more than any other fat depots or the waist circumference, correlates with vascular aging and subclinical atherosclerosis [67]. In fact, the enlarged and hypoxic epicardial fat becomes an active supplier of pro-inflammatory and pro-atherogenic cytokines to the subtending myocardium and vasculature. A high variability across populations and parameters considered in different studies makes it difficult to define a unique mechanistic association between epicardial fat thickness and cardiometabolic risk. Nevertheless, it is likely that epicardial fat has an influence on CAD disease, plaque composition, and lipid core, which are markers of plaque vulnerability.

Effects of diabetes and hypoglycemic drugs on cardiac fat and function

Cardiac fat is more extensive in type 2 diabetic than in similarly obese non-diabetic patients. This suggests a role of progressive insulin resistance, hyperinsulinemia, and hyperglycemia in the deterioration of cardiac adiposity. Consistently, the association of LV dysfunction and the amount of fat inside, or around, the heart has been described more frequently in diabetic than non-diabetic obese subjects. Hyperglycemia may provide an increased amount of glycerol, which is a substrate of TG synthesis, and may competitively impair the catabolism of FAs, thereby promoting their storage.

The addition of the antidiabetic drug pioglitazone to insulin treatment in T2D patients was shown to reduce intramyocardial triglyceride content without affecting heart function [68]. Moreover, in men with well-controlled T2D, the evidence is that hypoglycemic drugs pioglitazone and metformin improved LV diastolic function, and decreased cardiac work respectively, without changing cardiac TG content [69]. This markedly mitigates the role of intramyocellular fat in the pathogenesis of heart dysfunction. The pathophysiological implication of enlarged pericardial fat is not clear, since a correlation with cardiac function has not yet been demonstrated. In fact, treatment with pioglitazone for 24 weeks has been found to improve LV diastolic function in T2D patients, de-

spite increasing pericardial fat volume, whereas the administration of metformin did not result in any effect [70]. Direct drug influences on the metabolic and hemodynamic profiles, both of which have a recognized effect on cardiac function, may have masked the relationship between TG storage and cardiac outcome in these studies.

The controversial findings described above suggest a need for further studies to find out:

1. Whether differences in cardiac adiposity (in the range observed in humans with and without diabetes) have any clinically significant impact on heart function. Also, it may help to explain the high prevalence of cardiovascular disease associated with obesity and diabetes.
2. If hyperglycemia is implicated in the regulation of intramyocardial and epicardial fat.
3. If 2. is valid, then whether this form of glucose toxicity can be alleviated by hypoglycemic drugs.

Cardiac adiposity and inflammation

Intramyocardial fat

Cardiac adiposity and its related LV hypertrophy and dysfunction are positively associated with inflammatory markers. Myocardial FA intermediates, lysophosphatidic acid (LPA), phosphatidic acid (PA), diacylglycerol (DAG), and ceramides, resulting from impaired/saturated FA oxidation and/or biosynthetic pathways, are responsible for the inflammatory response caused by a prolonged cardiac FA overload. LPA, PA, and DAG are intermediate products that emerge during the synthesis of triglycerides. Ceramides are produced by the hydrolysis of sphyngomyelin sphingomielinase (SMase), or they are synthesized *de novo* by serine palmitolyltransferase (SPT) and ceramide synthase resulting from the condensation of palmitoyl-CoA with serine.

Ceramide has been shown to inhibit the mitochondrial respiratory chain by interacting with complex III in isolated cardiomyocytes [71]. In programmed cell death of neonatal rat cardiomyocytes, a progression has been observed from release of citochrome c and loss of membrane potential to ceramide accumulation, which finally results in the activation of caspase-3 like protease. All of these are documented as hallmarks of impending cell death [27]. Myriocin administration (SPT inhibitor) to a normal and lipotoxic heart of mice overexpressing LpL reduces ceramide concentration and improves cardiac function simultaneously with the normalization of substrate utilization [72].

Also, FA intermediates (e.g. DAG) can activate different serine/threonine-specific protein kinases which belong to the superfamily of mitogen-activated protein kinases (MAPK), IkB (IKK), and protein kinase C (PKC). These kinases play a critical role in apoptosis, inflammation, T cell differentiation, and insulin action. MAPK direct and integrate a complex signaling network which regulates the growth of myocytes and activates C-Jun N-terminal kinases (JNK). The network controls important cell function including cell growth, differentiation, and apoptosis [73]. Ceramides can inhibit Akt/PKB, another serine/threonine protein kinase, specially involved in glucose and protein metabolism and survival pathways inhibiting apoptosis [74]. Activation of serine kinases by FA intermediates might be significant, representing the mechanism of convergence between dysfunctional lipid overload and inflammation. In fact, activation of JNK and IKK promotes the nuclear factor κB (NF-κB) pathway, which induces transcription of several proteins causing proliferation, inflammation, and apoptosis.

Epicardial Fat

All the damage mechanisms occurring in the fatty myocardium, once exposed to an FA overflow, are amplified by the consensual expansion of epicardial adipose tissue. Lipid accumulation in epicardial adipocytes causes cell enlargement, which decreases oxygen delivery. The combination of local hypoxia and lipid overload triggers hypoxia-sensitive pathways in adipose tissue with ROS production, activation of JNK1 and IKK/NF-κB pathways, and induction of genes involved in the inflammatory response [75].

The hypoxic and inflammatory status is characterized by increased production and release of proinflammatory cytokines, such as TNFα, IL-6, and C-reactive protein. These cytokines activate the NF-κB pathway and a series of complex intracellular signals, which contribute to macrophage, and T cell, recruitment and tissue infiltration. Macrophages are responsible for the majority of TNFα and a significant part of IL6 expression in adipose tissue [76]. They also release other proinflammatory cytokines, resulting in a feed-forward cycle, which exacerbates the inflammatory status and its propagation to subtending tissues.

Adipokine, cytokine, and growth factors may propagate to the myocardium via simple diffusion.

Data from the Framingham study document that epicardial, but not pericardial (intrathoracic), fat volume is associated with CAD [77]. Recently, Sacks *et al*. found that the expression of 39 genes was increased in epicardial fat of patients with severe CAD compared with controls [78]. Seventeen mRNAs were linked to inflammatory processes. These included interleukin 8 (IL-8), mitogen-activated protein kinase 8 (Tpl2), lipocalin-2, monocyte chemoattractant proein-1 (MCP1/CCL2), p50 subunit of nuclear factor κB1 (NF-κB1), PAI-1, and TNFa. Seven mRNAs were related to oxidative stress, including GPX3, heme oxigenase 1, endothelial nitrix oxide sinthase (eNOS), and superoxide dismutase (SOD). Fifteen were involved in metabolism and angiogenesis, or they were specific to fat cells. These results support the hypothesis that expression of pro-inflammatory, redox, and angiogenic genes in epicardial fat may contribute locally to CAD and/or correlate with its severity.

Conclusions

The studies summarized in this review indicate that fatty heart is a typical finding in subjects with obesity and type 2 diabetes. Fatty heart correlates with LV dysfunction and systemic or local inflammation in several studies. However, not all studies confirm these associations. The level of association between severity of cardiac adiposity and cardiac damage is stronger in animal studies, in which myocardial metabolism can be manipulated in a more extreme (though unphysiological) fashion. Therefore, it is important to explore whether intra-myocardial TG stores are altered in human patients with heart disease, independent of diabetes and obesity.

Prospective studies are required to assess the prognostic role of a fatty heart in changing the risk, or the progression, of cardiovascular disease. It is important to recognize that the fatty heart likely reflects a dysregulation in the FA-trapping capacity of peripheral adipose tissue. The latter is a more likely the primary culprit, and possibly the best target for prevention and treatment related to the toxic and inflammatory consequences of cardiac adiposity.

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