Islet amyloid deposits preferentially in the highly functional and most blood-perfused islets

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Abstract

Islet amyloid and beta cell death in type 2 diabetes are heterogeneous events, where some islets are affected early in the disease process, whereas others remain visibly unaffected. This study investigated the possibility that inter-islet functional and vascular differences may explain the propensity for amyloid accumulation in certain islets. Highly blood-perfused islets were identified by microspheres in human islet amyloid polypeptide expressing mice fed a high-fat diet for three or 10 months. These highly blood-perfused islets had better glucose-stimulated insulin secretion capacity than other islets and developed more amyloid deposits after 10 months of high-fat diet. Similarly, human islets with a superior release capacity formed more amyloid in high glucose culture than islets with a lower release capacity. The amyloid formation in mouse islets was associated with a higher amount of prohormone convertase 1/3 and with a decreased expression of its inhibitor proSAAS when compared to islets with less amyloid. In contrast, levels of prohormone convertase 2 and expression of its inhibitor neuroendocrine protein 7B2 were unaltered. A misbalance in prohormone convertase levels may interrupt the normal processing of islet amyloid polypeptide and induce amyloid formation. Preferential amyloid load in the most blood-perfused and functional islets may accelerate the progression of type 2 diabetes.

Key Words
- islet amyloid
- pancreatic islets
- heterogeneity
- blood flow

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Introduction

Wide heterogeneities among and within pancreatic islets have previously been identified. Beta cells vary in size, granularity, proliferation, functionality and maturation (1, 2, 3). Some subsets of beta cells are more adaptable during pregnancy and metabolic challenges, and beta cells have also shown age-dependent variations (4, 5, 6). On an inter-islet level, we have previously described two subpopulations of islets distinguished by their differences in blood supply; one low blood-perfused, low-oxygenated population with dormant metabolic activity (7), and another population with a high blood supply exhibiting superior functionality and beta cell proliferation indexes (8). Noteworthy, the latter population of high-functioning islets exhibited an increased sensitivity to hypoxia and cytokines leading to reduced survival upon stress (9).

Also, islet lesions have been shown to be of a heterogeneous nature and the beta cell loss in type 2 diabetes (T2D) is not consistent between islets or between different parts of the pancreas (10). Islet amyloid, a clinical finding seen in approximately 90% of T2D patients, certainly affects islets in this irregular pattern; some islets may contain large amounts of amyloid in contrast

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to adjacent islets that appear unaffected (11). Aggregates of islet amyloid polypeptide (IAPP) are cytotoxic and associated with beta cell death both in T2D (12) and in islets after their transplantation (13, 14, 15). Many biological and chemical factors have been described to affect the aggregation of IAPP. In T2D, a major trigger for amyloid formation is the increased IAPP production and secretion. *In vitro* studies show that IAPP is co-secreted with insulin, and that an enhanced secretion may induce amyloid. In cultured islets, amyloid deposit formation is glucose dose dependent and high glucose concentrations stimulate the process (16). Other insulin and IAPP secretagogues agents like extendin-4, potassium chloride, leucine, tolbutamide, glutamine or alpha-ketoisocaproic acid also induce amyloid in cultured islets (17, 18). On the other hand, substances that decrease IAPP secretion like diazoxide or somatostatin limited amyloid deposit formation (18). *In vivo*, in transgenic mice overexpressing human IAPP (hIAPP), the overexpression is not enough for amyloid to develop; amyloid will only form if the animals are predisposed with a genetic or environmental trigger like genetic obesity and hypolipidemia (ob/ob), high-fat diet (HFD) or administration of glucocorticoids (19, 20, 21). IAPP itself has a significant role in the peptides misfolding and aggregation. ProIAPP is more prone to form aggregations than mature IAPP and a hypersecretion of proIAPP due to aberrant processing by the prohormones PC1/3 and PC2 may result in misfolding (22, 23). Also, ER stress induced by chronic hyperglycemia or compensatory insulin production, and glycosaminoglycans like heparan sulfates, have been suggested as important factors (23). Heparan sulfate is highly expressed in islets and important for islet function (24), but has also been identified in amyloid plaques in T2D (25). The glycosaminoglycan is located at the basement membrane of the beta cell and can interact with the N-terminal region of human proIAPP/IAPP, which will stabilize the peptide and thus form a nidus for amyloidogenesis (26, 27).

In our previous studies of highly blood-perfused islets, we have showed that these islets, besides an increased capacity for glucose-stimulated insulin release, also had substantial differences in gene expression when compared to other islets, including a higher IAPP gene expression (8). To investigate amyloid content and fibrillogenesis in highly functional islets we used in the present study, a mouse model expressing hIAPP, as well as single human islets cultured in high glucose. We hypothesized that highly functional islets would be more predisposed for amyloid formation.

Materials and methods

Experimental animals

Male mice deficient in mouse IAPP (mIAPP), but expressing hIAPP (mIAPP$^{-/-}$, hIAPP$^{+/+}$) (28) had free access to water and standard chow. Throughout the experimental studies, the animals additionally had free access to lard (HFD) (28). Experiments were performed after short-term (3-months) or long-term (10-months) HFD (28). All experimental procedures were approved by the Ethical Committee at Uppsala University.

Detection of highly blood-perfused islets

The animals were anesthetized with i.p. injections of avertin (Sigma-Aldrich) and placed on a heating pad to maintain body temperature. Polyethylene catheters were inserted into the ascending aorta for continuous blood pressure measurement and subsequent microsphere injection and into the left femoral artery for blood sampling. After the surgical procedure, the animals were allowed to stabilize their blood pressure for 10 min before injection of black (10 µm, E-Z Trac Ultraspheres; IMT, Stason Labs., Irvine, CA, USA) or fluorescent microspheres (10 µm, FluoSpheres Polystyrene Microspheres; Molecular Probes), into the ascending aorta. A reference blood sample was obtained by free flow from the femoral artery. It was collected for 60 s, with a start 5 s before microsphere injection, to enable quantitative determination of blood flow (8, 9). The pancreas was thereafter immediately retrieved for islet isolation or immunohistochemistry.

Islet isolation

Mouse islets were isolated by collagenase digestion, hand-picked and incubated in groups of 100 overnight at 37°C (air/CO_2, 95:5) in 5 mL RPMI-1640 medium (Sigma-Aldrich) supplemented with 2 mmol/L L-glutamine, 11.1 mmol/L glucose, 10% (v/v) fetal calf serum (FCS) and 0.1 mg/mL streptomycin (Roche Diagnostics Scandinavia) (29). The islets were thereafter dichotomously sorted in a fluorescence microscope. Microsphere-containing islets were size-matched to control islets without microspheres from the same mouse (8, 9).

Amyloid evaluation in mouse islets

Islets of animals fed 10-month HFD were following their isolation fixed in 10% (v/v) buffered formalin and

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embedded in paraffin. Consecutive sections (5 µm) were stained for amyloid with Congo red (Sigma-Aldrich) (30). Sections of size-matched islets with and without microspheres were photographed in a fluorescence microscope and the amyloid area was calculated as percentage of the total area of the islet. Image analysis was performed with ImageJ (NIH).

Location of microsphere-containing islets

From 3-month HFD microsphere-injected animals, the pancreas was oriented with a suture in the tail part, immobilized and consecutively sectioned (10 µm) perpendicularly to the longitudinal axis of the pancreas. Microsphere-containing islets were identified in a light microscope after hematoxylin–eosin staining. Whole section scanning of whole pancreases was performed by Aperio Scanscope AT (Leica Biosystems, Nussloch, Germany). The distance from the center of each microsphere- or non-microsphere-containing islet to the pancreatic center, defined as the origo of an image fitting the whole pancreatic section, was calculated with Aperio ImageScope software (Leica Microsystems, Wetzlar, Germany).

Mouse islet glucose-stimulated hormone release

Islets isolated from hIAPP animals fed 3-month HFD were sorted based on their microsphere content or not and incubated at low (1.67 mmol/L) and high glucose (16.7 mmol/L) concentrations for 60 min, respectively (31). The supernatants from both incubations were collected and analyzed for mouse insulin and human IAPP with ELISA (Mercodia, Uppsala, Sweden and EZHA-52K, Merck Millipore, respectively). Stimulation index was calculated accordingly (insulin release in high glucose)/(insulin release in low glucose). The islets were harvested to measure total hormone content and to ensure equal islet size in the groups by DNA measurement (Quant-iT PicoGreen dsDNAassay kit, Molecular Probes).

Mouse islet gene expression

RNA was extracted from isolated islets of animals fed 3-month HFD by the use of RNAeasy Plus Mini Kit (Qiagen) followed by first-strand cDNA synthesis by SuperScript III First-Strand Synthesis SuperMix (Invitrogen). qPCR was performed using a Light Cycler 480t (Roche Diagnostics) and Light Cycler FastStart DNA Master PLUS SYBR Green I kit (Roche Diagnostics). Gene expression was normalized to the reference gene Tata-box-binding protein (TBP). For primer information, see Supplementary Table 1 (see section on supplementary data given at the end of this article).

Prohormone convertase (PC) protein levels in mouse islets

Pancreas sections obtained from animals fed 3-month HFD were incubated with monoclonal mouse PC1/3 and PC2 antibodies (22) together with an insulin antibody (Fitzgerald, Acton, MA, USA). Heat-induced antigen retrieval (20 min) in 0.01 mol/L sodium citrate was performed for PC2 staining only. Fluorescent anti-mouse 488 (1:200, Jackson ImmunoResearch Laboratories) and anti-guinea pig 555 (1:1000 Molecular Probes) were used for primary antibody detection. To quantify PC protein levels on sections, images were acquired using a confocal laser scanner microscope (Zeiss LSM780) and analyzed with ImageJ (NIH).

Heparan sulfate quantification in mouse islets

In pancreases obtained from 3-month HFD animals, heparan sulfate (HS) glycosaminoglycans were identified with Alcian blue CEC staining (0.65 mol/L $MgCl_2$, pH 5.8, Sigma-Aldrich) (32). Reciprocal staining intensity was analyzed with ImageJ (NIH).

Human islets

Human islets from brain-dead, heart-beating donors, provided by the Nordic Network for Clinical Islet Transplantation (22–42 islets/donor, for donor data, Table 1), were individually cultured in CMRL 1066 supplemented with 10% (v/v) FCS, 2 mmol/L L-glutamine, 0.1 mg/mL streptomycin (Roche Diagnostics Scandinavia) and 5.5 or 20 mmol/L glucose. Experiments involving human islets were approved by the regional ethical board in Uppsala, Sweden.

Table 1 Human islet donor characteristics.

Donor	Sex	Age	BMI (kg/m^2)	HbA1C % (mmol/mol)
Donor 1	M	38	41.7	5.2 (33)
Donor 2	F	59	22.0	5.8 (40)
Donor 3	F	73	22.3	5.8 (40)
Donor 4	F	69	23.9	6.5 (47)

Data received from the Nordic Network for Clinical Islet Transplantation. BMI, body mass index; HbA1C, glycated hemoglobin.

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Human islet insulin release

Prior to high glucose culture (day 0), insulin release experiments were performed on individual islets to evaluate their functionality. Islets were pre-incubated in 1.67 mmol/L glucose for 30 min. Glucose-stimulated insulin release of single islets was then determined by incubation of them in 1.67 and 16.7 mmol/L glucose for 60 min, respectively. The supernatants were analyzed by an Ultrasensitive Insulin ELISA (Mercodia). Stimulation index was calculated accordingly: (insulin release in high glucose)/(insulin release in low glucose).

Islet amyloid quantification in human islets

Human islets were cultured individually (~30 islets/donor) in 20 mmol/L glucose for 18 days to stimulate IAPP secretion and subsequent amyloid formation (27). To visualize islet amyloid, the live islets were incubated with the amyloid-specific oligothiophene p-FTAA (30 µmol/L) (33, 34) for 60 min and thereafter imaged in a fluorescence microscope at 488 nm excitation. Image analysis was performed with ImageJ (NIH).

Statistical analysis

All values are given as means ± S.E.M. Parametrical data were analyzed by unpaired or paired Student's *t* test, and the distribution of amyloid between islets were calculated by a chi-square test. Gene expression analysis was performed on the ΔCT values for each gene. $P<0.05$ was considered significant.

Results

Animal characteristics and blood flow

hIAPP$^{+/+}$mIAPP$^{-/-}$ mice increased in weight during HFD treatment and weighed 33 ± 1 g ($n=23$) and 44 ± 1 g ($n=7$) after 3 and 10 months on HFD, respectively. The animals were all normoglycemic (6.7 ± 0.2 mmol/L; $n=30$). In the experiments, each microsphere corresponded to a blood flow of ≥0.21 ± 0.02 µL/min ($n=30$).

Amyloid in mouse islets

After 10-month HFD, amyloid deposits (≥0.1% islet area) were more frequent in islets with microspheres than in those without microspheres ($n=7$, Fig. 1A, B and C). The amyloid deposits were also twice as extensive (percentage of islet area) in the highly blood-perfused islets when compared to size-matched islets without microspheres ($n=7$, Fig. 1D).

Location of highly blood-perfused islets

Islets with microspheres were found throughout the pancreas. Islets with and without microspheres were similarly distributed in the central and peripheral parts of the gland (1.39 ± 0.18 vs 1.34 ± 0.17 mm to center, respectively, $n=4$).

Glucose-stimulated hormone release

No difference in islet size was measured between the investigated islets with and without microspheres (81.2 ± 13.5 vs 70.2 ± 12.1 ng DNA/islet, respectively, $n=6$ animals). There was neither any difference in insulin or IAPP content between the two groups of islets (6218 ± 1136 vs 6891 ± 1817 fmol insulin/islet and 377 ± 80 vs 375 ± 84 fmol IAPP/islet in islets with- and without microspheres, respectively, $n=6$). There was no difference between the two groups of islets in insulin release at a low glucose concentration (1.67 mmol/L), but in response to a high glucose concentration (16.7 mmol/L), the microsphere-containing islets secreted ~30% more insulin than the size-matched islets without microspheres (Fig. 1E). When calculating the glucose stimulation index (ratio of insulin release in high and low glucose concentration), islets with microspheres had a 44% higher ratio ($n=6$, Fig. 1F). No difference was however seen between the groups in IAPP release at either a low or a high glucose concentration (Fig. 1G). Islets with microspheres therefore had a ratio of released IAPP/insulin in response to glucose that was ~30% lower than other islets (Fig. 1H).

Amyloid in human islets

Little or no amyloid (<1% of islet area) was found at day 0 in the islets obtained from four different donors. After functional test of single islets, the islets were cultured individually for 18 days in high glucose to allow amyloid formation. At day 18, the islets had developed amyloid covering ~4% (range 0–30%) of the islet area. The islets were thereafter divided into two groups; islets with little or no (<1% of islet area) amyloid ($n=58$) and islets with amyloid (>1% of islet area) ($n=59$). The group of islets that formed amyloid after 18 days of high glucose culture was

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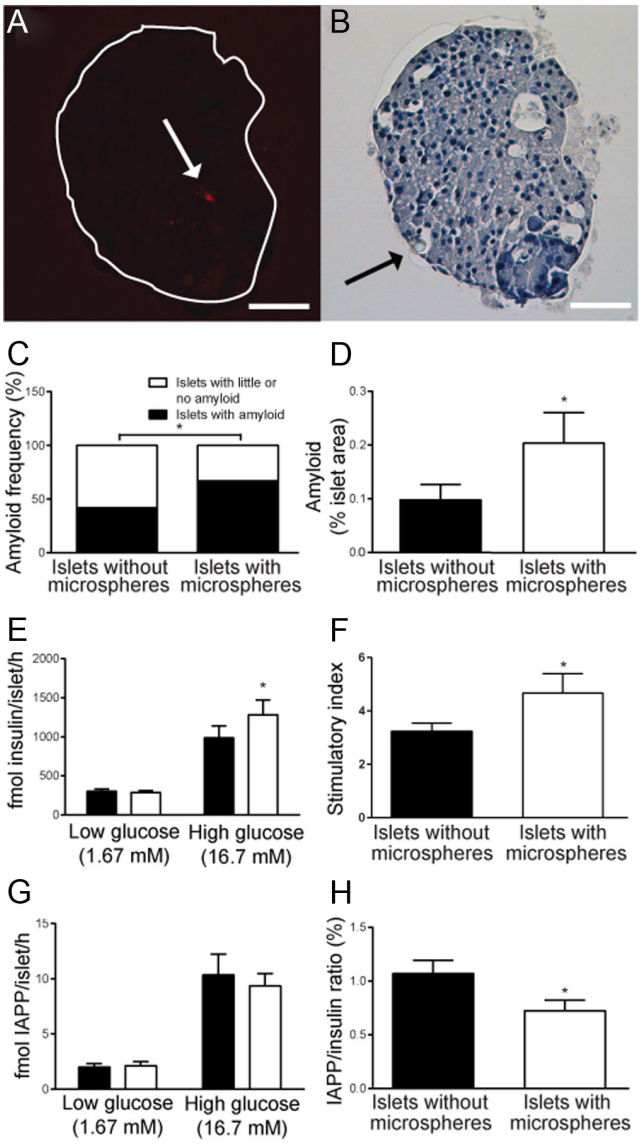

Figure 1
Function and amyloid content in high-fat diet (HFD)- fed mice expressing human islet amyloid polypeptide (hIAPP). Fluorescence (A) and brightfield (B) images of Congo red and hematoxylin-stained islet from mouse after 10-month HFD with amyloid deposit (white arrow) and microsphere (black arrow). Scale bar 50 µm. (C) In the group of islets with microspheres, the frequency of islets containing amyloid (≥0.1% islet area) was higher (closed bar segment) when compared to islets without microspheres. (D) The amyloid deposits were twice as extensive in highly blood-perfused islets (marked with microspheres) (open bar) when compared to size-matched islets without microspheres (closed bar). (E) After feeding of mice for three months with HFD, the glucose-stimulated insulin release was higher from islets with microspheres (open bar) vs control islets (closed bar). (F) Calculated glucose stimulation index was higher in islets with microspheres (open bar) vs control islets (closed bar). (G) There was no difference between control islets (closed bar) and microsphere-containing islets (open bar) with regard to IAPP release in either low (1.67 mmol/L) or high (16.7 mmol/L) glucose concentrations. (H) The ratio of released IAPP to insulin was lower in islets with microspheres when compared to control islets without microspheres. Values are given as means ± S.E.M. for 6 animals. *$P<0.05$ vs islets with microspheres.

found to have an ~50% better glucose-stimulated insulin release when measured prior to the high glucose culture (Fig. 2A). This was observed in islets from all four donors (Fig. 2B). In contrast, there was no visible amyloid in any of the islets before high glucose culture (day 0) (Fig. 2C, D, E, F, G, H, I and J).

ProIAPP processing enzymes

No difference in gene expression of the two prohormone convertases PC1/3 and PC2 ($n=6$, Fig. 3A and B) and the processing enzyme Carboxypeptidase E (CPE) (fold change 0.73 ± 0.20 in islets with microspheres, $n=5$) could be observed between islets with or without microspheres. However, the expression of the neuroendocrine peptide precursor proSAAS was ~50% lower in the microsphere-containing islets ($n=5$, Fig. 3C). 7B2, another neuroendocrine protein, did not differ between the two groups ($n=6$, Fig. 3D). PC1/3 immunofluorescence co-localized with insulin staining and was ~17% higher in islets with microspheres ($n=4$, Fig. 3E, G, H, I, J, K and L). In contrast, the intensity of PC2 staining in insulin-positive cells did not differ between the islets with and without microspheres ($n=4$, Fig. 3F, M, N, O, P, Q and R).

Highly sulfated glycosaminoglycans

The intensity of HS staining did not differ between the islets with or without microspheres ($n=4$, reciprocal intensity 136.6 ± 3.1 vs 131.2 ± 6.2 AU, respectively).

IAPP-degrading enzymes

A higher gene expression of the two matrix metalloproteinases MMP2 and MMP9 was observed in the islets with microspheres ($n=5$–6, Fig. 4A and B). No differences could be detected in the expression of the inhibitors tissue inhibitor of matrix metalloproteinases (TIMP) 1–3 between islet with or without microspheres ($n=6$, Fig. 4C, D and E). There was neither any difference in the expression of insulin-degrading enzyme (IDE) ($n=6$, fold change 1.54 ± 0.51 in islets with microspheres) and neprilysin ($n=4$, fold change 1.22 ± 0.67 in islets with microspheres).

ER stress induced gene expression

Gene expression of C/EBP-homologous protein (CHOP) ($n=5$, fold change 1.21 ± 0.37 in islets with microspheres)

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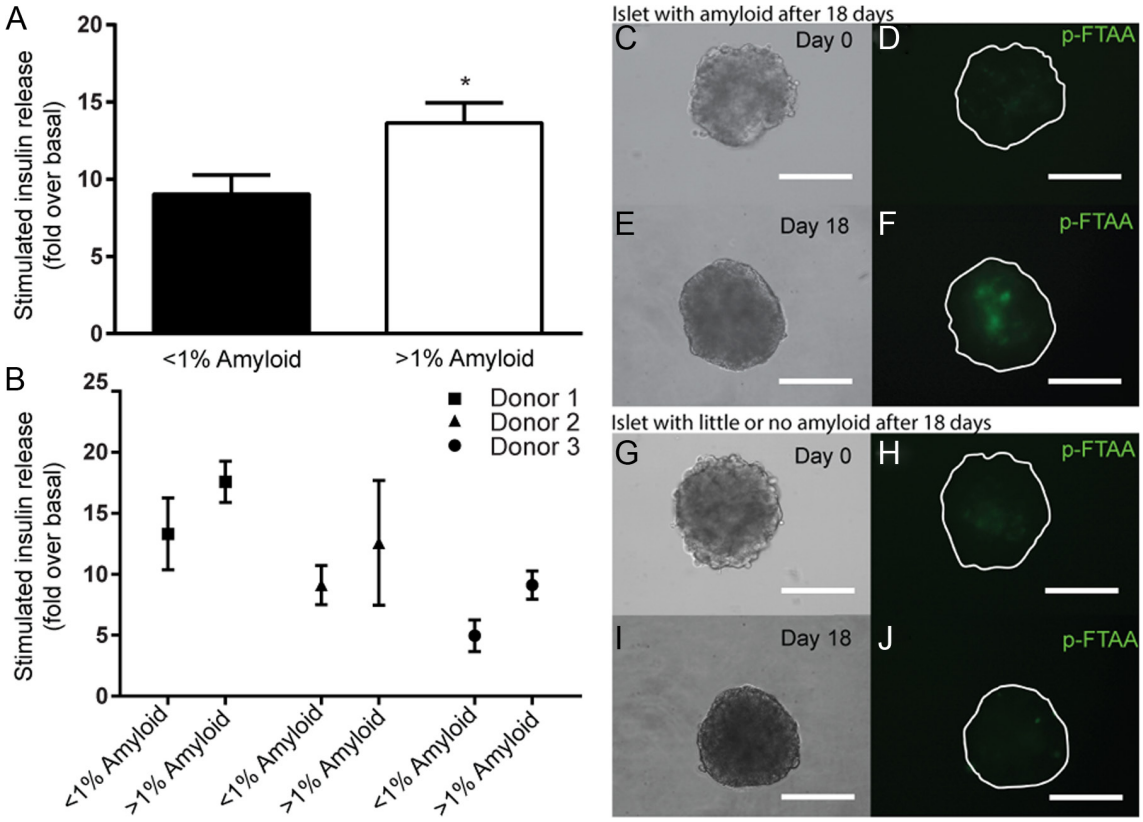

Figure 2
Glucose-stimulated insulin release and subsequent high glucose induced amyloid formation in human islets. (A) Human islets with amyloid formed after 18 days of high glucose culture had better glucose-stimulated insulin release prior to culture. (B) Similar findings were observed in islets from four different donors. Representative images of islet developing amyloid after high glucose culture at day 0 (C and D) and day 18 (E and F) and islet without amyloid after high glucose culture at day 0 (G and H) and day 18 (I and J), scale bar 100 µm. Values are given as means ± S.E.M. for islets from four different donors and ~120 islets were investigated in total. *$P<0.05$ vs islets with less than 1% amyloid after culture.

and immunoglobulin-binding protein (BiP) ($n=5$, fold change 1.21 ± 0.62 in islets with microspheres) did not differ between the islets with or without microspheres.

Discussion

The present study describes increased susceptibility for islet amyloid formation in a subpopulation of highly blood-perfused and functionally active islets of hIAPP mice. In addition, a subgroup of human islets with a better insulin release capacity developed more amyloid during high glucose exposure. Increased amyloid in the highly blood-perfused functionally active islets of hIAPP mice was associated with an imbalance in PC protein levels. This imbalance with the following incapacity of processing proIAPP to mature IAPP has previously been described to be of great importance for the development of amyloid deposits (22, 35).

Identification of highly blood-perfused, highly functional islets has previously been performed by the microsphere technique (8, 9, 36, 37). In order to be able to study the tendency for amyloid formation in these islets, we used mice expressing hIAPP due to the non-amyloidogenic nature of rodent IAPP (28). These mice lack mIAPP to avoid interference with amyloid fibril formation. Since amyloid formation does not develop spontaneously in the hIAPP mouse model, a trigger of e.g. HFD is needed (28). In order to investigate visible amyloid deposits, we used mice fed a diet high in fat for 10 months to allow sufficient time for the development of the deposits. Also, we tried to identify mechanisms preceding the fibrillation process, and for these experiments, we used animals that had been fed a HFD for three months.

After 10 months of HFD, amyloid was detected in islets from all animals. However, when comparing islets, we observed preferential amyloid development in the islets with a high blood perfusion as identified by

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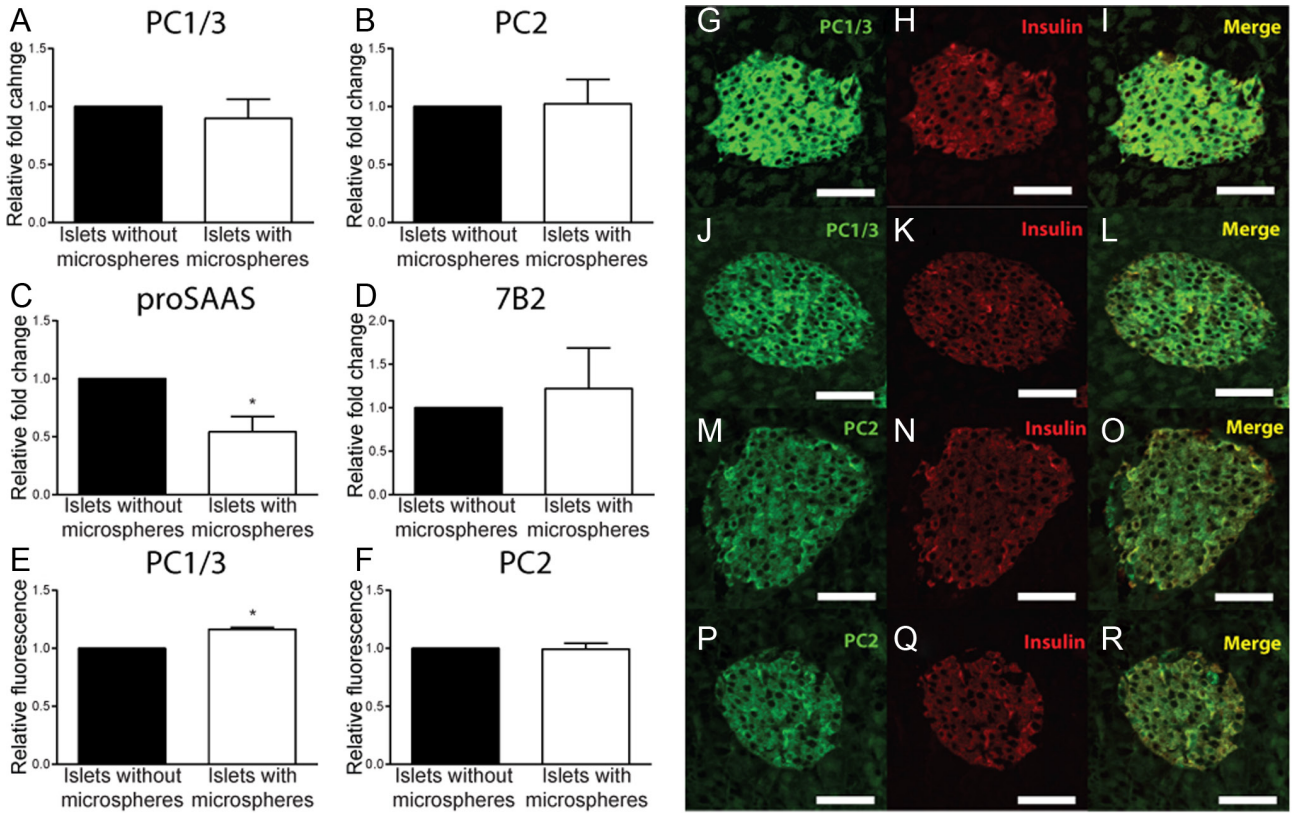

Figure 3

Prohormone convertase (PC) expression in islets from human IAPP-expressing mice fed for 3 months with high-fat diet (HFD). (A and B) The relative gene expression of PC1/3 and PC2 did not differ between the two groups of isolated islets with and without microspheres. (C) The expression of the inhibitor of PC1/3 activity, proSAAS, was decreased in islets with microspheres. (D) The expression of the corresponding inhibitor of PC2, 7B2, did not differ between the groups. PC protein levels evaluated as intensity of immunofluorescence of PC1/3 and PC2, showed higher amounts of PC1/3 in islets with microspheres (E, G, H and I) when compared to control islets without microspheres (E, J, K and L). No difference in PC2 levels was seen between islets with microspheres (F, M and N) when compared to control islets without microspheres (F, P, Q and R). Values are given as means ± S.E.M. for 4–6 animals. **P*<0.05 vs islets without microspheres.

microspheres. The amount of amyloid was also twice as extensive in these islets when compared to the group with lower blood perfusion.

Consistent with our previous study in rat (8), islets with high blood flow as identified by microspheres had an approximately 30% higher insulin release than other islets in response to high glucose. However, when measuring IAPP secretion from the same islets, no difference could be seen between the two groups in either high or low glucose concentrations.

Since the glucose stimulation index, a measure of insulin secretory capacity, was particularly augmented in the highly blood-perfused mouse islets marked by microspheres, we decided to use this index for studies on human islets as the microsphere technique is restricted to experimental animal models and cannot be used to identify highly perfused human islets. The glucose stimulation index of each single human islet was evaluated at day 0 and the islets were subsequently cultured in high glucose medium for 18 days to induce IAPP secretion and amyloid formation (27). After 18 days of high glucose culture, extensive amyloid was seen in numerous islets (amyloid deposits range 0–30% of islet area). When the islets were divided into no or low (<1%) or high (>1%) amyloid content, islets that developed amyloid were found to have an approximately 50% higher glucose stimulation index prior to the incubation irrespective of the donor.

To further investigate the mechanisms of amyloid formation in the highly blood-perfused, highly functional mouse islets, we studied the expression of the ER stress markers CHOP and BiP as ER stress has been described to be associated with amyloid formation in some (38), but not all studies (39). In this study, we did not see any indications of ER stress in the highly functional islets prone to develop amyloid. Another study has described a preferential location of human islets containing

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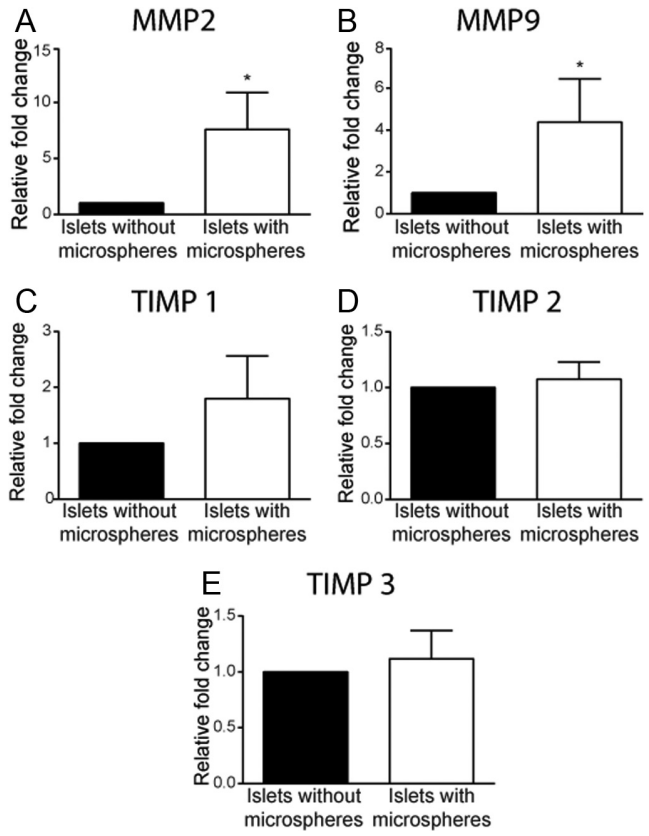

Figure 4
Gene expression analysis of matrix metalloproteinases (MMP) and their inhibitors in isolated islets of human IAPP expressing mice fed a high-fat diet (HFD) for 3 months. (A and B) The relative expression of the two metalloproteinases MMP2 and MMP9 was increased in islets with microspheres when compared to islets without microspheres. (C and E) The expression of the endogenous inhibitors TIMP1, TIMP2 and TIMP3 was similar between the two groups. Values are given as means ± S.E.M. for 5–6 animals. *$P<0.05$ vs islets without microspheres.

amyloid to the peripheral regions of the pancreas (40). We did, in our studies in mice, however, not find any regional differences of the position of microsphere-containing islets.

Processing deficiencies of proIAPP has been suggested to contribute to islet amyloid development in T2D (41, 42, 43, 44). An increased proinsulin-to-insulin ratio with a major proportion of partially processed des-31,32 proinsulin, a proinsulin intermediate partially processed by PC1/3 but not PC2, has also been identified in such patients (45). To investigate if proIAPP processing was altered in the highly blood-perfused islets, we performed gene expression analysis of PC1/3 and PC2, the two major processing enzymes of both IAPP and insulin precursor molecules. Since the regulation of the two prohormone convertases also has been shown to occur on a post-transcriptional level (46, 47), the levels of insulin, PC1/3 and PC2 were evaluated by immunohistochemistry as well. Although the intensity of insulin and PC2 did not differ, the PC1/3 intensity was increased with approximately 17% in islets containing microspheres. Although we were unable to investigate the activity of either PC1/3 or PC2, we also observed a decrease in the gene expression of the endogenous inhibitor of PC1/3, proSAAS, which couples to increased activity of PC1/3 (48). The corresponding inhibitor of PC2, 7B2, was unchanged.

PC1/3 appears to be more important for proinsulin processing than PC2, but both enzymes can process both the COOH- and NH_2-terminal of proinsulin, although not as efficient as the two enzymes combined (49, 50). In contrast, proIAPP requires PC2 for processing at the NH_2-terminus (41, 49). Interestingly, the proIAPP intermediate N-IAPP with an unprocessed NH_2-terminal has been suggested to be more amyloidogenic than mature IAPP (42) and has been identified in human amyloid deposits (43, 44). We were not able to measure N-terminal intermediate proIAPP, but in diabetic mice transplanted with mouse islets expressing human proIAAP, lack of PC2 in the islet transplants caused rapid graft failure with recurrence of hyperglycemia (51). The authors concluded that an increased production of prefibrillar proIAPP aggregates is plausible to have contributed.

N-IAPP has been described to have an increased affinity to HS, which is located at the cellular surface. This is suggested to cause a locally increased concentration of the amyloidogenic processing intermediate, which may seed amyloid formation (52). As HS is important for islet function (24), we hypothesized that highly functional islets may have an increased amount of HS facilitating the observed increase in amyloid formation. We did, however, not find any differences in HS intensity between the two groups of islets. Nevertheless, an increased amount of high-affinity N-IAPP in the microsphere-containing islets may by itself seed the process.

Amyloid peptide Aβ has been described to increase transcription of vascular MMP9 (53). This could be of importance for amyloid formation, since both MMP2 and MMP9 have shown the ability to degrade Aβ to soluble and less fibrillogenic fragments (54). MMP9, but not MMP2, has also been described to degrade IAPP (55). We could detect an increase in both MMP2 and MMP9 expression in the microsphere-containing islets, a feature which might be a response to an increased fibrillation process in these islets. However, the effects of islet amyloid on MMP2 and MMP9 expression or the degradation capacity of the two matrix metalloproteinases have not been studied. An increased

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expression may simply reflect the specific phenotype of the highly vascularized islets (56).

In conclusion, in the present study, we have found that the most functional islets of hIAPP mice and humans are more prone to develop amyloid than other islets. This may be explained by the observed imbalance between the two prohormone convertases PC1/3 and PC2 in these islets. As the subpopulation of islets that was affected by amyloid was the most functional one, the amyloid formation may result in unfavorable effects on long-term glucose homeostasis, which *per se* increases the metabolic demand and may speed up and worsen disease development.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EC-17-0148.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S U, S B, M O, P N, G T W and P O C designed the study and interpreted data. S U, S B and M O acquired and analyzed data. S U and P O C wrote the manuscript and S B, M O, P N and G T W revised the manuscript critically for intellectual content. All authors approved the final version of the paper.

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