Cardiac lipoprotein lipase: Metabolic basis for diabetic heart disease

Thomas Pulinilkunnil, Brian Rodrigues*

Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, British Columbia, Canada V6T 1Z3

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Abstract

The heart has a limited potential to synthesize fatty acid (FA), and, therefore, FA is supplied from several sources: lipolysis of endogenous cardiac triglyceride (TG) stores or from exogenous sources in the blood. Lipoprotein lipase (LPL), synthesized in cardiomyocytes, catalyzes the breakdown of the TG component of lipoproteins to provide FA to the heart. It is the vascular endothelial-bound LPL that determines the rate of plasma TG clearance, and, hence, it is also called heparin-releasable (HR) “functional” LPL. Functional LPL is regulated by numerous dietary and hormonal factors and is sensitive to pathophysiological alterations like those observed during diabetes. In this condition, absolute or relative lack of insulin impairs cardiac glucose transport and oxidation, resulting in FA becoming the preferred means of energy supply. To make available this increased requirement of the heart for FA, the diabetic heart upregulates its luminal LPL activity by posttranslational mechanisms. Chronically elevated cardiac LPL can result in abnormal FA supply and utilization by the heart tissue that could potentially initiate and sustain cardiac dysfunction during diabetes. As effective blood glucose control is difficult during diabetes, it is conceivable that a parallel increase in functional cardiac LPL activity may predispose people with diabetes to premature death from cardiac disease. By gaining more insight into the initial metabolic processes in the diabetic heart, we can attempt to piece together a part of the cascade of events leading to diabetic heart disease.

1. Clinical problem of diabetes

The World Health Organization estimates that approximately 170 million people suffer from diabetes worldwide, with this figure likely to more than double by 2030 (http://www.who.int/diabetes/en/). In patients diagnosed with diabetes, the Type 2 form (tissue insulin resistance followed by inadequate insulin secretion and ultimately, hyperglycemia) accounts for 90% of diagnosed cases when compared to Type 1 (pancreatic β cell death with catastrophic loss of circulating insulin and profound hyperglycemia). Among the numerous complications of diabetes, cardiovascular abnormalities like coronary vessel disease and atherosclerosis have been identified as the leading causes of mortality [1]. However, clinical [2] and experimental data [3] suggests the prevalence of an additional cardiac injury termed ‘diabetic cardiomyopathy’ that occurs in the absence of any change in coronary blood flow. Experimental diabetic cardiomyopathy consists of two major elements: an initial phase of short-term and severe modification in fuel metabolism [3,4] followed by progressive chronic myocardial damage that involves augmented stiffness of the left ventricular wall, accumulation of connective tissue and insoluble collagen, and abnormalities of various proteins that regulate ion flux (specifically intracellular calcium) [5]. After 4 to 6 weeks, these changes are reflected in a measurable contractile dysfunction [6] similar to patients with diabetes [7]. However, verification of the initial cardiac changes observed in animals has not been completely established in humans given the limited diagnostic use of sensitive techniques to measure changes in energy metabolism. This has led to widespread underestimation of these early cardiac events. Hence, in
human diabetic hearts, a preliminary metabolic malfunction may predispose the diabetic heart to accelerated damage when exposed to conditions of dyslipidemia, atherosclerosis, and hypertension.

2. Cardiac energy metabolism

2.1. Substrate supply and utilization

In the heart, cellular energy in the form of ATP is obtained via the oxidation of various substrates like FA, glucose, lactate and ketone bodies, with glucose and FA being the principal fuels [8–10] (Fig. 1). Following insulin dependent glucose uptake and glycolysis, pyruvate dehydrogenase (PDH) complex facilitates entry of pyruvate into the mitochondria and its oxidation eventually provides the heart with approximately 30% of its energy [11]. Compared to glucose, FA is the preferred substrate, and contributes approximately 70% of the ATP necessary for normal heart function [12,13]. Nevertheless, the heart has limited potential to synthesize this substrate. Therefore, FA delivery and utilization by the heart involve: i) release from adipose tissue and transport to the heart after complexing with albumin [14], ii) provision through the breakdown of endogenous cardiac TG stores [15], iii) internalization of whole lipoproteins [16], and iv) hydrolysis of circulating TG-rich lipoproteins to FA by LPL positioned at the endothelial surface of the coronary lumen [17]. Recently, LPL mediated hydrolysis of circulating lipoproteins to FA was suggested to be the principal source of FA for cardiac utilization [18]. This is particularly important given that the molar concentration of FA bound to albumin is ~10 fold less than that of FA in lipoprotein-TG [19].

During diabetes, adipose tissue lipolysis is increased [20], resulting in elevated circulating plasma FA and hepatic very low density lipoprotein (VLDL)-TG secretion [21]. Additionally, there is an enhanced activity of enzymes that catalyze the synthesis of TG, thus facilitating the accumulation of intracellular TG stores [22,23]. Ensuing hydrolysis of this TG store may lead to high tissue FA levels. LPL also increases to guarantee FA supply to diabetic heart in order to compensate for the diminished contribution of glucose as an energy source [24]. However, the dramatic increase in FA influx is not without negative consequences. In the heart, elevated FA and subsequent TG synthesis have been implicated in a number of metabolic, morphological, and mechanical changes, and more recently, in “lipotoxicity” [25]. During lipotoxicity, FA accumulates and can, either by themselves or via production of second messengers such as ceramides, provoke cell death [26]. Given the pivotal function of LPL in FA delivery, and FA’s contribution in mediating cellular lipotoxicity, examining the regulation of this enzyme is crucial for understanding the metabolic basis of diabetic heart disease.

Fig. 1. Fatty acid (FA) provision to the heart. FA is provided to the heart from three major sources. Adipose tissue lipolysis with release of FA into the plasma, LPL mediated breakdown of TG-rich lipoproteins from liver (VLDL) and gut (Chylomicron), and endogenous TG breakdown within the heart. The other major substrate that is utilized by the heart is glucose. HSL—hormone sensitive lipase; FAO—fatty acid oxidation.
2.2. Role of LPL in heart disease

Hypertriglyceridemia is a common feature in patients with insulin resistance, diabetes and obesity, and is positively correlated to deleterious tissue lipid deposition [27]. Diminished whole body LPL activity is suggested to be one mechanism resulting in impaired clearance of circulating lipoproteins, and ensuing hypertriglyceridemia [28]. Interestingly, administration of NO-1886, an LPL-activating agent, to high-fat fed animals, suppressed hypertriglyceridemia and lipid accumulation [29]. In transgenic rabbits that have global overexpression of LPL, attenuation of hypertriglyceridemia and reduced deposition of body fat were observed [30]. Taken together, these studies suggested that systemic increases in LPL could have beneficial effects on whole body lipid metabolism. Conversely, other studies have documented that tissue-specific augmentation of LPL can result in lipotoxic myopathy [31–33]. Thus, the question that emerges is how much of an increase in LPL activity is required for the enzyme to be either beneficial or pathological. In an attempt to address this issue, transgenic animals were developed that overexpressed LPL at low, moderate and high levels in skeletal and cardiac muscle [33]. High tissue-specific LPL overexpression was associated with insulin resistance and severe myopathy, characterized by elevated FFA and TG deposition, muscle fiber degeneration, and extensive proliferation of mitochondria and peroxisomes [33]. In a more recent study using genetically engineered mice that specifically overexpressed cardiomyocyte surface bound LPL, lipid oversupply and deposition were observed, together with excessive dilatation and impaired left ventricular systolic function (lipotoxic cardiomyopathy) [34]. These experiments clearly demonstrate that in the absence of any vascular defects, selective overexpression of LPL in the heart is sufficient to cause cardiac failure. Given that diabetes is associated with heart disease, and the unequivocal demonstration that LPL per se has the ability to accelerate cardiac muscle disease, specifically modulating LPL in the diabetic heart could have beneficial implications.

3. Lipoprotein lipase

3.1. Synthesis and activation

Although the functional location of LPL mediated lipoprotein hydrolysis is at the capillary endothelial cell surface, a number of approaches including in situ hybridization have failed to demonstrate LPL mRNA localization in endothelial cells [35]. Experimental data from the adult mouse heart suggest that LPL is synthesized and processed in cardiac myocytes, with distribution of immunoreactive LPL protein being observed in different myocardial compartments [17,36]. Thus, electron microscopic studies of immunogold-labeled sections of mouse myocardium demonstrated that 78% of total LPL was present in cardiac myocytes, 3–6% in the interstitial space, and 18% at the capillary endothelium [17,36]. Within the myocytes, LPL was found localized within the sarcoplasmic reticulum, Golgi complex and secretory vesicles [17]. In the human myocardium, LPL protein was also detected in interstitial cells [37]. Synthesis and activation of LPL in the heart occur by mechanisms similar to those of adipose tissue [38] (Fig. 2). LPL is synthesized as an inactive, monomeric proenzyme in the rough endoplasmic reticulum (ER), and is postulated to be activated somewhere between the ER and Golgi apparatus, then proceeding for either degradation or secretion [38–44].

3.2. Degradation and secretion

The process of LPL turnover by intracellular degradation represents an important posttranslational mechanism by which enzyme activity is rapidly regulated. In adipose tissue, this process is rapid, with a $t_{1/2}$ of about 40 min resulting in almost 80% of newly synthesized LPL being degraded prior to being secreted [39,45]. Degradation probably occurs in a leupeptin inhabitable, lysosomal or a tunicamycin sensitive, ER compartment [39]. Similar to the intracellular pool, adipocyte surface bound LPL also undergoes degradation after being internalized [46]. With surface bound LPL, only a fraction (<28%) undergoes lysosomal degradation following internalization of the enzyme [47]. Moreover, this degradation is dependent on both the number of heparan sulphate proteoglycans (HSPG) binding sites in addition to the extent of HSPG sulfation [48]. Whether the cardiomyocyte internalizes surface bound LPL for degradation and if so, do alterations in this process regulate cardiac LPL during physiological or pathological stress merits further investigation.

Following completion of enzyme synthesis, LPL is secreted as an active homodimer by either constitutive and/or regulated mechanisms [38]. Constitutive mechanisms involve spontaneous release of LPL, whereas regulated release occurs in response to a secretagogue (e.g., heparin) [24]. Majority of the cell types expressing LPL exhibit constitutive release. For regulated release to occur, the enzyme requires packaging and assembly in secretory vesicles. In cardiomyocytes, dimerized LPL binds to cell surface HSPG [38]. Subsequently, LPL is translocated across the interstitial space to the endothelial cell surface in the vascular lumen where it executes its function of hydrolyzing circulating lipoproteins. A number of observations support the idea that luminal HSPG bound LPL is not an outcome of uptake from the plasma pool but rather, requires transfer from the myocytes. For example, perfusion of isolated rat [24] or guinea pig hearts [49,50] with heparin causes release of LPL to occur in two phases: a) a rapid phase, occurring within seconds after heparin perfusion, probably representing detachment of LPL from the endothelial luminal (apical) surface, and b) a sustained release, likely occurring as a result of LPL mobilization from within
endothelial cells, interstitial space or cardiomyocytes. Additionally, when the luminal heparin-releasable (HR) LPL pool from isolated perfused hearts was depleted and allowed to recover for 1 h after enzyme removal, 40% of the normally available luminal LPL activity was recovered, again suggesting that an intrinsic mechanism persists to maintain coronary luminal LPL [51].

3.3. Transfer from myocyte to endothelial cells

Myocyte cell surface LPL is suggested to be transported towards the vascular endothelial cells by mechanisms that are currently unclear. In co-culture experiments using adipocytes and endothelial cells, heparanase like compounds secreted from endothelial cells have been suggested to cleave adipocyte bound HSPGs to release heparan sulphate oligosaccharides [52]. The oligosaccharides bound LPL non-covalently, likely preventing LPL degradation in interstitial fluid, and serve as extracellular chaperones, enabling transport of the enzyme to the apical surface of endothelial cells [53,54]. Subendothelial basement membranes likely sequester and stabilize this LPL secreted by myocytes [55]. The extracellular matrix in subendothelial basement membranes is composed of collagens, fibronectin, laminin and glycosaminoglycans like heparan, dermanatan and chondroitin sulphate proteoglycans, of which LPL binds mainly to HSPG [55]. Binding of LPL to HSPG at the basolateral surface of the endothelium was reported to be obligatory for efficient transport to the apical surface, an effect that was susceptible to heparinas (proteases that cleave HSPGs) [54]. In fibroblasts, as binding of LPL to HSPG aligns these proteoglycans along the actin cytoskeleton, disruption of actin filaments resulted in irregular distribution of HSPG on the fibroblast cell surface, with ensuing impairment of LPL secretion [56]. Whether this organization of HSPG along the actin cytoskeleton is involved in the transcytosis of LPL from the abluminal to the luminal surface in the coronary endothelial cell is presently unknown. In addition to HSPG, movement of LPL across the endothelial cell also requires VLDL receptors [57,58]. Hence, treatment of the basolateral surface with a receptor-associated protein inhibitor or specific antibodies against the VLDL receptor abolished LPL transcytosis [57]. Alternatively, employing adenoviral gene transfer to overexpress VLDL receptors [57] or transgenic deletion of these receptors [58] resulted in an increase or decrease in LPL activity, respectively.

3.4. Endothelial recycling

LPL turnover at the endothelial lumen can occur either by detachment from HSPG and subsequent hepatic degra-
dation [59], or internalization of the HSPG–LPL complex into an endothelial endocytotic compartment [60] (Fig. 2). Within the endothelial cell, an acidic pH enables LPL to remain bound to HSPG, promoting recycling of the internalized LPL complex back onto the luminal surface, thereby allowing endothelial cells to maintain an auxiliary pool of the enzyme [60]. The acidic pH in the endocytotic vesicles favors HSPG–LPL binding, thereby enabling the complex to be released back into the medium or inserted onto the cell surface [60]. Whether this mechanism of endothelial recycling takes place in the whole heart is yet to be ascertained.

4. Insulin mediated control of cardiac lipoprotein lipase

Potential sites for regulation of cardiac LPL include: a) nucleus (transcriptional control) [61], b) rough endoplasmic reticulum (maturation of LPL by glycosylation) [40,62], c) Golgi network (vesicular transport and secretion of LPL) [39,45], d) plasma membrane (LPL binding to the cell surface) [63], and e) vascular endothelial cell surface (vectorial transfer and recycling of the enzyme) [60]. Of these sites, the majority of studies have focused on enzyme regulation at the transcriptional and translational levels. The unique characteristic of LPL is that in some tissues like adipose and heart, changes in activity can occur independent of alterations in LPL mRNA [51]. Such changes would be desirable given that during conditions like fasting or diabetes wherein insulin levels are altered, and FA utilization is augmented, rapid increase in LPL activity may not match the slow turnover of LPL mRNA.

4.1. Fasting

Physiological hypoinsulinemia induced by fasting reduces but enhances LPL activity in the adipose and heart tissues, respectively [64,65]. The fasting induced decline in adipose LPL was secondary to degradation of newly synthesized LPL, suggesting posttranslational regulation of the enzyme [66,67]. More recently, fasting did not alter intradipocyte LPL even though the extracellular pool of enzyme was changed to the inactive form [66,67]. As the latter effect was sensitive to inhibition by transcription blockers, the authors suggested the presence of a gene whose product prevents the enzyme from becoming active even though synthesis of LPL protein continues unabated [67]. In the heart, fasting with ensuing hypoinsulinemia augments HR-LPL activity [65]. This was supported by immunocytochemical studies in the mouse myocardium, which revealed a 5 fold increase in LPL at the luminal projections of endothelium subsequent to fasting [17]. Changes in luminal LPL activity following fasting were independent of shifts in LPL mRNA or alterations in LPL protein and activity in cardiomyocytes, suggesting a posttranslational mechanism for this increase [65]. Some studies have suggested that this increase in HR-LPL could be attributed to increased uptake of LPL from the blood since uptake of exogenous 125I-LPL was augmented in the heart following fasting [68]. However, in perfused guinea pig hearts, newly synthesized LPL can move from myocytes to the vascular lumen within 30 min, and increased rate of LPL transfer could also explain this effect of fasting on HR-LPL [49,50].

4.2. Diabetes

Although extensively studied, the relative contribution of cardiac LPL activity to the delivery of FA to the diabetic heart is largely inconclusive [24,51,69–74] (Table 1). In part, this variability among different studies was due to the dosage and duration of STZ diabetes. For example, induction of severe diabetes (100 mg/kg STZ) reduced [24], whereas moderate diabetes (55 mg/kg STZ) augmented coronary HR-LPL within 4 days [51]. Other reasons for this inconsistency in LPL activity measurements occurred because LPL activity and protein were largely obtained using whole heart homogenates, which do not distinguish LPL localized on capillary endothelial cells from the myocyte pool (Table 1). Thus, moderate diabetes (Fig. 3A–B) induced with 55 mg/kg STZ did not alter LPL when activity measurements were carried out in heart homogenates [51]. However, using retrograde perfusion of these hearts with heparin to displace coronary LPL, we were the first to demonstrate significantly elevated HR-LPL activity following chronic and acute (Fig. 3C) diabetes [24,51]. The elevated HR-LPL peak could not be explained by an enhanced LPL synthesis, as both cellular and surface bound LPL activities in myocytes from 55 mg/kg STZ rats were low relative to control [24]. Next, we confirmed that: a) the increase in LPL protein originates mainly in capillary blood vessels, presumably within or at the luminal and abluminal surface of the endothelial cell [75], b) could be regulated by short-term changes in insulin [75] but was largely independent of hyperglycemia [51], and c) was capable of

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<tr>
<td>Rat</td>
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<td>Mice</td>
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<td>Mice</td>
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n/c = no change; ↓ = decreased; ↑ = increased; – = not evaluated; PHPLA = post heparin plasma lipolytic activity; DEX = dexamethasone.
hydrolyzing VLDL-TG. The amplified HR-LPL pool at the endothelial surface in the moderately diabetic rat heart could involve an increased number of HSPG sites on the endothelial surface, or uptake from the circulating plasma. With hypoinsulinemia following 24 h fasting, luminal HSPG binding sites are altered. However, indirect examination of coronary HSPG binding sites suggest that only a fraction of these sites are occupied by LPL in control hearts, and diabetes initiates rapid filling of these empty sites. The pool of enzyme required to fill these sites following diabetes does not involve uptake of enzyme from the plasma, but is obtained from the heart itself. Thus, rate of LPL transport could be one factor in explaining the diabetes-induced modification in HR-LPL activity.

One caveat associated with the above studies was that STZ, a pancreatic beta cell toxin, demonstrates a triphasic pattern of blood glucose following administration. This pattern is comprised of an initial brief hyperglycemia followed by a period of hypoglycemia before noticeable hyperglycemia is attained within 12–16 h. As metabolic switching in the heart from glucose to predominantly FA occurs rapidly (60–90 min) following hypoinsulinemia, an alternate model was used to examine LPL under conditions where insulin levels are acutely manipulated. Diazoxide (DZ), a selective KATP channel opener, hyperpolarizes the β cell membrane, decreases intracellular calcium, and leads to rapid inhibition of insulin secretion and hyperglycemia (within 1 h). Similar to STZ, DZ also demonstrated LPL increases in the coronary lumen that was negatively correlated to plasma insulin. This change remained unaffected by changes in systemic blood pressure, heart rate and pattern of contraction. Interestingly, 4 h following DZ administration, a 50–70% drop in surface bound myocyte LPL was observed at a time when luminal LPL activity was elevated. These findings suggest that following hypoinsulinemia, the heart switches to utilizing FA exclusively, and LPL is recruited from the myocyte to the myocardial endothelial lining.

### 4.3. Insulin resistance

The majority of Type 2 diabetic patients are initially insulin resistant (but not hyperglycemic, due to increased insulin secretion). With time and progressive decline in islet cell insulin secretion, insulin resistance cannot be overcome, and Type 2 diabetes ensues. Since hypoinsulinemia induced changes in LPL within 1 h, it was anticipated that short-term (within hours) insulin resistance, without the superimposition of hyperglycemia, would influence cardiac metabolism. Glucocorticoids play an important role in the development of peripheral insulin resistance by altering cellular glucose metabolism. Hence, both excess endogenous and exogenous glucocorticoids acutely impair insulin sensitivity and chronically promote development of the metabolic syndrome including obesity, diabetes and hypertension. Exploiting the euglycemic–hyperinsulinemic clamp, it was confirmed that the synthetic glucocorticoid hormone dexamethasone (DEX, 1 mg/kg) induced whole body and cardiac specific insulin resistance within 4 h, in the absence of changes in plasma glucose (Fig. 3B). Similar to hypoinsulinemia induced by STZ, hearts from insulin resistant DEX animals also demonstrated
enlargement of the coronary LPL pool [72] (Fig. 3C). However, unlike STZ diabetes, changes in HR-LPL in DEX hearts were accompanied by increases in LPL mRNA (Fig. 3D).

5. Regulation of cardiac lipoprotein lipase by metabolic outcomes of insulin deficiency

As described above, HR-LPL was determined to be sensitive to changes in plasma insulin. Whether the influence on coronary LPL was an outcome of direct changes in plasma insulin or was secondary to metabolic alterations associated with insulin lack was unclear. For instance, following insulin deficiency, in addition to other factors [79], elevated circulating glucose, FA and TG [69] could potentially influence regulation of LPL at the coronary lumen.

5.1. Hyperglycemia

Phloridzin, an inhibitor of the Na+-glucose co-transporter in proximal kidney tubules, reduces tubular reabsorption of glucose [80]. STZ rats treated with phloridzin restored normoglycemia, in the absence of any changes in plasma insulin [51]. Correction of hyperglycemia with phloridzin was unable to reduce cardiac LPL activity in hearts from STZ rats [51]. When DZ treated animals were administered graded doses (2 and 8 U of rapid-acting insulin intravenously) of insulin, there was induction of normoglycemia in these hyperglycemic rats. However, 2 U of insulin were unable to attenuate HR-LPL activity to control levels [51]. Furthermore, when DZ treated hearts were perfused for 1 h, either in the absence or presence of high glucose (25 mM), the high LPL activity in DZ treated hearts could not be maintained in vitro [81]. Finally, DEX treated animals also demonstrated augmented luminal HR-LPL in the absence of any changes in plasma glucose [72]. Collectively, these data suggest that coronary luminal LPL regulation is independent of circulating plasma glucose.

5.2. Fatty acids

In addition to hyperglycemia, lack of insulin also exposes cardiac tissue to high adipose derived FA, and exogenous (chylomicrons) and endogenous (VLDL) sources of lipoprotein-TG [82]. Previously, FA from TG hydrolysis was suggested to bind and competitively inhibit LPL activity, an effect that is abolished by incubation with albumin due to its higher affinity for FA [83]. Displacement of LPL from its binding sites is yet another potential mechanism by which LPL activity can be regulated by FA [84]. Adipose tissue LPL bound to endothelial cells can be displaced from its binding sites by oleic acid [85]. It was proposed that following LPL mediated TG hydrolysis, when supply of FA exceeds tissue demand, FA would probably bind to LPL and displace it from its binding sites, thereby regulating the enzyme activity via a negative feedback mechanism [84,85]. However, this effect was not observed in isolated cardiac myocytes or whole hearts [86]. Given that animals made hyperglycemic with DZ exhibited high LPL activity in the presence of augmented systemic FA [69], it is unlikely that either adipose or lipoprotein derived FA can directly influence cardiac LPL in vivo.

5.3. Lysophospholipids

In addition to FA, lysophosphatidylcholine (LPC), a component of lipoprotein phospholipids [87], is also released during LPL mediated lipolysis of TG-rich lipoproteins [88] and can be augmented under conditions of extensive lipoprotein-TG breakdown. Given that: a) LPL possesses TG hydrolase and phospholipase activity [89], b) LPC formed from lipoprotein breakdown was shown to either directly or indirectly, through the release of heparanase like compounds, displace surface bound LPL from adipocytes [52], and c) lipoproteins isolated from diabetic animals and patients have a higher LPC content [90,91], LPC would be expected to increase coronary LPL and cause lipid oversupply during diabetes. Exposure of control hearts to extremely low concentrations of LPC (1–100 nM) was able to enhance HR-LPL to levels comparable to those seen following STZ or DZ [69]. As only LPC and not FA, TG or glucose was able to sustain the high LPL activity in vitro in DZ hearts [81], the importance of LPC as a putative second messenger involved in LPL trafficking was confirmed. Using WR 1339 (a non-ionic detergent that physically alters lipoproteins making them inaccessible for LPL mediated hydrolysis) to inhibit circulating TG lipolysis, the ability of DZ to enhance luminal LPL was dramatically restricted [69], suggesting that lipoprotein hydrolysis and its byproducts like LPC are a key metabolic trigger that facilitates enzyme transfer in vivo. It should be noted that LPC can also be generated from cell membrane-derived phosphatidylcholine hydrolysis by phospholipase A2 (PLA2) [92], and from hydrolysis of the phospholipid monolayer enclosing the intracellular TG droplet [93]. Whether these sources of LPC also contribute towards the increase in luminal LPL following diabetes merits further investigation.

In co-culture of adipocytes and endothelial cells, LPC was shown to cleave and transport surface bound LPL to the endothelial surface [52]. Unlike adipocytes, LPC was unable to release coronary luminal LPL [69], suggesting alternate signaling mechanisms for LPL regulation. A recent report suggested that LPC contributes to the activation of a pro-inflammatory endothelial phenotype, an effect requiring inputs from protein kinase C (PKC) [94]. Interestingly, PKC has been implicated in the regulation of LPL in adipocytes [95] and macrophages [96]. Furthermore, in macrophages, leptin induced augmentation of LPL gene expression involved PKC activation [97]. Interestingly, the in vitro effects of LPC on cardiac LPL also occurred through
activation of endothelial PKC [81]. This mechanism involved formation of lysophosphatidic acid (LPA), and activation of myocyte G-protein coupled receptors (G12/13), likely facilitating cardiomyocyte LPL secretion [81] (Fig. 4). It should be noted that myocardial PKC activity increases following STZ diabetes [98].

Functional effects of LPA, through stimulation of G12/13 receptors [99], result in translocation of cytosolic RhoA [99,100], and its downstream effectors, Rho kinases (ROCK I and ROCK II), to the cell membrane, leading to cytoskeletal reassembly [99,100]. Actin cytoskeleton rearrangement has been implicated in cardiomyocyte LPL secretion [101]. Incubation of myocytes with LPA induced formation of a dense and organized network of thick and parallel F actin filaments, and augmented myocyte HR-LPL [102]. Since cytochalasin D (actin polymerization inhibitor) and Y-27632 (ROCK inhibitor) appreciably reduced this myocyte HR-LPL activity, LPA likely facilitates secretion of preformed LPL [102] (Fig. 4). Comparable to the effect of LPA on myocytes, hyperglycemia induced by DZ also caused significant membrane translocation of RhoA and ROCK I in whole hearts, an effect that was reversed by insulin [102]. Hence, a mechanism for the acute effect of hyperglycemia in augmenting cardiac HR-LPL could involve RhoGTPase activation and actin reorganization.

6. Conclusions

Despite insulin therapy, the risk of heart disease and hypertension is increased 2–4 fold in diabetic patients. Following hypoinsulinemia/hyperglycemia, when cardiac glucose utilization is impaired, the heart undergoes metabolic transformation wherein it switches energy production to exclusive oxidation of FA. This process is made possible by amplification of coronary LPL [103], thereby allowing uninterrupted FA supply to the diabetic heart. Since changes in cardiac LPL can result in abnormal FA supply and utilization by the heart tissue, it could potentially initiate and sustain cardiac dysfunction during diabetes. As effective blood glucose control is often compromised in patients with insulin resistance and diabetes, and as pharmaceutical management can never duplicate the exquisite control of glucose observed in healthy humans, it is likely that increased luminal LPL, and abnormal FA supply and utilization by the heart could result in lipotoxic events that will initiate a number of
metabolic, morphological, and mechanical changes, and eventually cardiac disease (Fig. 5).

Given the proposed role for LPL in initiating diabetic cardiomyopathy through its ability to enhance FA supply, restricting cardiac LPL translocation could be a therapeutic advantage as this would lead to metabolic switching to glucose utilization. Interestingly, a recent study demonstrated that cardiac specific LPL knockout mice displayed enhanced expression of GLUT-1, GLUT-4 and insulin receptor substrate 2 with a parallel decline in the expression of PDK-4 and insulin receptor substrate 1 [104]. This resulted in markedly enhanced basal glucose uptake in these hearts [104]. Future studies are required to establish conclusively that increased LPL in the diabetic heart is causally related to the development of cardiomyopathy. In the STZ diabetic rat, this awaits the discovery of pharmacological agents that specifically inhibit cardiac LPL activity in vivo. In terms of drug discovery and application of this research, development of cardiac specific lysosphospholipid receptor or RhoGTPase inhibitors would help in slowing LPL translocation to the coronary lumen thus interrupting FA supply to the heart.

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