Endothelial cells respond to hyperglycemia by increasing the LPL transporter GPIHBP1

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Chiu AP, Wang F, Lal N, Wang Y, Zhang D, Hussein B, Wan A, Vlodavsky I, Rodrigues B. Endothelial cells respond to hyperglycemia by increasing the LPL transporter GPIHBP1. Am J Physiol Endocrinol Metab 306:E1274–E1283, 2014. First published April 15, 2014; doi:10.1152/ajpendo.00007.2014.—In diabetes, when glucose uptake and oxidation are impaired, the heart is compelled to use fatty acids (FA) almost exclusively for ATP. The vascular content of lipoprotein lipase (LPL), the rate-limiting enzyme that determines circulating triglyceride clearance, is largely responsible for this FA delivery and increases following diabetes. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein [GPIHBP1; a protein expressed abundantly in the heart in endothelial cells (EC)] collects LPL from the interstitial space and transfers it across ECs onto the luminal binding sites of these cells, where the enzyme is functional. We tested whether ECs respond to hyperglycemia by increasing GPIHBP1. Streptozotocin diabetes increased cardiac LPL activity and GPIHBP1 gene and protein expression. The increased LPL and GPIHBP1 were located at the capillary lumen. In vitro, passing EC caused a loss of GPIHBP1, which could be induced on exposure to increasing concentrations of glucose. The high-glucose-induced GPIHBP1 increased LPL shuttling across EC monolayers. GPIHBP1 expression was linked to the EC content of heparanase. Moreover, active heparanase increased GPIHBP1 gene and protein expression. Both ECs and myocyte heparan sulfate proteoglycan-bound platelet-derived growth factor (PDGF) released by heparanase caused augmentation of GPIHBP1. Overall, our data suggest that this protein “ensemble” (heparanase-PDGF-GPIHBP1) cooperates in the diabetic heart to regulate FA delivery and utilization by the cardiomyocytes. Interrupting this axis may be a novel therapeutic strategy to restore metabolic equilibrium, curb lipotoxicity, and help prevent or delay heart dysfunction that is characteristic of diabetes.

heparanase; lipoprotein lipase; platelet-derived growth factor; glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein

with uninterrupted contraction being a unique feature of the heart, cardiac muscle has a high demand for energy. This organ demonstrates substrate promiscuity, enabling it to utilize multiple sources of energy such as fatty acids (FA), carbohydrates, amino acids, and ketones (2). Among these, carbohydrate and FA are the major participants from which the heart derives most of its energy. Accordingly, in a basal setting, glucose and lactate account for ~30% of energy, whereas 70% of ATP generation is derived from FA oxidation (25). Concerning FA, the heart has a limited capacity to synthesize this substrate and thus relies on 1) release of FA from adipose tissue and transport to the heart after complexing with albumin (24), 2) breakdown of endogenous cardiac triglycerides (TG) (14), and 3) lipolysis of circulating TG-rich lipoproteins to FA by lipoprotein lipase (LPL) positioned at the endothelial cell (EC) surface of the coronary lumen (26).

In diabetes, because glucose uptake and oxidation are impaired, the heart is coerced to use FA almost exclusively for ATP generation (33). 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 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 (31). Innate to the cardiac muscle, the uptake of albumin-bound FA is driven by plasma membrane FA transporters (for example, CD36), which increase following diabetes (21). Diabetes also enhances adipose TG lipase to mobilize the storage pool of TG within cardiomyocytes (43). Finally, the utilization of VLDL-TG as a FA source by the diabetic heart is influenced not only by elevated plasma VLDL concentrations but also the vascular content of LPL, a rate-limiting enzyme in circulating TG clearance (2). We were the first to report higher luminal LPL activity following diabetes (32).

The increase in cardiac LPL with diabetes was immediate and unrelated to gene expression and involved exaggerated processing to dimeric, catalytically active enzymes, an obligatory step for ensuing secretion (39). Transfer to the coronary lumen requires movement of LPL to the cardiomyocyte plasma membrane by AMP-activated protein kinase, protein kinase D, and p38 MAPK (18). Activation of these kinases following diabetes facilitated LPL vesicle formation in addition to promoting cytoskeleton rearrangement for secretion onto surface heparan sulfate proteoglycans (HSPG) (10). For its onward movement across the interstitial space to the basolateral side of vascular ECs, detachment of LPL from the myocyte surface is a prerequisite and is likely mediated by high-glucose-induced secretion of EC heparanase (38). This endoglycosidase, exceptional in its ability to degrade heparan sulfate (HS), instigates LPL release from cardiomyocyte HSPG (38). From here, LPL traverses the interstitial space, and glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) is suggested to deliver it across ECs onto the luminal binding sites of these cells, where the enzyme is functional (42). Thus, GPIHBP1, a protein expressed abundantly in the heart but only on capillary ECs, operates as a transporter, collecting LPL...
from the interstitial space and transferring it across ECs (7, 9, 15).

Given that GPIHB1 mRNA levels change rapidly (22), we tested whether this protein has a part to play in the transfer of LPL to the vascular lumen after diabetes. Our data suggest a novel mechanism in which high-glucose-induced secretion of heparanase not only liberates LPL from the myocyte cell surface but can also induce GPIHB1 expression, thus promoting LPL shuttling to the apical side of ECs.

MATERIALS AND METHODS

Experimental animals. This investigation adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and was approved by the Animal Care Committee of the University of British Columbia (certificate no. A13-0098). Adult male Wistar rats (250–320 g) were injected with streptozotocin (STZ), a β-cell-specific toxin. STZ (55 mg/kg) was administered intravenously as a single dose to generate a model of poorly controlled type 1 diabetes (19, 20). Hyperglycemia was confirmed in blood samples from the tail vein using a glucometer (AccuSoft) and glucose test strips (Accu-Chek Advantage; Roche). STZ animals were kept for 4 days before hearts were removed for both LPL and GPIHB1 measurement.

Heart perfusion and isolation of cardiomyocytes. Rats were anesthetized with 65 mg/kg ip pentobarbital sodium; the thoracic cavities were opened, and hearts were carefully excised. After cannulation of the aorta, the heart was secured by tying below the innominate artery and perfused retrogradely with Krebs-Henseleit buffer (37). Ventricular calcium-tolerant myocytes were prepared by way of a previously described procedure (34). Briefly, myocytes were made calcium tolerant by successive exposure to increasing concentrations of calcium. Cardiomyocytes were plated at a density of 200,000 cells/well on laminin-coated six-well culture plates. Cells were maintained using Medium-199 and incubated at 37°C in a 5% CO2 humidified incubator to 80–90% confluence (38). Cells were detached following trypsin digestion, and aliquots were used for seeding of a new culture. Cells from passages 5–10 were used for detection of GPIHB1 and heparanase.

EC culture. RAECs (Cell Applications) and bovine coronary artery ECs (BCAECs; Clonetics) were cultured at 37°C in a 5% CO2 humidified incubator to 80–90% confluence (38). Cells were detached following trypsin digestion, and aliquots were used for seeding of a new culture. Cells from passages 5–10 were used for detection of GPIHB1 and heparanase.

In vitro lipolysis of VLDL. Isolation of VLDL (without chylomicrons) by density gradient ultracentrifugation was achieved using serum from 16-h-fasted rats (6 PM to 10 AM). Fasting of rats for this duration led to the production of more VLDL and minimized the contribution from chylomicrons. In vitro lipidolysis of VLDL-TG was carried out on the basis of previously described methods (36). Briefly, various concentrations (0–0.8 mM) of VLDL-TG were incubated with heparin-releasable LPL at 37°C for 30 min. At the end of incubation the reaction was stopped by the addition of precooled 0.3 M Na2HPO4 (pH 6.9), and the tubes were immediately immersed in ice. From this reaction mixture, 50 μl was pipetted in triplicate to measure free fatty acids released by hydrolysis of VLDL-TG with a NEFA C kit (Wako Chemicals).

Cytokine array. Media were collected from RAECs (EC culture medium) incubated with DMEM containing either 5.5 or 25 mM glucose for 12 h. Myocytes (myocyte culture medium) incubated with DMEM or DMEM containing 500 ng/ml purified heparanase (HPA) were also collected. Media were centrifuged at 2,000 rpm for 5 min. Array membranes were incubated with blocking buffer at room temperature (RT), followed by a 1-h incubation with EC culture medium or myocyte culture medium at RT for 2 h. Membranes were washed (3 times for 5 min) and incubated with working primary antibody and anti-horseradish peroxidase, respectively, at RT for 2 h. Array membrane was detected with enhanced chemiluminescence (ECL) and Hyperfilm ECL Film (GE Healthcare).

Reagents and antibodies. Heparin (Hepalexan, 1,000 U/ml) was from Organon. [3H]Hirudin was purchased from Amersham Canada. Anti-HPA antibody monoclonal antibody 130 was from InSight (Rehovot, Israel). Anti-GPIHB1 antibody was from Novus Biologicals. Anti-PECAM (CD31) was from Chemicon (Millipore). Anti-β-actin (C4), goat anti-mouse antibody, and goat anti-rabbit antibody were from Santa Cruz Biotechnology. To measure free fatty acid released from VLDL-TG breakdown, a NEFA C assay kit was purchased from Wako. Purified active and latent heparanase were a kind gift from Dr. Israel Vlodavsky, Hadassah University Hospital, Jerusalem, Israel. Phosphatidylinositol-specific phospholipase C (PI-PLC), Alexa 555-labeled anti-mouse IgG, Alexa 488-labeled anti-rabbit IgG, Trizol, SuperScript III RT, GPIHB1 (Rn01503971_g1) and actin

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Cardiac LPL and GPIHBP1 after STZ diabetes. Following injection of STZ and maintenance of animals for 4 days, there was a pronounced increase in blood glucose (Fig. 1A, right). Because utilization of glucose is impaired following diabetes, the heart rapidly adapts to use FA exclusively, an effect determined largely by LPL (18). Indeed, perfusion of hearts with heparin indicated that LPL activity increased following hyperglycemia (Fig. 1A, top right). Interestingly, immunofluorescence detection of LPL revealed that most of the increased enzyme in diabetic hearts was located at the coronary lumen (Fig. 1A, bottom right). In the heart, LPL synthesized in cardiomyocytes is shuttled across ECs with the help of GPIHBP1 (12, 42). To determine whether the increased vascular content of LPL following diabetes is associated with GPIHBP1, its protein (Fig. 1B) and mRNA (Fig. 2B) expression were examined and determined to be augmented. Because the increased GPIHBP1 had an exclusive endothelial location (Fig. 2A), our data suggest that GPIHBP1 is important for myocyte to EC transfer of LPL to increase FA delivery to the diabetic heart.

Endothelial GPIHBP1 following cell passage and influence of high glucose. GPIHBP1 is expressed exclusively in endothelial cells (42). We determined the level of this glycoprotein in RAoECs and BCAECs and unexpectedly identified loss of this glycoprotein following passaging of these cells (Fig. 3A). In an attempt to mimic in vivo hyperglycemia, RAoECs were exposed to increasing concentrations of glucose for 12 h. Interestingly, in cells from later passages (in which GPIHBP1 expression had been suppressed), although concentrations of glucose between 10 and 15 mM increased GPIHBP1 mRNA (Fig. 3B, right) and protein (Fig. 3C, right), the increases were more robust with the higher concentration of glucose. Choosing 25 mM glucose and different times, high glucose increased GPIHBP1 mRNA expression, an effect that was pronounced after 12 h (Fig. 3B). Measurement of GPIHBP1 protein under these conditions uncovered an even more rapid increase within 4 h of incubation with high glucose (Fig. 3C). This increased EC GPIHBP1 was predominantly membrane bound (Fig. 4A). It should be noted that RAoECs and BCAECs from the earlier passages (e.g., passages 5–7) also responded to high glucose by increasing GPIHBP1 expression (data not shown). Overall,
these data imply that by sensing the impending loss of glucose transport, ECs respond by increasing GPIHBP1 to shuttle LPL.

**GPIHBP1-associated transfer of LPL across the endothelial monolayer.** To test the functional relevance of the high-glucose-induced augmentation of GPIHBP1, LPL transport from the basolateral to the apical side of RAOEC monolayers was determined. As expected, there was an increase in heparin-releasable LPL activity at the apical side of ECs exposed to high glucose (Fig. 4B), an effect that was blunted by PI-PLC (Fig. 4B, inset). The osmotic control mannitol had no influence on LPL transport (Fig. 4B). The LPL that had been shuttled following high glucose was functionally active, as exposure of this apical heparin-releasable enzyme to lipoprotein triglyceride was capable of increasing its hydrolysis to FA (Fig. 4C). Since prior exposure of cells to PI-PLC [which is expected to cleave GPIHBP1 (15)] and prevent LPL shuttling] abolished this TG breakdown, our data indicate that in ECs exposed to high glucose, enhancement of GPIHBP1 is an effective stimulus for increasing delivery of FA to cardiomyocytes to support energy requirements.

**GPIHBP1 expression is linked to endothelial content of HPA.** HPA, an EC endoglycosidase, can cleave HS side chains on HSPGs in the extracellular matrix and on the cell surface of cardiomyocytes to release LPL for transfer across the interstitial space to reach ECs (29). As described previously, exposure of RAOECs to high glucose rapidly increased the secretion of both active (Fig. 5A) and latent (data not shown) HPA. Intriguingly, in addition to its ability to rapidly liberate cardiomyocyte HSPG-sequestered LPL, exposure of ECs to active but not latent (data not shown) HPA for 12 h produced a robust increase in GPIHBP1 mRNA and protein (Fig. 5B). Because passaging of RAOECs progressively decreased HPA expression (Fig. 5C) similarly to loss of GPIHBP1, our data have uncovered a novel autocrine role (either direct or indirect) for active HPA to affect GPIHBP1 expression.

**Cardiomyocytes induce EC GPIHBP1.** Although GPIHBP1 is highly expressed in ECs in vivo, ECs in culture lose expression of this glycoprotein (1). Because ECs in the heart are closely appositioned to cardiomyocytes, we reasoned that a paracrine influence from cardiomyocytes may be responsible...
for EC expression of GPIHBP1. Using a coculture system to mimic the intact heart, we determined that in ECs in which GPIHBP1 had been silenced (passages 8–10), a simple introduction of cardiomyocytes into the vicinity restored GPIHBP1 expression to levels observed with early cell passages (Fig. 6, A and B). These results point toward paracrine factors from cardiomyocytes influencing EC GPIHBP1 expression. Assuming that the stimulus for the release of signaling mediators from myocytes could originate from ECs (likely HPA), we exposed the coculture to high glucose. Noteworthy, a further expression of EC GPIHBP1 was evident in this condition (Fig. 6C), suggesting that high-glucose-induced HPA released from ECs can affect GPIHBP1 by both autocrine and paracrine signaling. HPA-released platelet-derived growth factor from myocytes and ECs can induce GPIHBP1 expression. ECs and myocyte HSPG-anchored proteins can be liberated by HPA (38). Using a protein array and ECs exposed to high glucose or myocytes treated with active HPA, a number of proteins were increased in the culture medium. These included monocyte chemotactic protein-1, prolactin, receptor for advanced glycation end products, thymidylate kinase TMP-1 (data not shown), and platelet-derived growth factor (PDGF) (Fig. 7A). Given the acknowledged role of PDGF in FA metabolism (35), we treated EC with recombinant PDGF and discovered an induction of GPIHBP1 from passage 7 (which still express GPIHBP1) and passage 10 (in which there is a loss of GPIHBP1) (Fig. 7B). Nevertheless, the increase was more robust in cells from passage 10, as these cells had an extremely low initial expression of GPIHBP1. Our data suggest a mediator role (either autocrine or paracrine) for this growth factor in FA delivery to the diabetic myocyte.

DISCUSSION

Underutilization of glucose and overreliance on FA are hallmarks of the diabetic heart (33). Although albumin-bound circulating FA is
an important source of this substrate, its molar concentration is 
~10-fold less than that of lipoprotein TG (26). As such, the hydrol-
ysis of circulating TG is suggested to be the predominant source of
 FA for cardiac utilization during diabetes (3). Lipoprotein-TG break-
down occurs at the coronary lumen, with the assistance of LPL
positioned at apical surface of ECs. Following diabetes, in the ab-
sence of changes in LPL synthesis, coronary LPL activity is aug-
mented by posttranslational modifications. These include an
increase in LPL dimerization (39), vesicle transport (18), and
shuttling of the enzyme from the myocyte surface to the
basolateral side of ECs (6). Data from the current study present
the novel idea that in response to hyperglycemia, transport of
LPL to the apical side of ECs to assist in TG breakdown is
achievable through induction of GPIHBP1.

Fig. 4. GPIHBP1 induced by high glucose
increases LPL shuttling across endothelial cell
monolayers. A: RAOECs seeded on slides
were placed in a 6-well plate and treated with
5.5 (CON) or 25 mM glucose (HG) for 12 h.
Slides were then washed with ice-cold PBS,
fixed with methanol, and permeabilized using
0.2% Triton X in PBS. Anti-rabbit GPIHBP1
(1:100) and anti-mouse CD31 antibodies (1:
100) were used for immunofluorescence stain-
ing. Scale bar, 20 μm. B: in a separate exper-
iment, RAOECs were seeded on Transwell
inserts and grown until they formed a tight
monolayer. Cells were then treated with CON
or HG for 4 and 12 h, respectively. In some
experiments, this was followed by treatment
with or without phosphatidylinositol-specific
phospholipase C [PI-PLC; 1 U/ml for 1 h
(inset)]; 25 mM mannitol (Mnt) for 12 h was
used as an osmotic control. Following the
indicated times, these Transwell inserts were
removed and placed in a different 6-well plate
with DMEM on the basolateral side containing
10 μg/ml purified LPL. After incubation for 1
h and washing (3 times) with PBS, medium
containing heparin (8 U/ml) was used for 3
min to release apical surface-bound LPL. LPL
activity was determined by measuring the in
vitro hydrolysis of [3H]triolein substrate.
C: using the above protocol (with incubation of
HG for 12 h), the heparin-releasable LPL me-
dium was incubated with increasing concen-
trations of very-low density lipoprotein-tri-
glyceride (VLDL-TG; 0–0.8 mM) at 37°C for
30 min and the concentration of released free
fatty acid (FA) determined. One group of cells
was treated with 1 U/ml PI-PLC prior to incu-
bation with high glucose. *P < 0.05; n = 3.
Fig. 5. Endothelial heparanase (HPA) can increase GPIHBP1 gene and protein expression. 

A: RAOECs from passages 6–10 were used for determination of latent (L-HPA) and active heparanase (A-HPA). Cells from passages 7–10 were treated with HG (25 mM) for 12 h, and medium was collected. B: the medium was concentrated using an Amicon column at 4°C and subsequently centrifuged for 15 min at 14,000 g. HPA in this concentrated medium was determined by Western blot; n/H110054. C: recombinant HPA was used to treat RAOECs (passages 7–10) for 12 h, cell lysates were collected, and GPIHBP1 mRNA (top) and protein (bottom) were determined using TaqMan and Western blot, respectively; n = 3–4. *P < 0.05.

Fig. 6. Loss of endothelial GPIHBP1 expression can be restored on coculture with myocytes (Myo). A and B: RAOECs (A) or BCAECs (B) (passages 8–10) were seeded on Transwell inserts and cultured until 80–90% confluence. The Transwell insert was then transferred to a 6-well plate containing attached Myo and cocultured for 12 h. Isolation of Myo was achieved by collagenase digestion of rat hearts, and cells were attached to laminin-coated 6-well plates in M199 containing 0.1% BSA and kept overnight. After coculture with myocytes, endothelial cell protein was collected and used for Western blot of GPIHBP1.

C: in some experiments, endothelial cells (with or without coculture with Myo) were exposed to normal (5.5 mM) or high glucose (HG; 25 mM) for 12 h, and GPIHBP1 was determined; n = 3–4. *P < 0.05 compared with control; #P < 0.05 compared with normal glucose.
Following a single injection of a moderate dose (55 mg/kg) of STZ, there is an induction of hypoinsulinemia and hyperglycemia. Increasing the dose to 100 mg/kg also creates an environment of hyperlipidemia (39). In the former situation, coronary LPL activity is augmented, whereas with the latter setting and the presence of higher circulating FA, LPL is turned off (32). With D55 hearts, in the absence of any change in protein synthesis, the increase in LPL activity principally at the vascular lumen could be explained largely by posttranslational modifications that increased transfer of myocyte enzyme to the ECs (30). Not yet determined is whether the concluding step, moving LPL across EC, is also increased following diabetes. GPIHBP1, a glycosylphosphatidylinositol-anchored protein expressed exclusively in ECs (15), is a recent addition to the mechanism that transfers LPL across ECs (42). GPIHBP1 has a strong binding affinity for LPL, accepting it from the interstitial space and moving it to the coronary lumen (41). Intriguingly, the mRNA for GPIHBP1 changes much more rapidly than most mRNAs in mammalian cells (22). Our results present the novel observation that to guarantee FA supply to the diabetic heart, the LPL transporter GPIHBP1 increases rapidly in ECs.

In an attempt to elucidate the mechanism by which diabetes influences GPIHBP1, we exposed two different EC lines to glucose, a substrate whose concentrations increase rapidly following STZ. However, given that under standard cell culture conditions glucose does not alter the levels of GPIHBP1, we investigated whether glucose might increase GPIHBP1 levels indirectly, perhaps by promoting PDGF release from ECs. As shown in Fig. 7A, glucose significantly increased PDGF release from ECs (CON HG vs. CON A-HPA). We next examined whether glucose might increase GPIHBP1 levels in ECs by treating cells with PDGF (Fig. 7B). As shown in Western blot analysis, glucose increased GPIHBP1 levels in a dose-dependent manner (CON HG vs. PDGF). In addition, the level of the GPIHBP1 transcript was increased in ECs treated with PDGF (Fig. 7C). These results suggest that glucose may increase GPIHBP1 levels in ECs by promoting PDGF release.
conditions there has been an insinuation that EC GPIHBP1 is silenced following cell passaging (9, 42), we initially attempted to authenticate this speculation. To our surprise, both rat and bovine aortic ECs lose their ability to express GPIHBP1 protein with increasing subculturing of the cells. However, the introduction of increasing concentrations of glucose to the culture medium increased GPIHBP1 gene and protein expression in both the early and late cell passages. Because high glucose also had comparable effects on LPL shuttling across ECs in association with a greater ability to hydrolyze lipoprotein TG, data from the current study strongly implicate glucose as an important stimulus that influences EC GPIHBP1. Interestingly, high glucose has also been observed to turn on the FA transporters CD36 (13) and FA-binding protein 4 (11) in ECs. Thus, with the onset of hyperglycemia, ECs globally are well adapted to promote FA availability to cardiomyocytes.

With EC subculturing, we were interested to discover whether proteins other than GPIHBP1 are also switched off. We focused on heparanase, an endoglycosidase that specifically cleaves HS side chains on HSPGs (4). Intriguingly, in addition to this extracellular function to release bound ligands like LPL, heparanase, by entry into the nucleus to regulate histone acetylation/methylation, is also capable of modulating gene transcription. In this regard, tumor cells, by expressing higher levels of heparanase, are more invasive, as secreted heparanase can breakdown extracellular matrix in addition to promoting gene expression in adjacent cells that drives an aggressive tumor phenotype (27, 28). Similarly to the loss of GPIHBP1, EC passaging also caused nonappearance of heparanase, suggesting a potential connection between these two proteins. Indeed, because the addition of active heparanase to ECs augmented gene and protein expression of GPIHBP1, together with the fact that high glucose induced heparanase secretion, our data for the first time link heparanase to the entire process by which LPL progresses forward during diabetes from the apical side of cardiomyocytes to the apical side of ECs.

Notwithstanding the demonstrated impact of high glucose on GPIHBP1, we were unable to explain the disappearance of this glycoprotein on passaging of ECs. A recent study found that in the intact heart, there is a robust expression of EC GPIHBP1, and therefore, it is possible that paracrine signaling influences expression of this glycoprotein (42). Major cell types in the heart include fibroblasts, smooth muscles, and myocytes, with the latter making up almost 60% of the adult rat myocardium (5). We were impressed by our observations that ECs that had lost their ability to express GPIHBP1 regained this glycoprotein when cocultured with cardiomyocytes. Because another study (42) has also hinted at this possibility, our data indicate an interaction between cardiomyocytes and EC likely through paracrine signaling mediators. Currently, it is unclear whether the stimulus for the release of signaling mediators from myocytes could originate from ECs. ECs are first responders to hyperglycemia, releasing heparanase for ensuing hydrolysis of myocyte HSPG, which is linked to liberation of bound proteins. Given that exposure of the coculture to high glucose caused an even greater expression of EC GPIHBP1, our data suggest cellular cooperation between ECs (via heparanase) and myocytes (through unidentified proteins) that facilitates energy switching from glucose to FA in the diabetic heart.

HSPG are ubiquitously present in every tissue compartment, particularly the extracellular matrix, cell surface, intracellular granules, and nucleus (17). They consist of a core protein to which several linear HS side chains are covalently linked and function not only as structural proteins but also as anchors due to the high content of charged groups in HS (16). The latter property is implicitly used to electrostatically bind a number of different proteins (chemokines, coagulation factors, enzymes like LPL, and growth factors such as vascular endothelial growth factor) (23). 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 (4). Heparanase is an endoglycosidase, exceptional in its ability to degrade HS, thereby instigating ligand release. Using a cytokine array and incubation of cardiomyocytes with active heparanase, a number of cytokines were increased in the myocyte culture medium. We focused on PDGF, as this protein can regulate angiogenesis but also play a significant role in increasing the metabolic reliability of smooth muscle on FA (35). Favorably, PDGF increased EC GPIHBP1. Because exposure of ECs to high glucose also increased PDGF in the medium, likely through an autocrine stimulus of heparanase secretion with the release of PDGF bound to EC HSPG, our data suggest that this protein “ensemble” (heparanase-PDGF-GPIHBP1) cooperates in the diabetic heart to regulate FA delivery and utilization by the cardiomyocytes.

Overall, through their release of heparanase, ECs, which are the first responders to hyperglycemia, are responsible for regulating LPL-derived FA delivery to the cardiomyocytes. Although this mechanism 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).

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


