Cellular and Molecular Life Sciences (2021) 78:2771–2780
https://doi.org/10.1007/s00018-020-03660-2

Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

Dichotomic role of heparanase in a murine model of metabolic syndrome

Esther Hermano[1] · Françoise Carlotti[2] · Alexia Abecassis[1] · Amichay Meirovitz[1] · Ariel M. Rubinstein[1] · Jin-Ping Li[3] · Israel Vlodavsky[4] · Ton J. Rabelink[2] · Michael Elkin[1,5]

Received: 13 May 2020 / Revised: 17 September 2020 / Accepted: 28 September 2020 / Published online: 13 October 2020
© Springer Nature Switzerland AG 2020

Abstract

Heparanase is the predominant enzyme that cleaves heparan sulfate, the main polysaccharide in the extracellular matrix. While the role of heparanase in sustaining the pathology of autoimmune diabetes is well documented, its association with metabolic syndrome/type 2 diabetes attracted less attention. Our research was undertaken to elucidate the significance of heparanase in impaired glucose metabolism in metabolic syndrome and early type 2 diabetes. Here, we report that heparanase exerts opposite effects in insulin-producing (i.e., islets) vs. insulin-target (i.e., skeletal muscle) compartments, sustaining or hampering proper regulation of glucose homeostasis depending on the site of action. We observed that the enzyme promotes macrophage infiltration into islets in a murine model of metabolic syndrome, and fosters β-cell-damaging properties of macrophages activated in vitro by components of diabetogenic/obese milieu (i.e., fatty acids). On the other hand, in skeletal muscle (prototypic insulin-target tissue), heparanase is essential to ensure insulin sensitivity. Thus, despite a deleterious effect of heparanase on macrophage infiltration in islets, the enzyme appears to have beneficial role in glucose homeostasis in metabolic syndrome. The dichotomic action of the enzyme in the maintenance of glycemic control should be taken into account when considering heparanase-targeting strategies for the treatment of diabetes.

Keywords Heparanase · Diabetes · Obesity · Macrophages · Insulin resistance

Abbreviations

ECM	Extracellular matrix
HS	Heparan sulfate
AGE	Advanced glycation end products
HFD	High-fat diet
CD	Control diet
Hpse-KO	Heparanase-deficient mice
sFA	Saturated fatty acids
uFA	Unsaturated fatty acids

Esther Hermano, Françoise Carlotti, Alexia Abecassis contributed equally to this work.

✉ Michael Elkin
melkin@hadassah.org.il

[1] Department of Oncology, Sharett Institute, Hadassah-Hebrew University Medical Center, 91120 Jerusalem, Israel

[2] Division of Nephrology, Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

[3] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

[4] Cancer and Vascular Biology Research Center, The Rappaport Faculty of Medicine, Technion, Haifa, Israel

[5] Hebrew University Medical School, 91120 Jerusalem, Israel

Introduction

Extracellular matrix (ECM) and its remodeling emerged as essential determinants in the regulation of metabolic status [1–5]. The role of the ECM-degrading enzyme heparanase in diabetes and its complications has been actively investigated during the last decade [6–14]. Heparanase enzyme is the sole mammalian endoglycosidase degrading heparan sulfate (HS), the main polysaccharide component of the ECM, which is ubiquitously found in basement membranes and at the cell surface. HS and its enzymatic cleavage by heparanase was previously shown to be implicated in autoimmune diabetes [10–14] and diabetic complications [6–9]; and heparanase induction by several components of the diabetic milieu [i.e., high glucose, advanced glycation end products (AGE), free fatty acids [8, 15–20]] was described. In the setting of autoimmune diabetes, elevated levels of heparanase were detected in pancreas, and multiple roles for heparanase

in insulitis and beta cell damage were identified, including promotion of leukocyte migration/passage across the islet basement membrane, as well as depletion of HS, essential component of beta cell survival [10–12, 14, 21]. Several studies attested heparanase targeting as novel therapeutic approach for autoimmune diabetes [10–12].

Importantly, insulitis, in addition to its key role in the pathogenesis of autoimmune diabetes, contributes to progression of metabolic syndrome/type 2 diabetes [22, 23]. Metabolic syndrome is a cluster of conditions that includes insulin resistance, hyperglycemia, excess adiposity and dyslipidemia. In a significant fraction of patients, combined effects of the aforementioned conditions precipitate into type 2 diabetes. However, the clinical onset of type 2 diabetes does not occur until beta cells fail to secrete sufficient insulin to maintain normoglycemia in the face of insulin resistance. Thus, along with insulin resistance, beta cell dysfunction is a major component of type 2 diabetes pathology.

Islet inflammation acts as a key mechanism of beta cell damage [22, 24–27]. Obesity/metabolic syndrome represent low-grade inflammatory state characterized by chronic activation/recruitment of macrophages (among other factors—due to increased levels of free fatty acid that trigger TLR-4 signaling). Moreover, macrophages are the dominant immunocyte type that cause inflammation in type 2 diabetes islets [reviewed in [22, 23]]. Macrophages infiltrate islets in clinical and experimental type 2 diabetes [28–30] and are causally involved in beta cell dysfunction [22, 23, 25, 29, 31].

Notably, heparanase was recently shown to sustain macrophage reactivity in several obesity/diabetes-related [7, 9, 18, 32, 33] and non-related [34–36] inflammatory disorders.

Given the causal involvement of islet macrophages in the pathogenesis of type 2 diabetes [22, 23] and identification of heparanase as a mechanistic determinant of macrophage activation in obesity [32] we hypothesized that heparanase mediates the diabetogenic effect of excess adiposity by directing beta cell damaging action of macrophages in the setting of type 2 diabetes. However, our findings revealed a more complex mode of action of the enzyme in the maintenance of glycemic control, depending on the site of action (i.e., insulin-producing vs. insulin-target compartments).

Materials and methods

Mouse model of obesity

Sixteen-week-old male C57BL/6J mice and heparanase knock-out (Hpse-KO) mice on C57BL/6J background [37] were fed high-fat diet (HFD) (Teklad TD.06414, 60% of total calories from fat), or control diet (CD) (Teklad 2018S) for 12 consecutive weeks. Animals were then killed and tissue samples collected. All experiments were performed in accordance with the Hebrew University Institutional Animal Care and Use Committee.

Metabolic studies

Weight and fasting (5 h) blood glucose levels were monitored at indicated time points. For glucose tolerance tests, mice were fasted overnight before i.p. injection of 2 g/kg glucose. For insulin tolerance tests, mice were fasted for 2 h before injection of 0.75 units/kg recombinant human insulin i.p. (Humalog, Lilly). Glucose levels were measured in blood drawn from the tail vein with a portable glucometer (Accucheck) at the indicated time points.

Assessment of insulin sensitivity in vivo

Mice were intraperitoneally injected with human insulin (Novo Nordisk, Princeton, NJ, USA; 5 U/kg body weight) or saline (Control). Lysates of skeletal muscle (soleus and gastrocnemius), isolated 10 min after insulin administration, were analyzed by immunoblotting with antibodies directed against phospho-Akt, total Akt, phospho-insulin receptor and total insulin receptor.

Immunohistochemistry

Paraffin-embedded slides were deparaffinized and incubated in 3% H_2O_2. Antigen unmasking was carried out by treatment (5 min) with Pronase (for anti-F4/80 (AbD Serotec) staining). Slides were incubated with primary antibodies diluted in CAS-Block (Invitrogen) or with CAS-Block alone, as a control. Appropriate secondary antibodies (Nichirei) were then added and slides incubated at room temperature for 30 min. Color was developed using the DAB substrate kit (Thermo Scientific), followed by counterstaining with Mayer's hematoxylin. Staining with control IgG or without addition of primary antibody showed low or no background staining in all cases. Slides were visualized with a Zeiss axioscope microscope and number of F4/80-positive macrophages per islet was quantified in a blinded fashion (≥ 3mice per group).

Macrophage isolation and treatment

Primary mouse macrophages were isolated as previously [34]. After adherence, macrophages were incubated 2 h in standard culture conditions [34]; then the medium was replaced by serum free RPMI-1640 medium and macrophages were incubated (37 °C, 24 h) with 200 µM of fatty acids, [palmitic, stearic, and oleic acids (Sigma-Aldrich), diluted in 95% ethanol, and conjugated with fatty acid-free BSA at a 2:1 molar ratio, or with BSA (vehicle) alone]. Where indicated, macrophages were pre-treated with

recombinant heparanase (0.8 µg/ml) 2 h prior to addition of fatty acids/vehicle. In some experiments heat-inactivated heparanase [34, 35] was used as a control. Conditioned medium was collected after 24 h, filtered (0.2-µm filter, Sartorius Stedim Biotech) and used in further experiments, as indicated in the "Results" section.

Glucose-stimulated insulin secretion (GSIS)

After 1-h preincubation in glucose-free Krebs–Ringer buffer (KRB), MIN6 cells (1×10^5 cells/24 well) were incubated for 1 h in KRB (130-mM NaCl, 4.7-mM KCl, 1.2-mM KH_2PO_4, 1.2-mM $MgSO_4$, 2-mM $CaCl_2$, 5-mM $NaHCO_3$, 10-mM Hepes and 0.5% BSA) containing glucose (0.5 or 20 mM). Insulin secreted into KRB, and insulin contents were measured with insulin ELISA (Mercodia, Inc., Winston-Salem, NC). GSIS is expressed per 1×10^5 MIN6 cells.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from 1×10^6 cells using TRIzol (Invitrogen), according to the manufacturer's instructions, and quantified by spectrophotometry. After oligo (dT)-primed reverse transcription of 1 µg of total RNA, the resulting cDNA was amplified using the primers listed below. Real-time quantitative PCR (qRT-PCR) analysis was performed with an automated rotor gene system RG-3000A (Corbett Research). The PCR reaction mix (20 µl) was composed of 10-µl PerfeCTa SYBR Green FastMix (Quantabio), 5 µl of diluted cDNA (each sample in triplicate) and a final concentration of 0.3 µM of each primer. Hypoxanthine guanine phosphoribosyl transferase (HPRT) primers were used as an internal standard. The following primers were utilized: mouse HPRT sense: 5′-GTC GTG ATT AGC GAT GAA-3′, antisense: 5′-CTC CCA TCT CCT TCA TGA CAT C-3′; mIL-1b sense: 5′-CAA CCA ACA AGT GAT ATT CTC CAT G-3′; mIL-1b antisense: 5′-GAT CCA CAC TCT CCA GCT GCA-3′.

Immunoblotting

Pancreatic tissue lysates were processed for western blot analysis as previously (33). Briefly, equally loaded samples were subjected to SDS-PAGE (8% acrylamide) under reducing conditions and proteins were transferred to a polyvinylidene difluoride membrane (Millipore). Membranes were blocked with 3% BSA (1 h, room temperature) and probed (overnight, 4 °C) with anti-phospho-insulin receptor Tyr1150/1151, anti-phospho-AKT Ser 473, anti-total insulin receptor or anti-total AKT (Cell Signaling), followed by horseradish peroxidase-conjugated secondary antibody (KPL) and chemiluminescent substrate (Biological Industries). Band intensities were measured on the captured images using ImageJ.

Statistical analysis

Statistical analysis was assessed by unpaired two-tailed Student's *t* test. p values ≤ 0.05 were considered statistically significant.

Results

Heparanase deficiency prevents high fat diet-induced hyperglycemia and islet macrophage infiltration

First, we applied the model of metabolic syndrome, based on male C57/BL6 mice fed for 12 weeks with high-fat diet (HFD) [38, 39], utilizing wild-type (*wt*) and heparanase-deficient (Hpse-KO) mice on C57BL6 background. HFD-fed C57BL6 mice represent one of the best-studied models of obesity/type 2 diabetes and related metabolic abnormalities, including hyperglycemia and insulin resistance. Consistent with previous reports [38], following 12 weeks on HFD, the *wt* mice displayed significantly increased body weight and elevated levels of 5-h fasting glucose, as compared to control diet (CD)-fed mice (Fig. 1a, c). The levels of non-fasting glucose were also significantly increased in HFD-fed *wt* mice (217.3 mg/dl vs. 119.7 mg/dl, two-sided Student's *t* test $p < 0.001$), in agreement with the published data [39]. Similar to the *wt* animals, Hpse-KO mice displayed increased body weight following 12 weeks of HFD (Fig. 1b). However, there was no difference in fasting (Fig. 1d) and non-fasting (153.9 mg/dl vs. 144.16 mg/dl) glucose levels between HFD-fed vs. CD-fed Hpse-KO mice. It should be noted that even prior to initiation of HFD, blood glucose levels in Hpse-KO mice were higher than in *wt* animals (Fig. 1c, d, experimental week 0). However, elevated glucose in Hpse-KO mice appears not to be due to dysfunction of beta cells, since plasma insulin levels were actually higher in Hpse-KO, as compared to wt animals on experimental week 0 (0.47 ng/ml vs. 1.3 ng/ml, two-sided Student's *t* test $p = 9.2 \times 10^{-5}$).

Given the role of macrophage-driven beta cell damage in type 2 diabetes pathogenesis [22, 23], and involvement of heparanase in abnormal activation/pathologic effects of macrophages in diabetic complications, obesity and pancreatic tumors [7, 9, 32–34], we next set out to determine whether the enzyme influences mobilization/activation of macrophages in the islets (and, therefore, beta cell injury) in our model. To this end, we compared the number of macrophages localized to the islet compartment by IHC in *wt* vs. Hpse-KO mice fed with HFD or CD. As shown in Fig. 2a markedly increased number of macrophages was detected

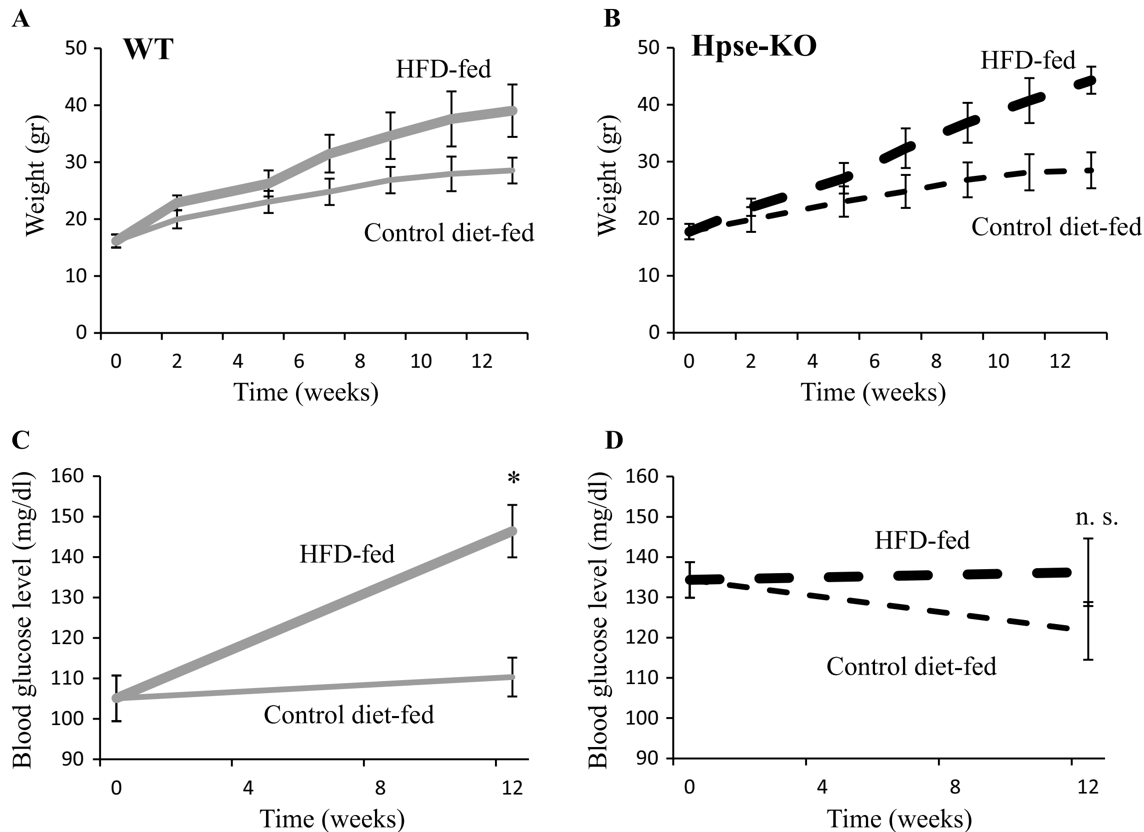


Fig. 1 HFD leads to hyperglycemia in wild-type but not in Hpse-KO mice. Wild-type (wt, gray lines) and Hpse-KO (dashed black lines) mice were fed HFD (thick lines) or control diet (thin lines) for 12 consecutive weeks. **a**, **b** Body weight of wt (**a**) and Hpse-KO (**b**) mice fed with HFD or control diet was monitored. Data are the mean ± SD. **c**, **d** Fasting blood glucose levels detected in *wt* mice (**c**) and Hpse-KO mice (**d**) following 12 weeks of HFD (thick lines) or control diet (thin lines). Data are the mean ± SD. Two-sided Student's *t* test $*p=0.0005$, $n \geq 5$ mice per condition; *n.s* not statistically significant. The experiment was performed three times and similar results were obtained

in HFD-fed *wt* mice, as compared to *wt* mice fed with control diet, in line with the previously reported data [22, 23]. Importantly, there was no increase in the number of macrophages in HFD-fed vs. CD-fed Hpse-KO mice (Fig. 2a), indicating that the enzyme deficiency prevents increase in mobilization of islet-associated macrophages under metabolic syndrome.

Heparanase augments beta cell damaging effect of macrophages in vitro

Based on these observations, along with the ability of heparanase to foster macrophage activation by diabetogenic substances, including fatty acids [9, 32, 40], we hypothesized that in the Hpse-KO HFD-fed animals lack of heparanase prevents adverse activation/mobilization of macrophages and, thus, macrophage-mediated beta cell damage. To validate this hypothesis in vitro, we compared the beta cell damaging effect of mouse macrophages, stimulated by saturated fatty acids (sFA, i.e., palmitate/stearate) in the absence or presence of heparanase enzyme. It was previously reported that sFA represent one of the key triggers of TLR4-mediated macrophage activation in the setting of obesity/type 2 diabetes. Moreover, experiments utilizing mouse beta cell line MIN6 (a reliable in vitro model of glucose-stimulated insulin secretion and beta cell function [22, 28]), confirmed that FA-activated macrophages, but not beta cells per se, produce inflammatory mediators that promote beta cell dysfunction [22, 28]. Thus, we isolated primary *wt* mouse macrophages (as in [34]), activated them by sFA (i.e., palmitate, stearate) in the absence or presence of recombinant active heparanase, and compared their ability to impair glucose-stimulated insulin secretion (GSIS) by MIN6 cells. As shown in Fig. 2b, MIN6 cells incubated with medium conditioned by *wt* macrophages activated by sFA in the presence of heparanase secreted significantly

less insulin than in the absence of heparanase, following glucose challenge, corroborating the contribution of heparanase to macrophage-mediated beta cell injury. Consistent with these observations, we detected augmented expression of IL-1β (key cytokine implicated in islet inflammation and beta cell dysfunction in type 2 diabetes [22, 23, 28] in *wt* macrophages stimulated by sFA in the presence of recombinant active heparanase (Fig. 2c). Notably, this effect was dependent on heparanase enzymatic activity, since no increase in IL-1β expression levels was detected in the presence of heat-inactivated heparanase.

Unlike sFA, unsaturated fatty acids (uFA) were previously reported to display only modest macrophage-stimulating ability [41]. In agreement with this notion, stimulation of *wt* macrophages with uFA (i.e., oleate) alone failed to induce an increase in IL-1β expression (Fig. 2c, white bars) and ability to impair GSIS of MIN6 cells (Fig. 2b, white bars). Notably, following addition of recombinant heparanase, *wt* macrophage stimulation with uFA resulted in increased IL-1β expression (Fig. 2c, gray bars). Additionally, medium conditioned by *wt* macrophages stimulated by uFA in the presence of heparanase decreased GSIS of MIN6 cells (Fig. 2b, gray bars), although the decrease did not reach statistical significance.

On the other hand, medium conditioned by sFA-stimulated heparanase-deficient macrophages (derived from Hpse-KO mice) had no effect on insulin secretion by MIN6 cells in response to glucose challenge (Fig. 2d). In agreement, following sFA stimulation, heparanase-deficient macrophages expressed significantly lower levels of IL-1β, as compared to *wt* macrophages (Fig. 2e).

In line with these findings, we detected higher insulin levels in the circulation of HFD-fed Hpse-KO mice, as compared to HFD-fed *wt* mice (15.3 ng/ml vs. 9.7 ng/ml, two-sided Student's *t* test $*p = 0.024$). Altogether, these observations support the proposed role of heparanase in fostering beta cell damaging effect of islet macrophages. In contrast, heparanase deficiency prevents macrophage-mediated islet damage, thereby preserving beta cell function.

Heparanase deficiency impairs insulin sensitivity in vivo

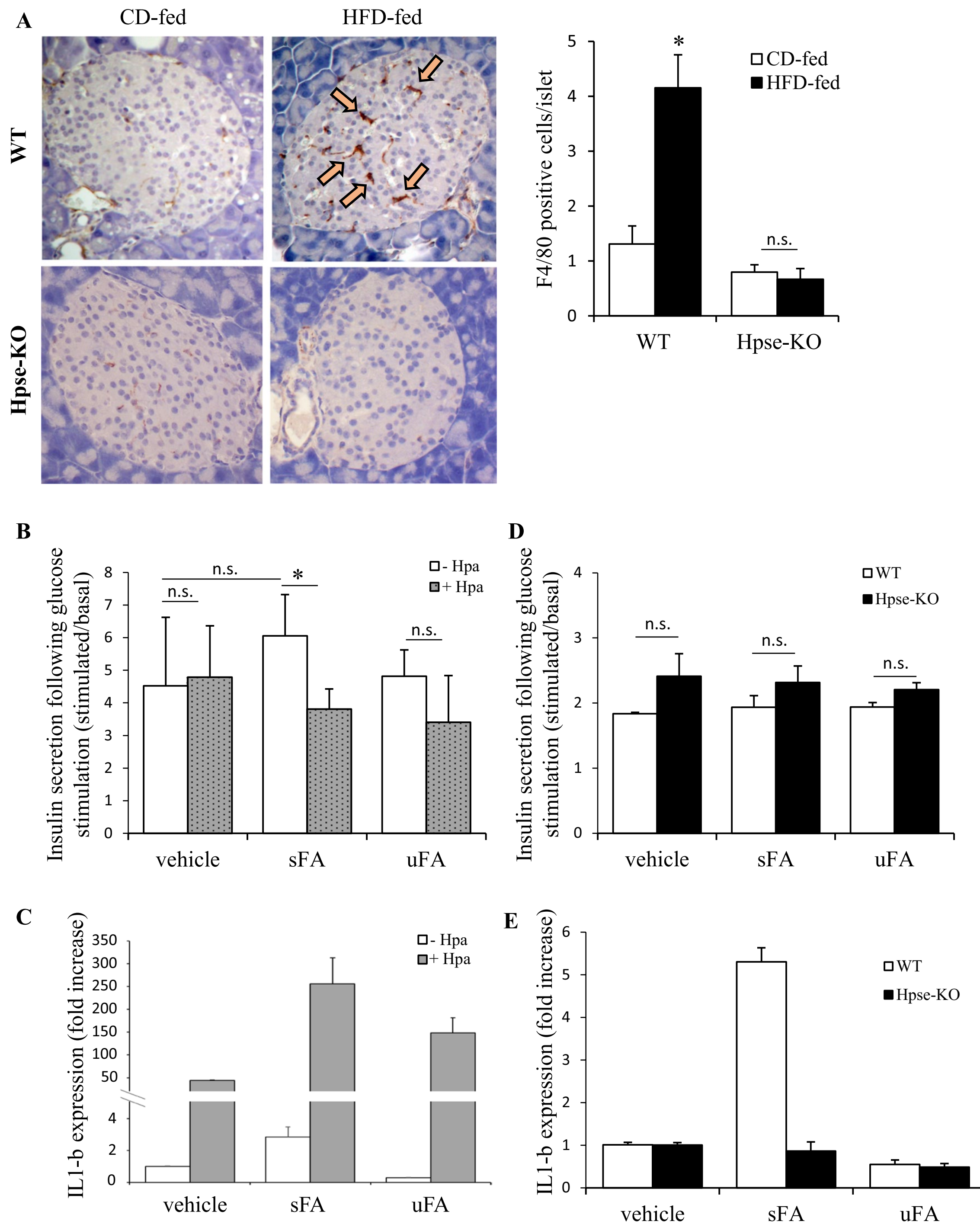
We next compared glucose tolerance in *wt* vs. Hpse-KO mice following 12 weeks of HFD/CD. Age-matched control diet-fed mice were used as controls. Despite the protective effects of heparanase deficiency on beta cell function observed in vitro (Fig. 2d, e), glucose tolerance test (GTT) revealed a higher degree of glucose intolerance in Hpse-KO mice as compared to *wt* mice (in both HFD-fed and CD-fed animals, Fig. 3a). Moreover, insulin tolerance test revealed that Hpse-KO mice (both HFD and CD fed) are less sensitive to exogenous insulin (Fig. 3b). These observations, along with the recent reports on contribution of heparanase to insulin receptor (InsR) triggering in malignant tumors of various origins (i.e., myeloma, breast carcinoma, synovial sarcoma) [42–44], led us to hypothesize that lack of heparanase in Hpse-KO mice did not allow for adequate InsR activation in diabetes-relevant insulin-target tissues (i.e., muscle), thus rendering Hpse-KO mice insulin resistant. To test this hypothesis, we assessed in vivo insulin response in *wt* and Hpse-KO mice, by analyzing activation of InsR signaling cascade in lysates of soleus skeletal muscle samples harvested 10 min after i.p. insulin administration (Fig. 4). We found that heparanase deficiency rendered skeletal muscle resistant to insulin: impaired insulin signaling (evidenced by the lack of insulin-stimulated phosphorylation of InsR and Akt) was observed in soleus skeletal muscle derived from Hpse-KO, but not *wt* mice (Fig. 4).

Altogether, these findings reveal a dichotomic action of heparanase in type 2 diabetes: while in islet compartment, the enzyme facilitates macrophage infiltration in vivo and fosters beta cell damaging properties of macrophages in vitro, in insulin-target tissue (i.e., muscle) heparanase is essential to ensure insulin sensitivity.

Discussion

Significant fraction of obese individuals with metabolic syndrome eventually develops type 2 diabetes. Progression to type 2 diabetes involves, along with insulin resistance, occurrence of an additional metabolic event—beta cell dysfunction. As a result, beta cells are unable to secrete excessive amounts of insulin to compensate for the insulin resistance, leading to hyperglycemia and onset of type 2 diabetes. Islet inflammation appears to contribute prominently to the beta cell dysfunction and adversely activated macrophages infiltrating pancreatic islets are key players in the beta cell damage [22, 23, 25, 29, 31].

Heparanase enzyme powers inflammation and macrophage reactivity in several pathological conditions triggered by obesogenic/diabetogenic milieu components [7, 9, 32]. Therefore, in the present study, we investigated involvement of the enzyme in pathogenesis of metabolic syndrome/type 2 diabetes, assuming that the enzyme (produced by islet-associated macrophages or islet cells [10, 11, 14, 47]) fosters macrophage-mediated islet damage, thus contributing to dysregulated glucose metabolism.



Indeed, our findings (Figs. 1and 2) initially suggested that in metabolic syndrome/type 2 diabetes the enzyme acts primarily in the islet compartment and exerts pro-diabetic effects via facilitation of beta cell injury by macrophages. Additional heparanase-driven phenomena initially reported in the setting of T1D (i.e., facilitation of leukocyte migration across the islet basement membrane and depletion of intra-islet HS) [10–12, 14, 21] can also contribute to beta cell damage in type 2 diabetes. However, further experiments (Figs. 3 and 4) revealed a more

◂**Fig. 2** Effect of heparanase on islets damaging properties of macrophages. **a** Pancreata from CD- and HFD-fed wt and Hpse-KO mice were processed for immunostaining with F4/80 antibody (left panel). Blind assessment of the number of F4/80-positive cells per islet in each group was then performed. Bar graph (right panel) represents statistics of the number of F4/80 + cells per islet (mean ± SD, n ≥ 3 mice per condition, two-sided Student's *t* test ***p** = 0.00026, *n.s* not statistically significant). **b**, **c** Primary wt mouse macrophages were stimulated by 200 µM of saturated (palmitate/stearate) or unsaturated (oleate) fatty acids (sFA or uFA, in accordance,) in the absence (white bars) or presence (gray bars) of recombinant heparanase enzyme (Hpa). As control, macrophages were treated with vehicle (BSA) alone. **b** Medium conditioned by the macrophages was then added to MIN6 cells (diluted at ratio 1:5) and glucose-stimulated insulin secretion (GSIS) was assessed 24 h later. Note that presence of heparanase significantly augmented beta cell-damaging properties of saturated FA-activated macrophages (Two-sided Student's *t* test ***p** = 0.04; *n.s* not statistically significant). Beta cell-damaging properties of uFA-activated macrophages were also increased in the presence of heparanase, but did not reach statistical significance. Note that when medium conditioned by vehicle-stimulated macrophages was used, presence of heparanase per se had no effect on GSIS, ruling out possible effect of carry-over of the enzyme via the conditioned medium. Also note the lack of statistically significant differences on GSIS exerted by medium conditioned by sFA-treated vs vehicle-treated macrophages, ruling out possible effect of carry-over of sFA via the conditioned medium. **c** Expression of IL-1β in macrophages stimulated by sFA or uFA in the absence or presence of active recombinant heparanase was assessed by qRT-PCR. **d**, **e** In a similar manner, primary macrophages derived from wt (empty bars) or Hpse-KO (black bars) mice were stimulated by sFA or uFA. Some macrophages were treated by vehicle (BSA) alone. **d** Medium conditioned by the macrophages was then added to MIN6 cells and their glucose-stimulated insulin secretion was assessed 24 h later. **e** Expression of IL-1β in wt (empty bars) or Hpse-KO (black bars) macrophages stimulated with sFA or uFA was assessed by qRT-PCR

complex picture: heparanase may either sustain or hamper proper regulation of glucose metabolism, depending on the site of action. While in islets heparanase appears to foster mobilization of beta cell damaging macrophages, in skeletal muscle (the prototypic insulin-target tissue), the presence of heparanase is a prerequisite for proper InsR signaling. The precise molecular mechanism underlying the action of heparanase in ensuring insulin sensitivity has not been fully elucidated, (clear limitation of the present study); nevertheless, emerging involvement of HS, enzymatic substrate of heparanase, in modulation of insulin responses in the target tissues offers possible explanation. Indeed, perlecan (the main HS proteoglycan in extracellular space) appears to regulate responsiveness to insulin, and perlecan-deficient mouse model demonstrated that loss of perlecan led to augmented insulin sensitivity in muscle [3]. Since heparanase activity reduces the amount of extracellular HS [35, 45], lack of enzymatic remodeling of perlecan in Hpse-KO mice can explain insulin resistance in Hpse-KO muscle tissue (Fig. 4).

Additionally, glypican-4 (cell surface HS proteoglycan) was shown to interact with and enhance InsR signaling [2]. This effect on InsR signaling appears to involve release of glypican from the cell surface by an enzymatically regulated process and its direct interaction with InsR [2], likely through HS chains. Of note, heparanase enzymatic activity drives shedding of cell surface HS proteoglycans, and releases cell surface-derived bioactive HS fragments that potentiate growth factor-receptor signaling [46].

Causal contribution of the enzyme to insulin sensitivity is further supported by the series of findings reported in pathologies other than metabolic dysregulation that involve activation of InsR (i.e., malignant tumor cells of both epithelial and mesenchymal origin (breast carcinoma, myeloma, sarcoma) [42–44]. In all of the aforementioned malignant cell types, heparanase promotes insulin–InsR signaling, while inhibition of the enzyme repressed InsR signaling cascade [42–44].

While further studies are warranted to fully elucidate the mechanism(s) of heparanase action in facilitation of InsR signaling, as well as regulation of the enzyme expression in insulin-target tissues (which is much less investigated then in islets [47]), our findings reveal the dichotomic role of heparanase in the maintenance of glucose homeostasis. We suggest that this dichotomy of the enzyme function is due to multiple mechanistic possibilities and diverse biological roles ascribed to HS, in the pancreas vs. peripheral insulin-target tissues. Our study implies that while considering heparanase-targeting strategies for treatment of diabetes or its complications [8–12, 47], one has to take into account its possible beneficial effect on insulin resistance. Thus, our results may offer rational and mechanistically informed basis to improve design of clinical testing of heparanase inhibitors in diabetic patients.

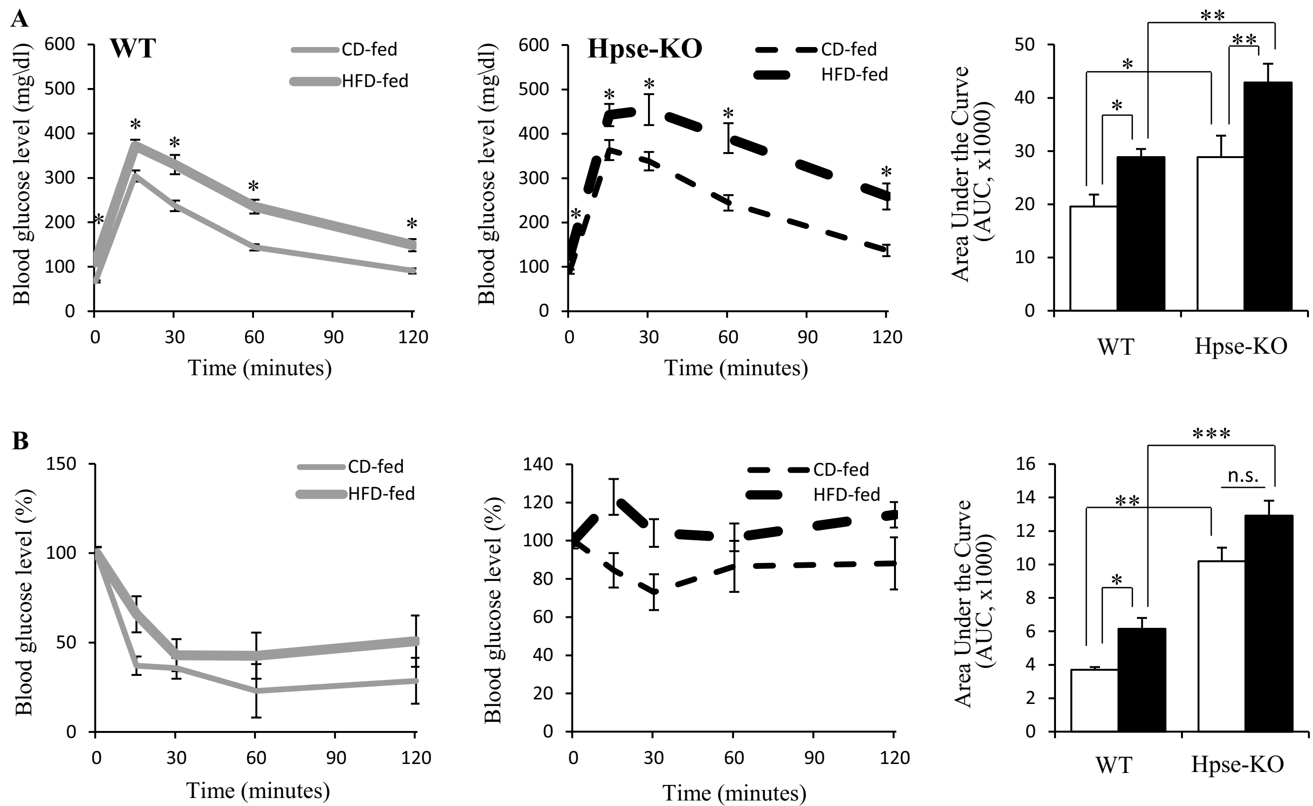


Fig. 3 Glucose and insulin tolerance in wt and Hpse-KO mice. **a** Glucose tolerance test was performed on *wt* (left panel) and Hpse-KO (middle panel) mice fed with HFD [thick lines] or control diet [thin lines]. Following an overnight fast (16 h), mice were given an intraperitoneal injection of glucose (2 g/kg body weight). At the indicated time points, blood glucose was measured as indicated in Methods. Results are mean ± SE; $n \geq 6$ per condition; Two-sided Student's *t* test $*p \leq 0.022$. Areas under the curves were compared and the respective bar graphs represent mean ± SE (right panel), two-sided Student's *t* test $*p \leq 6.8 \times 10^{-5}$, $**p \leq 0.007$. **b** Insulin tolerance test was performed on 2 h fasted *wt* (left panel) and Hpse-KO (middle panel) mice fed with HFD [thick lines] or control diet [thin lines], applying an i.p. injection of 0.75 units/kg body weight, human insulin. Blood glucose was measured as described in Methods. Data are mean ± SE; $n \geq 6$ per condition; Two-sided Student's *t* test $*p \leq 0.022$. Areas under the curve were calculated and bar graph represent mean ± SE (right panel); Two-sided Student's *t* test $*p = 0.012$, $**p = 4.16 \times 10^{-6}$, $***p \leq 1.16 \times 10^{-5}$, *n.s* not statistically significant

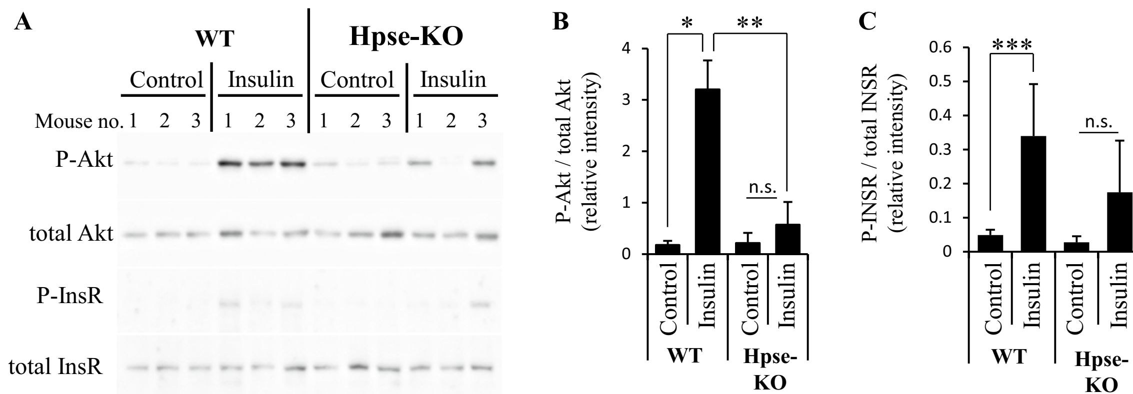


Fig. 4 Heparanase deficiency impairs insulin sensitivity in skeletal muscle. **a** Wild-type and Hpse-KO mice were injected (i.p.) with human insulin (Novo Nordisk, Princeton, NJ, USA; 5 U/kg body weight) or saline (Control). Lysates of skeletal muscle, isolated from the mice ten min after insulin administration, were analyzed by immunoblotting with antibodies directed against phospho-Akt (P-Akt), total Akt, phospho-InsR (P-InsR) and total InsR InsR. **b**, **c** The band intensity was quantified using ImageJ software; intensity ratios for P-Akt/total Akt (**b**) and P-InsR/total InsR (**c**) are shown. Data are the mean ± SD. Two-sided Student's *t* test $*p = 0.0007$, $**p = 0.003$, $***p = 0.03$; *n.s* not statistically significant, $n = 3$ mice per condition

Acknowledgements We thank Prof. Danielle Melloul (Department of Endocrinology, Hadassah-Hebrew University Medical Center) for her continuous help and collaboration.

Author contribution E.H., F.C., A.A., and A.M.R. conducted experiments; JP.L., I.V. generated/contributed experimental animals; E.H., F.C., A.M., T.J.R., M.E. analyzed data; E.H., F.C., T.J.R., M.E. designed research studies, E.H., F.C., A.M., I.V., T.J.R. reviewed and edited the manuscript. M.E. was responsible for conceptualization, study design, supervised the study, wrote the manuscript. M.E. is the guarantor of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding This study was supported by the Legacy Heritage Bio-Medical Program of the Israel Science Foundation (Grant no. 663/16).

Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All animal experiments were performed in accordance with the Hebrew University Institutional Animal Care and Use Committee.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

1. Huang G, Greenspan DS (2012) ECM roles in the function of metabolic tissues. Trends Endocrinol Metab 23(1):16–22. https://doi.org/10.1016/j.tem.2011.09.006
2. Ussar S, Bezy O, Bluher M, Kahn CR (2012) Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. Diabetes 61(9):2289–2298
3. Yamashita Y, Nakada S, Yoshihara T, Nara T, Furuya N, Miida T, Hattori N, Arikawa-Hirasawa E (2018) Perlecan, a heparan sulfate proteoglycan, regulates systemic metabolism with dynamic changes in adipose tissue and skeletal muscle. Sci Rep 8(1):7766. https://doi.org/10.1038/s41598-018-25635-x
4. Townsend SE, Gannon M (2019) Extracellular matrix-associated factors play critical roles in regulating pancreatic beta-cell proliferation and survival. Endocrinology 160(8):1885–1894. https://doi.org/10.1210/en.2019-00206
5. Kang L, Ayala JE, Lee-Young RS, Zhang Z, James FD, Neufer PD, Pozzi A, Zutter MM, Wasserman DH (2011) Diet-induced muscle insulin resistance is associated with extracellular matrix remodeling and interaction with integrin alpha2beta1 in mice. Diabetes 60(2):416–426. https://doi.org/10.2337/db10-1116
6. Abu El-Asrar AM, Alam K, Nawaz MI, Mohammad G, Van den Eynde K, Siddiquei MM, Mousa A, De Hertogh G, Geboes K, Opdenakker G (2015) Upregulated expression of heparanase in the vitreous of patients with proliferative diabetic retinopathy originates from activated endothelial cells and leukocytes. Invest Ophthalmol Vis Sci 56(13):8239–8247. https://doi.org/10.1167/iovs.15-18025
7. Baker AB, Chatzizisis YS, Beigel R, Jonas M, Stone BV, Coskun AU, Maynard C, Rogers C, Koskinas KC, Feldman CL, Stone PH, Edelman ER (2010) Regulation of heparanase expression in coronary artery disease in diabetic, hyperlipidemic swine. Atherosclerosis 213(2):436–442
8. Gil N, Goldberg R, Neuman T, Garsen M, Zcharia E, Rubinstein AM, van Kuppevelt T, Meirovitz A, Pisano C, Li JP, van der Vlag J, Vlodavsky I, Elkin M (2012) Heparanase is essential for the development of diabetic nephropathy in mice. Diabetes 61(1):208–216. https://doi.org/10.2337/db11-1024
9. Goldberg R, Rubinstein AM, Gil N, Hermano E, Li JP, van der Vlag J, Atzmon R, Meirovitz A, Elkin M (2014) Role of heparanase-driven inflammatory cascade in pathogenesis of diabetic nephropathy. Diabetes 63(12):4302–4313
10. Simeonovic CJ, Popp SK, Brown DJ, Li FJ, Lafferty ARA, Freeman C, Parish CR (2020) Heparanase and type 1 diabetes. Adv Exp Med Biol 1221:607–630. https://doi.org/10.1007/978-3-030-34521-1_24
11. Simeonovic CJ, Popp SK, Starrs LM, Brown DJ, Ziolkowski AF, Ludwig B, Bornstein SR, Wilson JD, Pugliese A, Kay TWH, Thomas HE, Loudovaris T, Choong FJ, Freeman C, Parish CR (2018) Loss of intra-islet heparan sulfate is a highly sensitive marker of type 1 diabetes progression in humans. PLoS ONE 13(2):e0191360. https://doi.org/10.1371/journal.pone.0191360
12. Simeonovic CJ, Ziolkowski AF, Wu Z, Choong FJ, Freeman C, Parish CR (2013) Heparanase and autoimmune diabetes. Front Immunol 4:471. https://doi.org/10.3389/fimmu.2013.00471
13. Zhang D, Wang F, Lal N, Chiu AP, Wan A, Jia J, Bierende D, Flibotte S, Sinha S, Asadi A, Hu X, Taghizadeh F, Pulinilkunnil T, Nislow C, Vlodavsky I, Johnson JD, Kieffer TJ, Hussein B, Rodrigues B (2017) Heparanase overexpression induces glucagon resistance and protects animals from chemically induced diabetes. Diabetes 66(1):45–57. https://doi.org/10.2337/db16-0761
14. Ziolkowski AF, Popp SK, Freeman C, Parish CR, Simeonovic CJ (2012) Heparan sulfate and heparanase play key roles in mouse beta cell survival and autoimmune diabetes. J Clin Invest 122(1):132–141. https://doi.org/10.1172/JCI46177
15. An XF, Zhou L, Jiang PJ, Yan M, Huang YJ, Zhang SN, Niu YF, Ten SC, Yu JY (2011) Advanced glycation end-products induce heparanase expression in endothelial cells by the receptor for advanced glycation end products and through activation of the FOXO4 transcription factor. Mol Cell Biochem 354(1–2):47–55
16. Masola V, Gambaro G, Tibaldi E, Onisto M, Abaterusso C, Lupo A (2011) Regulation of heparanase by albumin and advanced glycation end products in proximal tubular cells. Biochim Biophys Acta 1813:1475–1482. https://doi.org/10.1016/j.bbamcr.2011.05.004
17. Maxhimer JB, Somenek M, Rao G, Pesce CE, Baldwin D Jr, Gattuso P, Schwartz MM, Lewis EJ, Prinz RA, Xu X (2005) Heparanase-1 gene expression and regulation by high glucose in renal epithelial cells: a potential role in the pathogenesis of proteinuria in diabetic patients. Diabetes 54(7):2172–2178
18. Qin Q, Niu J, Wang Z, Xu W, Qiao Z, Gu Y (2013) Heparanase induced by advanced glycation end products (AGEs) promotes macrophage migration involving RAGE and PI3K/AKT pathway. Cardiovasc Diabetol 12(1):37
19. Han J, Hiebert LM (2013) Alteration of endothelial proteoglycan and heparanase gene expression by high glucose, insulin and heparin. Vascul Pharmacol 59(3–4):112–118
20. Qing Q, Zhang S, Chen Y, Li R, Mao H, Chen Q (2015) High glucose-induced intestinal epithelial barrier damage is aggravated by syndecan-1 destruction and heparanase overexpression. J Cell Mol Med. https://doi.org/10.1111/jcmm.12523

21. Parish CR, Freeman C, Ziolkowski AF, He YQ, Sutcliffe EL, Zafar A, Rao S, Simeonovic CJ (2013) Unexpected new roles for heparanase in type 1 diabetes and immune gene regulation. Matrix Biol 32(5):228–233. https://doi.org/10.1016/j.matbio.2013.02.007
22. Eguchi K, Nagai R (2017) Islet inflammation in type 2 diabetes and physiology. J Clin Invest 127(1):14–23. https://doi.org/10.1172/JCI88877
23. Ying W, Fu W, Lee YS, Olefsky JM (2020) The role of macrophages in obesity-associated islet inflammation and beta-cell abnormalities. Nat Rev Endocrinol 16(2):81–90. https://doi.org/10.1038/s41574-019-0286-3
24. Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 11(2):98–107
25. Nackiewicz D, Dan M, He W, Kim R, Salmi A, Rutti S, Westwell-Roper C, Cunningham A, Speck M, Schuster-Klein C, Guardiola B, Maedler K, Ehses JA (2014) TLR2/6 and TLR4-activated macrophages contribute to islet inflammation and impair beta cell insulin gene expression via IL-1 and IL-6. Diabetologia 57(8):1645–1654
26. Eheim A, Medrikova D, Herzig S (2014) Immune cells and metabolic dysfunction. Semin Immunopathol 36(1):13–25
27. Marzban L (2015) New insights into the mechanisms of islet inflammation in type 2 diabetes. Diabetes 64(4):1094–1096. https://doi.org/10.2337/db14-1903
28. Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, Yagi N, Ohto U, Kimoto M, Miyake K, Tobe K, Arai H, Kadowaki T, Nagai R (2012) Saturated fatty acid and TLR signaling link beta cell dysfunction and islet inflammation. Cell Metab 15(4):518–533
29. Ying W, Lee YS, Dong Y, Seidman JS, Yang M, Isaac R, Seo JB, Yang BH, Wollam J, Riopel M, McNelis J, Glass CK, Olefsky JM, Fu W (2019) Expansion of islet-resident macrophages leads to inflammation affecting beta cell proliferation and function in obesity. Cell Metab 29(2):457–474.e455. https://doi.org/10.1016/j.cmet.2018.12.003
30. Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, Fontana A, Reinecke M, Homo-Delarche F, Donath MY (2007) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56(9):2356–2370
31. Westwell-Roper CY, Ehses JA, Verchere CB (2014) Resident macrophages mediate islet amyloid polypeptide-induced islet IL-1beta production and beta-cell dysfunction. Diabetes 63(5):1698–1711. https://doi.org/10.2337/db13-0863
32. Hermano E, Goldberg R, Rubinstein AM, Sonnenblick A, Maly B, Nahmias D, Li JP, Bakker MAH, van der Vlag J, Vlodavsky I, Peretz T, Elkin M (2019) Heparanase accelerates obesity-associated breast cancer progression. Cancer Res 79(20):5342–5354. https://doi.org/10.1158/0008-5472.CAN-18-4058
33. Blich M, Golan A, Arvatz G, Sebbag A, Shafat I, Sabo E, Cohen-Kaplan V, Petcherski S, Avniel-Polak S, Eitan A, Hammerman H, Aronson D, Axelman E, Ilan N, Nussbaum G, Vlodavsky I (2013) Macrophage activation by heparanase is mediated by TLR-2 and TLR-4 and associates with plaque progression. Arterioscler Thromb Vasc Biol 33(2):e56–65. https://doi.org/10.1161/ATVBAHA.112.254961
34. Hermano E, Meirovitz A, Meir K, Nussbaum G, Appelbaum L, Peretz T, Elkin M (2014) Macrophage polarization in pancreatic carcinoma: role of heparanase enzyme. J Natl Cancer Inst 106(12):dju332
35. Lerner I, Hermano E, Zcharia E, Rodkin D, Bulvik R, Doviner V, Rubinstein AM, Ishai-Michaeli R, Atzmon R, Sherman Y, Meirovitz A, Peretz T, Vlodavsky I, Elkin M (2011) Heparanase powers a chronic inflammatory circuit that promotes colitis-associated tumorigenesis in mice. J Clin Invest 121(5):1709–1721
36. Masola V, Zaza G, Bellin G, Dall'Olmo L, Granata S, Vischini G, Secchi MF, Lupo A, Gambaro G, Onisto M (2018) Heparanase regulates the M1 polarization of renal macrophages and their crosstalk with renal epithelial tubular cells after ischemia/reperfusion injury. FASEB J 32(2):742–756. https://doi.org/10.1096/fj.201700597R
37. Zcharia E, Jia J, Zhang X, Baraz L, Lindahl U, Peretz T, Vlodavsky I, Li JP (2009) Newly generated heparanase knock-out mice unravel co-regulation of heparanase and matrix metalloproteinases. PLoS ONE 4(4):e5181
38. Montgomery MK, Hallahan NL, Brown SH, Liu M, Mitchell TW, Cooney GJ, Turner N (2013) Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding. Diabetologia 56(5):1129–1139
39. Pettersson US, Walden TB, Carlsson PO, Jansson L, Phillipson M (2012) Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLoS ONE 7(9):e46057. https://doi.org/10.1371/journal.pone.0046057
40. Xu G, Qin Q, Yang M, Qiao Z, Gu Y, Niu J (2017) Heparanase-driven inflammation from the AGEs-stimulated macrophages changes the functions of glomerular endothelial cells. Diabetes Res Clin Pract 124:30–40. https://doi.org/10.1016/j.diabres.2016.12.016
41. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116(11):3015–3025. https://doi.org/10.1172/JCI28898
42. Cassinelli G, Dal Bo L, Favini E, Cominetti D, Pozzi S, Tortoreto M, De Cesare M, Lecis D, Scanziani E, Minoli L, Naggi A, Vlodavsky I, Zaffaroni N, Lanzi C (2018) Supersulfated low-molecular weight heparin synergizes with IGF1R/IR inhibitor to suppress synovial sarcoma growth and metastases. Cancer Lett 415:187–197. https://doi.org/10.1016/j.canlet.2017.12.009
43. Goldberg R, Sonnenblick A, Hermano E, Hamburger T, Meirovitz A, Peretz T, Elkin M (2017) Heparanase augments insulin receptor signaling in breast carcinoma. Oncotarget 8(12):19403–19412. https://doi.org/10.18632/oncotarget.14292
44. Purushothaman A, Babitz SK, Sanderson RD (2012) Heparanase enhances the insulin receptor signaling pathway to activate ERK in multiple myeloma. J Biol Chem 287(49):41288–41296
45. Elkin M, Ilan N, Ishai-Michaeli R, Friedmann Y, Papo O, Pecker I, Vlodavsky I (2001) Heparanase as mediator of angiogenesis: mode of action. FASEB J 15(9):1661–1663. https://doi.org/10.1096/fj.00-0895fje
46. Ramani VC, Purushothaman A, Stewart MD, Thompson CA, Vlodavsky I, Au JL, Sanderson RD (2013) The heparanase/syndecan-1 axis in cancer: mechanisms and therapies. FEBS J 280(10):2294–2306
47. Song WY, Jiang XH, Ding Y, Wang Y, Zhou MX, Xia Y, Zhang CY, Yin CC, Qiu C, Li K, Sun P, Han X (2020) Inhibition of heparanase protects against pancreatic beta cell death in streptozotocin-induced diabetic mice via reducing intra-islet inflammatory cell infiltration. Br J Pharmacol. https://doi.org/10.1111/bph.15183

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.