Hyperglycemia-Induced Secretion of Endothelial Heparanase Stimulates a Vascular Endothelial Growth Factor Autocrine Network in Cardiomyocytes That Promotes Recruitment of Lipoprotein Lipase

Dahai Zhang, Andrea Wan, Amy Pei-Ling Chiu, Ying Wang, Fulong Wang, Katharina Neumaier, Nathaniel Lal, Michael J. Bround, James D. Johnson, Israel Vlodavsky, Brian Rodrigues

Objective—During diabetes mellitus, coronary lipoprotein lipase increases to promote the predominant use of fatty acids. We have reported that high glucose stimulates active heparanase secretion from endothelial cells to cleave cardiomyocyte heparan sulfate and release bound lipoprotein lipase for transfer to the vascular lumen. In the current study, we examined whether heparanase also has a function to release cardiomyocyte vascular endothelial growth factor (VEGF), and whether this growth factor influences cardiomyocyte fatty acid delivery in an autocrine manner.

Approach and Results—Acute, reversible hyperglycemia was induced in rats, and a modified Langendorff heart perfusion was used to separate the coronary perfusate from the interstitial effluent. Coronary artery endothelial cells were exposed to high glucose to generate conditioned medium, and VEGF release from isolated cardiomyocytes was tested using endothelial cell conditioned medium or purified active and latent heparanase. Autocrine signaling of myocyte-derived VEGF on cardiac metabolism was studied. High glucose promoted latent and active heparanase secretion into endothelial cell conditioned medium, an effective stimulus for releasing cardiomyocyte VEGF. Intriguingly, latent heparanase was more efficient than active heparanase in releasing VEGF from a unique cell surface pool. VEGF augmented cardiomyocyte intracellular calcium and AMP-activated protein kinase phosphorylation and increased heparin-releasable lipoprotein lipase.

Conclusions—Our data suggest that the heparanase-lipoprotein lipase-VEGF axis amplifies fatty acid delivery, a rapid and adaptive mechanism that is geared to overcome the loss of glucose consumption by the diabetic heart. If prolonged, the resultant lipotoxicity could lead to cardiovascular disease in humans. (Arterioscler Thromb Vasc Biol. 2013;33:2830-2838.)

Key Words: heparanase ■ hyperglycemia ■ lipoprotein lipase ■ metabolism ■ vascular endothelial growth factor A

therosclerotic cardiovascular disease is a leading cause of diabetes mellitus–related death.1-3 However, patients with diabetes mellitus also demonstrate a specific impairment of the heart muscle (diabetic cardiomyopathy),4 with changes in cardiac metabolism having a significant impact on its development. In this regard, cardiac glucose uptake, glycolysis, and pyruvate oxidation are impaired. Thus, the heart rapidly adapts to predominantly use fatty acids (FAs) for ATP.3 In the short term, this adaptation might be beneficial. Chronically, increased FA conversion to potentially toxic FA metabolites (ceramides, diacylglycerols, and acylcarnitines), paired with an increased formation of reactive oxygen species secondary to elevated FA oxidation, can promote cardiac cell death (lipotoxicity).4

Multiple adaptive mechanisms operate to make FA available to the diabetic heart, with cardiac lipoprotein lipase (LPL) being a major contributor, through its ability to hydrolyze lipoprotein-triglyceride into free FA.3 We were the first to report a robust expansion in the coronary pool of LPL after diabetes mellitus.4 This increase in LPL activity was immediate and unrelated to LPL gene expression.6 Its prelude included accelerated LPL processing to a dimeric, catalytically active enzyme, an obligatory step for ensuing secretion.7 Active enzyme transfer to the cardiomyocyte plasma membrane then involved LPL vesicle formation and actin cytoskeleton polymerization, thus providing LPL cargo with an appropriate transport infrastructure for secretion onto myocyte plasma membrane heparan sulfate proteoglycans (HSPGs).7 HSPGs are ubiquitous macromolecules consisting of a core protein to which several linear HS side chains are covalently linked. They function not only as structural...
proteins but also as anchors. In cardiomyocytes, the latter property is used to bind several different proteins (including enzymes such as LPL and growth factors such as vascular endothelial growth factor [VEGF]). Attachment of these bioactive proteins is an efficient arrangement, providing the cell with a rapidly accessible reservoir, precluding the need for de novo synthesis when the requirement for a protein is increased. Heparanase is an endoglycosidase, exceptional in its ability to degrade HS and release its associated proteins.

In endothelial cells (ECs), heparanase is initially synthesized as an inactive (latent; L-Hep) 65-kDa enzyme that is secreted followed by HSPG-facilitated reuptake. After undergoing proteolytic cleavage in the lysosome, a 50-kDa polypeptide is produced that is ≈100-fold more active than its latent form. In the presence of high glucose (25 mmol/L), we have previously reported that EC-released heparanase caused LPL detachment from the myocyte cell surface. Given their similar localization on the cardiomyocyte cell surface, it is possible that heparanase releases not only LPL but also VEGF, as observed in cancer cells. Such a function would be beneficial to the diabetic heart, given the capability of VEGF to induce angiogenesis, thus providing the heart with sufficient O₂ to accommodate increased FA oxidation (paracrine function). As VEGF's promotion of angiogenesis has been linked to AMP-activated protein kinase (AMPK) activation in EC, an AMPK-activating property of VEGF in cardiomyocytes (autocrine function) could also directly affect FA delivery (AMPK promotes LPL recruitment to cardiomyocyte cell surface). Interestingly, cardiac-specific overexpression of LPL is associated with severe myopathy, characterized by both muscle fiber degeneration and extensive proliferation of mitochondria and peroxisomes.

In genetically engineered mice that specifically overexpressed an anchored form of cardiomyocyte surface-bound LPL, lipid oversupply and deposition were observed, together with excessive dilatation and impaired left ventricular systolic function. Interestingly, loss of cardiac LPL also causes cardiomyopathy. Hence, although specific knockout of cardiac LPL in adult mice increased glucose metabolism, neither this effect nor albumin-bound FA could replace the action of LPL, and cardiac ejection fraction decreased. These experiments in genetically modified mice demonstrate that cardiac LPL is of crucial importance, and disturbing its innate function is sufficient to cause cardiac failure. The present study describes a novel pathway by which high glucose-induced secretion of endothelial heparanase stimulates a VEGF-LPL autocrine network in cardiomyocytes.

### Figure 1.
High glucose facilitates secretion of both latent heparanase (L-Hep) and active heparanase (A-Hep). To study heparanase release by high glucose in vitro, bovine coronary artery endothelial cells (bCAECs) were incubated with DMEM containing 25 mmol/L glucose. After an incubation period of 30 minutes, medium (endothelial cell conditioned medium) and cells were separated. Medium was concentrated and Western blot used for detection of L-Hep (A, left) and A-Hep (A, right). bCAECs were washed with PBS and intracellular L-Hep (B, left) and A-Hep (B, right) determined using Western blot (B, right). Glucose DMEM (5 mmol/L) was used as control. n=3 to 5 animals in each group. *Significantly different from Con, P<0.05.
Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

High Glucose Promotes Secretion of Latent and Active Heparanase From ECs

ECs are the predominant cell type in the heart to express heparanase. Using bovine coronary artery endothelial cells incubated with high glucose, we measured heparanase release into the incubation medium. Within 30 minutes, high glucose released ≈7-fold more L-Hep and active heparanase (A-Hep) as compared with control, with L-Hep being the major form secreted (Figure 1A). Measurement of residual heparanase revealed that ECs have a larger reserve of L-Hep as compared with A-Hep (Figure 1B, inset). On incubation with high glucose, although the intracellular content of both heparanase forms were reduced, this decrease was only statistically significant with A-Hep (Figure 1B). The mannitol osmolality control had no effect on medium or intracellular heparanase (data not shown). To model the in vitro observation of heparanase release after high glucose, we used diazoxide (DZ) to induce acute hyperglycemia. Blood glucose levels increased within 30 minutes and remained elevated for ≥2 hours after DZ injection (Figure 1A in the online-only Data Supplement). Assuming that high glucose stimulates heparanase secretion from the basolateral side of ECs to affect HSPG cleavage on the underlying myocytes, we used the modified Langendorff perfused heart to separate the coronary perfusate from the interstitial fluid and measured interstitial heparanase. There was a robust secretion of interstitial A-Hep within 30 minutes of DZ, an effect that closely mirrored the changes in blood glucose. Extending the duration of DZ for 2 hours lowered this active heparanase to values below control. Interestingly, this pattern of release was also observed with L-Hep (Figure 1B in the online-only Data Supplement).

Endothelial Cell Conditioned Medium Is an Effective Stimulus for Releasing Cardiomyocyte VEGF

As high glucose was effective in releasing both L-Hep and A-Hep into the incubation medium, we collected this endothelial cell conditioned medium (ECCM) after a 30-minute exposure to 25 mmol/L glucose. Exposure of cardiomyocytes to this ECCM caused a 5-fold release of VEGF into the medium, an effect that was only observed after 30 minutes of incubation (Figure 2A). Previously, we used heparin, a highly negatively charged molecule, to release proteins such as LPL from cell surface HSPG. Heparin-treated cardiomyocytes were also capable of releasing VEGF, but unlike ECCM, this effect of heparin was rapid, observed as early as 3 minutes after incubation and suggested an extracellular localization of VEGF. Extending the duration of heparin incubation further increased the amount of VEGF released (Figure 2B). To relate these findings to an in vivo model of hyperglycemia, cardiomyocytes were isolated from control and DZ-treated animals. As basal and heparin-releasable VEGF released into the medium was lower in DZ myocytes as compared with control, our data suggest that a relationship exists between acute hyperglycemia and VEGF secretion from the cardiomyocytes, possibly secondary to secreted EC heparanase.
It should be noted that increasing the duration of hyperglycemia (7 days) using streptozotocin increased the expression of cardiac VEGF (Figure IV in the online-only Data Supplement).

Latent Heparanase Is More Efficient Than Active Heparanase in Releasing Cardiomyocyte VEGF

We hypothesized that the delay of ≤30 minutes required for ECCM to release cardiomyocyte VEGF represented the time needed for A-Hep to hydrolyze HS side chains on the cardiomyocyte cell surface to liberate VEGF. Interestingly, although A-Hep was enzymatically active in releasing fibroblast growth factor (Figure 3A, right inset), its effect on releasing VEGF was statistically insignificant (Figure 3A, central inset). Remarkably, it was L-Hep that produced the most dramatic release of VEGF (Figure 3A), especially at concentrations between 0.1 and 1 μg/mL (Figure 3B). In addition, the release of VEGF with 1 μg L-Hep was unexpectedly fast, with significant amounts of VEGF being detected as early as 1 minute, increasing with the duration of incubation (Figure 3C).

Latent Heparanase Can Release VEGF From a Unique Cell Surface Pool

We treated myocytes with heparin, followed by L-Hep, anticipating a reduced release by the latter if the 2 treatments released VEGF from the same pool. L-Hep was still capable of releasing VEGF under these conditions (Figure 4A). This release was rapid, and unexpectedly, the amount of VEGF released by L-Hep was ≈2-fold higher than heparin (Figure 4A). To address incomplete displacement of VEGF by heparin, we used pharmacological concentrations of heparin and multiple treatments. Interestingly, neither the higher concentration (data not shown) nor the addition of a second incubation with heparin was able to release VEGF to the same extent as L-Hep (Figure 4B). Using an alternative
approach, we observed that adding increasing concentrations of recombinant VEGF yielded proportional cell surface binding after release by heparin or L-Hep (Figure II in the online-only Data Supplement). However, it should be noted that compared with heparin, L-Hep released higher amounts of recombinant VEGF at all concentrations used (Figure II in the online-only Data Supplement). Interestingly, prior incubation with heparin lowered the heparin-releasable pool of VEGF but had a limited effect on the L-Hep-releasable pool, suggesting that multiple VEGF cell surface binding domains exist (Figure 4C). Whether these pools originated extracellularly was tested using Matrigel to which recombinant VEGF was attached. Under these conditions, L-Hep still induced the highest amount of VEGF release compared with heparin and A-Hep (Figure 4D), suggesting a predominantly extracellular effect, and a unique L-Hep-releasable pool of VEGF on the cardiomyocyte cell surface. As L-Hep was more effective than heparin in releasing collagen-bound VEGF (Figure III in the online-only Data Supplement), our data suggest that VEGF could also be bound to non–HS-binding sites on the cardiomyocyte cell surface.

Cardiomyocyte Intracellular Calcium and AMPK Phosphorylation Are Augmented by VEGF and ECCM

Elevation in cytosolic calcium is known to increase calcium/calmodulin-dependent protein kinase kinase β phosphorylation and hence, AMPK activation. Cardiomyocytes treated with VEGF displayed a robust increase in intracellular free calcium, an effect that was immediate and returned to baseline within 5 minutes (Figure 5A, left). Although the amplitude of the peak calcium response induced by ECCM was comparable with that demonstrated by VEGF, the response was delayed (Figure 5A, right), and likely related to a requirement of ECCM to promote prior release of myocyte VEGF. As predicted, AMPK phosphorylation followed the rise in intracellular calcium observed with both VEGF and ECCM. High glucose itself had no influence on AMPK phosphorylation (Figure 5B). Bevacizumab reduced activation of AMPK observed with ECCM (Figure 5B, inset).
Cardiomyocyte Heparin-Releasable LPL Is Increased by VEGF
After AMPK activation, p38 mitogen-activated protein kinase is a downstream signaling molecule that regulates LPL transport in myocytes by facilitating rearrangement of the actin cytoskeleton. In cardiomyocytes treated with VEGF, there was a robust phosphorylation of p38 mitogen-activated protein kinase (Figure 6A, left), associated with an increase in the F/G actin ratio (Figure 6A, right). As this effect would be expected to accelerate LPL transport to the cell surface, we measured heparin-releasable enzyme. Interestingly, VEGF after 2 or 4 hours was capable of increasing heparin-releasable LPL secretion (Figure 6B, left). The increase in LPL protein with VEGF correlated to an increase in LPL activity, both enzymatic (Figure 6B, right) and functional (Figure V in the online-only Data Supplement).

Discussion
Given their strategic location, ECs are the first to detect and inform underlying myocytes about hyperglycemia after diabetes mellitus. In this regard, we have reported that high glucose is a potent stimulator of A-Hep secretion from the EC, which stimulated the myocyte to transfer LPL to the vascular lumen. In the current study, we demonstrated that in addition to A-Hep, L-Hep can also be secreted in response to high glucose to release cardiomyocyte HSPG-bound VEGF as a consequence. In doing so, VEGF facilitated cardiomyocyte LPL movement, an adaptive mechanism that is geared to overcome the loss of glucose consumption by the diabetic heart.

In hearts from diabetic animals or ECs exposed to high glucose, we reported elevated A-Hep secretion into the interstitial space and incubation medium. We also determined that ATP release, purinergic receptor activation, cortical actin disassembly, and stress actin formation were essential for high glucose–induced A-Hep secretion from lysosomes. As lysosomes contain both A-Hep and L-Hep, an anticipated consequence of high glucose ought to be their dual secretion. Indeed, the current study also identified higher amounts of L-Hep in the interstitial fluid of hearts from hyperglycemic animals, likely a result of polarized secretion toward the basolateral rather than the apical side of ECs. As high glucose promoted an increase of L-Hep in ECCM, our data suggest that ECs can rapidly secrete both A-Hep and L-Hep after diabetes mellitus.

Present on the cardiomyocyte surface are HSPGs, ubiquitous macromolecules that can strongly bind several proteins such as

Figure 5. Vascular endothelial growth factor (VEGF) and endothelial cell conditioned medium (ECCM) activate cardiomyocyte AMPK through calcium signaling. Cardiomyocytes were plated on glass bottom dishes coated with laminin and maintained using medium 199 at 37°C for 3 hours. In cardiomyocytes exposed to VEGF (1 µg/mL) or ECCM, changes in cytosolic Ca²⁺ over time were measured using Fura-2. Representative traces of individual cardiomyocytes are shown (A, bottom). The horizontal bars indicate an average response of 15 to 25 cardiac cells of 3 separate preparations. **Significantly different from Con, P<0.01 (A, top). To evaluate effects on AMPK, myocytes were treated with high glucose, VEGF, or ECCM for 1 hour, and protein extracted to determine phosphorylated and total AMPK using Western blot. *Significantly different from untreated control, P<0.05 (B). Results are mean±SE of 3 myocyte preparations in each group. The monoclonal antibody Bevacizumab (200 µg/mL) was used to neutralize VEGF in ECCM, and AMPK activation in myocytes subsequently determined (B, inset).
LPL, which has a heparin-binding domain. Such a location would allow for a rapid release when there is a requirement for LPL. We have reported that myocyte LPL can be released by A-Hep, facilitating its forward movement across the interstitial space to the apical side of vascular ECs, where LPL provides the heart with FA by breaking down circulating triglyceride (Figure 6C). Like LPL, VEGF also has a heparin-binding domain, and thus an affinity to HSPG. Using the property of heparin to enable ionic displacement of HSPG-bound proteins, we observed a rapid release of VEGF from the cardiomyocytes, suggesting that it resides extracellularly. Although ECCM had a comparable effect in releasing VEGF, the time to release was prolonged and likely related to a requirement for proteolytic displacement of VEGF by active heparanase. To confirm that this process occurs in an in vivo setting of hyperglycemia, myocytes from DZ animals were treated with heparin, and VEGF release was determined. As acute hyperglycemia caused a significant reduction in the heparin-releasable pool of VEGF, our results suggest that after diabetes mellitus, heparanase is a likely candidate that increases the availability of free VEGF, an effect that is supported by the ability of chronic hyperglycemia to increase the cardiac expression of VEGF.
To validate whether heparanase is responsible for releasing cardiomyocyte VEGF, we successively purified A-Hep and L-Hep, with expected molecular weights and activity. Unexpectedly, L-Hep produced a greater release of VEGF, with A-Hep having a minimal effect. Retrospectively this was explainable, given that L-Hep requires HSPG binding to undergo internalization and subsequent activation. As the release of VEGF by L-Hep was rapid and sustained, our results imply that L-Hep, through its time-dependent occupation of myocyte HSPG, is able to displace cell surface VEGF.

Considering an extracellular location for VEGF, we assumed that prior removal of this growth factor using heparin will lower the amount released by L-Hep. Intriguingly, L-Hep was still able to release VEGF after heparin, an effect that was rapid, and even more robust than the initial heparin treatment. Assuming incomplete displacement of VEGF with heparin, we used higher concentrations of heparin, together with multiple heparin treatments but were still unable to release comparable amounts of VEGF as seen with L-Hep. In addition, as prior incubation with heparin inhibited the amount of recombinant VEGF that could be released by heparin and not L-Hep, our data suggest that either heparin or L-Hep releases VEGF through different mechanisms or from different pools. To address the latter question, we used Matrigel to which VEGF was bound. In this setting, L-Hep still had the most robust effect in releasing VEGF compared with heparin or A-Hep, which was similar to our observations in the cardiomyocytes. Given that Matrigel consists of collagen IV, laminin, entactin, and HSPG (simulating the extracellular construct of cardiomyocytes), it is possible that any one of these components may bind VEGF and be accessible only to L-Hep. Indeed, compared with heparin, L-Hep was effective in releasing collagen-bound VEGF.

To address these questions, we used L-Hep to evaluate the role of LPL in VEGF release. Unexpectedly, L-Hep produced a greater release of VEGF, with A-Hep having a minimal effect. This was explainable, given that L-Hep requires HSPG binding to undergo internalization and subsequent activation. As the release of VEGF by L-Hep was rapid and sustained, our results imply that L-Hep, through its time-dependent occupation of myocyte HSPG, is able to displace cell surface VEGF.

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Altogether, our results demonstrate a function of L-Hep that L-Hep was effective in releasing collagen-bound VEGF. Altogether, our results demonstrate a function of L-Hep that is unique and not simply limited to its reuptake into ECs to generate active enzyme. It also addresses a fundamental question as to why a cell would secrete an inactive protein for reuptake and activation, which seems counterintuitive and wasteful.

After its release from the myocyte surface, VEGF has a paracrine function in ECs where it stimulates new blood vessel formation (to provide O2 needed for mitochondrial oxidative phosphorylation of FA) or promotes FA uptake and transport across the endothelial layer. However, this growth factor is also competent to provide autocrine signaling. For example, cardiomyocytes express VEGF receptors, and in these cells, VEGF induces a gene expression program of hypertrophy, in addition to evoking cardiomyocyte plasticity to match changes in capillary density. To test the existence of an autocrine pathway for VEGF control of metabolism, we focused on AMPK, a pivotal cellular energy sensor and regulator. At least in ECs, which are quiescent, AMPK can be stimulated by VEGF, an effect that would be more relevant in the highly metabolically active cardiomyocytes. Our data indicate that recombinant VEGF also has a capacity to induce AMPK activation in cardiomyocytes. Multiple pathways produce AMPK activation including an elevation in AMP levels or a Ca2+-dependent process involving phosphorylation by an upstream kinase, calcium/calmodulin-dependent protein kinase kinase β. Given that VEGF increases calcium mobilization in several cells, we determined intracellular Ca2+ concentrations in cardiomyocytes treated with VEGF. Interestingly, this growth factor caused significant Ca2+ influx in a rapid, but transient manner. As similar Ca2+ responses were also observed with ECCM, albeit delayed (likely because of a prior requirement for L-Hep to release VEGF), together with AMPK activation, our data for the first time suggest that cardiomyocyte-derived VEGF has a novel autocrine role in stimulating AMPK.

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, using this described mechanism, we observed that VEGF was able to increase LPL translocation to the myocyte cell surface. Our data on the ability of VEGF to promote LPL movement implicate this growth factor in the cascade of expanding actions that are geared to help the diabetic heart switch its substrate selection to predominately FA. Although Heinonen et al recently reported a negative correlation between LPL activity and VEGF, their study differs from ours in that LPL was measured in the plasma, mouse models of atherosclerosis fed a Western-type diet were used, and VEGF-A was overexpressed by adenovirus gene transfer.

In summary, heparanase, LPL and VEGF together, can amplify FA delivery to the diabetic heart in the short term (T1D patients who have poor glucose control, which leads to bouts of hyperglycemia; Figure 6C). If these events are prolonged, the resultant lipotoxicity could lead to cardiovascular disease in humans. Gaining more insight into the heparanase-LPL-VEGF axis may assist in devising novel therapeutic strategies to restore metabolic equilibrium, curb lipotoxicity, and help prevent or delay heart dysfunction seen during diabetes mellitus.

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Disclosures

None.

References

After diabetes mellitus, the heart uses predominantly fatty acid (FA) for energy and does so by increasing lipoprotein lipase. This amplification of vascular endothelial growth factor and its receptor occurs, leading to impaired myocardial angiogenesis, and an insufficient capacity to dispose of FA, with resulting triglyceride accumulation. If prolonged, the resultant lipotoxicity could lead to cardiovascular disease.
Hyperglycemia-Induced Secretion of Endothelial Heparanase Stimulates a Vascular Endothelial Growth Factor Autocrine Network in Cardiomyocytes That Promotes Recruitment of Lipoprotein Lipase

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Supplement Material

Experimental Animals
To induce acute, reversible hyperglycemia, diazoxide (DZ, 100 mg/kg) was injected as previously described\textsuperscript{19}. Hyperglycemia (as measured using a glucometer) was observed as early as 30 min after DZ injection, and remained 2 to 3 times higher than normal for the duration of the study. Animals were euthanized with pentobarbital sodium 4 h after DZ, and hearts removed for further experiments. To induce chronic hyperglycemia, rats were injected i.v. with 55 mg/kg streptozotocin (STZ). Animals were kept for 7 days after which their hearts were removed.

Modified Langendorff Heart Perfusion
To separate coronary perfusate from the interstitial effluent, a modified Langendorff retrograde perfusion technique was used\textsuperscript{8}. Briefly, hearts were carefully excised with the aorta, inferior vena cava, and lungs still attached. After the aorta was cannulated, the hearts were retrogradely perfused by the non-circulating Langendorff technique. The right and left branches of the pulmonary artery were cut before they entered the lungs. Subsequently, the inferior vena cava and branches of the right and left pulmonary veins were ligated, the lungs were removed, and the pulmonary artery was cannulated and tied. At this time, most of the perfusate began flowing through the pulmonary cannula (coronary perfusate), whereas a small amount of fluid (1-2\%) dripped down to the apex of the heart (interstitial transudate). The coronary and interstitial effluents were collected separately in timed fractions and assayed for A-Hep and L-Hep using Western blot.

Collagen bound VEGF
250 µl/mL of collagen IV in M199 was used to coat a 6-well plate, and incubated at 37°C to form a gel. 100 ng recombinant VEGFA was added to each well for 15 min, followed by heparin (5 U/mL) or L-Hep (1 µg/mL) treatments for 30 min. Medium collected was used to determine VEGF using ELISA.

Separation of lipoproteins
Blood was withdrawn from the inferior vena cava and serum obtained after centrifugation at 3,000 g for 20 min was then used for isolation of major lipoproteins. Briefly, ultracentrifugation was carried out at 40,000 rpm (288,000 g) for 18 h at 15°C. Lipoprotein layers were removed, and the volume of each layer measured. Different fractions were then measured for TG, cholesterol, and protein content. Isolation of VLDL without chylomicrons was achieved by using serum from 16-h fasted rats (6 PM–10 AM). Fasting of rats for this duration leads to the production of more VLDL and minimizes the contribution from chylomicrons.

Material and Reagents
Purified A-Hep and L-Hep were prepared as described\textsuperscript{22}. Heparin (Hepalean, 1000U/ml) was purchased from Organon, Canada. Anti-heparanase (mAb130) was obtained from InSight (Rehovot, Israel) and recognizes both the active (50 kDa) and latent form (65 kDa) of heparanase. VIVASPIN filter tubes (30,000 MWCO PES) were obtained from Sartorius Stedim Biotech. AMPK-α, phospho-AMPK (Thr-172), p38 MAPK, and phospho-p38 MAPK (Thr180/Tyr182), were purchased from Cell Signaling. A F/G actin biochem kit was obtained from Cytoskeleton (Denver, CO).
Supplemental Fig. IV

Supplemental Fig. V
Supplemental Figure Legends

Supplemental Figure I. Acute hyperglycemia augments the amount of active and latent heparanase in cardiac interstitial fluid. Rats were treated with diazoxide (DZ, 100 mg/kg, i.p.), and blood samples were collected at various times from the tail vein to determine blood glucose (A). At the indicated time points, hearts were also isolated and perfused using the modified Langendorff technique, which separates interstitial fluid from the coronary effluent. The amount of heparanase (active, A-Hep; latent, L-Hep) in the interstitial fluid was determined using Western blot. Normal rats were used as control (Con) (B).

Supplemental Figure II. Latent heparanase releases more recombinant myocyte bound VEGF than heparin. Cardiomyocytes were isolated and incubated with increasing concentrations of recombinant VEGF (15 min). Following this time, cells were treated with either 5 U/mL of heparin or 1 µg/mL of L-Hep (30 min), and VEGF released into the medium assayed using ELISA. *Significantly different from Con, #Significantly different from the heparin-treated group, P<0.05.

Supplemental Figure III. The extracellular matrix protein collagen can bind VEGF. Collagen IV was solidified on 6-well plates, and exogenous VEGF (100 ng) added for 15 min to allow binding onto this extracellular matrix protein. Following a PBS wash, heparin (5 U/mL) or L-Hep (1 µg) were added to the plates, and VEGF release was determined using ELISA. *Significantly different from Con.

Supplemental Figure IV. Increased VEGF expression is observed in STZ-induced chronically diabetic hearts. Male Wistar rats (250-320 g) were injected i.v. with 55 mg/kg STZ, and animals kept for 7 days before hearts were removed. Whole heart homogenates of control (Con) and STZ animals were used to determine VEGF expression by Western blot.

Supplemental Figure V. VEGF-induced increase in cardiomyocyte LPL is catalytically active and competent to breakdown VLDL-TG. Primary cardiomyocytes were treated with 100 ng/mL VEGF for 4 h, and heparin-releasable medium subsequently collected. This medium (rich in LPL) was then concentrated using VIVASPIN tubes, and incubated with increasing concentrations of VLDL-TG (0-0.8 mM). The concentration of released free fatty acids was determined after 30 min.