Role of Lipoprotein Lipase in Fatty Acid Delivery to the Heart

Andrea Wan and Brian Rodrigues

Abstract Diabetes is rampant across the globe, with 5–6 % of Canadians currently diagnosed with diabetes. In people with diabetes, inadequate pharmaceutical management predisposes the patient to heart failure, which is the leading cause of diabetes related deaths. One instigator for this cardiac dysfunction is change in fuel utilization by the heart. Thus, following diabetes, when cardiac glucose utilization is impaired, the heart undergoes metabolic transformation wherein it switches to using fats as an exclusive source of energy. Although this switching is geared to help the heart initially, in the long term, this has terrible end results. These include the generation of noxious by products which kill cardiac cells, reduce cardiac function and ultimately result in an increased morbidity and mortality. A key perpetrator that may be responsible for organizing this metabolic disequilibrium is lipoprotein lipase (LPL), the enzyme responsible for providing fat to the hearts. Either exaggeration or reduction in its activity following diabetes could lead to heart dysfunction. The objective of this article is to describe the biology of LPL during diabetes. By gaining more insight into the mechanism(s) by which cardiac LPL is regulated, new therapeutic strategies can be devised that may assist in restoring metabolic equilibrium, to help prevent or delay heart disease seen during diabetes.

Keywords Cardiomyocytes • HSPG • Heparanase • VEGF • GPIHBP1

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1 Introduction

The incidence of diabetes has reached epidemic proportions, and globally, approximately 366 million people are affected by this disease. This number is projected to grow to 552 million by 2030 (7.8 % of the adult population) (International Diabetes Federation). Cardiovascular disease is the leading cause of diabetes-related death [1]. Although atherosclerotic vascular disease is a primary reason for this cardiovascular dysfunction, a susceptibility to heart failure in patients with diabetes might also be an outcome of a specific impairment of the heart muscle itself (labeled diabetic cardiomyopathy) [2–4]. The etiology of diabetic cardiomyopathy is complex, with changes in cardiac metabolism being considered a principal culprit. The earliest change that occurs in the diabetic heart is reduced glucose consumption, with a switch to utilization of fatty acids (FA) predominantly as an energy resource [5]. A majority of FA provided to the heart comes from the breakdown of circulating lipoprotein-triglycerides (TG) [6], a process catalyzed by lipoprotein lipase (LPL) located at the vascular lumen. In models of moderate Type 1 diabetes, when circulating albumin-bound FA has yet to increase, an augmented vascular LPL activity is observed [7]. Although this adaptation might be beneficial in the short-term, it is potentially catastrophic over a protracted duration, given the malicious effects produced by increased FA oxidation and myocyte TG accumulation [8–10]. Intriguingly, following severe diabetes when circulating albumin-bound FA also increases, luminal LPL is "turned off" to avoid lipid overload [11]. Paradoxically, chronic cardiac-specific deletion of LPL is also associated with a decreased cardiac ejection fraction (LPL function is irreplaceable) [12–14]. In this chapter, we will examine the role of coronary vascular LPL in fatty acid delivery to the heart, and how an increase in its activity is a prelude for the cardiovascular complications seen with diabetes.

2 Cardiac Metabolism

With uninterrupted contraction being a unique feature of the heart, cardiac muscle has a high demand for energy. As a consequence, this organ demonstrates substrate promiscuity, enabling it to utilize multiple sources of energy, including FA, carbohydrates, amino acids and ketones [15]. Among these, carbohydrates and FA are the major participants from which the heart derives most of its energy. Accordingly, in a basal aerobic setting, glucose and lactate account for approximately 30 % of energy, whereas 70 % of ATP generation is through FA oxidation [15]. However, the heart has a limited capacity to synthesize FA, and thus relies on an exogenous supply. FA delivery and utilization by the heart involves: (a) release from adipose tissue and transport to the heart after complexing with albumin, (b) provision through breakdown of endogenous cardiac triglyceride (TG) stores, and (c) lipolysis of circulating TG-rich lipoproteins (VLDL and chylomicrons) to FA by lipoprotein lipase (LPL) positioned at the endothelial cell (EC) surface of the coronary lumen (Fig. 1).

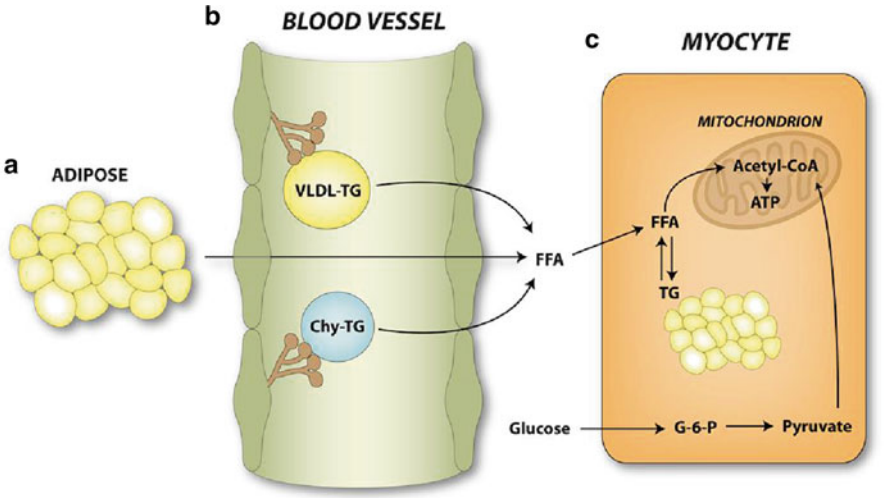

Fig. 1 Substrate utilization by the heart. Glucose entry into cardiomyocytes occurs through transporters. Once inside, it undergoes glycolysis and pyruvate is formed, which is transported into the mitochondria. At this location, pyruvate undergoes decarboxylation to acetyl-CoA, which is eventually used for ATP generation. The other major substrate that is utilized by the heart is fatty acid (FA). FA is delivered to the heart from three major sources: (**a**) adipose tissue lipolysis with release of FA into the plasma, (**b**) LPL mediated breakdown of TG-rich lipoproteins from the liver (VLDL) and gut (chylomicron), and (**c**) endogenous triglyceride (TG) breakdown within the heart. Specifically related to LPL, this enzyme is located at the apical side of endothelial cells, and hydrolyzes circulating TG to release FA that are taken up into the cardiomyocyte by FA transporters. This FA can be stored as TG or undergo β-oxidation to generate acetyl-CoA, which is further oxidized to generate ATP

3 Lipoprotein Lipase (LPL)

Regarding LPL, (a) greater than 90 % of plasma FA are contained within lipoprotein-TG, with LPL having a pivotal role in hydrolysis of this TG to FA, (b) compared to other tissues, the heart has the most robust expression of LPL, and (c) LPL-mediated lipolysis of TG-rich lipoproteins is suggested to be a principal source of FA for cardiac utilization. Lipoprotein-TG clearance by LPL proceeds at the apical surface of EC that line the coronary lumen. Despite this critical function, EC do not synthesize LPL [16]. Instead, this enzyme is synthesized in cardiomyocytes and processed to dimeric [17], catalytically active enzyme, an obligatory step for ensuing secretion. Transfer to the coronary lumen requires movement of LPL to the cardiomyocyte plasma membrane [18] by AMP-activated protein kinase [19], protein kinase D, and p38 MAPK [20]. Activation of these kinases facilitates LPL vesicle formation, in addition to promoting cytoskeletal rearrangement for secretion onto cell surface heparan sulfate proteoglycans (HSPG) [20], where the enzyme is

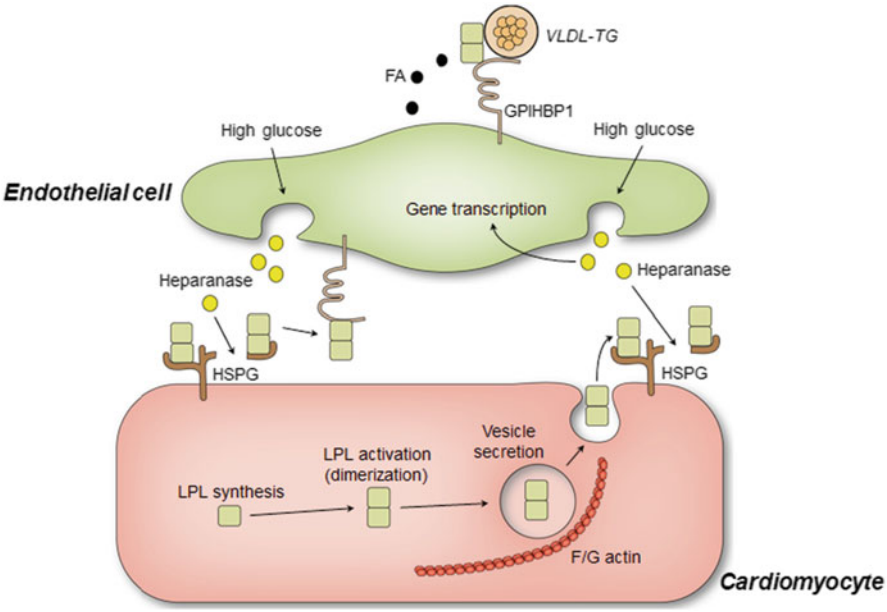

Fig. 2 Synthesis, activation and transport of LPL in the heart. Following its synthesis as an inactive monomer in cardiomyocytes, LPL undergoes several post-translational processes to be activated (dimerized). Fully processed dimeric LPL is sorted in vesicles that are targeted to the cell surface (for secretion) by moving along the actin cytoskeleton. At the plasma membrane, LPL docks with heparan sulfate proteoglycan (HSPG) binding sites. For its onward movement to the luminal side of the endothelial cell (EC), detachment of LPL from HSPG is a prerequisite. EC heparanase facilitates transfer of LPL from the cardiomyocyte to the vascular lumen. In the EC lysosomes, active heparanase is stored in a stable form. In normal conditions, heparanase released from the basolateral side of EC towards the cardiomyocyte can initiate cleavage of myocyte HSPG side chains. Following this, the oligosaccharide-bound LPL released into the interstitial space is then transported towards the apical side of EC. Out here, LPL is responsible for lipoprotein-TG hydrolysis. Following diabetes, increased circulating glucose can enhance heparanase secretion from the EC, which can cleave more HSPGs. The released LPL is transferred to the lumen. The end effect is to increase TG hydrolysis, bringing more FA to the cardiomyocyte, leading to lipotoxicity

momentarily located [21, 22] (Fig. 2). For its onward movement across the interstitial space to the apical side of vascular EC, detachment of LPL from the myocyte surface is a prerequisite, and is likely mediated by enzymatic cleavage of cardiomyocyte surface HSPG by heparanase [23, 24].

4 Heparanase

HSPG are ubiquitous macromolecules present in every tissue compartment but particularly the extracellular matrix, cell surface, intracellular granules and nucleus [25]. They consist of a core protein to which several linear heparan sulphate (HS)

side chains are covalently linked, and function, not only as structural proteins but also as anchors [26]. The latter property is implicitly used to bind a number of different proteins [chemokine's, coagulation factors, enzymes like LPL, and growth factors such as vascular endothelial growth factor (VEGF)] [27]. Attachment of these bioactive proteins is a clever arrangement, providing the cell with a rapidly accessible reservoir, precluding the need for *de novo* synthesis when the requirement for a protein is increased. Heparanase is an endoglycosidase, exceptional in its ability to degrade HS, thereby instigating release of ligands [28].

Heparanase is initially synthesized as a latent (inactive) 65 kDa proheparanase enzyme that undergoes cellular secretion followed by reuptake facilitated by HSPG [29, 30]. After undergoing proteolytic cleavage (removal of a 6 kDa linker peptide in lysosomes), a 50 kDa polypeptide is formed that is ~100-fold more active than the 65 kDa inactive proheparanase [31, 32]. Within the acidic compartment of lysosomes (that have a predominantly perinuclear localization), active heparanase is stored in a stable form until mobilized. In the presence of high glucose (HG, 25 mM), we reported a clear redistribution of lysosomal heparanase from a perinuclear location towards the plasma membrane of EC, together with an elevated heparanase secretion into the incubation medium (Fig. 2). We also determined that ATP release, purinergic receptor activation, cortical actin disassembly and stress actin formation were essential for HG-induced heparanase secretion [23].

Although a role for heparanase in physiology (e.g., embryonic morphogenesis) has been described, it was intensive research focused on cancer progression that hinted towards a unique responsibility in cardiac metabolism. In cancer, degradation of HS chains by the increased expression of heparanase is associated with extracellular matrix and basement membrane disruption [33]. The loss of this physical barrier facilitates tumor cell invasion [34]. However, it was the reported liberation of LPL anchored to HS that was the most provocative. In the heart, subsequent to its synthesis and transfer to the myocyte surface, it was uncertain as to how LPL journeys to its site of action, the vascular lumen. We established that LPL detachment from the cell surface is a requirement, and is made possible by heparanase. Given its strategic location, EC can operate as a first responder, informing the underlying myocytes about hyperglycemia following diabetes. We proved that HG is a potent stimulator of heparanase secretion from EC, and in so doing, empowered the myocyte to send LPL to the vascular lumen (in search of lipoprotein-TG) as an adaptation to contest the impending loss of glucose consumption. EC secretes both latent and active heparanase. We questioned why a cell would release inactive enzyme followed by reuptake and subsequent activation. This practice would appear counterintuitive and wasteful, unless latent heparanase has a function. Indeed, compelling data from our lab suggests that latent heparanase generates signals in myocytes to reload LPL from an intracellular pool, to replenish the surface reservoir released by active heparanase [24]. One interesting feature of myocyte HSPG is its capability to electrostatically bind proteins other than LPL. As such, we questioned whether these proteins could be released by heparanase, and further focused on those that would be able to aid in LPL-derived fatty acid delivery and oxidation. VEGF is one such protein that fits this profile.

5 Vascular Endothelial Growth Factor (VEGF)

Following diabetes, the heart rapidly increases LPL at the vascular lumen by transferring this enzyme from the underlying cardiomyocyte. We implicated heparanase in this process, an enzyme that cleaves HSPG to facilitate bioactive protein release from this attachment receptor. We reported that within 30 min of hyperglycemia, active heparanase was secreted into the interstitial space, and was able to detach LPL from the sub-endothelial myocyte cell surface, for onward movement to the vascular lumen. In the heart, cardiac myocytes are also a major source of VEGF. As the predominant forms of VEGF, VEGFA ($VEGF_{164}$) and VEGFB ($VEGF_{167}$) have a heparin binding domain, it is possible that in addition to an intracellular location, VEGF can be captured by myocyte cell surface HSPG following its secretion. Such a location would allow for a rapid release when there is a requirement for this growth factor. If true, then it is conceivable that given this comparable location of LPL and VEGF at the myocyte cell surface, heparanase will also dislodge VEGF. This would be an elegant mechanism, with VEGF assisting in LPL-derived FA uptake (by its effects on FABP and FATP), in addition to supporting cardiac flexibility that is desirable to oxidize these FA (through its promotion of angiogenesis; FA oxidation needs ample oxygenated blood).

Of the many different VEGF isoforms, VEGFA and VEGFB are notable standouts abundantly expressed in the myocardium, but more critically, are related to metabolic regulation [35, 36]. Upon secretion, they have a variable affinity for HSPG, an interaction that transpires by way of a heparin binding domain (HBD, amino acids rich in basic residues) on VEGF [37, 38]. Although this liaison protects VEGF against degradation, it also allows the cell matrix to retain a pool of readily accessible bioactive growth factors that can be freed, either by ionic displacement (employing heparin) or more physiologically, by matrix proteolysis (through the action of heparanase).

VEGFA: From a single human VEGFA gene, alternative splicing generates a number of isoforms. The predominantly expressed $VEGF_{165}$ (mouse $VEGF_{164}$) is the main effector of VEGF action, and is partially tethered (50–70 %) to the extracellular matrix by its HBD. VEGFA has a proven role in vascular permeability (through its formation of intracellular gaps and fenestrations), vasodilatation (due to the induction of eNOS and NO production), and blood vessel formation (encompassing angiogenesis, vasculogenesis, and arteriogenesis) [39, 40]. VEGFA is also capable of promoting FA binding protein 4 (FABP4) expression, a FA transporting protein abundantly expressed in microvascular EC in the heart [41].

VEGFB: Of the isoforms identified, $VEGF_{167}$ constitutes more than 80 % of the total VEGFB transcript, and also possesses a HBD. Unlike other VEGF family members, VEGFB is not required for physiological angiogenesis nor does it influence EC permeability [42, 43]. Nevertheless, in the heart, although located predominantly in myocytes, this cytokine executes a unique metabolic function in EC. By stimulating FA transport proteins (FATP3 and 4) in these cells, VEGFB promotes

FA uptake and transport across the EC layer. To recapitulate, the effects of VEGF would be expected to not only indirectly support LPL-derived FA transport across EC but to also provide O_2 needed for mitochondrial oxidative phosphorylation of FA, the main source of cardiomyocyte ATP.

Having been released by heparanase, LPL needs to be transferred from the basolateral to the apical (luminal) side of EC where the enzyme is functional. This transfer across the EC is facilitated by glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1).

6 Glycosylphosphatidylinositol-Anchored High Density Lipoprotein-Binding Protein 1 (GPIHBP1)

GPIHBP1 is a glycoprotein abundantly expressed in the heart, exclusively on capillary EC [44, 45]. On the apical side of these cells, its ability to avidly bind both lipoprotein-TG and LPL allows it to serve as a platform for TG (chylomicrons, VLDL) lipolysis along the luminal surface of capillaries [46–48]. At the basolateral side of EC, GPIHBP1 operates as a transporter, collecting LPL from the interstitial spaces surrounding myocytes and shuttling it across EC to the capillary lumen [47] (Fig. 2). Given these critical functions, complete absence or mutations of GPIHBP1 cause mislocalization of LPL (enzyme remains trapped in interstitial space) and severe hypertriglyceridemia [49]. The earliest evidence of regulated GPIHBP1 expression came from experiments investigating the effects of fasting/refeeding [50]. Fasting amplifies coronary luminal LPL activity [19] and cardiac GPIHBP1 expression, effects that are reversed 6 h after refeeding [50]. As GPIHBP1 levels change quickly, it is conceivable that to increase FA delivery to the diabetic heart, this protein participates in the accelerated transfer of LPL from the cardiomyocyte to the vascular lumen (Fig. 2). We tested whether EC respond to hyperglycemia by increasing GPIHBP1, and our preliminary evidence indicates that diabetes stimulates cardiac LPL activity and GPIHBP1 gene and protein expression.

7 Aberrant Fuel Metabolism in Diabetes

In diabetes, as glucose uptake and oxidation are impaired, the heart is compelled to use FA exclusively for ATP generation. Multiple adaptive mechanisms, either whole body or intrinsic to the heart, operate to make this achievable. These include augmented adipose tissue lipolysis, where breakdown of stored TG in fat cells increases circulating FA that are transported to the heart. If delivered to the liver, these FA can raise circulating lipoprotein concentrations as hepatic FA availability is a rheostat for VLDL synthesis. In so doing, VLDL-TG is an additional and major resource to increase FA delivery to the heart for oxidation. Innate to the cardiac muscle, the uptake of albumin-bound FA is driven by plasma membrane FA transporters

(for example CD36), which are increased following diabetes. Diabetes also enhances adipose triglyceride lipase leading to mobilization of the storage pool of TG within cardiomyocytes. Finally, the utilization of VLDL-TG as a FA source by the diabetic heart is influenced, not only by elevated plasma concentrations of VLDL, but also by the vascular content of LPL, the rate-limiting enzyme in circulating TG clearance. We were the first to report higher luminal LPL activity following diabetes [51].

8 Physiological and Pathophysiological Regulation of LPL

LPL synthesis and activity are altered in a tissue specific manner by physiological conditions like cold exposure, lactation, or feeding and fasting [52, 53]. In fasting, with ensuing hypoinsulinemia, LPL activity decreases in the adipose tissue but increases in the heart. As a result, FA from circulating TG is diverted away from storage to meet the metabolic demands of cardiomyocytes. Hence, LPL fulfills a "gate-keeping" role by regulating the supply of FA to meet the metabolic requirements of different tissues.

We described a robust expansion in the coronary pool of LPL following diabetes, likely an effect to address the urgent need for FA to compensate for decreased ATP production from glucose. This increase in LPL activity was immediate and unrelated to LPL gene expression [54]. We consequently described potential mechanisms to explain this novel finding in the diabetic heart. Its prelude includes exaggerated LPL processing to dimeric, catalytically active enzyme, an obligatory step for ensuing secretion [17]. Transfer of this active enzyme to the coronary lumen however requires movement of LPL to the cardiomyocyte plasma membrane [18]. The signaling behind the passage of LPL in cardiomyocytes was the responsibility of AMP-activated protein kinase (AMPK) [19]. Downstream, transit control of LPL by AMPK embraced a) activation of protein kinase D, whose zinc-finger domain is known to interact with trans Golgi membranes, allowing for LPL vesicle formation [7], and b) p38 MAPK activation and actin cytoskeleton polymerization, thus providing the LPL cargo a transport infrastructure for secretion onto myocyte plasma membrane HSPG [20]. For its onward movement across the interstitial space to the apical side of vascular EC, LPL detachment from the myocyte cell surface is a prerequisite, and is likely mediated by enzymatic cleavage of cardiomyocyte HSPG by high-glucose induced heparanase release [23].

9 Cardiomyopathy in Diabetes

Heart disease is not only a leading cause of death, but also a substantial driver of health care costs among people with diabetes. Coronary vessel disease and atherosclerosis are the primary reasons for this increased incidence of cardiovascular dysfunction. However, Type 1 (T1D) and Type 2 (T2D) patients have also been

diagnosed with reduced or low-normal diastolic function and left ventricular hypertrophy in the absence of coronary heart disease (cardiomyopathy) [55]. Evidence of cardiomyopathy has also been reported in animal models of T1D and T2D. Cardiomyopathy is a complicated disorder, and several factors have been associated with its development. These include an accumulation of connective tissue and insoluble collagen, impaired endothelium function and sensitivity to various ligands (e.g., β-agonists), and abnormalities of various proteins that regulate ion flux, specifically intracellular calcium [56, 57]. The view that diabetic cardiomyopathy could occur as a consequence of early alterations in cardiac metabolism has also been put forward, and linked to LPL.

10 Role of LPL in Cardiomyopathy

Cardiac-specific deletion [14] or overexpression [6, 58, 59] of LPL is associated with heart dysfunction. In this context, consider that although the loss of cardiomyocyte LPL in adult mice increased glucose metabolism (typically, increased FA uptake by cells slows down glucose oxidation pathways), neither this effect, nor albumin bound FA could replace the action of LPL, and cardiac contractility decreased [14]. Cardiac specific overexpression of LPL caused severe myopathy characterized by lipid oversupply and deposition, muscle fiber degeneration, excessive dilatation, and impaired left ventricular function [6, 58, 59]. The latter experiments demonstrate that in the absence of any vascular defects, selective overexpression of LPL in the heart is sufficient to cause lipotoxicity and cardiac failure, a situation comparable to that seen with diabetic cardiomyopathy. Although this increase of LPL in diabetes serves to guarantee FA supply and consumption when glucose utilization is compromised, it unintentionally provides a surfeit of FA to the diabetic heart, sponsoring a setting where FA uptake exceeds the mitochondrial oxidative capacity. Chronically, the resulting increase in the conversion of FA to potentially toxic FA metabolites, including ceramides, diacylglycerols, and acylcarnitines, paired with increased formation of reactive oxygen species secondary to elevated FA oxidation, can provoke cardiac cell death (lipotoxicity).

11 Conclusion

Pharmaceutical management of diabetes can never completely duplicate the exquisite control of glucose observed in healthy humans and hence, patients with diabetes who have imperfect control of their glucose are exposed to repeated bouts of hyperglycemia. As a consequence, changes in cardiac metabolism (decreased glucose utilization forces the heart to increase LPL at the vascular lumen to augment FA) arise, are predicted to occur rapidly, and can influence the development of cardiovascular disease if sustained. The amplification in LPL emerges as an

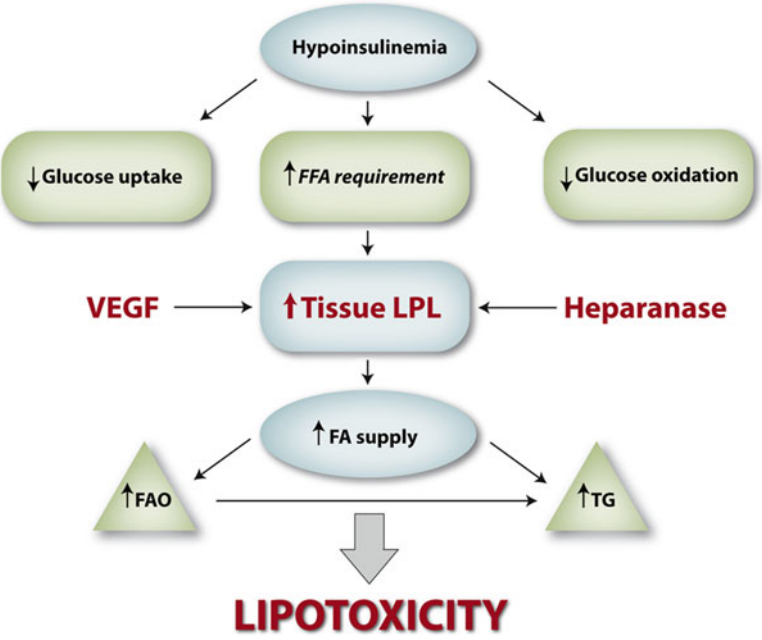

Fig. 3 Metabolic basis for diabetic heart disease. The earliest change that occurs in the diabetic heart is altered energy metabolism. Hence, in the presence of reduced glucose utilization, the heart switches to predominantly use FA for energy. The protein "ensemble" (heparanase-VEGF-LPL) cooperates in the diabetic heart to make this possible. This abnormal FA utilization by cardiac tissue may have lethal consequences under the umbrella called "lipotoxicity". Specifically, FA accumulates and can, either by themselves or via the production of second messengers such as ceramides, provoke cell death. Understanding how heparanase cooperates with its accomplices, VEGFs and LPL, would be valuable to restore metabolic equilibrium and limit lipotoxicity and diabetes-related cardiac damage

outcome of enzyme movement to the cardiomyocyte cell surface, with subsequent forward transfer to the coronary lumen. We suggest that this occurs due to high glucose induced secretion of endothelial heparanase (the first responder) that has concurrent functions. These include stimulation of myocyte HSPG bound LPL, and VEGF release. The latter effect contributes towards augmenting LPL action, and amplifying FA delivery and utilization by the diabetic heart. At the apical side of EC, LPL is responsible for VLDL-TG hydrolysis to FA (the preferred substrate for the diabetic heart), which we propose can result in lipotoxicity (Fig. 3). By gaining further insight into the mechanism(s) by which diabetes alters endothelium-bound LPL, we can attempt to piece together a part of the cascade of events leading to diabetic heart disease. Appreciating the mechanism of how the heart regulates LPL following diabetes should allow the identification of novel targets for therapeutic intervention, to restore metabolic equilibrium and limit lipotoxicity and diabetes-related cardiac damage.

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References

1. Sowers JR, Epstein M, Frohlich ED (2001) Diabetes, hypertension, and cardiovascular disease: an update. Hypertension 37(4):1053–1059
2. Francis GS (2001) Diabetic cardiomyopathy: fact or fiction? Heart 85(3):247–248
3. Picano E (2003) Editorial comment—diabetic cardiomyopathy: the importance of being earliest. J Am Coll Cardiol 42(3):454–457
4. An D, Rodrigues B (2006) Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. Am J Physiol Heart Circ Physiol 291:H1489–H1506
5. Kim MS, Wang Y, Rodrigues B (2012) Lipoprotein lipase mediated fatty acid delivery and its impact in diabetic cardiomyopathy. Biochim Biophys Acta 1821:800–808
6. Voshol PJ, Jong MC, Dahlmans VEH et al (2001) In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. Diabetes 50(11):2585–2590
7. Kim MS, Wang F, Puthanveetil P et al (2008) Protein kinase D is a key regulator of cardiomyocyte lipoprotein lipase secretion after diabetes. Circ Res 103:252–260
8. Poirier P, Bogaty P, Garneau C et al (2001) Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care 24:5–10
9. Bertoni AG, Tsai A, Kasper EK et al (2003) Diabetes and idiopathic cardiomyopathy: a nationwide case–control study. Diabetes Care 26:2791–2795
10. Struthers AD, Morris AD (2002) Screening for and treating left-ventricular abnormalities in diabetes mellitus: a new way of reducing cardiac deaths. Lancet 359:1430–1432
11. Kim MS, Wang F, Puthanveetil P et al (2009) Cleavage of protein kinase D after acute hypoinsulinemia prevents excessive lipoprotein lipase-mediated cardiac triglyceride accumulation. Diabetes 58:2464–2475
12. Augustus A, Yagyu H, Haemmerle G et al (2004) Cardiac-specific knock-out of lipoprotein lipase alters plasma lipoprotein triglyceride metabolism and cardiac gene expression. J Biol Chem 279:25050–25057
13. Noh HL, Okajima K, Molkentin JD et al (2006) Acute lipoprotein lipase deletion in adult mice leads to dyslipidemia and cardiac dysfunction. Am J Physiol Endocrinol Metab 291: E755–E760
14. Augustus AS, Buchanan J, Park TS et al (2006) Loss of lipoprotein lipase-derived fatty acids leads to increased cardiac glucose metabolism and heart dysfunction. J Biol Chem 281: 8716–8723
15. Lopaschuk GD, Ussher JR, Folmes CD et al (2010) Myocardial fatty acid metabolism in health and disease. Physiol Rev 90:207–258
16. Camps L, Reina M, Llobera M et al (1990) Lipoprotein lipase: cellular origin and functional distribution. Am J Physiol 258:C673–C681
17. Wang Y, Puthanveetil P, Wang F et al (2011) Severity of diabetes governs vascular lipoprotein lipase by affecting enzyme dimerization and disassembly. Diabetes 60:2041–2050
18. Pulinilkunnil T, Abrahani A, Varghese J et al (2003) Evidence for rapid "metabolic switching" through lipoprotein lipase occupation of endothelial-binding sites. J Mol Cell Cardiol 35:1093–1103
19. An D, Pulinilkunnil T, Qi D et al (2005) The metabolic "switch" AMPK regulates cardiac heparin-releasable lipoprotein lipase. Am J Physiol Endocrinol Metab 288:E246–E253

20. Kim MS, Kewalramani G, Puthanveetil P et al (2008) Acute diabetes moderates trafficking of cardiac lipoprotein lipase through p38 mitogen-activated protein kinase-dependent actin cytoskeleton organization. Diabetes 57:64–76
21. Eckel RH (1989) Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N Engl J Med 320:1060–1068
22. Enerback S, Gimble JM (1993) Lipoprotein lipase gene expression: physiological regulators at the transcriptional and post-transcriptional level. Biochim Biophys Acta 1169:107–125
23. Wang F, Kim MS, Puthanveetil P et al (2009) Endothelial heparanase secretion after acute hypoinsulinemia is regulated by glucose and fatty acid. Am J Physiol Heart Circ Physiol 296: H1108–H1116
24. Wang Y, Zhang D, Chiu AP et al (2013) Endothelial heparanase regulates heart metabolism by stimulating lipoprotein lipase secretion from cardiomyocytes. Arterioscler Thromb Vasc Biol 33:894–902
25. Iozzo RV, San Antonio JD (2001) Heparan sulfate proteoglycans: heavy hitters in the angiogenesis arena. J Clin Invest 108:349–355
26. Iozzo RV (2001) Heparan sulfate proteoglycans: intricate molecules with intriguing functions. J Clin Invest 108:165–167
27. Ma S, Tang KH, Chan YP et al (2010) miR-130b Promotes CD133(+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. Cell Stem Cell 7:694–707
28. Bame KJ (2001) Heparanases: endoglycosidases that degrade heparan sulfate proteoglycans. Glycobiology 11:91r-98r
29. Fairbanks MB, Mildner AM, Leone JW et al (1999) Processing of the human heparanase precursor and evidence that the active enzyme is a heterodimer. J Biol Chem 274:29587–29590
30. Gingis-Velitski S, Zetser A, Kaplan V et al (2004) Heparanase uptake is mediated by cell membrane heparan sulfate proteoglycans. J Biol Chem 279:44084–44092
31. Pikas DS, Li JP, Vlodavsky I et al (1998) Substrate specificity of heparanases from human hepatoma and platelets. J Biol Chem 273:18770–18777
32. Abboud-Jarrous G, Rangini-Guetta Z, Aingorn H et al (2005) Site-directed mutagenesis, proteolytic cleavage, and activation of human proheparanase. J Biol Chem 280:13568–13575
33. Eccles SA (1999) Heparanase: breaking down barriers in tumors. Nat Med 5:735–736
34. Vlodavsky I, Beckhove P, Lerner I et al (2012) Significance of heparanase in cancer and inflammation. Cancer Microenviron 5:115–132
35. Testa U, Pannitteri G, Condorelli GL (2008) Vascular endothelial growth factors in cardiovascular medicine. Cardiovasc Med (Hagerstown) 9:1190–1221
36. Lan L, Wilks A, Morgan TO et al (1995) Vascular endothelial growth factor: tissue distribution and size of multiple mRNA splice forms in SHR and WKY. Clin Exp Pharmacol Physiol Suppl 22:S167–S168
37. Krilleke D, DeErkenez A, Schubert W et al (2007) Molecular mapping and functional characterization of the VEGF164 heparin-binding domain. J Biol Chem 282:28045–28056
38. Li X (2010) VEGF-B: a thing of beauty. Cell Res 20:741–744
39. Weis SM, Cheresh DA (2005) Pathophysiological consequences of VEGF-induced vascular permeability. Nature 437:497–504
40. Tammela T, Enholm B, Alitalo K et al (2005) The biology of vascular endothelial growth factors. Cardiovasc Res 65:550–563
41. Elmasri H, Karaaslan C, Teper Y et al (2009) Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. FASEB J 23:3865–3873
42. Nash AD, Baca M, Wright C et al (2006) The biology of vascular endothelial growth factor-B (VEGF-B). Pulm Pharmacol Ther 19:61–69
43. Li X, Kumar A, Zhang F et al (2012) Complicated life, complicated VEGF-B. Trends Mol Med 18(2):119–127
44. Ioka RX, Kang MJ, Kamiyama S et al (2003) Expression cloning and characterization of a novel glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein, GPI-HBP1. J Biol Chem 278:7344–7349

45. Young SG, Davies BS, Voss CV et al (2011) GPIHBP1, an endothelial cell transporter for lipoprotein lipase. J Lipid Res 52:1869–1884
46. Weinstein MM, Yin L, Tu Y et al (2010) Chylomicronemia elicits atherosclerosis in mice—brief report. Arterioscler Thromb Vasc Biol 30:20–23
47. Davies BS, Beigneux AP, Barnes RH 2nd et al (2010) GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. Cell Metab 12:42–52
48. Young SG, Davies BS, Fong LG et al (2007) GPIHBP1: an endothelial cell molecule important for the lipolytic processing of chylomicrons. Curr Opin Lipidol 18:389–396
49. Beigneux AP, Davies BS, Gin P et al (2007) Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. Cell Metab 5:279–291
50. Kroupa O, Vorrsjo E, Stienstra R et al (2012) Linking nutritional regulation of Angptl4, Gpihbp1, and Lmf1 to lipoprotein lipase activity in rodent adipose tissue. BMC Physiol 12:13
51. Rodrigues B, Cam MC, Jian K et al (1997) Differential effects of streptozotocin-induced diabetes on cardiac lipoprotein lipase activity. Diabetes 46:1346–1353
52. Doolittle MH, Ben-Zeev O, Elovson J et al (1990) The response of lipoprotein lipase to feeding and fasting. Evidence for posttranslational regulation. J Biol Chem 265:4570–4577
53. Kitajima S, Morimoto M, Liu E et al (2004) Overexpression of lipoprotein lipase improves insulin resistance induced by a high-fat diet in transgenic rabbits. Diabetologia 47: 1202–1209
54. Sambandam N, Abrahani MA, St Pierre E et al (1999) Localization of lipoprotein lipase in the diabetic heart: regulation by acute changes in insulin. Arterioscler Thromb Vasc Biol 19: 1526–1534
55. Rodrigues B, Cam MC, McNeill JH (1995) Myocardial substrate metabolism: implications for diabetic cardiomyopathy. J Mol Cell Cardiol 27:169–179
56. Pulinilkunnil T, Rodrigues B (2006) Cardiac lipoprotein lipase: metabolic basis for diabetic heart disease. Cardiovasc Res 69:329–340
57. Fang ZY, Prins JB, Marwick TH (2004) Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. Endocr Rev 25:543–567
58. Kim JK, Fillmore JJ, Chen Y et al (2001) Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 98:7522–7527
59. Levak-Frank S, Radner H, Walsh A et al (1995) Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. J Biol Chem 96:976–986