



Treatment with semaglutide, a GLP-1 receptor agonist, improves extracellular matrix remodeling in the pancreatic islet of diet-induced obese mice

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ABSTRACT

Aims: The extracellular matrix (ECM) is fundamental for the normal endocrine functions of pancreatic islet cells and plays key roles in the pathophysiology of type 2 diabetes. Here we investigated the turnover of islet ECM components, including islet amyloid polypeptide (IAPP), in an obese mouse model treated with semaglutide, a glucagon-like peptide type 1 receptor agonist.

Main methods: Male one-month-old C57BL/6 mice were fed a control diet (C) or a high-fat diet (HF) for 16 weeks, then treated with semaglutide (subcutaneous 40 µg/kg every three days) for an additional four weeks (HFS). The islets were immunostained and gene expressions were assessed.

Key findings: Comparisons refer to HFS vs HF. Thus, IAPP immunolabeling and beta-cell-enriched beta-amyloid precursor protein cleaving enzyme (*Bace2*, -40 %) and heparanase immunolabeling and gene (*Hpse*, -40 %) were mitigated by semaglutide. In contrast, perlecan (*Hspg2*, +900 %) and vascular endothelial growth factor A (*Vegfa*, +420 %) were enhanced by semaglutide. Also, semaglutide lessened syndecan 4 (*Sdc4*, -65 %) and hyaluronan synthases (*Has1*, -45 %; *Has2*, -65 %) as well as chondroitin sulfate immunolabeling, and collagen type 1 (*Col1a1*, -60 %) and type 6 (*Col6a3*, -15 %), lysyl oxidase (*Lox*, -30 %) and metalloproteinases (*Mmp2*, -45 %; *Mmp9*, -60 %).

Significance: Semaglutide improved the turnover of islet heparan sulfate proteoglycans, hyaluronan, chondroitin sulfate proteoglycans, and collagens in the islet ECM. Such changes should contribute to restoring a healthy islet functional milieu and should reduce the formation of cell-damaging amyloid deposits. Our findings also provide additional evidence for the involvement of islet proteoglycans in the pathophysiology of type 2 diabetes.

1. Introduction

It has long been recognized that interactions between pancreatic islet cells and the surrounding extracellular matrix (ECM) are fundamental for their normal physiological functions and for maintaining islet architecture, both during development and in adults [1–3]. Although not readily apparent in conventional histological preparations, islet ECM is structurally and functionally complex and is mainly associated with the

cell surface of endocrine cells and the periphery of blood vessels [4]. Islet transplantation, which is currently a viable alternative for the treatment of type 1 diabetes [5], has further emphasized the importance of this stroma, as several methods have been devised to better preserve or even enhance ECM components while isolating islets, thereby reducing functional loss in the endocrine cells [6,7].

Islet ECM components include heparan sulfate proteoglycans (HSPG), chondroitin/dermatan sulfate proteoglycans (CSPG), collagens,

Abbreviations: *Bace2*, beta-amyloid precursor protein cleaving enzyme; *Col*, collagen; CSPG, chondroitin/dermatan sulfate proteoglycans; DIO, diet-induced obese; ECM, extracellular matrix; GAG, glycosaminoglycan; GLP1, glucagon-like peptide type 1; *Has*, hyaluronan synthase; *Hpse*, heparanase; *Hspg*, heparan sulfate proteoglycans; *Hspg2*, perlecan; IAPP, islet amyloid polypeptide; *Lox*, lysyl oxidase; *Mmp*, metalloproteinase; *Sdc4*, syndecan 4; T2D, type 2 diabetes; *Vegfa*, vascular endothelial growth factor A.

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and adhesion glycoproteins, which are variously involved in pancreatic diseases [2,4,8]. For example, HSPG and/or their heparan sulfate glycosaminoglycan (GAG) chains have protective roles for beta-cells by reducing the damaging effects of both cytotoxic proteins and hydrogen peroxide [9,10]. In addition, HSPG [11–13] and the free GAG hyaluronan [14] can form aggregates with the beta-cell hormone islet amyloid polypeptide (IAPP), which is thought to be a key event in the pathophysiology of type 2 diabetes (T2D) [15]. HSPG can also promote the accumulation of soluble IAPP close to beta-cells, which can then be disruptive to their cell membranes [10], while culturing beta-cells on a collagen I matrix prevents cell damage by hypoxia [16], an insult that occurs in the T2D islet [17].

Over the past few decades, T2D has become a major global health issue [18] and many therapeutic strategies are currently available or under development, which also address the management of obesity and other associated risks [19]. Recent advances in the pharmacological treatment of T2D include glucagon-like peptide 1 (GLP-1) receptor agonists such as Semaglutide, which exert their beneficial effects by modifying various pathways involved in glucose metabolism, especially insulin and glucagon secretion [20]. Additionally, we have shown recently that Semaglutide reduces several adverse alterations in pancreatic islets found in diet-induced obese (DIO) and hyperglycemic mice, including disruption of cell organization, apoptosis, low hormone levels, and inflammation [21]. However, little is known about whether Semaglutide modulates the expression and metabolism of the islet ECM.

Using a mouse model of DIO and T2D [22,23], we here investigated whether the ensuing metabolic alterations would also affect the components of the islet ECM and factors involved in their turnover. We also evaluated if Semaglutide would prevent or diminish an eventual ECM remodeling.

2. Materials and methods

2.1. Animals, diets, and experimental protocol

All animal procedures followed the Guide's Recommendations for the Care and Use of Laboratory Animals (National Academies Press, 8th Edition, Washington, DC, 2011) and ARRIVE [24] and were approved by the local Animal Experimentation Ethics Committee (protocol number CEUA/054/2018). We housed five animals per cage in a ventilated NexGen system (Allentown Inc., PA, USA) under controlled conditions: $20 \pm 2^\circ\text{C}$, 12 h/12 h dark/light cycle, and free access to food and water.

Forty male mice (C57BL/6, one month of age) were randomly assigned to two dietary groups ($n = 20/\text{group}$) that were fed for 16 weeks a control diet or a high-fat diet. The diets were manufactured by Pragsolucoes (Jau, SP, Brazil) based on the AIN-93G (until three months old) and AIN93M recommendations [25,26]. After week 16, the animals were studied for an additional four weeks, separated into the groups: C, control diet ($n = 10$, received the same volume of vehicle as semaglutide group subcutaneously); CS, control diet plus semaglutide ($n = 10$); HF, high-fat diet ($n = 10$, received the same volume of vehicle as semaglutide group subcutaneously); HFS, a high-fat diet plus semaglutide ($n = 10$).

Semaglutide (Ozempic, Novo Nordisk, Bagsvaerd, Denmark) was administered subcutaneously to mice at 40 $\mu\text{g}/\text{kg}$ once every three days, corresponding to 1.2 μg of the drug per animal, assuming an average body weight of 30 g in young adult mice. The stock Semaglutide solution (Ozempic, 1.34 mg/mL) was diluted in sterile 0.9 % NaCl to 6.0 $\mu\text{g}/\text{mL}$ so that an injection volume of 200 μL would contain 1.2 μg of the drug. Furthermore, it was reported that the semaglutide half-life in rodents is shorter than in humans [27]. Therefore, we treated our mice once every three days instead of once a week.

2.2. Pancreas dissection and immunohistochemistry

Once the animals were deeply anesthetized (150 mg/kg

intraperitoneal sodium pentobarbital), the pancreas was dissected ($n = 5/\text{group}$) and fixed in 4 % w/v formaldehyde in phosphate buffer 0.1 M, pH 7.2, for 48 h at room temperature. Next, the pancreas was processed for light microscopy and embedded in Paraplast Plus (Sigma-Aldrich Co., St Louis, MO, USA). For immunohistochemistry, deparaffinized and hydrated 5- μm -thick sections were treated with citrate buffer, pH 6.0, at 60 °C for 20 min for antigen retrieval and then incubated with 2.5 % horse serum to block non-specific bindings (Vector Laboratories, CA, USA). The slides were then incubated for 2 h at room temperature with the following primary antibodies diluted in 1.5 % horse serum: anti-IAPP (Cusabio, CSB-PA12224AORb, 1:100), anti-chondroitin sulfate (Sigma-Aldrich, SAB4200696, 1:100), anti-CD31 (Abcam, AB28364, 1:100), anti-perlecan (Cusabio, CSB-PA967416, 1:100), and anti-heparanase (Cusabio, CSB-PA010716, 1:100). Next, the slides were incubated with the secondary biotinylated pan-specific antibody (10 min, Vector Laboratories, CA, USA), followed by incubation with streptavidin and peroxidase (5 min), and revealed with 3,3'-diaminobenzidine tetrahydrochloride (DAB), 3 min per slide (Vectastain Universal Quick HRP Kit, peroxidase, PK-7800, Vector Laboratories, CA, USA). The slides were finally counterstained with hematoxylin and mounted in Entellan (Merck, Darmstadt, Germany). Negative controls consisted of tissue sections processed in the same way, except that the primary antibody was replaced by 1.5 % horse serum. Digital images were obtained with a Nikon microscope, model 80i, and a DS-Ri1 digital camera (Nikon Instruments, Inc., New York, 133 USA).

2.3. Islet isolation and real-time quantitative polymerase chain reaction

The bile duct was cannulated in another set of animals ($n = 5/\text{group}$). The pancreas was inflated with cold Hanks' solution (14025126, Invitrogen, CA, USA) containing 1 mg/mL bovine serum albumin and 0.8 mg/mL collagenase fraction V (c9263, Sigma-Aldrich, St. Louis, MO, USA). The pancreas was then removed, put in a bottle containing the same collagenase solution, and incubated in a 37 °C water bath for 20 min. Finally, the islets were collected manually with automatic micropipettes under a stereomicroscope (Luxeo 4d Stereo Zoom Microscope, Labomed, CA, USA) [28].

The total RNA from islets was extracted (Trizol, Invitrogen, CA, USA), and its concentration was determined on a spectrophotometer (Nanovue, GE Life Sciences, USA). Then, one microgram of RNA was treated with DNase 1, and Oligo (DT) primers for mRNA and superscript 3 reverse transcriptase (Invitrogen, CA, USA) were used for the synthesis of the first-strand cDNA (Biorad Cfx96 Thermocycler, Bio-Rad, Hercules, CA, USA) with the Sybr green mix (Invitrogen, CA, USA). The endogenous control to correct the expression of target genes was the beta-actin gene. Primer sequences were designed by Primer3 software (<http://bioinfo.ut.ee/primer3-0.4.0/>) (Table 1). The efficiency of RT-qPCR, calculated from a series of cDNA dilutions, was approximately equal to the endogenous control for the target gene. Finally, the relative mRNA expression ratio was calculated using the $2^{-\Delta\Delta\text{ct}}$ eq. ($2^{-\Delta\text{ct}}$ expresses the difference between the target gene number of cycles, ct , and the endogenous control) [29].

2.4. Data analysis

Numerical data were first tested for normality (Shapiro-Wilk test for a small sample) and homogeneity of variances (Bartlett test) and expressed as mean and standard deviation. Next, the differences among groups were tested using a two-way ANOVA followed by Tukey's multiple comparison test. Graphpad prism v.9.5 for Windows (Graphpad Software, San Diego, CA, USA) was used for all statistical analyses, and differences were considered significant when $P < 0.05$.

Table 1
Primers.

Gene	FW	RV
<i>Actb</i>	TGTTACCAACTGGGACGACA	GGGGTGTGAAGGTCCTCAA
<i>Bace2</i>	TCAATCCCACCCAGGACAAG	ACAAGTGGTCCGGCTAAAGT
<i>Col1a1</i>	ACGTCTGGTGAAGTGGTC	CAGGAAAGCCTCTTCTCCT
<i>Col6a3</i>	GCAATGCATGAGACCCTCTG	ACTCAAGGCCCTCTGACTC
<i>Has1</i>	GGCGAGCACTCACGATCATC	AGGAGTCCATAGCGATCTGAAG
<i>Has2</i>	TGTGAGAGGTTCTATGTGTCCT	ACCGTACAGTCCAATGAGAAGT
<i>Hpse</i>	GACAAGGAACCGACTTCCGAAGAA	CTGTAGAGCATGTCCACTGAGCTT
<i>Hspg2</i>	TACCGGTCATTGAG	AGATCCCGTCCGATTC
<i>Iapp</i>	TGTGCTCTCTGTTGCAATTGA	GGATCCCACGTTGAGATG
<i>Lox</i>	AGCTTGCTTGTGGCCTTCA	CCACAGCATGGACGAATTCA
<i>Mmp2</i>	ACAAGTGGTCCGGCTAAAGT	GTAACACAAGGCTTCATGGGG
<i>Mmp9</i>	GCCGACTTTTGTGGCTTCC	GGTACAAGTATGCCTCTGCCA
<i>Sdc4</i>	CCCTCCCTGAAGTGATTGA	AGTTCTTGGGCTCTGAGG
<i>Sulfatase1</i>	GCCAAGGCCATGATGAG	TTCCACCGCTCTGGCTGACT
<i>Vegfa</i>	ATCTTCAAGCCGCTCTGTGT	TATGTGCTGGCTTGGTGAG

Abbreviations: *Actb*, Beta-actin; *Bace*, beta-secretase; *Col1a1*, collagen type I alpha 1; *Col6a3*, collagen type VI alpha 3; *Has*, Hyaluronan synthase; *Hpse*, heparanase; *Hspg2*, perlecan; *Iapp*, islet amyloid polypeptide; *Lox*, Lysyl oxidase; *Mmp*, matrix metalloproteinase; *Sdc4*, syndecan; *Vegfa*, vascular endothelial growth factor A.

3. Results

3.1. Semaglutide modulates IAPP and BACE2 in pancreatic islets of obese and diabetic mice

The IAPP protein was usually expressed in both control groups (C and CS), as shown by their positive immunoreactions (Fig. 1). The labeling was more prominent in the HF islets (Fig. 1) but was mildly reduced in HFS animals (Fig. 1). Moreover, the immunolabelings are in agreement with the gene expressions of *Iapp* (Fig. 2A) and *Bace2* (beta-site APP-cleaving enzyme 2) (Fig. 1B), whose values in the HFS group were diminished by 40 % in both *Iapp* and *Bace2*, compared to the HF animals.

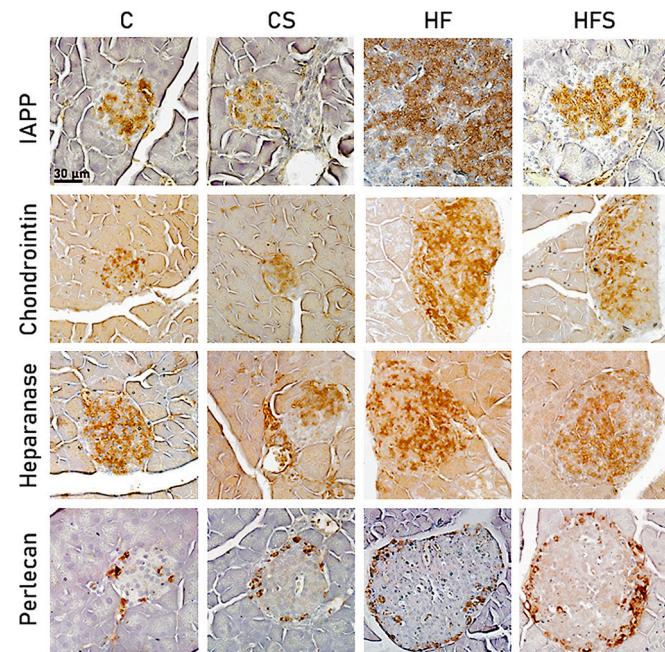


Fig. 1. Immunohistochemistry panel of proteoglycans in the pancreatic islet of obese mice and treatment with semaglutide. Perlecan was identified in the islet crown, while IAPP, chondroitin sulfate, and heparanase were located at the islet core. In general, the islets in the HF group were hypertrophied (photomicrographs were obtained at the same magnification). Groups: C, control diet; HF, high-fat diet; S, semaglutide treatment.

3.2. Semaglutide affects the expression of heparan sulfate-degrading enzymes in pancreatic islets of obese mice

The heparanase immunolabeling (Fig. 1) was similar to the gene expression of heparanase (*Hpse*) (Fig. 2D), with an increase in the HF group but a reduction in the HFS group. In addition, the message for *Sulfatase 1* and *Hpse*, two enzymes involved in the degradation of heparan sulfate chains, responded differently to a HF diet. Thus, *Sulfatase 1* diminished (~35 %, Fig. 2C), while *Hpse* increased (+40 %, Fig. 2D) in the HF group compared to the C group. However, the HFS group, compared to the HF group, showed enhanced *Sulfatase 1* (+90 %) and diminished *Hpse* (~40 %). *Hpse* was also reduced in CS vs. C (~70 %).

3.3. Semaglutide improves perlecan immunolabeling and HSPG2 and VEGFa gene expressions in pancreatic islets of obese mice

The immunolabeling of perlecan, a basement membrane proteoglycan, showed a distinctly capillary location in the pancreatic islets (Fig. 1), and as such changes in its expression might be related to angiogenesis. Measuring the gene expression of the VEGF (Vegfa) angiogenic factor and immunolabeling islet capillaries with the CD31 endothelial marker (Fig. 3A–D) confirmed this possibility, and showed that this proteoglycan is located chiefly close to blood vessels. There was a significant decrease in perlecan gene (*Hspg2*) expression in HF vs. C (~60 %), but an increase in CS vs. C (+100 %) and HFS vs. HF (+900 %) (Fig. 3E). *Vegfa* expression had a similar pattern (CS vs. C, +115 %; HFS vs. HF, +420 %) (Fig. 3F), demonstrating a favorable action of Semaglutide in increasing *Hspg2* and *Vegfa*. Altogether these results imply that Semaglutide might have a role in stimulating vessel neoformation.

3.4. Semaglutide modulates the expression of syndecan 4, hyaluronan synthase 1, and hyaluronan synthase 2 in pancreatic islets of obese mice

In addition to perlecan seen in Fig. 3, the expression of syndecan 4 (*Sdc4*), another HSPG, was also addressed. *Sdc4* increased in HF vs. C (+230 %) but was reduced in HFS vs. HF (~65 %) (Fig. 4A). The hyaluronan synthase isoforms 1 (*Has1*, +35 %) and 2 (*Has2*, +345 %) increased in HF vs. C, but decreased in HFS vs. HF (~45 % and ~65 %, respectively). Besides, *Has1* was reduced in CS vs. C (~25 %) (Fig. 4B, C).

3.5. Semaglutide regulates the turnover of other ECM components of the pancreatic islet of obese mice

To determine whether obesity/T2D and the semaglutide treatment would broadly affect the islet ECM or be more selective to the previous

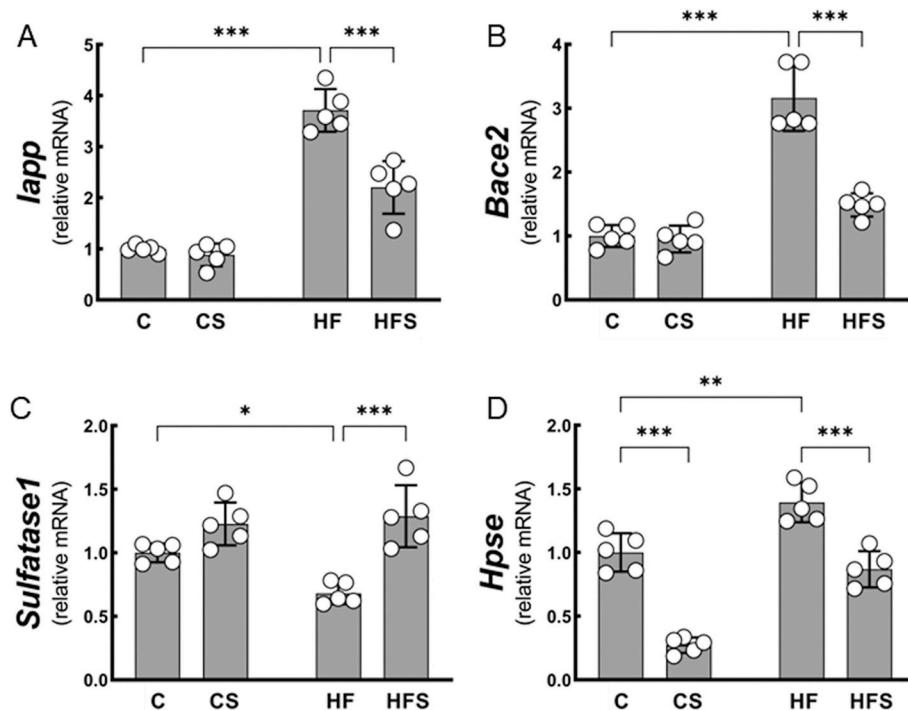


Fig. 2. Relative mRNA expression of *Iapp*, *Bace2*, *Sulfatase1*, and *Hpse* in the pancreatic islet of obese mice and treatment with semaglutide. A - islet amyloid polypeptide, B - beta-cell-enriched beta-site amyloid precursor protein cleaving enzyme 2, C - Sulfatase 1 (E); D - heparanase. Groups: C, control diet; HF, high-fat diet; S, semaglutide treatment. Statistical significance, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

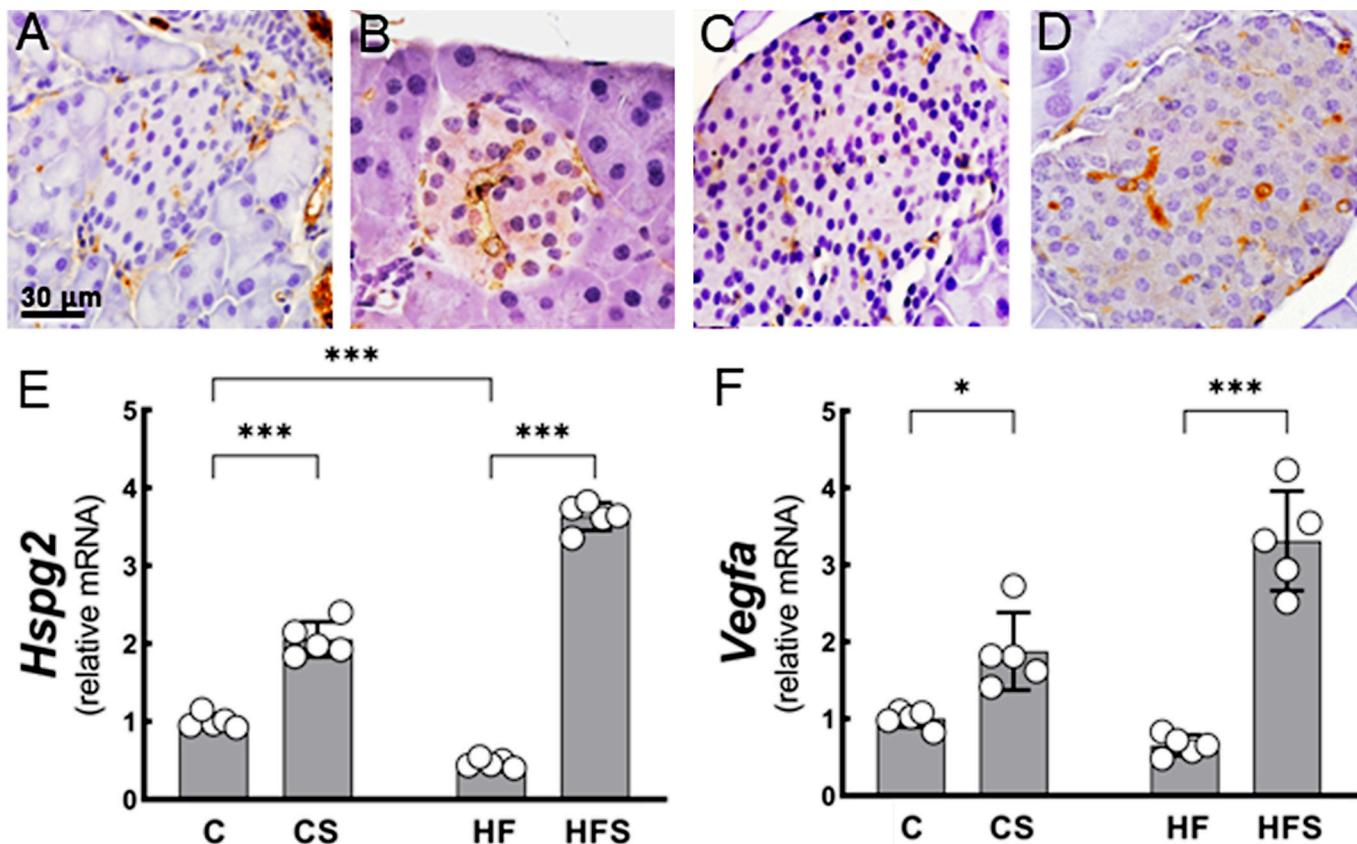


Fig. 3. CD31 immunolabeling and relative mRNA expression of *Hspg2* and *Vegfa* in the pancreatic islet of obese mice and treatment with semaglutide. A-D – representative photomicrographs of CD31 immunolabeling (same magnification): A - group C; B- group CS; C - group HF; D - group HFS; E - perlecan gene expression; F - vascular endothelial growth factor A gene expression. Groups: C, control diet; HF, high-fat diet; S, semaglutide treatment. Statistical significance, * $P < 0.05$; *** $P < 0.001$.

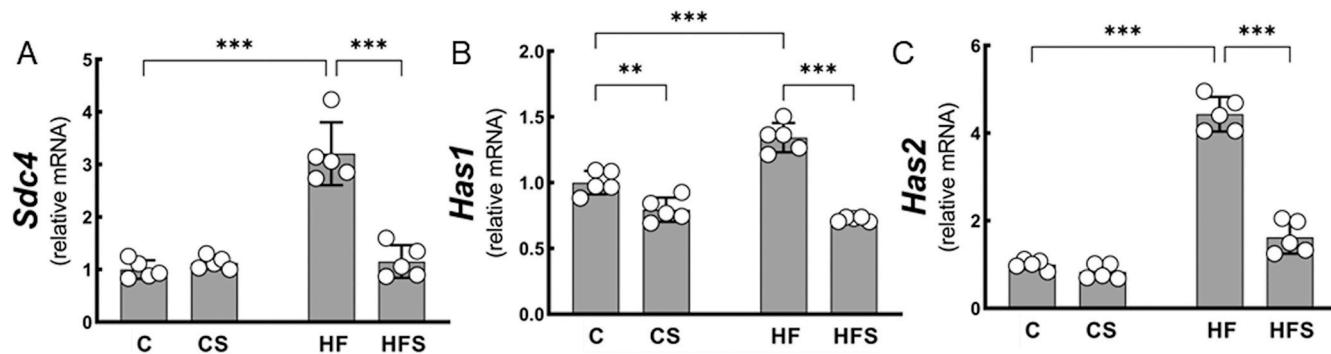


Fig. 4. Relative mRNA expression of Syndecan and hyaluronan synthases and interstitial extracellular matrix components in the pancreatic islet of obese mice and treatment with semaglutide. A - syndecan 4; B - hyaluronan synthase 1; C - hyaluronan synthase 2. Groups: C, control diet; HF, high-fat diet; S, semaglutide treatment. Statistical significance, **P < 0.01; ***P < 0.001.

classes of proteoglycans and GAGs, we analyzed the turnover of other stromal components. For example, we estimated the overall synthesis of chondroitin/dermatan sulfate proteoglycans by immunolabeling the chondroitin sulfate GAG chain (Fig. 1). The labelings showed that these proteoglycans are broadly distributed in the islets, irrespective of their size, and that the staining was enhanced in HF and diminished in HFS (Fig. 1).

The interstitial collagen type I (*Col1a1*, +500 %), the alpha 3 chain of collagen type VI (another major collagenous component of the islet ECM, *Col6a3*, +250 %), and the collagen cross-linking enzyme lysyl oxidase (*Lox*, +340 %) were much enhanced in HF vs. C, but reduced in HFS vs. HF (−60 %, −15 %, and −30 %, respectively) (Fig. 5A–C). Likewise, the collagen-degrading enzymes *Mmp2* (+80 %) and *Mmp9* (+45 %) were increased in HF vs. C but lessened in HFS vs. HF (−45 % and −60 %, respectively) (Fig. 5D, E).

4. Discussion

The mouse model we used replicates many metabolic dysfunctions and pancreatic islet structural changes which occur in obesity and T2D, as shown previously [23,30,31]. In addition, in previous publications, we give details of the metabolic alterations of the animals that received identical diets for the same period of experimentation [21,32]. The study on liver metabolism under obesity and semaglutide treatment [32] included pair-fed control groups, which showed few or none of the metabolic changes we observed in animals treated with the drug. We here used pancreata from these animals, so that the pancreatic effects of semaglutide we report should be independent from weight loss alone.

One possible limitation of this model, however, is that native murine IAPP would not undergo oligomerization and fibrillization [33], which are thought to depend on species-specific motifs in the amino acid sequence of the peptide chain [34]. Consequently, progression of the disease into amyloid-induced beta-cell apoptosis and the resulting

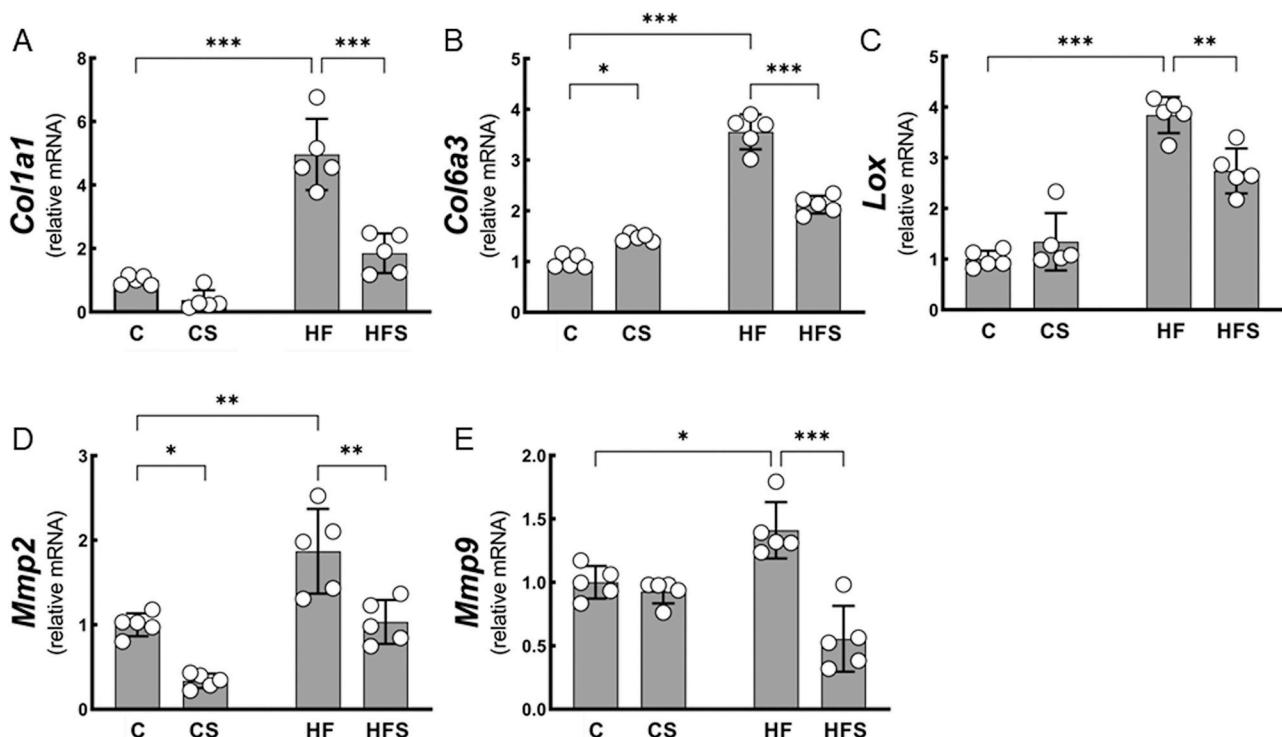


Fig. 5. Relative mRNA expression of interstitial extracellular matrix components in the pancreatic islet of obese mice and treatment with semaglutide. A - collagen I chain alpha 1; F - collagen VI chain alpha 3 (endotrophin); C - lysyl oxidase; D - matrix metalloproteinase 2; E - matrix metalloproteinase 9. Groups: C, control diet; HF, high-fat diet; S, semaglutide treatment. Statistical significance, *P < 0.05; **P < 0.01; ***P < 0.001.

decrease in insulin secretion, which are hallmarks in the pathophysiology of T2D, would be lacking. On the other hand, based on *in vitro* binding kinetics and electron microscopy, it has later been reported that native murine IAPP does form oligomers and amyloid fibrils, although at a slower rate than the human peptide. But most importantly, these murine IAPP aggregates are cytotoxic to the INS-1 pancreatic cell line [35].

Because islet proteoglycans [11–13] and hyaluronan [14] are required for IAPP aggregation, we wondered if semaglutide would affect the turnover of these extracellular molecules in such a way that would impair oligomerization and amyloid fibril formation. Indeed, directly interfering with IAPP fibrillization using pharmacological agents is an approach currently under investigation to treat T2D [5,36]. We thus focused our analyses on the expression of HSPG and hyaluronan, which are known to interact with IAPP. To further implicate these proteoglycans as potential mediators of the beneficial effects of semaglutide on the pancreatic islet, we also evaluated GAG degradative enzymes whose activities would diminish the binding affinity between heparan sulfate chains and IAPP.

Our findings showed that, overall, the turnover of the islet ECM was markedly affected in our animal model. However, semaglutide treatment, which improves various metabolic dysfunctions associated with obesity and T2D in the same model, irrespective of weight loss [21,32], partially or totally reverted these alterations in the islet ECM.

Although the present study did not aim to address the kinetics of amyloid formation, we measured in our groups the availability of the aggregate precursor IAPP in the pancreatic islet. IAPP is a soluble neuroendocrine peptide hormone that is co-secreted with insulin by beta-cells and regulates digestive functions and glucose homeostasis [37]. BACE2, a transmembrane protease expressed by beta-cells, is likewise involved in glucose metabolism [38]. However, the post-translational cleavage of IAPP by BACE2 is an event that contributes to the formation of cytotoxic amyloid deposits in the pancreatic islet [39]. As expected, the expressions of both IAPP and BACE2 were increased in our HF group, which should result not only in greater availability of precursors, but also in favorable conditions for the formation of amyloid aggregates. Of note, semaglutide treatment similarly reduced the expressions of IAPP and BACE2.

In agreement with previous data [40], our results showed that the HSPG perlecan has a limited distribution in the islet, since, as a basement membrane proteoglycan, it is essentially located in the endothelium. The HF diet had little or no effect on perlecan, yet its expression was noticeably increased in semaglutide-treated control and obese animals. This conspicuous capillary location of the perlecan immunolabeling on the mouse islet is in line with previous findings [40,41].

To check if the increase in perlecan expression in HFS and CS would be associated with blood vessel neoformation in the islet, we assessed the message for the angiogenic factor VEGFA and immunolabeled capillaries with the CD31 endothelial marker. VEGFA followed the same pattern as perlecan, with pronounced increases in HFS and CS. At the same time, the staining locations of this proteoglycan and CD31 were similar, which implies that semaglutide affects perlecan expression and angiogenesis. It is noteworthy that, although the incretin hormone GLP-1 has angiogenic properties [42], there are no specific data on such effects concerning the GLP-1 receptor agonist semaglutide. This putative angiogenic effect of semaglutide is however consistent with its cardiovascular protective action [42]. On the other hand, *in vitro* experiments have shown that the chemically similar GLP-1 agonist liraglutide activates, in human endothelial cells, the JAK2/STAT3 signaling pathway, leading to the upregulation of VEGF, basic fibroblast growth factor (bFGF), and endothelial nitric oxide synthase (eNOS), which are known angiogenic factors [43].

Despite an elevated synthesis of perlecan in the islet as a result of the semaglutide treatment, this HSPG would be restricted to capillary basement membranes. In contrast, heparan sulfate chains would greatly increase in the islet of HF animals due to the much enhanced gene

expression of the cell surface HSPG syndecan-4, which is widely distributed in the islet and is expressed mainly by beta-cells in mice [40]. The availability of HS chains would then markedly diminish due to the effects of semaglutide.

To determine whether the effects of semaglutide on HSPGs also included alterations in GAG turnover, we evaluated the expressions of sulfatase 1 and heparanase. These enzymes cleave different domains of the polysaccharide chain and could therefore affect their function and/or interaction with other molecules.

The GAG-degrading enzyme sulfatase 1, a 6-O-endosulfatase, specifically hydrolyzes sulfate ester groups from heparan sulfate, and the resulting under-sulfated chain has decreased binding affinity for ligands such as growth factors and interleukins [44]. Indeed, in the case of lipoproteins, the degree of this affinity is closely associated with the density and position of sulfate groups in the different GAG species [45]. In the presence of heparan sulfate, IAPP behaves similarly, with aggregation and amyloid formation being dependent on the sulfate content of the GAG chain. Accordingly, the binding affinity between IAPP and the GAG chain is higher for heparan sulfate than for chondroitin sulfate or dermatan sulfate [46]. In our HF group, sulfatase 1 expression slightly decreased compared to the controls, which should nonetheless impair base-level desulfation. In contrast, in HFS animals, the expression of sulfatase 1 was greatly enhanced, which should result in a higher content of undersulfated heparan sulfate chains in the pancreatic islet. These chains should bind less avidly to IAPP, and as a consequence, smaller amounts of toxic oligomers and amyloid would form in the islet.

Heparanase is an *endo*- β -D-glucuronidase that degrades heparan sulfate of both cell surface and interstitial proteoglycans, and its activity also affects the interaction between the GAG chain and soluble factors, including inflammatory mediators [47]. However, in the pancreatic islet, its role in cell function and dysfunction is variable. Thus, in isolated islets from transgenic mice overexpressing heparanase, heparan sulfate chains had reduced length and the amount of amyloid deposits was decreased [11]. On the other hand, a study using a mouse model of type 1 diabetes showed that the maintenance of normal beta-cell function depends on islet heparan sulfate chains, and that loss of these cells in diabetic animals was accompanied by higher heparanase expression. Notably, inhibiting heparanase activity with specific blockers prevented this damage to islet cells [48]. Our results are in line with these latter findings, as increased heparanase expression in the HF group was associated with a T2D metabolic profile. Our findings are also consistent with data showing that the upregulation of heparanase is linked with the hyperglycemic state and with an enhanced inflammatory response in humans and rodents [49]. Semaglutide treatment, however, reduced heparanase expression, which was accompanied by an attenuation of these adverse metabolic alterations. In other words, by reducing heparan sulfate enzymatic cleavage, semaglutide should diminish islet cell damage and restore normal beta-cell function.

In addition to specifically interacting with IAPP and promoting the deposition of cytotoxic amyloid fibrils in the islet and nervous tissue, as discussed herein, it has been recently reported that GAG chains of cell surface proteoglycans can also trap soluble IAPP close to the plasma membrane, which is damaging to the cell [10]. Therefore, by modulating the degradation and/or lessening the synthesis of GAG chains and IAPP, semaglutide would have this additional protective effect on islet cells, which again is consistent with its pharmacological effects on T2D.

Hyaluronan is a ubiquitous component of the ECM, having various functions related to cell migration and inflammation [50]. Existing physiologically as a free GAG chain, hyaluronan is synthesized by cell membrane-bound hyaluronan synthase enzymes [51]. In the healthy mouse pancreatic islet, hyaluronan is located mostly at the periphery and around blood vessels [52]. Our results showed that the gene expression of the two hyaluronan synthase isoforms was increased in HF animals, which should be due to an enhanced expression of inflammatory mediators [21]. There is in addition evidence that the accumulation of hyaluronan in the islet colocalizes with IAPP deposits, which suggests

that this GAG is also involved in IAPP aggregation and amyloid formation [14]. By restoring the expression of hyaluronan synthases 1 and 2 in HF animals to normal values, semaglutide should diminish the hyaluronan concentration in the islet, thereby reducing IAPP aggregation.

In short, the above results indicate that, in semaglutide-treated obese animals, a moderately reduced availability of IAPP together with marked changes in the turnover of HSPGs, heparan sulfate chains, and hyaluronan should lead, in the pancreatic islet, to lesser amounts of amyloid deposits and better cell-protective effects.

We showed previously that inflammatory factors such as IL-1 beta, IL-6, and TNF-alpha are increased in the pancreatic islets of obese mice and that semaglutide restored their values to those of controls [21]. Many of these factors, however, differentially modulate the expression of ECM components, including HSPGs and GAGs [53]. Thus, to determine whether the effects of semaglutide on islet ECM would be specific to these proteoglycans and GAGs, or would also affect other components of the islet stroma, we investigated the expression of CSPG, collagen types I and VI, lysyl oxidase, MMP2, and MMP9.

The CSPGs secreted by islet cells have been identified as decorin, biglycan [12], and versican [52]. The anti-chondroitin sulfate antibody reacts with these proteoglycans, and our immunolabelings were conspicuous in all groups. This is consistent with these previously reported *in vitro* findings, in which CSPGs represent an appreciable portion of the total islet proteoglycans. Further, the staining pattern in the animal groups approximately followed the gene expressions of collagen I and lysyl oxidase, so that there were increases in the HF group, with its heightened inflammatory responses, which is in agreement with the roles these interstitial proteoglycans play in collagen fibrillogenesis during connective tissue remodeling [54]. The metalloproteinases MMP2 and MMP9, known as gelatinases, degrade several ECM components during connective tissue remodeling and inflammation, and both are expressed in the pancreas [55]. As expected, their expression was enhanced in the HF group, but semaglutide suppressed this effect.

In adipose tissues, the alpha 3 chain of collagen type VI may undergo proteolytic cleavage by the action of MMP2 and MMP9 [56], generating a biologically active peptide named endotrophin [57,58]. Expression of endotrophin is enhanced in obese individuals, and in the adipose tissue it promotes fibrosis, inflammation, and insulin resistance, while in tumors and hepatic diseases it is implicated in stromal enlargement [59]. Although collagen type VI is abundant in the pancreatic islet, particularly at the exocrine-endocrine interface and close to capillaries [60], the presence of endotrophin has not yet been shown in this tissue. Nevertheless, the decrease in the expressions of islet collagen VI-alpha 3, MMP2, and MMP9 following the semaglutide treatment is consistent with the beneficial effects of this drug on diabetes, given how endotrophin affects the adipose tissue.

These results concerning collagen types I and VI, and associated enzymes for their synthesis and degradation, therefore imply that semaglutide broadly affects the turnover of the islet ECM, which should underlie the structural and functional improvements in the pancreatic islet we previously reported [21].

Although the message for the various proteins provided compelling evidence for the modulation of islet ECM turnover by semaglutide, protein expression was analyzed immunohistochemistry, and not Western blotting, as the latter often yields less reliable quantitative results [61]. This is nonetheless a limitation of the present study.

5. Conclusions

In conclusion, semaglutide changed the turnover of islet HSPGs, their heparan sulfate side chains, and hyaluronan. In addition, the turnover of CSPGs and collagens in the islet ECM was likewise improved. Overall, such changes not only contribute to restoring a healthy islet functional milieu, but should also reduce the formation of amyloid deposits and the ensuing endocrine-cell damage. Our findings also provide additional evidence for the involvement of islet proteoglycans and GAGs in the

pathophysiology of T2D.

CRediT authorship contribution statement

LEMC, TSM, FFM, MBA, performed research and analyzed data; LEMC, MBA, TSM, FFM, wrote the paper; CAML, designed research, analyzed data, and project administration, supported the research, and edited the manuscript's final version. All authors discussed the results, commented on the manuscript, and approved the article's final version.

Declaration of competing interest

The authors declare no competing interests.

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References

- [1] R.N. Wang, L. Rosenberg, Maintenance of beta-cell function and survival following islet isolation requires re-establishment of the islet-matrix relationship, *J. Endocrinol.* 163 (2) (1999) 181–190, <https://doi.org/10.1677/joe.0.1630181>.
- [2] J.C. Stendahl, D.B. Kaufman, S.I. Stupp, Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation, *Cell Transplant.* 18 (1) (2009) 1–12, <https://doi.org/10.3727/096368909788237195>.
- [3] M.T. Adams, B. Blum, Determinants and dynamics of pancreatic islet architecture, *Islets* 14 (1) (2022) 82–100, <https://doi.org/10.1080/19382014.2022.2030649>.
- [4] S. Vigier, H. Gagnon, K. Bourgade, K. Klarskov, T. Fulop, P. Vermette, Composition and organization of the pancreatic extracellular matrix by combined methods of immunohistochemistry, proteomics and scanning electron microscopy, *Curr. Res. Transl. Med.* 65 (1) (2017) 31–39, <https://doi.org/10.1016/j.retram.2016.10.001>.
- [5] Y. Shi, Y.Z. Zhao, Z. Jiang, Z. Wang, Q. Wang, L. Kou, et al., Immune-protective formulations and process strategies for improved survival and function of transplanted islets, *Front. Immunol.* 13 (2022), 923241, <https://doi.org/10.3389/fimmu.2022.923241>.
- [6] D.M. Tremmel, J.S. Odorico, Rebuilding a better home for transplanted islets, *Organogenesis* 14 (4) (2018) 163–168, <https://doi.org/10.1080/15476278.2018.1517509>.
- [7] D.M. Tremmel, S.D. Sackett, A.K. Feeney, S.A. Mitchell, M.D. Schaid, E. Polyak, et al., A human pancreatic ECM hydrogel optimized for 3-D modeling of the islet microenvironment, *Sci. Rep.* 12 (1) (2022) 7188, <https://doi.org/10.1038/s41598-022-11085-z>.
- [8] M. Bogdani, E. Korpos, C.J. Simeonovic, C.R. Parish, L. Sorokin, T.N. Wight, Extracellular matrix components in the pathogenesis of type 1 diabetes, *Curr. Diab. Rep.* 14 (12) (2014) 552, <https://doi.org/10.1007/s11892-014-0552-7>.
- [9] C.J. Simeonovic, S.K. Popp, L.M. Starrs, D.J. Brown, A.F. Ziolkowski, B. Ludwig, et al., Loss of intra-islet heparan sulfate is a highly sensitive marker of type 1 diabetes progression in humans, *PLoS One* 13 (2) (2018), e0191360, <https://doi.org/10.1371/journal.pone.0191360>.
- [10] N. Quittot, M. Fortier, M. Babych, P.T. Nguyen, M. Sebastian, S. Bourgault, Cell surface glycosaminoglycans exacerbate plasma membrane perturbation induced by the islet amyloid polypeptide, *FASEB J.* 35 (2) (2021), e21306, <https://doi.org/10.1096/fj.202001845R>.
- [11] M.E. Oskarsson, K. Singh, J. Wang, I. Vladavsky, J.P. Li, G.T. Westermark, Heparan sulfate proteoglycans are important for islet amyloid formation and islet amyloid polypeptide-induced apoptosis, *J. Biol. Chem.* 290 (24) (2015) 15121–15132, <https://doi.org/10.1074/jbc.M114.631697>.
- [12] S. Potter-Perigo, R.L. Hull, C. Tsoli, K.R. Braun, S. Andrikopoulos, J. Teague, et al., Proteoglycans synthesized and secreted by pancreatic islet beta-cells bind amylin, *Arch. Biochem. Biophys.* 413 (2) (2003) 182–190, [https://doi.org/10.1016/s0003-9861\(03\)00116-4](https://doi.org/10.1016/s0003-9861(03)00116-4).
- [13] A. Abedini, S.M. Tracz, J.H. Cho, D.P. Raleigh, Characterization of the heparin binding site in the N-terminus of human pro-islet amyloid polypeptide: implications for amyloid formation, *Biochemistry* 45 (30) (2006) 9228–9237, <https://doi.org/10.1021/bi0510936>.
- [14] R.L. Hull, M. Bogdani, N. Nagy, P.Y. Johnson, T.N. Wight, Hyaluronan: a mediator of islet dysfunction and destruction in Diabetes? *J. Histochem. Cytochem.* 63 (8) (2015) 592–603, <https://doi.org/10.1369/0022155415576542>.

- [15] J.J. Aguilera, F. Zhang, J.M. Beaudet, R.J. Linhardt, W. Colon, Divergent effect of glycosaminoglycans on the in vitro aggregation of serum amyloid a, *Biochimie* 104 (2014) 70–80, <https://doi.org/10.1016/j.biochi.2014.05.007>.
- [16] A. Zbinden, M. Urbanczyk, S.L. Layland, L. Becker, J. Marzi, M. Bosch, et al., Collagen and endothelial cell coculture improves beta-cell functionality and rescues pancreatic extracellular matrix, *Tissue Eng. A* 27 (13–14) (2021) 977–991, <https://doi.org/10.1089/ten.TEA.2020.0250>.
- [17] P.A. Gerber, G.A. Rutter, The role of oxidative stress and hypoxia in pancreatic Beta-cell dysfunction in diabetes mellitus, *Antioxid. Redox Signal.* 26 (10) (2017) 501–518, <https://doi.org/10.1089/ars.2016.6755>.
- [18] Y. Zheng, S.H. Ley, F.B. Hu, Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, *Nat. Rev. Endocrinol.* 14 (2) (2018) 88–98, <https://doi.org/10.1038/nrendo.2017.151>.
- [19] M.J. Davies, V.R. Aroda, B.S. Collins, R.A. Gabbay, J. Green, N.M. Maruthur, Management of hyperglycaemia in type 2 diabetes, 2022. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), *Diabetologia* 65 (12) (2022) 1925–1966, <https://doi.org/10.1007/s00125-022-05787-2>.
- [20] M.A. Nauck, D.R. Quast, J.J. Wevers, J.J. Meier, GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art, *Mol. Metab.* 46 (2021), 101102, <https://doi.org/10.1016/j.molmet.2020.101102>.
- [21] T.S. Marinho, F.F. Martins, L.M. Cardoso, M.B. Aguila, C.A. Mandarim-de-Lacerda, Pancreatic islet cells disarray, apoptosis, and proliferation in obese mice. The role of Semaglutide treatment, *Biochimie* 193 (2022) 126–136, <https://doi.org/10.1016/j.biochi.2021.101910>.
- [22] P.H. Reis-Barbosa, T.S. Marinho, C. Matsuura, M.B. Aguila, J.J. de Carvalho, C. A. Mandarim-de-Lacerda, The obesity and nonalcoholic fatty liver disease mouse model revisited: liver oxidative stress, hepatocyte apoptosis, and proliferation, *Acta Histochem.* 124 (7) (2022), 151937, <https://doi.org/10.1016/j.acthis.2022.151937>.
- [23] J.C. Fraulob, R. Ogg-Diamantino, C.F. Santos, M.B. Aguila, C.A. Mandarim-de-Lacerda, A mouse model of metabolic syndrome: insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet, *J. Clin. Biochem. Nutr.* 46 (3) (2010) 212–223, <https://doi.org/10.3164/jcbn.09-83>.
- [24] N. Percie du Sert, V. Hurst, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, et al., The ARRIVE guidelines 2.0: updated guidelines for reporting animal research, *Exp. Physiol.* 105 (9) (2020) 1459–1466, <https://doi.org/10.1113/EP088870>.
- [25] P.G. Reeves, F.H. Nielsen, G.C. Fahey Jr., AIN-93 purified diets for laboratory rodents: final report of the american Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, *J. Nutr.* 123 (11) (1993) 1939–1951, <https://doi.org/10.1093/jn/123.11.1939>.
- [26] M.B. Aguila, F. Ornellas, C.A. Mandarim-de-Lacerda, Nutritional research and fetal programming: parental nutrition influences the structure and function of the organs, *Int. J. Morphol.* 39 (1) (2021) 327–334, <https://doi.org/10.4067/s0717-95022021000100327>.
- [27] R.G. Peterson, C.V. Jackson, K.M. Zimmerman, J. Alsina-Fernandez, M.D. Michael, P.J. Emmerson, et al., Glucose dysregulation and response to common anti-diabetic agents in the FATZ/O/Pco mouse, *PLoS One* 12 (6) (2017), e0179856, <https://doi.org/10.1371/journal.pone.0179856>.
- [28] D.S. Li, Y.H. Yuan, H.J. Tu, Q.L. Liang, L.J. Dai, A protocol for islet isolation from mouse pancreas, *Nat. Protoc.* 4 (11) (2009) 1649–1652, <https://doi.org/10.1038/nprot.2009.150>.
- [29] X. Rao, D. Lai, X. Huang, A new method for quantitative real-time polymerase chain reaction data analysis, *J. Comput. Biol.* 20 (9) (2013) 703–711, <https://doi.org/10.1089/cmb.2012.0279>.
- [30] C.A. Mandarim-de-Lacerda, M. Del Sol, B. Vazquez, M.B. Aguila, Mice as an animal model for the study of adipose tissue and obesity, *Int. J. Morphol.* 39 (6) (2021) 1521–1528, <https://doi.org/10.4067/S0717-95022021000601521>.
- [31] F. Ornellas, I. Karise, M.B. Aguila, C.A. Mandarim-de-Lacerda, Pancreatic islets of langerhans: adapting cell and molecular biology to changes of metabolism, in: J. Faintuch, S. Faintuch (Eds.), *Obesity and Diabetes*, Springer International Publishing, Cham, 2020, pp. 175–190. <https://www.doi.org/>.
- [32] R.M. Pontes-da-Silva, T.S. Marinho, L.E.M. Cardoso, C.A. Mandarim-de-Lacerda, M. B. Aguila, Obese mice weight loss role on nonalcoholic fatty liver disease and endoplasmic reticular stress treated by a GLP-1 receptor agonist, *Int. J. Obes.* 46 (1) (2022) 21–29, <https://doi.org/10.1038/s41366-021-00955-7>.
- [33] A.V. Matveyenko, P.C. Butler, Islet amyloid polypeptide (IAPP) transgenic rodents as models for type 2 diabetes, *ILAR J.* 47 (3) (2006) 225–233, <https://doi.org/10.1093/ilar.47.3.225>.
- [34] J.S. Fortin, M.O. Benoit-Biancamano, Wildlife sequences of islet amyloid polypeptide (IAPP) identify critical species variants for fibrillation, *Amyloid* 22 (3) (2015) 194–202, <https://doi.org/10.3109/13506129.2015.1070824>.
- [35] L.C.S. Erthal, L. Jotha-Mattos, F.A. Lara, S.A. Dos Reis, B.M.O. Pasarelli, C. M. Costa, et al., The toxic nature of murine amylin and the immune responsiveness of pancreatic islet to conformational antibody in mice, *Mol. Cell. Biochem.* 447 (1–2) (2018) 1–7, <https://doi.org/10.1007/s11010-018-3288-x>.
- [36] J.S. Fortin, M.O. Benoit-Biancamano, C.G. R, Discovery of ethyl urea derivatives as inhibitors of islet amyloid polypeptide fibrillation and cytotoxicity, *Can. J. Physiol. Pharmacol.* 94 (3) (2016) 341–346, <https://doi.org/10.1139/cjpp-2015-0204>.
- [37] S. Asthana, B. Mallick, A.T. Alexandrescu, S. Jha, IAPP in type II diabetes: basic research on structure, molecular interactions, and disease mechanisms suggests potential intervention strategies, *Biochim. Biophys. Acta Biomembr.* 1860 (9) (2018) 1765–1782, <https://doi.org/10.1016/j.bbamem.2018.02.020>.
- [38] G. Alcarraz-Vizan, C. Castano, M. Visa, J. Montane, J.M. Servitja, A. Novials, BACE2 suppression promotes beta-cell survival and function in a model of type 2 diabetes induced by human islet amyloid polypeptide overexpression, *Cell. Mol. Life Sci.* 74 (15) (2017) 2827–2838, <https://doi.org/10.1007/s00018-017-2505-1>.
- [39] Y.C. Yen, A.M. Kammeyer, J. Tirlangi, A.K. Ghosh, A.D. Mesecar, A structure-based discovery platform for BACE2 and the development of selective BACE inhibitors, *ACS Chem. Neurosci.* 12 (4) (2021) 581–588, <https://doi.org/10.1021/acscchemneuro.0c00629>.
- [40] J.Y. Cheng, J. Whitelock, L. Poole-Warren, Syndecan-4 is associated with beta-cells in the pancreas and the MIN6 beta-cell line, *Histochem. Cell Biol.* 138 (6) (2012) 933–944, <https://doi.org/10.1007/s00418-012-1004-6>.
- [41] K. Fukuchi, M. Hart, Z. Yan, J.R. Hassell, L. Li, Transgenic mice overexpressing both amyloid beta-protein and perlecan in pancreatic acinar cells, *Histol. Histopathol.* 19 (3) (2004) 845–852, <https://doi.org/10.14670/HH-19.845>.
- [42] M.P. Maskery, C. Holscher, S.P. Jones, C.I. Price, W.D. Strain, C.L. Watkins, et al., Glucagon-like peptide-1 receptor agonists as neuroprotective agents for ischemic stroke: systematic scoping review, *J. Cereb. Blood Flow Metab.* 41 (1) (2021) 14–30, <https://doi.org/10.1177/0271678X20952011>.
- [43] Y. Di, J. He, P. Ma, N. Shen, C. Niu, X. Liu, et al., Liraglutide promotes the angiogenic ability of human umbilical vein endothelial cells through the JAK2/STAT3 signaling pathway, *Biochem. Biophys. Res. Commun.* 523 (3) (2020) 666–671, <https://doi.org/10.1016/j.bbrc.2020.01.004>.
- [44] Y. Takashima, K. Keino-Masu, H. Yoshiro, S. Hara, T. Suzuki, T.H. van Kuppevelt, et al., Heparan sulfate 6-O-endosulfatases, Sulf1 and Sulf2, regulate glomerular integrity by modulating growth factor signaling, *Am. J. Physiol. Ren. Physiol.* 310 (5) (2016) F395–F408, <https://doi.org/10.1152/ajprel.00445.2015>.
- [45] L.E. Cardoso, P.A. Mourao, Glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to plasma LDL, *Arterioscler. Thromb. 14 (1) (1994) 115–124*, <https://doi.org/10.1161/01.atv.14.1.115>.
- [46] G.M. Castillo, J.A. Cummings, W. Yang, M.E. Judge, M.J. Sheardown, K. Rimvall, et al., Sulfate content and specific glycosaminoglycan backbone of perlecan are critical for perlecan's enhancement of islet amyloid polypeptide (amylin) fibril formation, *Diabetes* 47 (4) (1998) 612–620, <https://doi.org/10.2337/diabetes.47.4.612>.
- [47] K.M. Jayatilleke, M.D. Hulett, Heparanase and the hallmarks of cancer, *J. Transl. Med.* 18 (1) (2020) 453, <https://doi.org/10.1186/s12967-020-02624-1>.
- [48] A.F. Ziolkowski, S.K. Popp, C. Freeman, C.R. Parish, C.J. Simeonovic, Heparan sulfate and heparanase play key roles in mouse beta cell survival and autoimmune diabetes, *J. Clin. Invest.* 122 (1) (2012) 132–141, <https://doi.org/10.1172/JCI46177>.
- [49] N. Arfian, W.A.W. Setyaningsih, M.M. Romi, D.C.R. Sari, Heparanase upregulation from adipocyte associates with inflammation and endothelial injury in diabetic condition, *BMC Proc.* 13 (Suppl. 11) (2019) 17, <https://doi.org/10.1186/s12919-019-0181-x>.
- [50] K.T. Dicker, L.A. Gurski, S. Pradhan-Bhatt, R.L. Witt, M.C. Farach-Carson, X. Jia, Hyaluronan a simple polysaccharide with diverse biological functions, *Acta Biomater.* 10 (4) (2014) 1558–1570, <https://doi.org/10.1016/j.actbio.2013.12.019>.
- [51] I. Caon, A. Parnigoni, M. Viola, E. Karousou, A. Passi, D. Vigetti, Cell energy metabolism and hyaluronan synthesis, *J. Histochem. Cytochem.* 69 (1) (2021) 35–47, <https://doi.org/10.1369/0022155420929772>.
- [52] R.L. Hull, P.Y. Johnson, K.R. Braun, A.J. Day, T.N. Wight, Hyaluronan and hyaluronan binding proteins are normal components of mouse pancreatic islets and are differentially expressed by islet endocrine cell types, *J. Histochem. Cytochem.* 60 (10) (2012) 749–760, <https://doi.org/10.1369/0022155412457048>.
- [53] G. Schmidt, F. Sirosi, Y. Anini, L.M. Kauri, C. Gyamera-Acheampong, E. Fleck, et al., Differences of pancreatic expression of 7B2 between C57BL/6J and C3H/HeJ mice and genetic polymorphisms at its locus (Sgne1), *Diabetes* 55 (2) (2006) 452–459, <https://doi.org/10.2337/diabetes.55.02.06.db05-0733>.
- [54] J. Halper, Proteoglycans and diseases of soft tissues, *Adv. Exp. Med. Biol.* 802 (2014) 49–58, https://doi.org/10.1007/978-94-007-7893-1_4.
- [55] G.A. Cabral-Pacheco, I. Garza-Veloz, C. Castruita-De la Rosa, J.M. Ramirez-Acuna, B.A. Perez-Romero, J.F. Guerrero-Rodriguez, et al., The roles of matrix metalloproteinases and their inhibitors in human diseases, *Int. J. Mol. Sci.* 21 (24) (2020), <https://doi.org/10.3390/ijms21249739>.
- [56] W. Jo, M. Kim, J. Oh, C.S. Kim, C. Park, S. Yoon, et al., MicroRNA-29 ameliorates fibro-inflammation and insulin resistance in HIF1alpha-deficient obese adipose tissue by inhibiting endotrophin generation, *Diabetes* 71 (8) (2022) 1746–1762, <https://doi.org/10.2337/db21-0801>.
- [57] Y.S. Lee, The mechanism for adipose endotrophin production, *Diabetes* 71 (8) (2022) 1617–1619, <https://doi.org/10.2337/db22-0008>.
- [58] J. Oh, C.S. Kim, M. Kim, W. Jo, Y.H. Sung, J. Park, Type VI collagen and its cleavage product, endotrophin, cooperatively regulate the adipogenic and lipolytic capacity of adipocytes, *Metabolism* 114 (2021), 154430, <https://doi.org/10.1016/j.metabol.2020.154430>.
- [59] L. Williams, T. Layton, N. Yang, M. Feldmann, J. Nanchahal, Collagen VI as a driver and disease biomarker in human fibrosis, *FEBS J.* 289 (13) (2022) 3603–3629, <https://doi.org/10.1111/febs.16039>.
- [60] A. Hughes, C. Jessup, C. Drogemuller, D. Mohanasundaram, C. Milner, D. Rojas, et al., Gene therapy to improve pancreatic islet transplantation for type 1 diabetes mellitus, *Curr. Diabetes Rev.* 6 (5) (2010) 274–284, <https://doi.org/10.2174/157339910793360897>.
- [61] L. Pillai-Kastoori, A.R. Schutz-Geschwend, J.A. Harford, A systematic approach to quantitative Western blot analysis, *Anal. Biochem.* 593 (2020) 113608, <https://doi.org/10.1016/j.ab.2020.113608>.