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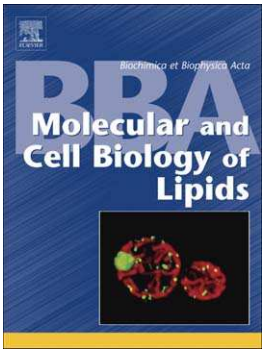

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The role of triacylglycerol in cardiac energy provision

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Abstract

Triacylglycerols (TAG) constitute the main energy storage resource in mammals, by virtue of their high energy density. This in turn is a function of their highly reduced state and hydrophobicity. Limited water solubility, however, imposes specific requirements for delivery and uptake mechanisms on TAG-utilising tissues, including the heart, as well as intracellular disposition. TAGs constitute potentially the major energy supply for working myocardium, both through blood-borne provision and as intracellular TAG within lipid droplets, but also provide the heart with fatty acids (FA) which the myocardium cannot itself synthesise but are required for glycerolipid derivatives with (non-energetic) functions, including membrane phospholipids and lipid signalling molecules. Furthermore they serve to buffer potentially toxic amphipathic fatty acid derivatives. Intracellular handling and disposition of TAGs and their FA and glycerolipid derivatives similarly requires dedicated mechanisms in view of their hydrophobic character. Dysregulation of utilisation can result in inadequate energy provision, accumulation of TAG and/or esterified species, and these may be responsible for significant cardiac dysfunction in a variety of disease states. This review will focus on the role of TAG in myocardial energy provision, by providing FAs from exogenous and endogenous TAG sources for mitochondrial oxidation and ATP production, and how this can change in disease and impact on cardiac function.

Key words:

heart; triacylglycerol; very-low-density lipoprotein; VLDL; chylomicron; lipid droplet

Highlights:

- Triacylglycerols are supplied to the myocardium within chylomicrons and VLDL
- Heart assimilates triacylglycerol-rich lipoproteins by LPL and receptor-mediated routes
- Exogenous triacylglycerol-derived fatty acids enter an intracellular lipid pool
- Intracardiac triacylglycerol is an important source of fatty acids for oxidation
- Dysregulation of triacylglycerol metabolism is associated with cardiac dysfunction

Abbreviations:

Apo	apolipoprotein
ATGL	adipose triacylglycerol lipase
CM	chylomicron
DGAT	diacylglycerol acyltransferase
FA	fatty acid
FABP	fatty acid binding protein
FAT	fatty acid translocase
FATP	fatty acid transport protein
GPAT	glycerol phosphate acyltransferase
H-LPL	heart lipoprotein lipase
KO	knockout
LD	lipid droplet
LPL	lipoprotein lipase
mTAG	myocardial triacylglycerol
NEFA	non-esterified fatty acid
PPAR	peroxisome proliferator-activated receptor
STZ	streptozotocin
TAG	triacylglycerol
TGRLP	triacylglycerol-rich lipoprotein
VLDL	very-low-density lipoprotein
VLDLR	very-low-density lipoprotein receptor
ZDF	Zucker diabetic fatty

1. Introduction

The heart assimilates lipids from the plasma avidly. The myocardium has a high and unremitting demand for energy, and early studies suggest that cardiac ATP synthesis is derived principally from glucose utilisation (about 30%) and FA oxidation (about 70%)(see [1]. The FAs may be supplied as non-esterified FA (NEFA), originating principally from adipose tissue lipolysis (though with some derived from "spillover" through the action of lipoprotein lipase (LPL)) or as TAG available in the hydrophobic cores of the TAG-rich lipoproteins (TGRLP): chylomicrons (CM), synthesised in the intestine from exogenous (dietary) fat, and very-low-density lipoprotein (VLDL), synthesised by the liver from endogenous lipid (derived in part from *de novo* lipogenesis of FA). Plasma TAG concentration is highly variable: fasting plasma [TAG] is typically ~ 0.6-0.7mM, increasing to > 1.5-3.0mM following a mixed meal. By contrast, plasma [NEFA] is typically ~ 0.3mM, although it rises in markedly in fasting (and diabetes), an effect partly compensated by the accompanying increased tissue uptake. Since TAG yields 3xFA upon complete hydrolysis, plasma TAG-FA availability greatly (>90%) exceeds NEFA availability. The heart also contains significant amounts of TAG within lipid droplets (LD). However, defining myocardial preference for lipids has proved difficult. Cardiac substrate utilisation changes depending on plasma availability, and in disease, but maladaptive substrate utilisation by the heart may itself cause cardiac dysfunction. The question therefore is the extent to which TAGs are efficient myocardial energy providers, and necessary for optimal cardiac function.

2. Myocardial exogenous triacylglycerol utilisation

2.1. Cardiac chylomicron-TAG utilisation

Measurement of TGRLP utilisation by the heart *in vivo* is technically challenging, but estimates of myocardial TAG-FA uptake generally support substantial cardiac CM-TAG utilisation.

Arterio-venous (A-V) differences measured by coronary sinus sampling are small, and early studies measuring total FA (NEFA + TAG-FA) across the human heart found that significant amounts of TAGs were extracted by the myocardium, and indirectly (by calculated difference) estimated that about half of the FAs utilised were TAG-derived (esterified). Subsequent A-V studies on non-diabetic humans found that TAG-emulsion accounted for only ~ 17% of cardiac FA uptake; moreover, the heart accounted for only 3% of systemic TAG uptake [2]. Studies in dog heart found that 56% myocardial oxygen consumption (mVO_2) was accounted for by total (NEFA+TAG) FA oxidation, of which 71% was calculated to be from TAG (hence about 40% of mVO_2 was TAG oxidation)[3].

Early experiments with rodents *in vivo* reported substantial cardiac uptake of labelled CM-TAG (more than any tissue examined besides liver)[4], and substantial CM-TAG oxidation was observed in rat heart, related to cardiac LPL activity; at physiological TAG concentrations (0.9mM) CM-TAG oxidation was able to provide about two-thirds of cardiac energy requirements [5]. Infusion of [^{14}C]palmitate-labelled CM into rats demonstrated substantial CM-TAG oxidation which was decreased only slightly (16%) by co-administration of glucose, implying a preference for CM-TAG oxidation [6]. High rates of label retention of CM prepared from chyle lymph were found in heart (more than skeletal muscle though less than white adipose tissue), and about 3 fold higher than NEFA; the ratio of TAG retention to NEFA retention was higher in heart than other tissues, suggesting a strong cardiac preference for TAG [7]. Gousios [8] perfused rabbit hearts with d<1.006 lipoproteins containing labelled TAG (a density corresponding to both CM and VLDL) and found fractional extraction rates of 15% in the fed state and 30% in hearts from fasted animals. Significant cardiac utilisation of d<1.006 lipoprotein-TAG (CM) was also found in isolated rat heart [9] – TAG oxidation accounted for 40% of CO_2 production (equivalent to that of NEFA oxidation)[10] (and in broad agreement with earlier studies [11]). Further evidence for a role for

CM-TAG in cardiac oxidative metabolism was the demonstration that CM increased mVO_2 of perfused rat hearts, and decreased (halved) their glucose utilisation [12]. Interestingly, whilst oxygen is essential for metabolism, oxygen availability may not dictate uptake of CM-TAG. Indeed, high rates of TAG emulsion uptake (and LPL activity) in mouse heart *in vivo* were not affected by chronic intermittent hypoxia [13] suggesting a potential mismatch between CM-TAG uptake and utilisation. Mouse hearts perfused with rat CM (0.4mM) utilised large amounts of CM-TAG [14] and achieved high degrees of cardiac power; equal amounts of CM-TAG assimilated were oxidised and esterified into tissue lipids (mainly TAG) and CM-TAG oxidation rates were similar to NEFA (0.4mM palmitate) oxidation, though proportionately more NEFA was oxidised than esterified. Furthermore, NEFA did not inhibit CM-TAG utilisation by the mouse heart [14]. Rat hearts perfused with rat CM utilised CM-TAG to about the same extent as NEFA when each were compared as sole lipid substrates [15, 16] and CM could maintain cardiac mechanical function; most (83%) of the CM-TAG assimilated was oxidised, the remainder was esterified mostly into cellular TAG [15, 16]. NEFA did inhibit CM-TAG utilisation when co-perfused, and this was entirely due to decreased CM-TAG oxidation, but CM did not suppress myocardial NEFA uptake and metabolism [16]. Together, these observations imply that NEFA-derived FA are more likely to be oxidised and CM-TAG-derived FAs more likely to be esterified [14] suggesting that there may be discrete partitioning between non-esterified and CM-TAG-derived FA. Mouse hearts clear CM-TAG (by lipolysis) more rapidly than they assimilate core (cholesteryl ester) lipid: about 5% of injected CM were recovered in hearts within 2 min, indicating rapid CM uptake by this tissue, but deficiency in heart LPL (H-LPL) decreases CM binding ("margination") to <1%, whilst mice that express LPL in heart alone doubled cardiac CM binding - the amount of LPL expressed governing both CM binding and lipid assimilation [17]. Mice expressing LPL only in heart maintained essentially normal plasma TAG concentration and clearance, indicating both the importance of myocardial LPL for plasma TAG metabolism and the large cardiac utilisation of TGRLP [17, 18].

Infusion of labelled CM-TAG into mice found that 80-90% of the TAG-FA was recovered from plasma, and TAG lipolysis contributed 10-20% of total plasma FA turnover [7]; given the substantial cardiac TAG-FA assimilation in these experiments, the authors proposed a model of very high local FA released from CM-TAG lipolysis [7]. This concept of heart acting as a "sink" for CM-TAG is seen in physiologic and pathologic situations of metabolic perturbation and redistribution of plasma substrates. Perfused hearts from lactating rats showed a significant increase in NEFA utilisation but suppression of CM-TAG utilisation, consistent with decreased heparin-releasable LPL activity and "redirection" of CM-TAG away from heart and towards lactating mammary gland [19]. Hence, the heart does make a significant metabolic drain on CM-TAG and this must be suppressed in the face of additional metabolic demand, e.g. from lactating mammary gland.

Determining the role of CM-TAG in cardiac metabolism in disease states has also proved difficult. In diabetes, TGRLP, including CM, are structurally altered, and this may change their efficacy as substrates [20-23]. Myocardial lipid metabolism is also increased (and glucose utilisation suppressed) in diabetes (see [1, 24]), so both substrate and cellular factors are likely to be important. CM-TAG from streptozotocin (STZ)-diabetic rats were apo-E deficient, and cleared from the plasma of non-diabetic rats more slowly than control CM-TAG [20]; furthermore incubation of diabetic CM with HDL increased their apo-E content and increased their uptake by heart [20]. Similarly, slow (whole body) plasma clearance of TGRLP by STZ-diabetic rats in the fed state was reported [25]. There was no difference between STZ-diabetic and control rats in plasma clearance of normal CMs (though TAG clearance did depend on CM-TAG concentration administered) but there was a significant increase (trebling) in CM binding to diabetic rat cardiac endothelium [26], potentially reflecting aberrant energy requirement of the diabetic myocardium. Non-diabetic rat CM-TAG were oxidised 3.4-fold higher by STZ-diabetic mouse hearts, and 2.3-fold higher by db/db

(type 2 diabetic) mouse hearts, compared to control hearts, coupled with higher incorporation of CM-TAG into cellular lipids in both STZ-mouse hearts and db/db mouse hearts [27]. By comparison, NEFA oxidation in these experiments was about 3-fold higher than CM-TAG oxidation by control hearts (and hence comparable to CM-TAG oxidation by diabetic hearts)[27] suggesting a general dysregulation of fatty acid beta-oxidation in the absence of glucose uptake. STZ-diabetic rat hearts utilised rat CM-TAG to a greater extent (about 50% more) than control rat hearts, consistent with increased cardiac LPL activity [22], whilst STZ-diabetic rat CMs were utilised even more avidly by both diabetic and non-diabetic rat hearts (about doubled), suggesting functional/composition differences in STZ-CM [22]. Similar studies with type 2 diabetic Zucker (ZDF) rats found comparable results: diabetic hearts utilised diabetic CM to a greater extent than controls, due principally to increased TAG-FA oxidation rather than esterification of TAG-derived FA into cellular lipids [23], and was associated with increased LPL activity in diabetic hearts [23]. PET studies have also suggested that heart TAG utilisation and metabolism are increased in rat type 2 diabetes, coupled with impaired diastolic function [28]. Taken together, these observations suggest that increased cardiac reliance on lipid substrates in diabetes is accompanied by increased CM-TAG utilisation by the diabetic heart, possibly as a result of altered particle size/apolipoprotein composition and myocardial LPL status.

2.2. Cardiac VLDL-TAG utilisation

VLDL provides the major source of endogenous TAG supply to peripheral (extrahepatic) tissues although the physiological role of this substrate is contentious. In the well-fed state it certainly represents the major transport form of endogenous TAG-FA, synthesised by *de novo* hepatic lipogenesis and exported to adipose tissue for storage; however, in the post-absorptive state it is assembled in the liver from adipose-derived NEFA, and it is unclear why NEFA cannot supply FA direct to oxidative tissues such as heart. Indeed, this pathway certainly occurs, but the issue of the

respective roles of NEFA and VLDL-TAG in furnishing FA as metabolic substrate to the myocardium remains. VLDL are less efficient substrates for LPL than CM, by virtue of their lesser size and susceptibility to lipolysis [29]. Several studies have therefore examined the role of VLDL in cardiac TAG-FA provision, but these are technically difficult, requiring preparation and isolation of sufficient appropriately labelled, physiologically relevant, and species-specific VLDL particles to use in test systems. Artificial TAG emulsions more closely resemble CM; VLDLs have been prepared from donor plasma by centrifugation with labelling either by preinjection of FA, or post-isolation *in vitro* FA/TAG radiolabel transfer, or by liver perfusion. Unlike the episodic influx of CM, which increases the plasma TAG concentration by an order of magnitude in the post-absorptive – post-prandial transition, plasma VLDL-TAG concentration is more constant (0.3-0.4mM).

Studies of cardiac A-V (coronary sinus) differences in non-diabetic patients undergoing cardiac catheterisation (usually in the post-absorptive state) found that some hearts extracted plasma (VLDL)TAG but not others (as previously noted [30]) whilst infused labelled TAG was consistently extracted (extraction fraction ~11%)[2]. TAGs and NEFAs accounted for ~17% and ~83% respectively of myocardial FA uptake; myocardial spillover of lipolysis-derived FAs was 35%, suggesting that VLDL-TAG may be a minor contributor to energy production, but that luminal lipolysis may also release significant NEFA to the systemic circulation (see [7]). In this study the myocardium was only a minor contributor to total systemic TAG (VLDL) uptake (~3%) and systemic NEFA production (~0.5%)[2]. Previous studies had also suggested only limited VLDL-TAG utilisation by the human heart – with about 10-20% of cardiac energy deriving from VLDL [31]), suggesting that in human heart, at least in the postabsorptive state, NEFAs may be more important cardiac substrates than VLDL-TAG [30, 32]. Since human hearts can assimilate and oxidise large amounts of glucose when deprived of NEFA (and rodent hearts can function normally despite being LPL deficient [33]) these observations suggest that plasma VLDL-TAG is not an essential cardiac fuel. In

contrast to human studies, rat hearts perfused with rat plasma VLDL at physiological concentration (0.18mM) show substantial cardiac VLDL-TAG utilisation: ~40% of available VLDL-TAG was utilised by 40 min and ~75% by 120 min [34] (although much lower rates of VLDL-TAG utilisation by isolated rat heart have also been reported under similar conditions [35]). High rates of VLDL-TAG utilisation by rat heart have also been reported (oxidation rate about 30nmol/min/g wet wt; about 60% of the VLDL-TAG utilised) [36]), with TAG-lipid removal double that of ^{125}I-apoprotein assimilation [34], and in some studies even higher rates were found – about 60nmol TAG/min/g wet wt, representing >90% VLDL-TAG hydrolysis of [37]. Rat hearts also utilised human VLDL avidly [38, 39], and to the same extent as rat VLDL [39], suggesting that species selectivity may be less of an issue than previously considered. Synthetic VLDL-sized TAG emulsions administered intravenously to mice were only poorly assimilated by heart (about 5-fold less than adipose tissue or skeletal muscle), potentially reflecting the availability of apolipoproteins (though fractional catabolic rate increased with increasing particle size)[40]. Indeed, intestinal VLDL-TAG (iVLDL; isolated from chyle lipids of d<1.006 of fasted rats and lacking apoE and apoCII) was utilised by rat hearts [41], and oxidised at twice the rate of NEFA [42], but about half the rate of CM-TAG oxidation. Less iVLDL-TAG than NEFA was recovered in cellular lipids [42] suggesting greater partitioning towards oxidation. Rat VLDL prepared by rat liver perfusion was utilised by isolated rat hearts (~ 18nmol FA/min/g), but to a significantly lower extent than NEFA (~ 60nmol FA/min/g)) and CM (~ 75nmol FA/min/g) when each was compared as sole lipid substrate (0.4mM)[16]. This limited VLDL utilisation was not suppressed by NEFA when co-perfused, unlike CM utilisation which was decreased by NEFA [16], suggesting distinct pathways of utilisation. Of the VLDL-TAG-FA assimilated, about one quarter was incorporated into tissue lipids whilst the remainder was oxidised; by contrast, about 90% of CM-TAG was oxidised; the metabolic partitioning was not affected by NEFA [16]. Comparable results have also been reported [15, 22, 23]. However, other authors demonstrate competition between NEFA and VLDL-TAG. Mouse

hearts perfused with human plasma VLDL or a synthetic TAG emulsion of similar size to CM utilised and oxidised TAG readily [43]; both VLDL and the CM-like emulsion decreased NEFA utilisation (by about half) and oxidation (by about 70%) and this effect was LPL-dependent [43]. These authors suggest that TGRLP-TAG is a major cardiac substrate and competes directly with NEFA utilisation, proposing a 'common' FA pool within the cardiomyocyte. The concept of a single cardiomyocyte FA pool is supported by the demonstration that heart-specific LPL knockout (KO) mice have increased plasma [TAG] with decreased plasma TAG clearance and decreased cardiac VLDL uptake but unchanged [33] or even decreased [44] cardiac NEFA uptake, surprising in view of potential competition from FA originating in LPL-mediated TAG lipolysis [33]; the finding that NEFA oxidation is decreased (whilst glucose oxidation is increased) in LPL-KO mouse hearts infers that TAG and NEFA metabolism are interlinked. Whilst synthetic CM-like TAG emulsion is utilised by mouse heart more than liver, skeletal muscle or adipose tissue, only about half this rate was found with mouse VLDL injected into mice *in vivo* [45], whilst NEFA uptake is comparable to other tissues, implying that myocardial TAG utilisation exceeds that of NEFA [45]. Together, these findings support the notion that plasma TAG are a quantitatively important cardiac substrate, especially in view of the compromised cardiac function in heart-specific cardiac LPL-KO mice with accompanying impaired cardiac VLDL utilisation [44], though acute studies in isolated rat hearts failed to show a significant influence of TGRLPs on cardiac function [16]. The importance of plasma TAG as a muscle fuel was shown in mice overexpressing LPL in muscle alone: this rescues the global LPL-KO mice from neonatal (hyperlipidemic) death [46], normalising plasma VLDL [47]. In addition, mutant mice expressing LPL in cardiac muscle but not skeletal muscle or adipose tissue have normal plasma TAG [18], suggesting cardiac LPL alone can rescue lethal LPL-KO hypertriglyceridemia and demonstrating the importance of plasma TAG as a bulk cardiac substrate and of the heart in regulating plasma TAG.

The role of insulin in cardiac TGRLP metabolism has proved difficult to elucidate, reflecting the difficulty in defining the role of insulin in cardiac LPL regulation. Rat heart assimilation of VLDL-TAG was comparable to adipose tissue and skeletal muscle, and was not significantly affected by insulin, but hearts of functionally hepatectomised rats assimilated VLDL-TAG more rapidly [48]. STZ-diabetic rat hearts lipolysed intestinal VLDL-TAG (0.32mM) to less than half the extent of control hearts [21, 41]; this was reversible with insulin, suggesting LPL recruitment to capillary endothelium requires functional insulin [41]. Lipolysis of plasma VLDL-TAG prepared from STZ-diabetic rats was only 63% that of plasma VLDL-TAG from non-diabetic animals [21]. "Diabetic" VLDL perfused into diabetic hearts underwent lipolysis to less than a quarter of control VLDL in non-diabetic hearts [21], suggesting that both VLDL particle (composition) and myocardial factors are influenced by insulin. By contrast, Sambandam *et al* found that normal plasma VLDL (0.3mM TAG) was utilised at a significantly faster rate by STZ-diabetic rat hearts than controls [49], and associated with increased LPL activity in the STZ-diabetic hearts [50]. VLDL-TAG metabolic rate in these studies was relatively low (control: 0.5nmol/min/heart) but comparable to other reports [21] whilst VLDL assimilation in diabetic heart was twice this rate (0.93nmol/min/heart)[49]. VLDL from perfused rat livers (0.4mM) showed similar results: STZ-diabetic hearts utilised about 50% more (control) VLDL-TAG than non-diabetic hearts. Interestingly, diabetic VLDL-TAG (from perfused STZ-diabetic rat livers) was utilised only poorly by control hearts, but diabetic hearts utilised diabetic VLDL-TAG comparably to control VLDL-TAG (and CM-TAG) [22]. As with CM, myocardial factors (uptake and disposal mechanisms) and lipoprotein factors (LP particle composition) are both likely to be important in diabetes. LPL regulation in diabetes has been recently reviewed [51]. Both glucocorticoids and insulin are essential for LPL translation [52]. Increased, unchanged, and decreased H-LPL activity have all been reported in diabetes; currently, at least in rodent models, the favoured paradigm is mediation of coronary luminal LPL by post-

translational (?heparanase-mediated) mechanisms, with increased H-LPL in diabetes [22, 23] as a consequence of the hyperglycemia [53].

VLDL utilisation in type 2 diabetes has also been examined. Micro-PET techniques in a rodent model of type 2 diabetes provided indirect evidence that increased myocardial FA oxidation was from plasma TAG rather than NEFA (although the specific contributions of VLDL and CM were not defined) [54]. ZDF-diabetic rat hearts utilised about 50% more VLDL-TAG prepared from ZDF rat liver perfusion than controls [23]; this increased utilisation was entirely accounted for by increased VLDL-TAG-FA oxidation with no increase in accumulation of myocardial lipids [23], further supporting the phenotype-specific nature of both VLDL-TAG synthesis and its utilisation by heart.

2.3. Cardiac TGRLP utilisation in disease

The relationship between TGRLP metabolism and cardiac function is complex – cardiac substrate (including TAG) selection is altered in hypertrophy/failure, but changes in substrate utilisation can themselves lead to cardiac dysfunction/failure – the concepts of adaptive and maladaptive responses and lipotoxicity. Hypertrophy is characterised by chronic increase in cardiac work and hence energy requirement. The conventional paradigm is that hypertrophy and heart failure are accompanied by increased glucose, and decreased FA utilisation, with downregulation of fatty acid oxidation-related genes [1]. However, cardiac LPL activity (and hence continued supply of TGRLP-FA for cardiac utilisation) are critical for the normal cardiac response to β-agonist stimulation and hypertension [55]. Furthermore, LPL-KO hearts are susceptible to failure because of low TAG hydrolysis and despite increased glucose utilisation [44]. Increased FA (and TAG) delivery to the heart by high-fat diets is beneficial in rats with MI and pressure-overload cardiac dysfunction [56]. Cold acclimation, a chronic physiologic stimulus to increase cardiac work, causes hypertrophy and increases heart LPL (but not VLDL-R) activity in rats, with increased TAG uptake; the increased LPL

is not related to AMPK activation [57, 58] suggesting that both acute and chronic mechanisms for upregulation of LPL may be active.

The composition of VLDL (apoprotein complement; lipid subclass content) varies in certain (metabolic) diseases, including diabetes [21], and sepsis [59]. Structurally variant VLDL synthesised by the liver in endotoxemia is a better substrate for myocardium (about double VLDL-TAG oxidation rate)[59]; furthermore, sepsis/endotoxemia is also associated with increased hepatic VLDL synthesis and secretion, increasing VLDL availability. Since cardiac work increased in increased in (early) sepsis as a result of the hyperdynamic circulation and augmented cardiac output, this may represent a mechanism to increase supply of endogenous lipid specifically to the working myocardium under these pathological conditions.

3. Myocardial triacylglycerol uptake

CM and VLDL TAG must enter the myocardium through specialised pathways. Two established routes of entry are 1. through lipolysis by lipoprotein lipase (EC 3.1.1.34) and 2. by particle or remnant particle uptake through lipoprotein receptors. Both pathways occur [15], but are functionally inter-related through “bridging” interactions at the coronary endothelium, hence their respective contributions to TAG (both CM and VLDL) uptake remain disputed. This may be a specious dispute.

Bharadwaj *et al* [60] demonstrated the importance of lipolysis in TGRLP core lipid uptake, and using heart specific LPL-KO and CD36-KO mice they further demonstrated that VLDL-derived TAG-FA entered the cell through a CD36-mediated channel (low capacity high affinity), whereas CM-derived TAG-FA entered through a non-CD36-mediated route (possibly the lower affinity non-saturable/receptor “flip-flop” mechanism. The details of TAG-FA channelling are however still uncertain (Fig. 1).

3.1. Lipoprotein lipase – mediated myocardial TAG uptake

Despite contributing less than 0.5% of total body mass, myocardium possesses 5% of total heparin-releasable LPL - LPL expression in the heart (H-LPL) is the highest (per g) of any tissue [61] suggesting 1. it is a quantitatively important mechanism of cardiac energy capture (sufficient for cardiac LPL activity to modulate circulating plasma TAG levels [33]) and 2. TAG must be an important cardiac oxidative fuel. LPL-mediated lipolysis is generally considered rate-limiting for tissue TAG utilisation. It has a complex cell biology which is not yet fully elucidated (see [51, 52, 62]. The attachment of mature, active LPL to EC-surface HSPG/GPIHBP1 constitutes a "platform for lipolysis" [63] permitting plasma TAG hydrolysis [64]. As well as tissue uptake of the liberated FA, there is FA "spillover" into the plasma [7]. In heart, a substantial proportion of LPL-released FA are assimilated by cardiomyocytes, through pathways that may or may not be shared with NEFA uptake mechanisms (e.g. CD36/FAT; FABP; FATP)[15, 60], but cardiac LPL may make a substantial contribution to plasma NEFA through luminal lipolysis of plasma lipoproteins. LPL regulation is highly tissue-specific, but its control in myocardium has proved difficult to define (see [51, 62]). The LPL gene has multiple upstream cis-acting regulatory elements including PPRE, LXR, RXR, CT element, sterol regulatory element-2, IFNγ-responsive element and anterior protein-1 (AP-1)-like element (see [52]). Cardiac LPL appears inversely related to adipose LPL but does not vary so profoundly. However, two features of H-LPL regulation are clear: 1. it is related to cardiac energy status and 2. it is influenced by plasma-borne factors which may be associated with TGRLP particles. H-LPL increases on fasting [65], and decreases on refeeding [61](though to only relatively limited extents). AMPK stimulation increases H-LPL activity and increases cardiac TAG uptake [66, 67]. It is likely that there is constitutive enzyme expression, perhaps reflecting the availability of VLDL-TAG regardless of nutritional status. Plasma TAG may modulate LPL and hence cardiac TAG assimilation [58, 68], and TGRLPs modified heparin-releasable H-LPL activity [16]. Other

glycerolipids can also regulate H-LPL activity: palmitoyl lyso-PC [69] and LPA [70] stimulate LPL translocation and activity. Cardiac LPL activity may be regulated directly: angptl-3 and angptl-4 are angiopoietin-like growth factor/cytokine related proteins secreted into plasma with pleiotropic effects, including inhibition of LPL [71]. Their expression changes with nutritional status - fasting induces cardiac angptl-4 [72, 73]. Loss of angptl-4 is associated with decreased plasma TAG through increased LPL-mediated clearance, whilst increased angptl-4 expression is associated with inhibited H-LPL and increased plasma TAG, together with cardiac dysfunction [74]. A similar mechanism has also been noted following high-fat feeding, which increases angptl-4 [58], suggesting a mechanism of cardiac LPL regulation *in situ* related to TAG-FA flux. However, chronic intermittent hypoxia has no effect on H-LPL activity or cardiac TAG uptake, despite inhibiting adipose LPL through upregulating angptl-4 [13]. Apolipoproteins also modify LPL activity directly: apo-CII is an obligatory activating co-factor of LPL whilst apo-CI and apo-CIII are inhibitors [75]. TGRLPs bind and stabilise LPL and protect it from angptl-4 inhibition; apo-CI and CIII inhibit this stabilisation [75]. Apo-E inhibits [76] and apo-AV stimulates [77] LPL and plasma TAG clearance. The physiological role of these effects is currently uncertain, but support the concept of TGRLPs regulating LPL and their own disposal.

Naturally-occurring low LPL-activity mutants including mink (*Mustela vison*) and domestic cat (*Felis catus*) have hyperlipidema in both fed and fasted states [78], supporting significant cardiac TGRLP uptake. Human LPL deficiency patients have severe hypertriglyceridemia, with increased cardiac glucose uptake (as with mouse H-LPL-KOs [33]), but do not show cardiac dysfunction, suggesting TGRLPs are a major but not essential cardiac energy source [79]. Whole body LPL-KO leads to severe hyperlipidemia and neonatal death; however, expression of LPL solely in heart was sufficient to prevent hypertriglyceridemia and lethality [18, 46]. Depletion of cardiac LPL by GPIHBP1-KO [64] or H-LPL-KO [60] also increased plasma TAG. LPL activity is required for TAG

sequestration within myocardial LDs – chemical inhibition of LPL or H-LPL deletion inhibited cardiac LD formation [80]. Augustus *et al* found rapid TAG clearance by mouse hearts (proportionately more than any other tissue examined) with broadly similar rates for synthetic CM-like TAG emulsion and VLDL-TAG utilisation [45]. Cardiac TAG uptake was highly dependent on LPL rather than receptor-dependent pathways: uptake was decreased by 88% in LPL-KO mice rescued with liver LPL, and heparin and the LPL inhibitor THL inhibited cardiac TAG uptake by 64% and 82% respectively (although residual uptake occurred through non-LPL mechanisms) [45]. In rat hearts, CM-TAG uptake was twice as rapid as VLDL-TAG, but both were inhibited to a greater extent by THL than by the LP receptor antagonist suramin [15]. Together, these studies support the role of the heart as an effective 'sink' for plasma TAG and this is primarily a function of LPL lipolysis and not its bridging function.

Less well characterised is the importance of LPL to cardiac performance. Mice lacking H-LPL survive, and cardiac function has been reported as normal in these mice [80]. However, NEFA or glucose alone may not be sufficient for optimal cardiac function [44]. Systolic dysfunction and hypertriglyceridemia were noted in H-LPL-KO mice [60, 81], and these mice failed to compensate for increased afterload [55]. Loss of H-LPL forced hearts to increase glucose utilisation [82], but available plasma NEFA are insufficient for optimum cardiac function, arguing that LPL-derived TAG-FAs are more important than NEFA for maintaining normal cardiac function. GLUT-1 overexpression on a background of H-LPL deletion increased glucose utilisation and corrected cardiac energetics and rescued function [83]; similarly, increased lactate utilisation and NEFA availability in exercise-trained mice could partially compensate for H-LPL deletion and limited TGRLP utilisation [83]. Defective energy intake can therefore be a contributor to cardiac dysfunction, though carbohydrates may substitute for FA under certain conditions.

However, LPL depleted mouse hearts had altered gene expression characterised by decreased FA metabolism and increased glucose utilisation, associated with decreased PPARα activation, implying a more generalised effect on metabolism [33, 44]. A specific role for LPL in the release of PPARα ligands has recently been proposed [84], suggesting that specifically VLDL-derived FAs activate myocardial PPARα-responsive genes to a greater extent than NEFA, by generating very high concentrations of unbound FA locally [85].

3.2. Lipoprotein receptor - mediated cardiac TAG uptake

Receptor-mediated assimilation of TGRLP has been reported for many tissues including heart [15, 60]; the principal cardiac lipoprotein receptor is believed to be the VLDL receptor (VLDLR), a highly conserved type 1 transmembrane glycoprotein member of the LDL receptor superfamily. It binds and internalises VLDL and other apo-E containing lipoproteins, including CM, in concert with LPL and apo-E (see [86-89]), suggesting VLDLR has a LPL-facilitating function. VLDLR is highly-expressed in tissues with high FA utilisation (i.e. a similar tissue distribution to LPL) including cardiomyocytes and to a limited extent endothelial cells [90] [91]. VLDLR is important for LD formation in cardiomyocytes as VLDLR knockdown diminishes LD-TAG [92]. $Vldlr^{-/-}$ mice fed high fat diets show decreased (~35%) cardiac uptake of [^{3}H]TAG [93] despite having no marked phenotype. VLDLR likely provides a significant route of glycerolipid uptake by heart [45] but its dependence on LPL status has been challenged [92], and $vldlr^{-/-}$ mice do not show altered plasma LP levels, despite decreased LPL expression [94], although stressing FA metabolism in these animals by fasting or high fat feeding [95] or cross-breeding on a hyperlipidemic ob/ob [95] or $ldlr^{-/-}$ [96] or $lpl^{-/+}$ [88] background does reveal a hypertriglyceridemic phenotype. VLDLR may substitute for bulk cardiac TAG uptake in LPL-KO models, and mediate the uptake of lipoprotein remnant (including CM remnant) particles [86, 97], again facilitated by LPL and apo-E [87]. Hence VLDLR may provide an accessory function to LPL, facilitating TGRLP anchoring to LPL by increasing the affinity of

lipoproteins for endothelium and enhancing lipolysis and TAG assimilation. The increased (VLDL)-TAG in VLDLR-KO mice was exacerbated when LPL was also partly deficient ($vldlr^{-/-}$ crossed with $lpl^{+/-}$), with halved VLDL turnover rates [88]. VLDLR deficient mice had low LPL levels [98] and inhibition of VLDLR with RAP (a folding and trafficking chaperone for VLDLR [99]) reduced LPL activity in wild type but not VLDLR-KO mice, suggesting VLDLR is required for normal LPL regulation. Cardiac VLDLR expression is increased during growth and fasting [100], with cardiac LD-TAG accumulation, but this is accompanied by increased LPL, FAT/CD36, H-FABPpm, acyl-CoA synthase and long chain acyl-CoA dehydrogenase mRNAs, supporting a more generalised elevation in lipid metabolism rather than a specific rise in VLDLR-mediated TAG uptake alone [100]. By contrast, Jokinen *et al* [101] noted no effect of fasting on rat heart VLDLR expression.

Interestingly, a 50% decrease in cardiac VLDLR expression occurs in STZ-diabetes, with decreased post-heparin plasma LPL activity together with hypertriglyceridemia, suggesting that this may represent a mechanism to diminish excessive flux of TGRLP into cardiomyocytes [102]. This is supported by conditions such as nephrosis which also increase plasma TAG concentrations and decrease VLDLR [103]. Ventricular VLDLR expression is decreased in hypertrophy [104](spontaneously hypertensive rat, together with decreased LPL expression), consistent with decreased FA (and increased glucose) utilisation [1], but no change in VLDLR in rat hearts subjected to increased workload (aortic constriction [105] or cold acclimation [57] -induced) was seen (despite increased cardiac LPL). In experimental sepsis, endotoxin decreased both cardiac VLDLR (via IL-1 β) expression and lipid accumulation in fasting mice; downregulation of VLDLR expression by LPS was mediated by IL-1β [106]. VLDLR is upregulated in hypoxic/ischemic conditions in rodent cardiomyocytes, hearts, and human hearts, via HIF-1α and (non-classical) HRE in the VLDLR promoter [92, 107, 108] with increased VLDL uptake and myocellular lipid

accumulation [108], and this mechanism may contribute to the TAG accumulation observed in hypoxia [109, 110].

4. Myocardial intracellular triacylglycerol utilisation

Intracellular cardiomyocyte TAG (mTAG) is a source of glycerolipids for structural/membrane (e.g. phospholipid) and signalling (e.g. DAG) functions [111], and mTAG-derived FAs may be metabolic signals/PPAR ligands [112](Fig. 1). mTAG buffers excess, potentially toxic FAs (especially at times of increased NEFA flux-uptake and/or limited FA oxidation), hence its putative role in lipotoxicity [113, 114]. However, its utilisation for ATP synthesis depends on oxidative metabolism, and the oxygen required for this must be derived from the coronary blood – this raises a paradox, since if blood flow is sufficient to provide oxygen for mTAG-FA oxidative metabolism, then it should also be able to provide blood-borne substrates. This discussion will focus specifically on mTAG as an oxidative substrate.

Studies specifically examining mTAG as an energy substrate are scarce, but an important role for mTAG as an oxidative substrate is emerging. Most exogenous FAs are rapidly oxidised by the cardiomyocyte [15, 16], and substantial cycling through mTAG prior to oxidation would require that mTAG synthesis and lipolysis are very active pathways, (see [111, 115]); indeed the concept of mTAG-FA pool turnover now appears to be central to its physiological role in energy provision as well as cardiac disease. The lipid droplet, previously considered an inert pool of storage TAG, is now known to be a highly dynamic FA resource (see [116, 117]), a major contributor to basal myocardial oxidation, and essential for normal cardiac function [118]. Myocardium shows the fastest TAG turnover of any tissue (see [119]), and this appears to be PPAR-driven [120]. Mouse heart TAG turnover rate actually exceeds the rate of FA oxidation, suggesting that most exogenous FAs are cycled through intracellular TAG prior to oxidation [120](supporting earlier findings of cardiac TAG-FA provision [121]). This may serve to protect the myocardium from excessive PPAR

activation from FA-CoA esters (putative PPAR ligands) and maintain myocardial metabolic flexibility and endogenous substrate mobilisation (e.g. on adrenergic stimulation [122]). mTAG as an energy resource is facilitated by the proximity of the LD to mitochondria [123]. The LD resembles plasma lipoproteins in structure, comprising a phospholipid monolayer shell containing multiple lipases, regulatory and scaffold proteins [119] related to core lipid metabolism, and a hydrophobic core [117]. Myocardial LDs are small with large surface areas facilitating rapid TAG mobilisation, and LD-TAG pathways are associated with the outer mitochondrial membrane and this may be related to intracellular channelling.

4.1. Myocardial triacylglycerol synthesis

Glycerol phosphate acyltransferase (GPAT) initiates TAG synthesis and is rate-limiting for this pathway. GPAT1 and GPAT2 are both expressed in cardiac outer mitochondrial membranes whilst GPAT3 and GPAT4 are found in the SR [115]. Cardiac GPAT activity and increases with fasting - likely a mechanism to buffer incoming FAs and/or to act as an internal FA store for subsequent β-oxidation [124]. Interestingly, cardiac GPAT activity also increases with adrenergic stimulation [125] potentially increasing TAG-FA availability for β-oxidation by increasing TAG pool turnover during increased cardiac work. Paradoxically, augmenting mTAG synthesis (by over-expression of diacylglycerol acyltransferase (DGAT-1)) promotes FA oxidation [113] (and lipolysis of mTAG directly releases FA for oxidation [120, 126]) implying that a TAG pool is required to promote FA oxidation and pool size may be critical. Hence TAG turnover is intimately related to FA oxidation. Interestingly, depletion of TAG synthetic capacity (by lipin-1/PAP depletion) causes less mTAG lipolysis and hence less reliance on mTAG for energy in FA-free perfusions [127].

4.2. Myocardial triacylglycerol lipolysis

Recent advances elucidating the mechanism of LD-mTAG hydrolysis have yielded important insights into the function of cellular TAG. Adipose triglyceride lipase (ATGL; PNPLA2; desnutrin;

calcium-independent PLA2ζ [128, 129]) is rate-limiting for the mobilisation of mTAG for β-oxidation. ATGL expression is increased by FAs, exercise, high fat feeding [130], starvation [131] and STZ-diabetes [132] suggesting that plasma-derived FAs activate lipolysis. However, ATGL activity may also be inhibited by long chain FA-CoA [133] – possibly a protective feedback mechanism preventing lipotoxic damage [134]{Pulinilkunnil, 2013 #25389}. ATGL-deficient mouse hearts have increased mTAG, together with increased glucose utilisation and cardiac dysfunction [129, 135, 136], implying that defective mTAG-FA provision may impair cardiac energetics. Loss of activity mutations in human ATGL cause neutral lipid storage disease, with cardiomyopathy [137]. Moreover, PPARα and PPARδ gene expression are downregulated in rodent ATGL deficiency [112], presumably due to decreased FA-PPAR ligands [135, 136], and this is associated with impaired mitochondrial oxidation [112], supporting the role of mTAG-derived FAs as oxidative substrates together with significant shuttling of incoming FAs through the LD-TAG pool prior to lipolysis and FA release for β-oxidation [120, 126, 138]. Heart-specific ATGL overexpression decreased mTAG, and surprisingly, decreased cardiac FA oxidation, supporting a role for a mTAG pool to facilitate FA flux to mitochondria. In addition, improved cardiac systolic function and protection from pressure-overload stress remodelling was seen in older mice overexpressing ATGL [114]. ATGL overexpression maintained cardiac energetics (ATP; PCr:ATP) and function in high fat fed mice, but exogenous NEFA oxidation was halved, suggesting preferential utilisation of endogenous lipids from the mTAG pool [139]. Perilipin-5 (PLIN5) binds ATGL on the LD surface [140], shielding TAG from lipolysis; ectopic PLIN5 expression increased mTAG levels and decreased FA oxidation, again suggesting rates of FA oxidation may be determined by mTAG-FA turnover [141, 142]. PLIN5 is also present on mitochondria, where it facilitates the tightly-coupled association of mitochondria with LDs [141], controlling both LD-TAG-derived FA release and facilitating its delivery to mitochondria for β-oxidation [141, 143, 144]. Together, these observations indicate that mTAG pool turnover may dictate rates of FA oxidation as the majority of FA cycles through mTAG. Therefore factors

that modulate the mTAG pool size may ultimately dictate rates of FA oxidation without directly affecting mitochondrial function.

4.3. Myocardial triacylglycerol-fatty acid oxidation

Measurements of myocardial TAG-FA oxidation have been attempted by a variety of techniques, including: 1. estimates based on changes in mTAG content 2. difference estimates based on total and exogenous FA oxidation and mVO_2 measurements 3. pulse-chase radiolabelling experiments and 4. NMR techniques, estimating TAG pool turnover.

General evidence for the importance of mTAG in FA provision for oxidation and efficient cardiac metabolism is the huge (>20 fold) increase in mTAG seen in inborn errors [145, 146] and mouse models [146, 147] of defective mitochondrial β-oxidation, especially as this is associated with cardiac dysfunction. Similar observations were noted for human subjects with neutral lipid storage disease [137].

A compelling demonstration of the importance of mTAG in energy provision is seen in *ex vivo* working rat hearts subject to acute increases in workload. Increased "exercise" (work) increased total β-oxidation (mVO_2) due to increased FA availability from both exogenous ([^{3}H]-oleate) and endogenous (calculated) sources– in exercise there was increased mTAG oxidation with no change in mTAG content – implying TAG turnover increased [148]. Exercise produces a switch to mTAG as energy substrate (mTAG oxidation increasing from about 10% of total FA oxidation to about 20% during contractile stimulation); in addition, lactate may also be permissive in this state, high lactate stimulating TAG turnover [149]. Subsequently, indirect estimates of mTAG oxidation found that mouse hearts lacking functional ACC2 had increased exogenous NEFA oxidation (22%) but also decreased mTAG content (and normal LV systolic function); however, mVO_2 measurements suggested increased oxidation of mTAG [150]. Hence, removing the inhibition of FA flux through

CPT1 facilitated utilisation of mTAG for FA oxidation as a component for increased 'global' FA oxidation in the heart.

Pulse-chase radiolabelling experiments provide direct evidence for the importance of mTAG to cardiac β-oxidation, with two thirds of ^{14}C-labelled mTAG utilised by rat hearts in the absence of exogenous FA; addition of NEFA preserved most (though not all – about 65%) of the labelled mTAG, indicating that endogenous mTAG makes a significant contribution to myocardial oxidative metabolism [151]. Catecholamines stimulated mTAG lipolysis and oxidation [152, 153], but only in the absence of exogenous NEFA [153, 154], and mTAG oxidation increased with cardiac work in the same model [154].

mTAG and exogenous NEFA oxidation were measured in mTAG-prelabelled working hearts perfused with no, low (0.4mM), or high (1.2mM) concentrations of palmitate. Endogenous mTAG oxidation rates were high (nearly one half of maximal NEFA oxidation rates; ~60% of myocardial ATP synthesis) in the absence of NEFA, with corresponding loss of mTAG content, but mTAG oxidation rate was suppressed and mTAG stores maintained when NEFA (1.2mM) was available. Interstingly, mTAG still contributed 12-20% of total FA oxidation, representing ~11% of cardiac ATP synthesis, indicating the obligate nature of mTAG for ATP production [126]. Furthermore, under these conditions loss of ^{14}C label from the [^{14}C]mTAG pool was matched by incorporation of ^{3}H label from exogenous [^{3}H]NEFA, indicating conservation of mTAG pool turnover and size. Interestingly, the initial mTAG oxidation rate was higher than subsequent rates [126, 155, 156], suggesting multiple intracellular TAG pools.

Recently, NMR techniques have provided important insights into myocardial TAG metabolism and pool dynamics in humans, confirming findings in animal models of metabolic disease [157, 158]. Dynamic mode NMR using ^{13}C-palmitate shows an initial, saturable exponential incorporation into mTAG in the intact heart, representing sarcolemmal receptor-mediated FA uptake, and a slower

linear rate, determined by the rate of cellular metabolism and sensitive to inhibitors of LCFA metabolism, reflecting TAG turnover [118, 120, 159-161]. TAG pool turnover is a function of mTAG pool size and FA availability: TAG turnover increases with high fat diet (in association with increased DGAT1 and ATGL expression [120, 130]), in diabetes [160] and in PPARα and PGC1α overexpressing mouse hearts [120, 162], but is decreased in cardiac hypertrophy with diminished PPAR activity [118]. In mouse hearts mTAG pool turnover measured by ^{13}C-NMR was 3.75 times faster than palmitate oxidation, suggesting that palmitoyl units for oxidation were preferentially derived from cellular TAG rather than exogenous NEFA (and hence exogenous FAs must first be routed through the TAG pool)[120]. Mouse hearts over-expressing PPARα (a model with diabetic-like phenotype) had even higher rates of mTAG pool turnover compared to (similar) exogenous NEFA oxidation rates (12.5-fold faster), implying that almost all incoming FAs are channelled through TAG; the mTAG pool size was comparable, but enzymes of TAG synthesis were increased in line with the increased TAG pool turnover rates [120]. This suggests that TAG pool turnover rate is more important than tissue TAG content for cardiomyocyte FA metabolism; both exogenous FA and TAG contribute to the accessible pool of cellular FA-CoA. Therefore, LD-mTAG turnover couples and matches FA uptake to utilisation through both TAG synthesis and lipolysis, regulating energy provision and potentially cardiac function [118, 161, 163]. Failure of this dynamic relationship between exogenous FA metabolism and lipid deposition is a potential cause of the maladaptive changes that contribute to cardiac dysfunction [164].

4.4. Myocardial triacylglycerol oxidation and cardiac disease:

To date, limited information only is available on the role of mTAG as an oxidative substrate in cardiac disease. Myocardial lipid accumulation with decreased FA oxidation in heart failure [1] suggests optimal cardiac function requires mTAG energy provision, although caution is required with this interpretation since diabetes is associated with increased mTAG, ATGL expression and FA

oxidation, but also cardiac dysfunction [132, 156, 165, 166]. TAG turnover is uncoupled from energy demand in heart failure [118, 163]. With rat aortic banding, mTAG-FA oxidation was decreased to zero in early hypertrophy/failure, in line with decreased mTAG pool turnover (and mTAG content), and increased glucose oxidation; NEFA oxidation was not increased to compensate the decreased mTAG oxidation [118]. Adrenergic stimulation doubled mTAG oxidation in proportion to workload in healthy hearts, but not in failing hearts (which had lower mTAG turnover rates relative to cardiac work), with the proportion of TAG metabolism to ATP production unchanged (about 10% - similar to [126])[118]; since FA oxidation decreases in response to adrenergic stimulation in heart failure, it is likely that abnormalities of cellular FA oxidation are early events in the development of cardiac dysfunction.

mTAG has also been shown to be an important energy source during reperfusion [167, 168]. Turnover rates of mTAG (based on glycerol measurements) increased during ischemia, but decreased on reperfusion [168]. Rat hearts reperfused in the presence of high (1.2mM, pathologically relevant) NEFA had increased NEFA oxidation rates, but only an initial increase in mTAG-FA oxidation; mTAG lipolysis was unchanged but mTAG synthesis was increased, expanding the mTAG pool and potentially protecting against toxic intracellular FAs [155]. In the absence of NEFA, mTAG oxidation increased on reperfusion and this was associated with improved functional recovery [155], suggesting that mTAG-derived FAs do not contribute to IR injury, as has been suggested for exogenous FA (though potentially arguing against incoming NEFAs having to obligatorily turnover through the TAG pool, at least in reperfusion). Increased mTAG content and turnover (induced by DGAT1 overexpression) were associated with normal cardiac energetics and function, but in the presence of exogenous NEFA, increased mTAG turnover was cardioprotective following ischemia, with improved function during reperfusion [169].

Diabetes is characterised by increased cardiac FA metabolism, with increased mTAG content [156, 165, 170] and impaired LV function [170]. TAG increases in diabetic hearts on acute fasting accompany diastolic dysfunction [171], though prolonged fasting improves insulin sensitivity, decreases mTAG and improves cardiac function, suggesting a diversion of TAG-derived FA to β-oxidation [172]. However, rates of mTAG oxidation in diabetic hearts have been reported as similar to controls [156, 165], suggesting that associated elevated NEFA concentrations may increase mTAG pool size but have only modest effects on mTAG pool turnover. STZ-diabetic rat hearts have increased mTAG content and turnover when perfused with high NEFA (1.2mM palmitate); at lower NEFA (0.5mM) concentrations, diabetic hearts still have increased TAG turnover, but mTAG content is unchanged [160]. mTAG oxidation contributed about 16% of ATP synthesis in diabetes, but surprisingly no contribution of mTAG to mitochondrial ATP synthesis was found in healthy hearts [160] (basal mTAG oxidation in healthy hearts accounted for only about 10% of total ATP synthesis, in good agreement with previous findings [126, 173]). Fractional enrichment of TAG was lower in diabetic hearts, suggesting TAG compartmentalisation in the diabetic myocardium (other reports have also suggested multiple TAG compartments with different turnover rates [126, 151, 174, 175]). STZ-diabetic rat hearts had increased mTAG content and lipolysis, but unchanged mTAG oxidation compared to controls (mTAG oxidation contributing about 10% of ATP production in control and diabetic hearts) when perfused with 1.2mM palmitate; however, in the absence of NEFA, mTAG oxidation increased in diabetic myocardium, providing some 70% of ATP synthesis [173]. Non-obese type 2 diabetic (GK) rats also have increased mTAG, and LV dysfunction; FA metabolism (assessed by PET) was increased, mostly a result of increased FA oxidation from mTAG, since genes of both TAG synthesis and lipolysis were increased [28].

5. Concluding remarks:

It is finally emerging that TAGs are indeed major providers of cardiac energy; furthermore, alterations in myocardial TAG utilisation are clearly associated with impaired cardiac function. However, outstanding questions remain, including the teleological role of VLDL as a FA carrier and NEFA substitute (i.e. VLDL as a metabolic signal), the regulation of myocardial LPL activity in relation to metabolic (and particularly plasma TAG) status, the possibility of differential uptake pathways and intracellular channelling of FAs from different sources (NEFA; CM-TAG; VLDL-TAG), together with the requirement for incoming FAs to cycle through mTAG before oxidation. In particular, defining the precise mechanism(s) of myocellular FA assimilation, together with the extent to which TGRLP-derived FAs contribute to mTAG pool turnover, compared to the requirement of NEFA to cycle through mTAG (hence putative intracellular FA channelling streams) remains a challenge.

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Conflicts of interest:

No conflicts of interest to declare

Figure legends:

Figure 1. Utilisation of triacylglycerol (TAG) by the cardiomyocyte. Triacylglycerol is available for mitochondrial β-oxidation from exogenous supply (CM: chylomicrons; VLDL: very-low-density lipoprotein) and endogenous myocardial "stores" (mTAG) within lipid droplets. Current controversies include (1) the degree to which fatty acid (FA) for oxidation is supplied from these various sources, in competition with non-esterified FA (NEFA), in the normal and diseased heart (2) the respective importance of lipoprotein receptor (VLDL-R)-mediated and LPL-mediated exogenous TAG uptake (3) the specific uptake route for FA derived from lipoprotein lipase (LPL)-mediated TAG lipolysis and that of NEFA (facilitated uptake or "flip-flop" FA translocation across the sarcolemma), and whether these are shared or separate (4) the existence of distinct intracellular FA pools derived from the various FA sources and uptake mechanisms (5) the extent to which intracellular FAs must be obligatorily cycled through intracellular mTAG prior to utilisation as respiratory fuel, or signalling and structural functions. Lipid droplet and mitochondria are in close anatomical and functional proximity. EC: endothelial cell; DAG: diacylglycerol; GPAT: glycerol-phosphate acyltransferase; DGAT: diacylglycerol acyltransferase; ATGL: adipose triacylglycerol lipase; PLIN5: perilipin-5; PPAR: peroxisome proliferator-activated receptor; VLDLR: VLDL-receptor; FATP: fatty acid transport protein; FAT: fatty acid translocase; FABP: fatty acid binding protein.

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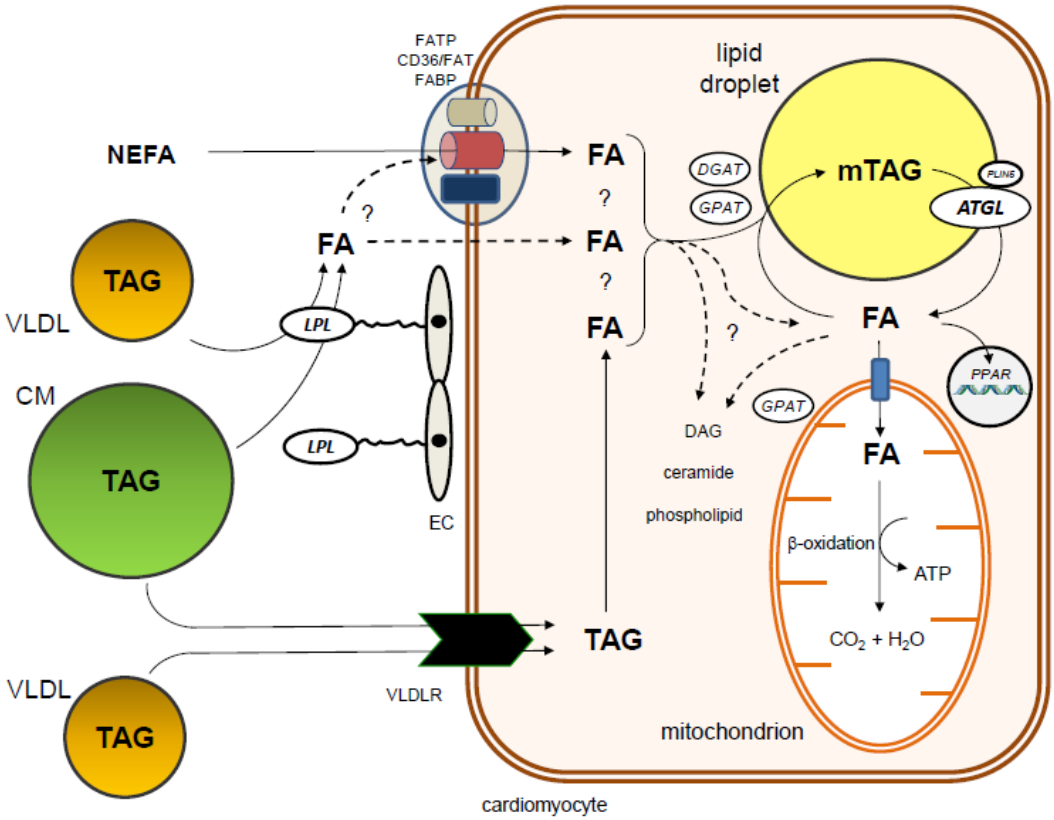

Figure 1

Highlights for Review: Evans & Hauton BBALIP-16-36

Highlights:

- Triacylglycerols are supplied to the myocardium within chylomicrons and VLDL
- Heart assimilates triacylglycerol-rich lipoproteins by LPL and receptor-mediated routes
- Exogenous triacylglycerol-derived fatty acids enter an intracellular lipid pool
- Intracardiac triacylglycerol is an important source of fatty acids for oxidation
- Dysregulation of triacylglycerol metabolism is associated with cardiac dysfunction