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Loss of perlecan heparan sulfate glycosaminoglycans lowers body weight and decreases islet amyloid deposition in human islet amyloid polypeptide transgenic mice

Andrew T. Templin[1,†,*], Mahnaz Mellati[1,†], Raija Soininen[2], Meghan F. Hogan[1], Nathalie Esser[1], J. Josh Castillo[1], Sakeneh Zraika[1], Steven E. Kahn[1], and Rebecca L. Hull[1]

[1]Division of Metabolism, Endocrinology and Nutrition, Veterans Affairs Puget Sound Health Care System and University of Washington, 1660 South Columbian Way, Seattle, 98108, Washington, USA [2]Oulu Center for Cell-Matrix Research, Biocenter Oulu and Faculty of Biochemistry and Molecular Medicine, University of Oulu, Pentti Kaiteran Katu 1, Linnanmaa, Oulu, Finland

*To whom correspondence should be addressed. E-mail: templin@uw.edu

†These authors contributed equally to this work

‡PEDS Board Member Responsible for Editing: Daniel Raleigh

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Abstract

Islet amyloid is a pathologic feature of type 2 diabetes (T2D) that is associated with β-cell loss and dysfunction. These amyloid deposits form via aggregation of the β-cell secretory product islet amyloid polypeptide (IAPP) and contain other molecules including the heparan sulfate proteoglycan perlecan. Perlecan has been shown to bind amyloidogenic human IAPP (hIAPP) via its heparan sulfate glycosaminoglycan (HS GAG) chains and to enhance hIAPP aggregation *in vitro*. We postulated that reducing the HS GAG content of perlecan would also decrease islet amyloid deposition *in vivo*. hIAPP transgenic mice were crossed with *Hspg2*$^{\Delta 3/\Delta 3}$ mice harboring a perlecan mutation that prevents HS GAG attachment (hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$), and male offspring from this cross were fed a high fat diet for 12 months to induce islet amyloid deposition. At the end of the study body weight, islet amyloid area, β-cell area, glucose tolerance and insulin secretion were analyzed. hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice exhibited significantly less islet amyloid deposition and greater β-cell area compared to hIAPP mice expressing wild type perlecan. hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice also gained significantly less weight than other genotypes. When adjusted for differences in body weight using multiple linear regression modeling, we found no differences in islet amyloid deposition or β-cell area between hIAPP transgenic and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice. We conclude that loss of perlecan exon 3 reduces islet amyloid deposition *in vivo* through indirect effects on body weight and possibly also through direct effects on hIAPP aggregation. Both of these mechanisms may promote maintenance of glucose homeostasis in the setting of T2D.

Key words: perlecan, heparan sulfate proteoglycan, islet amyloid, type 2 diabetes

Introduction

Islet amyloid deposits are pathologic protein aggregates found in the majority of individuals with type 2 diabetes (T2D) (Clark *et al*., 1988; Westermark, 1972; Hull *et al*., 2004). In humans, the degree of islet amyloid deposition is directly correlated with the rate of β-cell apoptosis and inversely correlated with β-cell mass (Jurgens *et al*., 2011). Islet amyloid polypeptide (IAPP, also known as amylin), a normal β-cell secretory product (Kahn *et al*., 1990), is a unique protein constituent of islet amyloid deposits (Westermark *et al*., 1987; Cooper *et al*., 1987). Human IAPP (hIAPP) and rodent IAPP (rIAPP) differ in a few critical amino acid residues, which results in hIAPP being amyloidogenic and cytotoxic, while rIAPP is not (Westermark *et al*., 1990; Lorenzo *et al*., 1994). Thus, hIAPP transgenic mice have been developed to facilitate *in vitro* and *in vivo* studies of cytotoxic hIAPP aggregation and β-cell loss (D'Alessio *et al*., 1994; Matveyenko and Butler, 2006). Numerous studies have examined approaches to decrease hIAPP aggregation or increase its clearance in order to reduce hIAPP-induced β-cell cytotoxicity in T2D (Meng *et al*., 2010; Zraika *et al*., 2010; Hopping *et al*., 2014; Oskarsson *et al*., 2018).

Relatively few studies have focused on the non-hIAPP components of islet amyloid deposits, such as serum amyloid P (Pepys *et al*., 1994), apolipoprotein E (Chargé *et al*., 1996; Vidal *et al*., 2003) and the heparan sulfate proteoglycan (HSPG) perlecan (Young *et al*., 1992). However, reducing the level of these molecules may be another approach to lessen the degree of amyloid formation and decrease the severity of this pathology. Although other HSPG molecules may play a role, perlecan is well-placed to participate in the process of islet amyloid formation *in vivo* as it is localized to the peri-capillary extracellular matrix in islets (Cross *et al*., 2017; Irving-Rodgers *et al*., 2008), and this is the site of islet amyloid deposition (Hull *et al*., 2004; Verchere *et al*., 1996; de Koning *et al*., 1994). *In vitro* studies have shown perlecan binds amyloidogenic hIAPP, but not non-amyloidogenic rIAPP (Castillo *et al*., 1998; Potter-Perigo *et al*., 2003). This high affinity binding was found to occur through interaction of hIAPP with heparan sulfate glycosaminoglycan (HS GAG) chains attached to the perlecan core protein (Castillo *et al*., 1998; Potter-Perigo *et al*., 2003; Abedini *et al*., 2006; Hull *et al*., 2012) and to result in increased amyloid formation (Castillo *et al*., 1998; Abedini *et al*., 2006; Hull *et al*., 2012). Further, heparin, a naturally occurring glycosaminoglycan, has been shown to increase hIAPP aggregation in cell-free systems and to increase amyloid deposition in hIAPP expressing islets (Potter *et al*., 2015). Conversely, heparinase treatment, which reduces HS content (Potter *et al*., 2015), and interventions that block HS synthesis (Hull *et al*., 2007; Oskarsson *et al*., 2015) or increase HS GAG chain degradation (Oskarsson *et al*., 2015) have been shown to decrease islet amyloid formation in cultured islets. Additionally, it has been shown that HS deficient cells are protected from hIAPP-induced cell death (Oskarsson *et al*., 2015). However, no studies to date have determined whether decreased expression of perlecan HS GAGs can reduce islet amyloid deposition *in vivo*.

Several mice with deletion mutations at different sites in the perlecan gene (*Hspg2)* have been developed. Deletion of either exon 6 or exon 7 prevents perlecan core protein formation, and mice homozygous for either of these mutations demonstrate embryonic or early neonatal lethality (Arikawa-Hirasawa *et al*., 1999; Costell *et al*., 1999), consistent with the critical role of perlecan during embryogenesis and early development. Another mouse model was developed that lacks exon 3 of *Hspg2* ($Hspg2^{\Delta 3/\Delta 3}$), resulting in production of a nearly full-length perlecan core protein deficient in HS GAG attachment sites and loss of HS GAG chains on the perlecan core protein (Rossi *et al*., 2003). In contrast to the other models, homozygous $Hspg2^{\Delta 3/\Delta 3}$ mice survive to adulthood and have no major developmental abnormalities aside from mild ophthalmic deformities and some changes in their skin and cartilage (Walz et al., 1997; Rossi et al., 2003; Shu et al., 2016). With the knowledge that perlecan HS GAGs enhance hIAPP fibril formation *in vitro (Castillo et al., 1998; Abedini et al., 2006)*, and that they are a component of islet amyloid *in vivo (Young et al., 1992; Kahn et al., 1999)*, we generated mice with β-cell hIAPP expression and the $Hspg2^{\Delta 3/\Delta 3}$ mutation (hIAPP;$Hspg2^{\Delta 3/\Delta 3}$) to examine for the first time whether loss of perlecan HS GAGs reduces islet amyloid formation and β-cell loss *in vivo*.

Materials and Methods

Animals

Homozygous $Hspg2^{\Delta 3/\Delta 3}$ mice on a C57BL/6 background were obtained from Dr. Karl Tryggvason (Karolinska Institutet, Stockholm) and bred with hemizygous hIAPP mice ($hIAPP^{+/0}$) on a DBA/2 background at the VA Puget Sound Health Care System (VAPSHCS). hIAPP transgene expression was driven by the rat insulin II promoter to express hIAPP specifically in β cells. This initial cross generated heterozygous $Hspg2^{+/\Delta 3}$ mice with and without the hIAPP transgene. The $hIAPP^{+/0}$;$Hspg2^{+/\Delta 3}$ animals were then crossed to produce siblings harboring the four study genotypes: wild type, WT ($hIAPP^{0/0}$; $Hspg2^{+/+}$, $n = 12$), $Hspg2^{\Delta 3/\Delta 3}$ ($hIAPP^{0/0}$;$Hspg2^{\Delta 3/\Delta 3}$, $n = 10$), hIAPP ($hIAPP^{+/0}$;$Hspg2^{+/+}$, $n = 10$) and hIAPP;$Hspg2^{\Delta 3/\Delta 3}$ ($hIAPP^{+/0}$;$Hspg2^{\Delta 3/\Delta 3}$, $n = 6$). All mice underwent pancreas histology evaluation, glucose tolerance testing and body weight determination. Mice were housed and studied at VAPSHCS following approval by the Institutional Animal Care and Use Committee.

Genotyping

Presence of the hIAPP transgene was determined by polymerase chain reaction of tail DNA using specific primers as previously described (Andrikopoulos *et al*., 2000). For $Hspg2^{\Delta 3}$ mice, the wild type *Hspg2* gene was amplified with a 5′-GGG GAC ACT TGT CAT CCT CT-3′ sense primer and an 5′-GCC GAG GCC ATC TGC AAG AA-3′ antisense primer. The mutant $Hspg2^{\Delta 3}$ allele was amplified with a 5′-TGT CAT CTC ACC TTG CTC CTG-3′ sense primer and a 5′-TCA AGA AGG CGA TAG AAG GCG-3′ antisense primer.

In vivo studies

We previously showed that 81% of male hIAPP transgenic mice develop amyloid *in vivo* compared to only 11% of females (Verchere *et al*., 1996). Therefore, only male mice were used in this study. At 8–10 weeks of age, male mice from all four study genotypes were placed on a high fat diet (HFD) containing 45% of calories from fat, 35% from carbohydrates and 20% from protein (D12290; Research Diets, New Brunswick, NJ). Mice were fed HFD for 1 year to induce amyloid formation *in vivo* as we have done previously (Hull *et al*., 2003). Body weight was determined at the beginning and the end of the study.

IPGTT, glucose and insulin assays

After 12 months of HFD feeding, mice were fasted overnight and then anesthetized with sodium pentobarbital. A baseline blood sample was

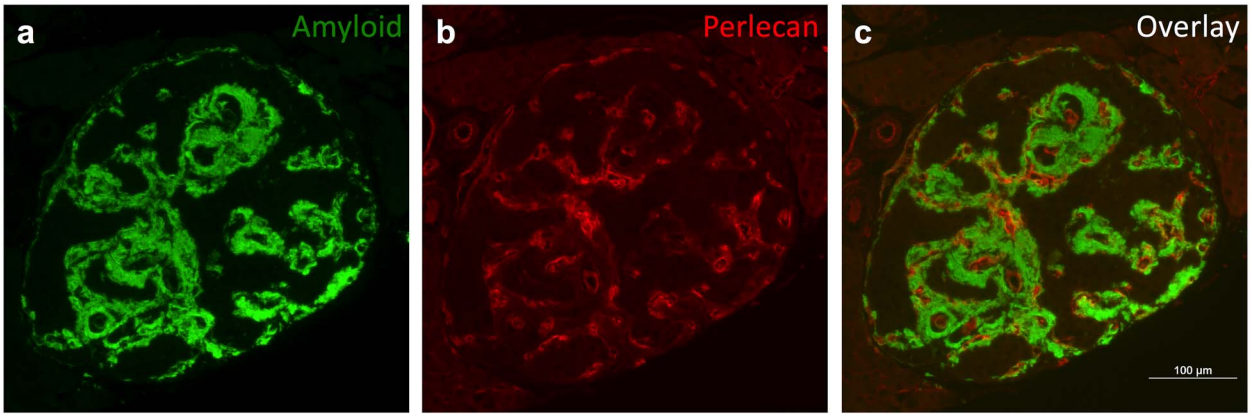

Fig. 1 Perlecan colocalizes with islet amyloid deposits in hIAPP transgenic mice a) islet amyloid deposits were visualized in hIAPP transgenic mouse pancreata by thioflavin S staining (green) following 1 year of HFD feeding. b) Perlecan immunostaining (red) is present in the islet peri-capillary space, and c) colocalizes with amyloid deposits in hIAPP transgenic mouse islets. Representative images are shown. Scale bar equals 100 µm

taken via the retro-orbital sinus and an intraperitoneal glucose bolus (1 g/kg body weight) was administered. Blood samples were then drawn at 5, 15, 30, 60, 90 and 120 min after glucose administration. Centrifugation for plasma separation was then performed and samples were stored at −20°C for subsequent analysis.

Plasma glucose concentration was determined using a plate based colorimetric assay utilizing the glucose oxidase method, and plasma insulin was measured using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay (Alpco, Salem, NH) (Aston-Mourney *et al*., 2013).

Histology

Pancreata were fixed in 4% (wt/vol) phosphate-buffered paraformaldehyde, processed and embedded in paraffin. Four micrometer pancreas sections were cut and stained with insulin antibody (I-2018, 1:2000, Sigma-Aldrich, St. Louis, MO) followed by Cy3-conjugated anti-mouse IgG to visualize β cells, and thioflavin S (T-1892, diluted 0.5% wt/vol; Sigma-Aldrich, St. Louis, MO) counterstain to visualize amyloid deposits (Hull *et al*., 2003; Aston-Mourney *et al*., 2013; Wang *et al*., 2001). Perlecan was visualized with an antibody against perlecan core protein (ab2501, 1:150, Abcam, Cambridge, MA) followed by Cy3-conjugated anti-rat IgG. Histological assessment was performed using an epifluorescence microscope (Eclipse NiE or Eclipse E800; Nikon, Japan), and images were analyzed using computer-based quantitative imaging software (NIS Elements, Nikon). Islet area, thioflavin S-positive (amyloid) and insulin-positive (β cell) areas were determined as described previously (Hull *et al*., 2003; Aston-Mourney *et al*., 2013; Wang *et al*., 2001). The number of islets analyzed for each group is as follows [mean ± SEM, range]: WT, 38.1 ± 3.3, 24–59; *Hspg2*$^{\Delta 3/\Delta 3}$, 46.8 ± 2.2, 34–58; hIAPP, 40.7 ± 5.9, 23–71; hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$, 38.7 ± 6.1, 23–61.

Calculations and statistical analysis

Amyloid prevalence was defined as the proportion of islets per mouse containing amyloid. Amyloid severity was defined as (Σ amyloid area/Σ islet area) × 100% for all islets from each mouse (Hull *et al*., 2003; Aston-Mourney *et al*., 2013; Wang *et al*., 2001). β-cell area was calculated as (Σ β-cell area/Σ islet area) × 100% for each mouse. Plasma glucose and insulin concentrations during the intraperitoneal glucose tolerance test (IPGTT) were analyzed by general linear model. β-cell secretory function was assessed by calculating the change in insulin from time 0 to 30 min, divided by the change in glucose from time 0 to 30 min. Comparisons of changes between groups were performed by Student's *t*-test for parametric data (body weight), or by Mann–Whitney U test for non-parametric data (amyloid deposition, β-cell area, plasma glucose and plasma insulin), and Bonferroni post-test was applied where appropriate. Multiple linear regression was performed to assess whether changes in the variables of interest (amyloid prevalence, amyloid severity and β-cell area) were related to the observed changes in body weight. Square root transformation was performed to satisfy normality assumptions for linear regression. Data are presented as mean ± SEM with $P \leq 0.05$ was considered significant.

Results

Perlecan is a component of islet amyloid deposits in hIAPP transgenic mice

To confirm that perlecan is a component of islet amyloid deposits in hIAPP transgenic mice *in vivo*, as has been shown in other models, we analyzed islet amyloid deposits in these mice following 1 year of HFD feeding (Fig. 1a). Islet perlecan staining (Fig. 1b) was found to colocalize with amyloid staining (Fig. 1c) in hIAPP mouse islets, again suggesting a role for perlecan HS GAGs in hIAPP aggregation and islet amyloid deposition *in vivo*.

Loss of perlecan heparan sulfate glycosaminoglycans reduces islet amyloid deposition and ameliorates amyloid-induced β-cell loss

In total, 8 out of 10 male hIAPP mice developed islet amyloid after 1 year of HFD feeding, compared with only two out of six hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice (Fig. 2a). As expected, WT and *Hspg2*$^{\Delta 3/\Delta 3}$ mice did not develop islet amyloid since they express mouse IAPP that is not amyloidogenic, with hIAPP mice exhibiting significantly higher islet amyloid prevalence (Fig. 2b) and amyloid severity (Fig. 2c) than WT mice. In contrast, hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice lacking perlecan HS GAGs exhibited significantly lower islet amyloid prevalence (Fig. 2b) and amyloid severity (Fig. 2c) compared to hIAPP mice. Furthermore, β-cell area (Fig. 2d) was decreased in islet amyloid-laden hIAPP compared to WT mice, and this reduction in β-cell area was

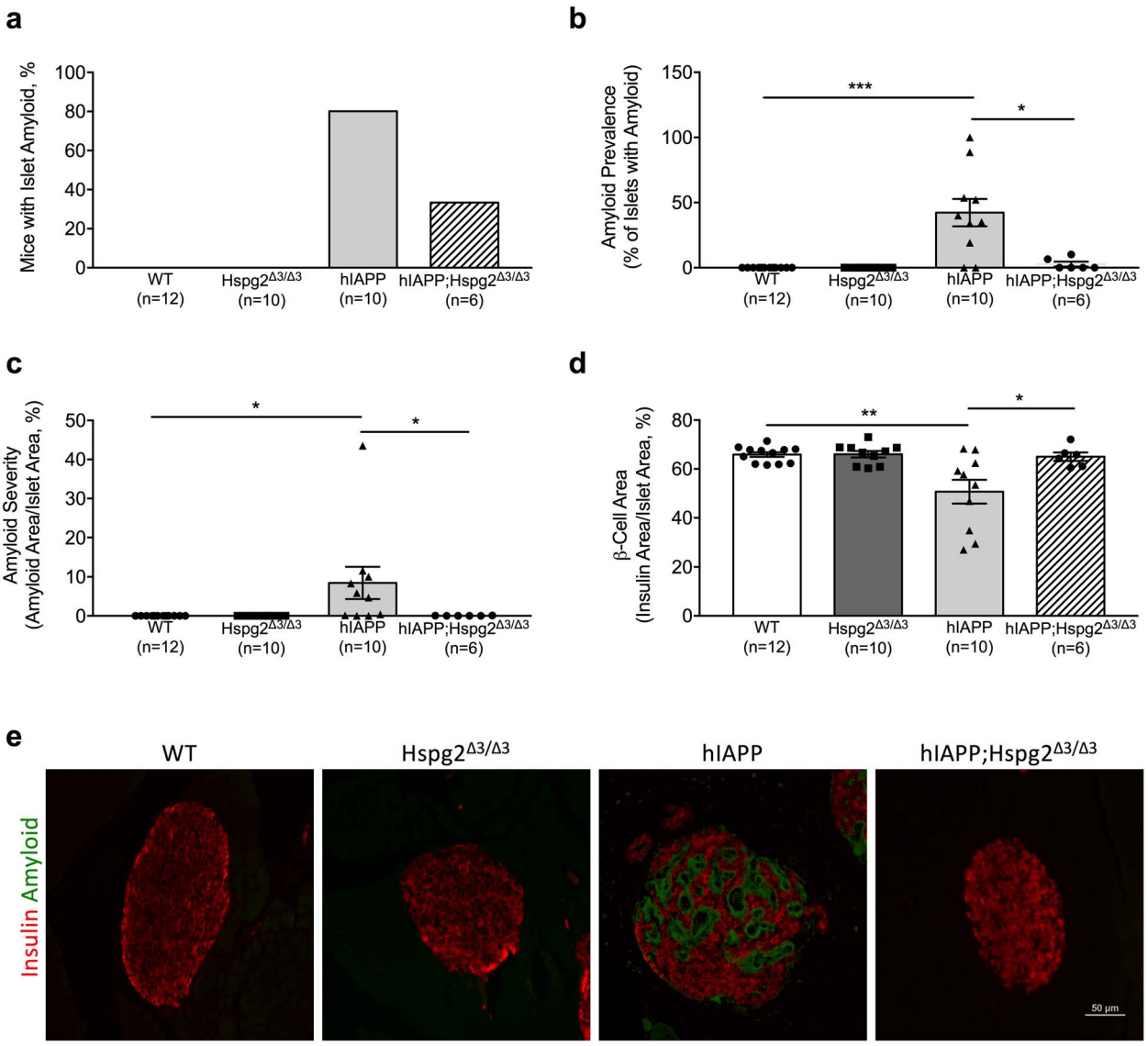

Fig. 2 *Hspg2*$^{\Delta 3/\Delta 3}$ genotype is associated with reduced islet amyloid deposition and β-cell loss in hIAPP transgenic mice a) Expression of perlecan lacking exon 3 reduces incidence of islet amyloid in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ vs hIAPP transgenic mice. b) Islet amyloid prevalence (% of islets containing amyloid) and c) islet amyloid severity (% of islet area occupied by amyloid) were significantly higher in hIAPP mice compared to both WT and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice after 1 year of HFD feeding; $*P < 0.05$, $***P < 0.001$. d) β-cell area was significantly reduced in hIAPP mice compared to WT mice and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice after 1 year of HFD feeding; $*P < 0.05$, $**P < 0.01$. e) Representative images are shown from each genotype (insulin immunostaining, red; amyloid staining with thioflavin S, green). Scale bar equals 50 μm. $n = 6$–12

rescued in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice. In WT and *Hspg2*$^{\Delta 3/\Delta 3}$ mice lacking hIAPP expression, β-cell area was not different, indicating that *Hspg2*$^{\Delta 3/\Delta 3}$deletion alone does not affect β-cell area (Fig. 2d). Representative immunohistochemistry images illustrate the amyloid deposition and reduced β-cell area observed in hIAPP islets (Fig. 2e).

Loss of perlecan heparan sulfate glycosaminoglycans does not alter glucose tolerance or insulin secretion

As expected, hIAPP mice with islet amyloid deposition displayed impaired glucose tolerance (Fig. 3a) and reduced insulin secretion (Fig. 3b and c) in response to an intraperitoneal glucose bolus compared to WT mice. Loss of perlecan HS GAGs did not alter glucose tolerance (Fig. 3a) or insulin secretion (Fig. 3b and c) in the absence of β-cell hIAPP expression. Despite our observation that loss of perlecan HS GAGs reduces amyloid deposition in hIAPP transgenic islets, hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice did not display improved glucose tolerance (Fig. 3a) or insulin secretion (Fig. 3b and c) compared to hIAPP mice.

Loss of perlecan heparan sulfate glycosaminoglycans leads to decreased weight gain in hIAPP mice fed a HFD

At 8–10 weeks of age, body weight was not significantly different among study groups regardless of genotype (Fig. 4a). At the end of the study, however, hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice weighed significantly less than hIAPP mice (Fig. 4b). hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice also exhibited significantly decreased body weight gain in response to HFD feeding compared to hIAPP mice (Fig. 4c).

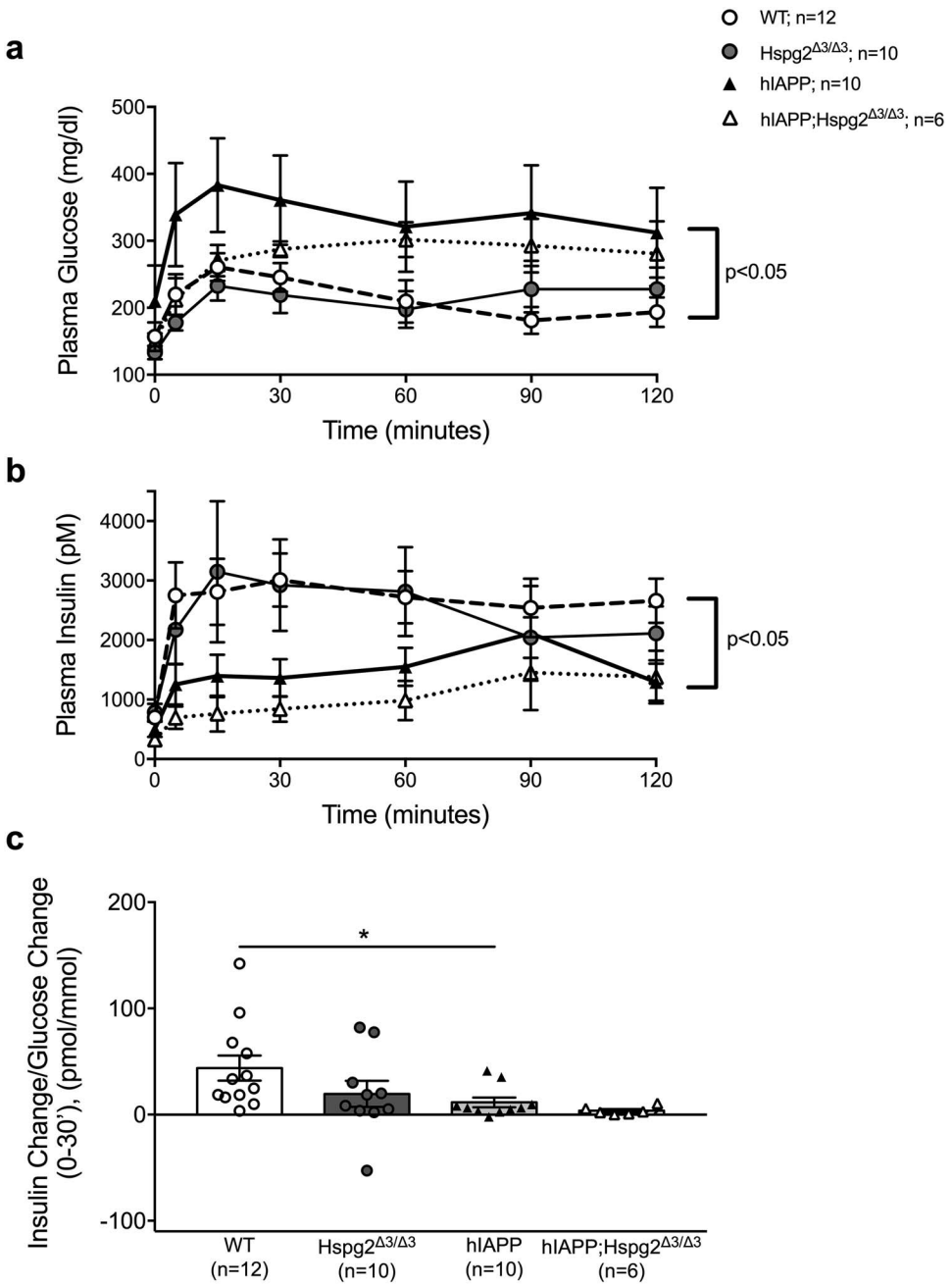

Fig. 3 *Hspg2*$^{\Delta 3/\Delta 3}$ genotype does not alter glucose tolerance or insulin secretion assessed by an IPGTT a) Glucose tolerance in response to intraperitoneal glucose was significantly poorer in hIAPP mice (filled triangle) vs WT mice (open circle) over 120 min; $P < 0.05$. Glucose tolerance was not different between hIAPP mice (filled triangle) and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice (open triangle). b) Insulin response to intraperitoneal glucose was decreased in hIAPP (filled triangle) compared to WT (open circle) mice; $P \leq 0.05$. Insulin response was not different between hIAPP mice (filled triangle) and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice (open triangle. c) Insulin secretion was decreased in hIAPP mice (filled triangle) compared to WT mice (open circle); $*P < 0.05$. Insulin secretion was not different between hIAPP mice (filled triangle) and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice (open triangle). $n = 6$–12

When adjusted for body weight, islet amyloid deposition and β-cell area are not different between groups

Because we observed lower weight gain and final body weight in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ compared to hIAPP mice, we adjusted the associations between genotype and islet morphology parameters for body weight differences using multiple linear regression analysis. After this adjustment, amyloid prevalence, amyloid severity and β-cell areas were no longer significantly different between hIAPP and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice (Table I).

Discussion

The heparan sulfate proteoglycan perlecan is known to be a component of islet amyloid in humans with T2D (Young *et al*., 1992; Kahn *et al*., 1999), and we found this is also the case in islet amyloid

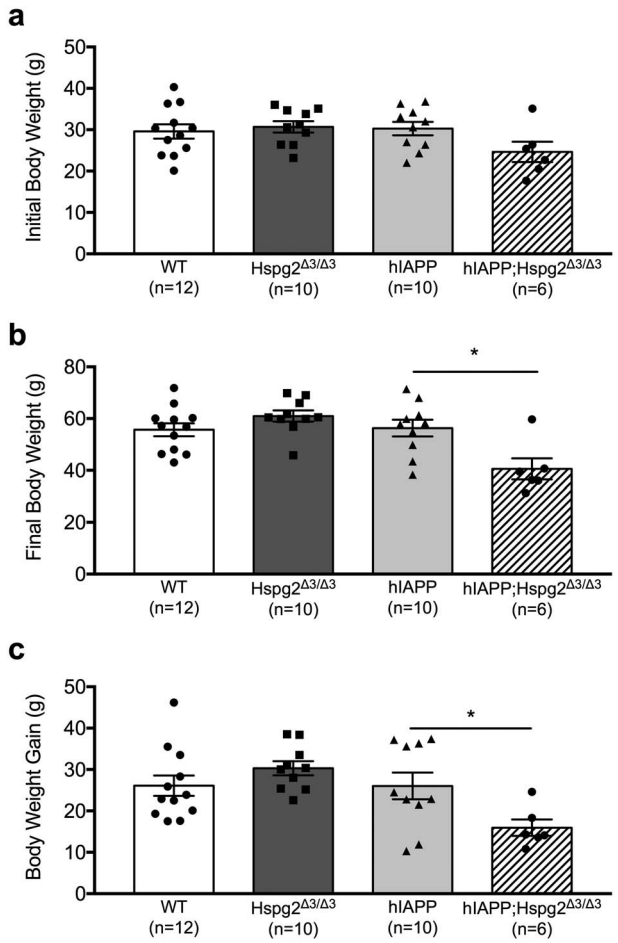

Fig. 4 *Hspg2*$^{\Delta 3/\Delta 3}$ genotype is associated with decreased weight gain in hIAPP transgenic mice fed a HFD a) Body weight was not different among WT, *Hspg2*$^{\Delta 3/\Delta 3}$, hIAPP and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ groups at the beginning of the study. b) Following 1 year of HFD feeding, hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice displayed lower body weight compared to hIAPP mice; $*P < 0.05$. c) hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice also exhibited less weight gain compared to hIAPP mice after 1 year of HFD feeding; $*P < 0.05$. $n = 6$–12

Table I. Unadjusted and body weight-adjusted *P*-values for islet morphological parameters

hIAPP vs hIAPP; *Hspg2*$^{\Delta 3/\Delta 3}$ comparison	Unadjusted *P*-value	Body weight-adjusted *P*-value
Amyloid prevalence	0.01	0.40
Amyloid severity	0.01	0.20
β-Cell area	0.05	0.99

deposits of hIAPP transgenic mice (Fig. 1). Each perlecan molecule contains up to three HS GAG chains attached to its *N*-terminal region (Castillo *et al*., 1998; Abedini *et al*., 2006), and previous work has revealed that HS GAGs enhance hIAPP amyloid formation *in vitro* (Castillo *et al*., 1998; Potter-Perigo *et al*., 2003; Hull *et al*., 2012; Potter *et al*., 2015). In this study, we determined for the first time whether perlecan HS GAG deficiency decreases islet amyloid formation and β-cell loss *in vivo*.

In line with our hypothesis, we found that genetic deletion of the perlecan HS GAG attachment site in hIAPP,*Hspg2*$^{\Delta 3/\Delta 3}$ mice was associated with decreased islet amyloid deposition compared to hIAPP mice expressing wild type perlecan *in vivo*. This finding is consistent with previous *in vitro* work showing that the interaction between hIAPP and perlecan is mediated through HS GAGs (Castillo *et al*., 1998; Potter-Perigo *et al*., 2003) and that inhibition of GAG synthesis decreases islet amyloid deposition (Hull *et al*., 2007). Other work observed decreased islet amyloid deposition *in vitro* in hIAPP transgenic islets with heparinase treatment (Potter *et al*., 2015) or overexpression of heparanase (Oskarsson *et al*., 2015), an endoglycosidase that cleaves HS chains. In the present study, we found that the decreased amyloid deposition observed in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ versus hIAPP mice was also associated with increased β-cell area. This is consistent with the well-known cytotoxic nature of oligomeric hIAPP aggregates, and the inverse relationship between amyloid deposition and β-cell area that has been described in numerous studies and in multiple amyloid-prone species (Jurgens *et al*., 2011; Wang *et al*., 2001; Guardado-Mendoza *et al*., 2009; Howard, 1986). Our data suggest that loss of perlecan HS GAGs reduces the aggregation of hIAPP species into cytotoxic structures, thereby reducing amyloid deposition and ameliorating β-cell loss. It is unlikely that the increase in β-cell area observed in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ compared to hIAPP mice occurred due a direct effect of loss of perlecan HS GAGs on the β cell, as we observed no difference in β-cell area between WT and perlecan HS GAG deficient *Hspg2*$^{\Delta 3/\Delta 3}$ mice.

Consistent with the known association between islet amyloid formation, glucose intolerance and impaired insulin secretion (Jurgens *et al*., 2011; Verchere *et al*., 1996; Hull *et al*., 2003; Howard, 1986; Montane *et al*., 2017; Westwell-Roper *et al*., 2015), we observed glucose intolerance and impaired insulin secretion in islet amyloid-prone hIAPP mice compared to amyloid-free WT mice. However, we found no difference in glucose tolerance or insulin secretion between hIAPP and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice. Given the significant decrease in islet amyloid observed in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice, improvements in these parameters were anticipated in this group. One potential explanation for this finding could be the presence of a hypovolemic response during the IPGTT in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice, which weighed significantly less than their hIAPP littermates and may have resulted in increased hepatic glucose production, decreased insulin secretion, and/or decreased insulin-mediated glucose utilization (Sherwin *et al*., 1980). Our failure to observe improvements in glucose tolerance and insulin secretion in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ compared to hIAPP mice may also be explained by the variability in plasma glucose and insulin observed within groups during the IPGTT.

As discussed, hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice exhibited significantly less weight gain and thus lower body weight compared to other study groups after 1 year of HFD feeding. We did not collect data on food intake or energy expenditure during this study, so the decreased body weight observed in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice could be due to decreased food intake, increased energy expenditure or other factors. This difference in body weight could be due to mutation of the perlecan gene in a tissue other than the pancreas since we used global *Hspg2* exon 3 knockout mice in this study. For example, previous studies have shown that perlecan is required for fibroblast growth factor 2 (FGF2) signaling in the central nervous system (Kerever et al., 2014; Ornitz and Itoh, 2015) and FGF2 levels are positively associated with fat mass (Hao et al., 2006). However, we did not observe decreased body weight in *Hspg2*$^{\Delta 3/\Delta 3}$ compared to WT mice, indicating that

an interaction between hIAPP and loss of perlecan HS GAGs likely underlies this observation.

Interventions that are associated with reduced body weight have previously been shown to decrease β-cell insulin/IAPP secretion and islet amyloid deposition (Hull et al., 2005; Aston-Mourney *et al*., 2013). In this study, the reduced body weight and lower weight gain observed in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice was again associated with reduced islet amyloid deposition. Consistent with a weight driven phenotype, when we adjusted measures of amyloid deposition and β-cell area for body weight using multiple linear regression modeling, we found that the observed differences in islet morphology between hIAPP and hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice were confounded by the lower final body weight observed in hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice. Given that linear regression modeling is a statistical correction, this result suggests that our findings are related to changes in body weight, but does not rule out the possibility that perlecan HS GAGs directly promote hIAPP aggregation *in vivo*.

We believe it is unlikely that the reduced body weight observed in these mice resulted from a toxic effect of combining hIAPP transgene expression and the *Hspg2*$^{\Delta 3/\Delta 3}$ mutation *per se*. Mice with the hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ genetic modification appeared healthy, gained weight during the study (albeit to a lesser extent than the other genotypes), and all hIAPP;*Hspg2*$^{\Delta 3/\Delta 3}$ mice enrolled completed the 1 year *in vivo* study. Additionally, both hIAPP and *Hspg2*$^{\Delta 3/\Delta 3}$ mouse models have previously been crossed with several other genetically altered mouse models without adverse effects (Andrikopoulos et al., 2000; Celie et al., 2007; Wijesekara et al., 2016). This includes the crossing of hIAPP transgenic mice with mice that overexpresses heparanase, resulting in a model similar to the one in the present study in its reduced HS content and hIAPP expression (Oskarsson *et al*., 2015).

In summary, we have shown that loss of perlecan HS GAGs in hIAPP;Hspg2$^{\Delta 3/\Delta 3}$ mice significantly reduces islet amyloid deposition, and that this is accompanied by an increase in β-cell area compared to hIAPP mice. These effects appear to be related to the lower body weight and weight gain observed in hIAPP;Hspg2$^{\Delta 3/\Delta 3}$ mice. Given the known role of HS GAGs to bind hIAPP (Castillo *et al*., 1998; Potter-Perigo *et al*., 2003) and promote amyloid aggregation (Abedini *et al*., 2006; Hull *et al*., 2012), the effects of perlecan HS GAGs on islet amyloid formation *in vivo* may also include direct effects related to hIAPP aggregation in addition to indirect effects related to body weight. Therapeutics targeting these effects may reduce islet amyloid deposition in the setting of T2D.

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Conflict of Interest

The authors declare that they have no relevant conflicts of interest.

Ethical Approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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