Endothelial cell–cardiomyocyte crosstalk in diabetic cardiomyopathy

Andrea Wan and Brian Rodrigues*

Faculty of Pharmaceutical Sciences, The University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC, Canada V6T 1Z3

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Abstract

The incidence of diabetes is increasing globally, with cardiovascular disease accounting for a substantial number of diabetes-related deaths. Although atherosclerotic vascular disease is a primary reason for this cardiovascular dysfunction, heart failure in patients with diabetes might also be an outcome of an intrinsic heart muscle malfunction, labelled diabetic cardiomyopathy. Changes in cardiomyocyte metabolism, which encompasses a shift to exclusive fatty acid utilization, are considered a leading stimulus for this cardiomyopathy. In addition to cardiomyocytes, endothelial cells (ECs) make up a significant proportion of the heart, with the majority of ATP generation in these cells provided by glucose. In this review, we will discuss the metabolic machinery that drives energy metabolism in the cardiomyocyte and EC, its breakdown following diabetes, and the research direction necessary to assist in devising novel therapeutic strategies to prevent or delay diabetic heart disease.

Keywords

Endothelial cell metabolism • Cardiomyocyte metabolism • Heparanase • Lipoprotein lipase • Vascular endothelial growth factor

1. Introduction

The incidence of diabetes has reached pandemic proportions, with ~347 million people affected globally. The prediction from the World Health Organization is that by 2030, diabetes will be the seventh leading cause of death.1 To manage this chronic disease, patients with diabetes require substantial resources and currently use up a prodigious 15% of national healthcare budgets.1 Treatment of this condition requires appropriate glucose and HbA1c monitoring and subsequent medication management. However, this practice does not exquisitely match the physiological control of glucose homeostasis. As a result, people with diabetes are prone to developing long-term complications including retinopathy, neuropathy, nephropathy, and most importantly cardiovascular disease, which accounts for 50–80% of diabetes-related deaths.2 – 5 It should be noted that several large trials assessing HbA1c cardiovascular disease, which accounts for 50–80% of diabetes-related deaths. Although atherosclerotic vascular disease is a primary reason for this cardiovascular dysfunction, heart failure in patients with diabetes might also be an outcome of an intrinsic heart muscle malfunction (labelled diabetic cardiomyopathy). Changes in cardiomyocyte metabolism, which encompasses a shift to exclusive fatty acid utilization, are considered a leading stimulus for this cardiomyopathy. In addition to cardiomyocytes, endothelial cells (ECs) make up a significant proportion of the heart, with the majority of ATP generation in these cells provided by glucose. In this review, we will discuss the metabolic machinery that drives energy metabolism in the cardiomyocyte and EC, its breakdown following diabetes, and the research direction necessary to assist in devising novel therapeutic strategies to prevent or delay diabetic heart disease.

2. Diabetic cardiomyopathy

Heart disease is a leading reason for death in patients with diabetes, with coronary vessel disease and atherosclerosis being primary causes for the increased incidence of cardiovascular dysfunction.13 In this patient population, the comorbidities of hypertension, abnormal cholesterol, and hypertriglyceridaemia significantly contribute to a more severe cardiovascular pathology and a greater mortality risk.14 However, Type 1 (T1D) and Type 2 (T2D) patients have also been diagnosed with reduced or low-normal diastolic function, left ventricular hypertrophy, and clinically overt congestive heart failure independent of vascular defects and hypertension.15 These observations suggest a specific impairment of heart muscle (termed diabetic cardiomyopathy). Evidence of cardiomyopathy has also been reported in rodent models of T1D16,17 and T2D,18,19 which are comparable with that seen in human diabetic patients. Given that rodents are resistant to atherosclerosis, these models provided strong evidence for the occurrence of diabetic cardiomyopathy. Cardiomyopathy is a complicated disorder, and several factors have been associated with its development. These
include (i) an increased stiffness of the left ventricular wall (associated
with an accumulation of connective tissue and insoluble collagen),20 (ii)
impaired sensitivity to various ligands (e.g. β-agonists and insulin),21,22
(iii) depressed autonomic function (accompanied by alterations in myo-
cardial catecholamine concentrations),23 (iv) compromised endothelium
function (related to reduced nitric oxide activity and augmented
synthesis of vasoconstrictors),24 (v) abnormalities of various proteins
that regulate ion flux (specifically intracellular calcium),25 and (vi) al-
terations in coronary microcirculation (as a consequence of both struc-
tural and functional modifications).24 The view that diabetic
cardiomyopathy could occur as a consequence of early alterations in
cardiac metabolism has also been put forward.

3. Conventional cardiomyocyte
metabolism

With uninterrupted contraction being a unique feature of the heart, the
cardiomyocyte has the highest demand for energy. This cell demon-
strates substrate promiscuity, enabling it to utilize multiple sources
for energy, including fatty acids (FAs), carbohydrates, amino acids, lac-
tate, and ketones.26 Among these, 95% of the energy generated is de-

erived from carbohydrates and FA through mitochondrial metabolism.

3.1 Glucose

In a basal setting, glucose and lactate supply ~30% of ATP generation,
whereas 70% of ATP generation is through FA oxidation (FAO).26 Glu-
cose metabolism requires uptake, glycolysis, and mitochondrial
oxidation to yield ATP (Figure 1). Cardiac glucose uptake is dependent
on the plasma membrane content of glucose transporters (GLUT1 and
GLUT4).27 GLUT1 has predominant plasma membrane localization and
accounts for basal glucose uptake, whereas GLUT4 is the dominant
transporter in the adult heart. In non-stimulated conditions, a majority
of GLUT4 is located in an intracellular pool. However, on stimulation
by insulin or contraction-associated activation of AMP-activated pro-
tein kinase (AMPK), GLUT4 is redistributed to the plasma membrane
to mediate glucose uptake.17,28 The delivered glucose undergoes gly-
colysis (catalysed by phosphofructokinase-1, PFK-1) to form pyruvate,
which then enters the mitochondria for oxidation, a process controlled
by the activity of pyruvate dehydrogenase (PDH).8 It should be noted
that functions of glucose extend beyond its primary use for energy gen-
eration and glycogen storage in the cardiomyocyte. Glucose can also be
used for ribose formation or enter into the hexosamine biosynthetic
pathway.29

3.2 Fatty acids

The cardiomyocyte has a limited capacity to synthesize FA and thus re-

dies on an exogenous and endogenous supply (Figure 1).8 Exogenous FA
delivery to the heart involves (i) release from adipose tissue and trans-
port to the heart after complexing with albumin, (ii) lipolysis of circu-
lating triglyceride (TG)-rich lipoproteins (VLDL, chylomicrons) to FA
by lipoprotein lipase (LPL) positioned at the EC surface of the coronary
lumen,5 and (iii) uptake by sarcolemmal FA transporters including FA
translocase (FAT/CD36), FA binding protein (FABPpm), and FA trans-
porter protein (FATP).10 Endogenous FA provision is through the

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/111/3/172/2595041)

**Figure 1** Cardiomyocyte metabolism. In the cardiomyocyte, two of the major substrates that are used for energy generation include glucose and FAs. Glucose uptake into the cardiomyocyte is predominantly a GLUT4 event. Following its entry into the cell, glucose can either be stored as glycogen or undergo glycolytic and oxidative metabolism to generate ATP. FA is the preferred energy substrate of the cardiomyocyte. Its entry is through a number of FA transporters including CD36, FABPpm, and FATP. FA can also undergo storage in the form of TGs or enter into the mitochondria to be oxidized for ATP.
breakdown of endogenous cardiac TG stores. Two essential enzymes involved in TG hydrolysis include adipose TG lipase (ATGL) and hormone-sensitive lipase (HSL). Delivered FA can enter the biosynthetic or oxidative pathways. In the latter, FAs are oxidized by conversion to fatty acyl-CoA, which is transported into the mitochondria through carnitine palmitoyltransferase (CPT1/CPT2). Inside the mitochondria, fatty acyl-CoA undergoes β-oxidation to generate acetyl-CoA, which is oxidized in the tricarboxylic acid cycle to yield ATP. When activated, AMPK, peroxisome proliferator-activated receptor-α (PPARα), and malonyl-CoA decarboxylase (MCD), all have substantial roles in modulating FA delivery and oxidation.

4. Alterations in cardiomyocyte metabolism following diabetes

We and others propose that interruptions in glucose and FA metabolism in the heart are the geneses of diabetic cardiomyopathy. Following diabetes, myocardial GLUT4 gene and protein expression are reduced. However, hyperglycaemia sustains glucose uptake by the diabetic heart, such that glucose influx into the cardiomyocyte remains comparable with control. In addition to hyperglycaemia, multiple adaptive mechanisms, at the level of the whole body and intrinsic to the heart, also operate to augment FA supply to the cardiomyocyte. These include adipose tissue lipolysis and plasma lipoprotein-TG, vascular LPL activity, and myocyte sarcolemmal FA transporters (e.g. CD36 and FABPpm), all of which increase following diabetes. The above changes in cardiac FA are under the supervision of AMPK (early) and PPARs (late). For example, following acute diabetes, rapid activation of AMPK is an adaptation that would ensure adequate cardiac energy production. It does so by increasing FA delivery through its activation of LPL in addition to repositioning CD36 to the sarcolemma, an FA transporter that is significantly increased in hearts from STZ-diabetic animals. AMPK also participates in the utilization of FA by phosphorylating and inhibiting acetyl-CoA carboxylase, relieving its inhibition on CPT1, hence promoting FAO. However, in chronic diabetes, with the addition of augmented plasma and heart lipids, AMPK activation is prevented, and control of FAO is through PPARα. Activation of cardiac PPARα has been reported in models of chronic diabetes (STZ-induced diabetic rats, ZDF rats, and db/db mice), promoting the expression of genes involved in various steps of FAO. For example, box family and subfamily O of transcription factors (FoxOs) are also known to regulate whole-body energy metabolism due to their effect in various tissues such as the liver, adipose tissue, skeletal muscle, and beta cells. However, their value in moderating cardiac substrate utilization, especially in conditions like insulin resistance/diabetes, is unknown. On the basis of our results, we believe that unlike AMPK and PPARα, which are time-dependent (early and late phases of diabetes) regulators of cardiac metabolism, FoxO is a key arbitrator of cardiac metabolism, whose activity is enhanced the instant there is a drop in systemic insulin levels. We have linked an increase in cardiomyocyte FoxO1 to augmented CD36 in the plasma membrane through actin cytoskeleton rearrangement. Collectively, all of the above mechanisms operate to increase FA supply and oxidation. In doing so, augmented FA utilization can significantly reduce cardiac glucose oxidation, and to a lesser extent glycolysis and glucose uptake, by increasing (i) citrate (a recognized inhibitor of PFK-1, the rate-limiting enzyme in glycolysis), (ii) pyruvate dehydrogenase kinase 4 (PDK4) expression (known to phosphorylate and inhibit PDH), and (iii) acetyl-CoA (which inhibits PDH, either allosterically or through activation of PDK4). Consequently, intracellular concentrations of glucose and its metabolites accumulate (glucotoxicity) to potentiate O-linked protein glycosylation and interfere with protein functionality.

When glucose utilization is compromised and surplus FAs are provided to the cardiomyocyte irrespective of its requirements, the damage to the cardiac muscle is severe. In gauging the impact of these excess FAs, two key points to consider are that mitochondrial metabolism of FA requires more oxygen than glucose, and diabetic cardiomyopathy is characterized by decreased capillary density and impaired myocardial perfusion. Additionally, chronic diabetes is associated with a significant decrease in PPARα expression and its associated genes. This sponsors a setting where oxygen delivery and augmented FA uptake cannot match its utilization, leading to their storage as TG in cardiomyocytes. Chronically, the conversion of TG to FA and potentially toxic metabolites, including ceramides, diacylglycerols, and acylcarnitines can provoke cardiomyocyte death (lipotoxicity). Interestingly, increasing FA uptake through the overexpression of cardiac human LPL or FA transport protein results in a cardiac phenotype resembling diabetic cardiomyopathy. Conversely, normalizing cardiac metabolism in diabetic animals reverses the development of cardiomyopathy. It should be noted that FAs also play a significant role in promoting cellular insulin resistance.

In addition to the cardiomyocyte, the heart also requires the ECs to perform a myriad of roles, pre-eminently increasing angiogenesis and capillary density, and the secretion of regulatory proteins, obligations that are crucial for sustaining cardiomyocyte function. As angiogenesis is and the secretion of regulatory proteins are largely dependent on glycolytic energy, it becomes pivotal to understand EC metabolism and its projected contribution to diabetic cardiomyopathy, information that is surprisingly sparse and incomplete.

5. Conventional endothelial cell metabolism

Prior to defining their metabolism, it is important to appreciate that although ECs from capillaries, large arteries and veins share some common qualities, these cells are exceptionally heterogeneous. Thus, based on the vessel type (arterial compared with venous and macrocompared with microvascular ECs), anatomic location, and environment, ECs behave differently in their responses to growth and migration stimuli and also exhibit characteristic clusters of genes. The other caveat is that because the isolation of primary ECs and their long-term maintenance in culture have often proved difficult, EC metabolism is commonly studied in vitro, using EC lines. Hence, the data obtained from one EC may not be universally applicable, nor might it be completely relevant to the intact endothelium in the heart.

Unlike the cardiomyocyte, FA generates only 5% of the total amount of ATP in ECs, with the majority being provided by glucose. Consequently, glycolytic flux is many folds higher than glucose or FAO, providing the ECs with about 85% of its ATP. Interestingly, a recent report suggested that ECs oxidize FAs primarily to feed the tricarboxylic acid cycle (Figure 2). In the majority of cells that utilize glucose, the initial step, glycolysis, is followed by oxidation in the mitochondria, the latter being the dominant pathway for ATP generation (Figure 2). However, ATP supply in ECs is relatively independent of the mitochondrial oxidative pathway.
Remarkably, at physiological concentrations, 99% of glucose is metabolized in the glycolytic pathway, with only 1% going into the Krebs cycle in the mitochondria. The dependence of ECs on glycolysis has multiple advantages. First, glycolytic enzymes are exquisitely positioned in the cytosol, in close proximity to the actin cytoskeleton. This organization provides an immediate supply of ATP for actin rearrangement to facilitate angiogenesis and vesicular secretion. Second, by utilizing the glycolytic pathway to generate ATP, ECs spare oxygen and FA to fuel the underlying cardiomyocytes. Myocytes have a high demand for oxygen, given that their major substrate for energy production is through the oxidative phosphorylation of FA. Thus, despite being in immediate contact with oxygen in the bloodstream, ECs still primarily utilize glycolysis to generate energy. Third, even though the mitochondrial oxidative pathway produces greater amounts of ATP per mole of glucose, the faster rate of glycolysis can match this ATP production. Fourth, ECs are reliant on the glycolytic pathway to generate not only energy but also the required intermediates necessary for cell growth, migration, and angiogenesis. Finally, an additional benefit of such an adaptation is that the EC is protected from mitochondrial electron leakage and generation of chemically reactive molecules [e.g. reactive oxygen species (ROS)] that would otherwise cause EC damage.

For glycolysis to occur, glucose enters into the cell with the help of transporters. Of the many different glucose transporters present in ECs, GLUT1 is the major isoform. This plasma membrane unipporter facilitates not only glucose influx into the ECs, from its luminal side, but also its efflux across the abluminal membrane border. Intriguingly, there is some evidence suggesting that the GLUT1 distribution is disproportionately towards an abluminal locale. However, this information is not always reliable, nor applicable, across different ECs (primary ECs vs. cell lines, microvascular vs. macrovascular ECs, and ECs from diverse tissues). If verified in microvascular ECs, this adaptation will permit faster glucose extrusion than uptake. This will allow the ECs to move glucose to the cardiomyocyte to power the high metabolic requirements of this subjacent cell. Once, inside the ECs, glucose is metabolized by key glycolytic enzymes (e.g. hexokinase II, PFKFB3) to pyruvate, of which <1% is metabolized by the TCA cycle, whereas the majority is converted to lactate. As a result, oxidative pathways account for a minimal amount of ATP generation within the ECs.

6. Aberrant endothelial cell metabolism in diabetes

In ECs, the entry point for glycolysis is glucose uptake by glucose transporters, the predominant one being GLUT1. Traditionally, GLUT1 was thought of as an insulin-independent transporter, and in response to diabetes, ECs are glucose-blind, with GLUT1 expression remaining unresponsive to hyperglycaemia. However, this idea has recently been questioned as ECs would conceivably attempt to protect themselves against the damaging effects of excessive glucose influx by reducing GLUT1 expression. One caveat is that, although GLUT1 reduction at the luminal side may be a favourable response, its depletion at the abluminal side will cause inadequate glucose expulsion to the cardiomyocyte. By extension, this will result in an unpredictably high concentration of intracellular glucose, leading to ROS generation and glycolytic inhibition. More recent data suggest that ECs exposed to high glucose (HG) have reduced GLUT1 expression and glucose uptake. This evidence, together with the reported reduction in enzymatic activity within the glycolytic pathway (e.g. phosphofructokinase), could explain the acknowledged decrease in glycolytic flux during diabetes.

Stalled glycolytic flux means that glycolytic intermediates accumulate and are shuttled into different metabolic pathways. These include the polyol pathway with the formation of sorbitol and fructose, hexosamine biosynthesis pathway that impedes angiogenesis, methylglyoxal...
pathway, protein kinase C activation, and defects in mitochondrial biogenesis and fragmentation. The net result is excess ROS and reactive nitrogen species production, and advanced glycation end product (AGE) synthesis, mediators of ECs and potentially cardiovascular dysfunction.

Mechanisms to explain the altered GLUT1 under conditions of hyperglycaemia include the thioredoxin-interacting protein (TXNIP) system. TXNIP, by directly binding to GLUT1, induces its endocytosis and its subsequent breakdown in lysosomes (an acute effect), in addition to reducing the level of GLUT1 mRNA (a chronic one). TXNIP levels are suppressed in many tumours, as cancer cells require high GLUT1 expression to sustain elevated glycolysis. Interestingly, TXNIP is an exquisitely glucose-sensitive gene and is induced in response to low insulin or HG. Whether diabetes is associated with increased TXNIP expression in ECs derived from hearts of diabetic animals is yet to be determined. An alternate pathway is one where TXNIP can reversibly bind to thioredoxin-1 (TRX1), an interaction that is weakened by ROS, and allows TXNIP to dissociate from oxidized TRX1 to down-regulate GLUT1. It is conceivable that with HG and ROS generation, not only is the expression of TXNIP increased but also its dissociation from TRX1, permitting its increased availability for GLUT1 interaction. Finally, a recent study has suggested that cardiomyocyte-derived exosomes, by sending glucose transporters and the associated glycolytic enzymes to the ECs, can modulate endothelial glucose transport and metabolism. Whether this process is repressed during hyperglycaemia would be of particular interest as an additional mechanism to explain altered glucose uptake and metabolism in the EC during diabetes.

In other cell types like the cardiomyocyte, limited glucose utilization provokes the overconsumption of FA to ensure adequate energy production. For this to happen, FA requires uptake and oxidation, processes that are augmented in the cardiomyocyte following diabetes, but have yet to be unequivocally substantiated in EC. In culture, ECs have been demonstrated to have reserve oxidative capacity and can increase oxidation under conditions of high metabolic demand or stress. Under conditions of glycolytic inhibition following diabetes, there is an expectation that the increased provision of FA would lead to an augmented oxidation of this substrate. However, the verification that FAO increases in the ECs will prompt other more complex questions. For example, is the increase in FAO geared towards ATP generation or nucleotide synthesis? Remarkably, in ECs, acetyl-CoA from FAO is used for DNA synthesis and cell multiplication. If excess FA is used for energy production, will its associated undesirable effects in ECs be even more deleterious than that seen in cardiomyocytes? ROS generated by FAO will impede glucose transport and glycolytic enzymes (e.g. GAPDH) and may further reduce glycolysis under HG conditions. To what magnitude are ECs teleologically equipped to handle excess FAO? To explain, the organelle responsible for FAO, mitochondria, make up only 2–6% of the ECs compared with their volume in hepatocytes (28%) or cardiomyocytes (32%). Thus, excess FA entering the ECs may be associated with a progressively smaller, incremental effect on oxidation—a ceiling effect, such that this substrate will either migrate through these cells or be stored as TG. The latter outcome is especially problematic, given the negative consequences of stored TG in cells other than adipocytes, in addition to the detrimental consequences of excessive FA utilization. Changes in metabolism and function of EC can participate not only in its own demise but also in cardiomyocyte dysfunction and cell death.

### 7. Endothelial cell: cardiomyocyte crosstalk

#### 7.1 Endothelial cell control of cardiomyocyte metabolism

Heparan sulfate proteoglycans (HSPGs) consist of a core protein to which several linear heparan sulfate (HS) side chains are covalently linked and function not only as structural proteins but also as temporary docking sites due to the high content of charged groups in HS. The latter property is implicitly used to electrostatically bind a number of different proteins including LPL and vascular endothelial growth factor (VEGF). Attachment of these bioactive proteins is a clever arrangement, providing the cell with a rapidly accessible auxiliary reservoir, precluding the need for de novo synthesis when the requirement for a particular protein is urgent. The action of EC heparan on cardiomyocyte HSPG releases LPL and VEGF, the major players in increasing FA delivery and utilization by the cardiomyocyte to overcome its lack of glucose utilization.

ECs have also been implicated in providing functional support to subjacent cardiomyocytes and do so by communicating via soluble paracrine factors. For example, in response to environmental cues like hyperglycaemia, the strategically located ECs act as ‘first responders’ to this cellular disturbance and react by secreting heparanase. Heparanase is synthesized as an inactive, latent (L-Hep) 65 kDa enzyme that undergoes cellular secretion followed by HSPG-facilitated reuptake. After undergoing proteolytic cleavage in lysosomes, a 50 kDa polypeptide is formed that is ~100-fold more active (A-Hep) than L-Hep. Within the acidic compartment of lysosomes, A-Hep is stored until mobilized (Figure 3). In the presence of HG, we reported redistribution of lysosomal heparanase from a perinuclear location towards the plasma membrane of ECs, together with elevated secretion into the medium. We also determined that ATP release, purinergic receptor activation, cortical actin disassembly, and stress actin formation were essential for HG-induced A-Hep secretion.

With respect to LPL in the heart, it was suggested that coronary ECs do not synthesize LPL despite its critical function at the vascular lumen. However, more recent observations have reported LPL mRNA expression in pure heart EC to amounts that were 25% of those present in pure cardiomyocytes. Nevertheless, in the heart, the majority of this enzyme is produced in cardiomyocytes—cardiac tissue that has the highest expression of this enzyme. Following its maturation by lipase maturation factor 1 (LMF1), LPL is subsequently secreted onto HSPG (syndecan 1) binding sites on the myocyte cell surface, where the enzyme is momentarily located. From here, LPL is transported across the interstitial space to the luminal surface of ECs where it is bound to HSPG and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) through an ionic linkage; LPL has an abundant amount of positively charged domains. At the lumen, LPL actively metabolizes the TG core of lipoproteins to FA, which are then transported into the heart for numerous metabolic and structural functions (Figure 4). Following diabetes and activation of post-translational mechanisms, the heart rapidly increases LPL at the vascular lumen by transferring this enzyme from the underlying cardiomyocyte. We implicated EC heparanase in this process, through a mechanism by which this enzyme detaches LPL from the myocyte surface, for onward movement to the vascular lumen.
**Figure 3** Cardiomyocyte–EC crosstalk. In the EC, latent heparanase is first secreted, followed by re-uptake and conversion to active heparanase. The secretion of both latent and active heparanase is dependent on glycolytically produced ATP. Both forms of heparanase are able to liberate myocyte surface-bound proteins including VEGFA and VEGFB. These growth factors have a paracrine influence on the EC, to facilitate angiogenesis through endothelial migration and proliferation. HSPG: heparan sulfate proteoglycan.

**Figure 4** Synthesis and transport of LPL. Following gene transcription, LPL mRNA is translated to inactive LPL proteins. The inactive monomer undergoes glycosylation, and several other post-translational processes to be dimerized in the endoplasmic reticulum (ER). The fully processed LPL is sorted into vesicles that are targeted towards the cell surface for secretion. This process occurs by the movement along the actin cytoskeleton. Subsequently, it docks with the cell surface and releases LPL onto HSPG-binding sites on the plasma membrane. At the basolateral side of the EC, glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) captures LPL in the interstitial space and transfers it across to the apical side of the EC. GPIHBP1 functions as a platform to enable LPL to hydrolyse lipoprotein-TG and release FAs. More recent data suggest that EC can also synthesize LPL, albeit at a limited capacity.
is the increased provision of FA to the diabetic cardiomyocyte through FA transporters like CD36. In addition to LPL, VEGF is another myocyte HSPG-bound protein that is released through heparanase action. Of the different VEGF isoforms, VEGFA and VEGFB are notable standouts, abundantly expressed in the cardiomyocyte. From a single human VEGFA gene, alternative splicing generates a number of isoforms. The predominantly expressed VEGFA165 is the main effector of VEGF action and is tethered (50–70%) to the cardiomyocyte extracellular matrix. With respect to VEGF, VEGFB167 constitutes more than 80% of the total VEGFB transcript and is also bound to HSPG. Upon secretion, each has variable affinity for HSPG-binding sites on the myocyte cell surface, an interaction made possible by VEGF heparin-binding domains (amino acids rich in basic residues). Not only does this liaison protect VEGF against degradation, but it also allows the myocyte matrix to retain a pool of readily readable growth factors (Figure 3).

Following its release by heparanase, VEGFA is competent to provide autocrine signalling. To test the existence of an autocrine pathway for VEGF control of metabolism, we focused on AMPK, a pivotal cellular energy sensor and regulator. We reported that recombinant VEGF has a capacity to induce AMPK activation in cardiomyocytes, likely through activation of a calcium-/calmodulin-dependent protein kinase (amino acids rich in basic residues). Interestingly, AMPK activation governs LPL recruitment to the myocyte surface for forward movement to vascular lumen. The mechanisms underlying LPL recruitment embraces p38 mitogen-activated protein kinase activation with subsequent phosphorylation of the heat-shock protein (Hsp) 25. Actin monomers are released from phosphorylated Hsp25 and self-associate to form fibrillar actin. Vesicles containing LPL then move along the actin filament network to bind to HSPG on the cardiomyocyte plasma membrane. Strikingly, we observed that VEGF was able to increase LPL translocation to the myocyte cell surface. Our data on the ability of VEGFA to promote LPL movement implicated this growth factor in the cascade of expanding actions that are geared to help the acutely diabetic heart switch its substrate selection to predominately FA. Whether this mechanism persists following chronic diabetes is currently unknown. Animals with long-term STZ-induced diabetes are characterized by reduced cardiac expression of VEGFA and its receptors. Additionally, other pathways like increased mobilization of fat from adipose tissue mediated by HSL and ATGL could surpass the efforts of LPL, after chronic diabetes.

Following its release from the cardiomyocyte, VEGFB may also have an autocrine signalling action, but related to a mechanism that prevents heart failure rather than modifying cell metabolism. VEGFB has been shown to promote cell survival and produce physiological cardiac hypertrophy. Indeed, multiple models of heart failure have indicated a significant drop in VEGFB, which may be due to decreased VEGFB protein or possibly due to impaired release of VEGFB from HSPG. Whether there is a role for decreased VEGFB in diabetic cardiomyopathy is currently unknown. Overall, under conditions of hyperglycaemia and the associated release of heparanase from the EC, myocyte cell-surface LPL is liberated for onward movement to the vascular lumen to promote FA delivery to the cardiomyocyte. Heparanase-releasable VEGFA aids in the replenishment of HSPG-bound LPL to facilitate the metabolic switching of the cardiomyocyte to FA. As this excessive use of FA could be detrimental to the heart, the VEGFB released ensures cardiomyocyte survival.

In addition to the above-mentioned regulatory mechanisms, it should be recalled that EC secretes nitric oxide (NO), which regulates vascular tone by relaxing vascular smooth muscle. However, NO can also influence the contractile function of cardiomyocytes through beta-adrenergic and muscarinic control. Intriguingly, NO has been reported to control cardiac substrate utilization. Another signalling communicator between EC and cardiomyocytes is neuregulin-1, a growth factor released from EC that has several cardioprotective functions. In isolated cardiac myocytes, neuregulin-1 has been linked to glucose uptake. Finally, given the metabolic flexibility of the cardiomyocyte, and its ability to use a number of substrates for ATP production (high-energy phosphate storage within the cardiomyocyte is minimal), it is possible that the products of EC glycolysis, like pyruvate and lactate, can be used by the cardiomyocyte when they become available. In cancer, tumour cells that are highly glycolytic secrete high amounts of lactate, which can be taken up by neighbouring cells and channelled into the TCA cycle. Whether this situation is possible in the heart, with cardiomyocytes taking up lactate produced by the EC, has yet to be identified.

### 7.2 Cardiomyocyte control of endothelial cell metabolism

Following its release from the cardiomyocyte in response to heparanase, LPL traverses the interstitial space and binds to its transporter GPIHBP1, a glycoprotein expressed exclusively on capillary EC. GPIHBP1 facilitates LPL relocation from the basolateral to the apical (luminal) side of the EC. Out here, it can also act as a platform, binding lipoproteins. This allows LPL to actively metabolize the lipoprotein-TG core, thereby liberating FAs that are transported to the cardiomyocytes. We described a novel mechanism in which heparanase-induced VEGFA released from the cardiomyocyte, in a paracrine manner, activated Notch signalling in the EC. This resulted in enhanced GPIHBP1 expression, promoted LPL translocation across the EC, and can regulate FA delivery to the cardiomyocytes. It is unclear whether cardiomyocyte-VEGFA also assists in FA transport across the EC. VEGFA is known to promote FA-binding protein 4 expression, an FA-transporting protein abundantly expressed in microvascular EC in the heart. Similarly, VEGFB released from the cardiomyocyte could also influence EC lipid transport by controlling the expression of vascular EC FATPs, and Vegfb/- mice have less uptake and accretion of lipids in the heart. Consistent with these observations in mice, VEGFB transgenic rat hearts exhibit increased Fatp4 RNA concentrations, whereas VEGFB knockout rat hearts display reduced amounts of Fatp4 RNA. However, as there was no change in FA uptake between TG, knock-out, and wild-type hearts, the authors concluded that in rats, VEGFB is dispensable for normal cardiac function under unstressed conditions and for high fat diet-induced metabolic changes.

As FAs traverse the ECs, there is a likelihood that this cell deviates to use this substrate as an alternate energy source during diabetes. However, it is not in the nature of the ECs to increase their consumption of FAs. As such, the ECs can sustain substantial damage when exposed to FAs. As described above, whether there is a role for cardiomyocyte VEGFB in protecting EC against excessive FA use is currently unknown. VEGFB has been shown to decrease expression of proteins involved in FAO. Increased FA delivery and decreased glucose oxidation in the cardiomyocyte increase FAO. To match the significant increase in FAO, an abundant supply of oxygen to cardiomyocyte is required to efficiently consume these FAs through oxidative phosphorylation. Cardiomyocytes respond by promoting
angiogenesis and vasculogenesis to enhance oxygen supply to the myocardium and do so by secreting the necessary paracrine signals that include cardiomyocyte surface-bound VEGFA and VEGFB.

VEGFA has a proven role in blood vessel formation, which encompasses angiogenesis, vasculogenesis, and arteriogenesis. VEGFA can bind to both VEGF receptors 1 and 2 (VEGFR1 and VEGFR2), but only by binding VEGFR2 does VEGFA activate downstream signals such as ERK, promoting EC migration and angiogenesis. As a member of the VEGF family, initial studies with VEGFB focused on its role in angiogenesis. Surprisingly, VEGFB does not appear to play a role in angiogenesis under normal conditions or even when overexpressed. However, although VEGFB may not directly have a role in angiogenesis, it may play a part in sensitizing ECs to VEGF-induced angiogenesis. It has been suggested that the binding of VEGFB to VEGFR1 leads to less VEGFR1 being available to bind VEGFA, allowing more VEGFA to activate signals via VEGFR2. In addition to this sensitizing effect, coronary vasculature in VEGFB overexpressing hearts had up to a 5-fold increase in the number of arteries, whereas adiponoviral overexpressing rats had a 2.5-fold increase, an effect likely related to EC survival. Overall, this dual action allows VEGFB to indirectly increase angiogenesis to permit tolerable levels of FAO by the cardiomyocytes.

Under the condition of diabetes when there is a failure to release VEGFA and VEGFB from the cardiomyocyte, its associated impact on the vasculature will result in an ‘oxygen shortfall’—a situation in which oxygen consumption related to FAO surpasses oxygen delivery, leading to FA storage in the cardiomyocyte and progressive cardiac dysfunction. It should be noted that in addition to this paracrine control of angiogenesis, intrinsic mechanisms within the EC, like FoxO1, have recently been implicated in control of the vascular architecture. FoxO1, a prominent member of the Forkhead box family and subfamily O transcription factors, has been known to play an important role in cell survival, oxidative stress resistance, energy metabolism, cell cycle arrest, and cell death. FoxO1 is activated in conditions such as fasting, nutrient excess, insulin resistance, diabetes, inflammation, sepsis, and ischaemia. Additionally, overexpression of endothelial FoxO1 resulted in restricted vascular expansion. Whether the increase in FoxO1 that we observed in diabetic cardiomyocytes is also a feature of EC from diabetic hearts is currently unknown. If proved, this could provide an additional mechanism by which the lack of angiogenesis leads to diabetic cardiomyopathy.

8. Significance

Inadequate glucose control leads to repeated bouts of hyperglycaemia, which is associated with ineffective glucose uptake (through GLUT1), metabolism, and deficiency in ATP generation within the EC. Additionally, as insulin signalling (via phosphatidylinositol 3-kinase, mitogen-activated protein kinase kinase and endothelial nitric oxide synthase phosphorylation) stimulates EC insulin transport and capillary recruitment in skeletal muscle, inadequate insulin action in EC is associated with a reduction in insulin delivery, and lower glucose uptake. Should this mechanism be present in the heart, this, together with the incompetence of GLUT4-mediated glucose transport into the cardiomyocyte means that this cell type will begin to use FA exclusively. One way by which the cardiomyocyte handles this increased FA utilization is to deploy LPL to the vascular lumen (with the help of EC heparanase), to break down circulating lipoprotein-TGs. The FA released by this process requires passage through the EC. As FAs traverse the ECs, there is an opportunity for this cell type to utilize FA, but is not without undesirable outcomes. ECs are both structurally and morphologically geared towards glycolysis, rather than FA utilization [amount of mitochondria in EC is small (< 10% of the cytoplasm) compared with cell types that have higher energy demands (e.g. cardiomyocytes, where mitochondria contribute up to 32% of the cytoplasmic volume)]. Cardiomyocyte-released growth factors (VEGF) in response to hyperglycaemia could protect EC against FA overload by turning on genes protective against cell death, or supplement the oxygen necessary for cardiomyocyte-mediated FAO by promoting angiogenesis (VEGFA). We propose that under the conditions of diabetes, there is a disruption of the harmonious dialogue between the EC and cardiomyocytes that results in lipotoxicity that can accelerate cardiovascular disease. Gaining more insight into mechanisms that (i) limit EC FA utilization and (ii) ensure adequate oxygen delivery to the cardiomyocyte by accelerating angiogenesis may delay cardiac failure and provide long-term management of this complication during diabetes.

9. Potential therapeutic strategies

To restore metabolic equilibrium and curb lipotoxicity, targeting the protein ‘ensemble’, heparanase-LPL-VEGF may help prevent or delay heart dysfunction seen during diabetes.

9.1 Heparanase

Heparanase, with a repertoire of functions, can be released from EC in response to HG. It affects myocyte metabolism and does so by interacting with its partners, VEGF and LPL. In the short term (e.g. diabetic patients who have poor control of glucose leading to bouts of hyperglycaemia), it can amplify FA delivery and utilization by the diabetic heart. If these events are prolonged, the resultant lipotoxicity could lead to cardiovascular disease in humans. Globally, inhibitors of heparanase (both active and latent-current strategies to modulate heparanase are aimed exclusively at blocking activity) could be expected to provide critical tools for managing the cardiac complications of diabetes. These include roneparstat (SST0001, a non-anticoagulant chemically modified heparin) and mupafostat (PI88, a mixture of oligosaccharides mimicking HS). Although we anticipate a delayed or milder cardiomyopathy using these agents, this is not an absolute. Agents that impede heparanase activity may prove to be less effective, especially as HG can also increase secretion of latent inactive heparanase that can fulfill its own functions in releasing LPL.

9.2 Lipoprotein lipase

Cardiac-specific overexpression of LPL causes a severe myopathy characterized by lipid oversupply and deposition, muscle fibre degeneration, excessive dilatation, and impaired left ventricular function in the absence of vascular defects, a situation comparable with diabetic cardiomyopathy. Thus, there is a potential benefit to lowering cardiac LPL following diabetes. Angiopoietin-like protein 4 (Angptl4) is known to convert dimeric active LPL at the vascular lumen into inactive monomers. Similarly, apolipoprotein C-III can inhibit LPL by displacement of the enzyme from the lipid droplet. The therapeutic advantage of stimulating Angptl-4 or apo C-III may, however, be limited by the possibility to also develop hypertriglyceridaemia. Recent studies have suggested that patients with inactivating mutations in ANGPTL4 have lower levels of TGs and a lower risk of coronary artery disease, linking TG to cardiac disease.
9.3 Vascular endothelial growth factor

Reduction of myocardial VEGFA,157 and its association with impairesed collateral formation in cardiac tissue,16 is a pivotal event in impaired cardiomyopathy. Conversely, VEGFA has been implicated in diabetic cardiomyopathy. Given the dissimilar global effects of VEGFA, one would anticipate the use of targeted gene therapy to restore VEGFA in the myocardium (local concentrations), rather than an approach to augment systemic levels. Findings on VEGFB in the diabetic heart are lacking. Anticipate the use of targeted gene therapy to restore VEGFA in the diabetic heart.


