Use of the PET ligand florbetapir for in vivo imaging of pancreatic islet amyloid deposits in hIAPP transgenic mice

Andrew T. Templin^{1,2} · Daniel T. Meier^{1,2} · Joshua R. Willard^{1,2} · Tami Wolden-Hanson¹ · Kelly Conway³ · Yin-Guo Lin³ · Patrick J. Gillespie⁴ · Krister B. Bokvist⁴ · Giorgio Attardo³ · Steven E. Kahn^{1,2} · Donalyn Scheuner⁴ · Rebecca L. Hull^{1,2}

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Abstract

Aims/hypothesis Islet amyloid deposits contribute to beta cell dysfunction and death in most individuals with type 2 diabetes but non-invasive methods to determine the presence of these pathological protein aggregates are currently not available. Therefore, we examined whether florbetapir, a radiopharmaceutical agent used for detection of amyloid- β deposits in the brain, also allows identification of islet amyloid in the pancreas.

Methods Saturation binding assays were used to determine the affinity of florbetapir for human islet amyloid polypeptide (hIAPP) aggregates in vitro. Islet amyloid-prone transgenic mice that express hIAPP in their beta cells and amyloid-free non-transgenic control mice were used to examine the ability of florbetapir to detect islet amyloid deposits in vitro, in vivo and ex vivo. Mice or mouse pancreases were subjected to autoradiographic, histochemical and/or positron emission tomography (PET) analyses to assess the utility of florbetapir in identifying islet amyloid.

Results In vitro, florbetapir bound synthetic hIAPP fibrils with a dissociation constant of 7.9 nmol/l. Additionally, florbetapir bound preferentially to amyloid-containing hIAPP transgenic vs amyloid-free non-transgenic mouse pancreas sections in vitro, as determined by autoradiography ($16,475 \pm 5581$ vs 5762 ± 575 density/unit area, $p < 0.05$). In hIAPP transgenic and non-transgenic mice fed a high-fat diet for 1 year, intravenous administration of florbetapir followed by PET scanning showed that the florbetapir signal was significantly higher in amyloid-laden hIAPP transgenic vs amyloid-free non-transgenic pancreases in vivo during the first 5 min of the scan (36.83 ± 2.22 vs 29.34 ± 2.03 standardised uptake value $\times$ min, $p < 0.05$). Following PET, pancreases were excised and florbetapir uptake was determined ex vivo by γ counting. Pancreatic uptake of florbetapir was significantly correlated with the degree of islet amyloid deposition, the latter assessed by histochemistry ($r = 0.74$, $p < 0.001$).

Conclusions/interpretation Florbetapir binds to islet amyloid deposits in a specific and quantitative manner. In the future, florbetapir may be useful as a non-invasive tool to identify islet amyloid deposits in humans.

Keywords Amyloid · Diabetes · Florbetapir · Islet · PET

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✉ Andrew T. Templin
templin@uw.edu

Abbreviations

CT	Computed tomography
GLP-1	Glucagon-like peptide-1
HFD	High-fat diet
hIAPP	Human islet amyloid polypeptide
PET	Positron emission tomography
SUV	Standardised uptake value

¹ Department of Medicine, VA Puget Sound Health Care System, 1660 S. Columbian Way, Seattle, WA 98108, USA

² University of Washington, Seattle, WA, USA

³ Avid Radiopharmaceuticals, Inc., Philadelphia, PA, USA

⁴ Eli Lilly Research Laboratories, Indianapolis, IN, USA

Introduction

Islet amyloid deposits are present in most individuals with type 2 diabetes and play a pathological role in beta cell loss

Research in context

What is already known about this subject?

- Islet amyloid deposits are a hallmark of type 2 diabetes, being present in most individuals with the disease, and are associated with beta cell loss
- Recently, the radiopharmaceutical agent florbetapir has been used to identify amyloid- β deposits in the brain using positron emission tomography (PET)
- Some PET ligands designed to bind brain amyloid deposits are also capable of binding synthetic islet amyloid in vitro and ex vivo

What is the key question?

- Can amyloid-binding PET ligands be used to specifically identify endogenous islet amyloid in the pancreas in vivo?

What are the new findings?

- In vitro, florbetapir binds to amyloid-containing mouse pancreas tissue to a greater degree than to amyloid-free tissue
- Intravenous administration of florbetapir followed by PET scanning showed a significantly higher signal in amyloid-laden hIAPP transgenic vs amyloid-free non-transgenic mouse pancreases in vivo during the first 5 min of the scan
- Following PET, pancreatic uptake of florbetapir ex vivo was found to be strongly correlated with the degree of islet amyloid deposition as assessed by histochemistry

How might this impact on clinical practice in the foreseeable future?

- The ability to identify islet amyloid deposits in vivo could provide a new diagnostic tool to guide diabetes treatment decisions

[1–4]. Post mortem examination of human tissue sections has shown that islet amyloid deposition is associated with increased rates of beta cell apoptosis and decreased beta cell area [1, 2]. Amyloid has also been linked to beta cell failure in transplanted human islets [5, 6]. Human islet amyloid polypeptide (hIAPP), the unique peptide component of islet amyloid [7, 8], is a peptide hormone synthesised by the beta cell and is co-secreted with insulin [9]. In vitro and in vivo models of islet amyloidosis have demonstrated that hIAPP aggregation results in oxidative and inflammatory stress leading to beta cell death [10–12] and various lines of research are now aimed at preventing islet amyloid formation and the resulting cytotoxicity [13–16]. However, no diagnostic test is available to identify and/or quantify islet amyloid in individuals with type 2 diabetes.

Positron emission tomography (PET) probes have been proposed as tools to facilitate in vivo assessment of islet morphology and to evaluate changes in islet structure over time [17–19]. Many of these probes are directed at beta cell biomarkers, such as the glucagon-like peptide-1 (GLP-1) receptor and glucokinase, with the intent to quantify beta cell mass [17, 20]. Ex vivo studies have determined that pancreatic uptake of radiolabelled exendin-4, a GLP-1 receptor agonist, is reduced upon destruction of beta cells in rats [20]. Although in vivo quantification of beta cell mass using PET has been more difficult to achieve [17, 18, 20], this approach has been used to demonstrate decreased beta cell mass in individuals with

type 1 diabetes [21]. A complementary approach that has not been fully explored is to determine the degree of islet amyloid present in vivo. Amyloid imaging probes have been designed for PET detection of amyloid- β deposits in the brain [22–24] and, since brain and islet amyloid deposits share structural similarities [25], these probes have been proposed as tools to detect islet amyloid as well. Limited studies have found that some amyloid- β amyloid imaging probes bind synthetic hIAPP in vitro and ex vivo [26, 27]. To our knowledge, no studies to date have tested whether these probes can identify and quantify endogenous islet amyloid in vivo.

Recently, the ^{18}F -labelled radiopharmaceutical florbetapir was approved for detection of amyloid- β -derived amyloid deposits in the brain for diagnosis of Alzheimer's disease [28, 29]. We sought to determine whether florbetapir may also be used to detect islet amyloid in vivo. We used an hIAPP transgenic mouse model of islet amyloidosis in which transgenic expression of hIAPP in beta cells leads to islet amyloid deposition, reduced beta cell mass and hyperglycaemia over 1 year of high-fat diet (HFD) feeding compared with non-amyloid-prone, non-transgenic littermate control mice that were also fed HFD and gained the same amount of weight [10, 30, 31]. This model was used to examine whether florbetapir could detect endogenous islet amyloid in vitro, in vivo and ex vivo and thus may prove to be a useful tool to identify islet amyloid in humans. This strategy could allow detection of islet amyloid in individuals with type 2 diabetes

and aid in determining the most effective treatments for individuals afflicted with this disease.

Methods

In vitro saturation binding assay and dissociation constant determination To prepare fibrillar hIAPP for binding studies, 30 $\mu\text{mol/l}$ hIAPP (no. 60804; Anaspec, Fremont, CA, USA) was dissolved in 10 mmol/l phosphate buffer and 5 mmol/l NaCl and incubated overnight at room temperature with agitation. For saturation binding assays, concentrations of synthetic hIAPP fibrils and radiolabelled florbetapir were determined empirically, starting with concentration ranges based on the literature and the authors' experience with amyloid- β peptide [32]. Concentration-dependent binding assays were carried out using 0.5, 0.25, 0.125, 0.0625, 0.03125 or 0.015 μg of synthetic hIAPP fibrils and 0.15 nmol/l [^{18}F]florbetapir in 250 μl PBS, eliciting binding of between 0 and 20% of the radioligand. Reaction mixtures contained IAPP fibrils (0.2 μg ; probe sonicated) and [^{18}F]florbetapir (0.9 nmol/l) in 250 μl PBS. Non-specific binding was defined as the binding of the radioligand in the presence of 10 $\mu\text{mol/l}$ 2-(4'-methylaminophenyl)benzothiazole (BTA-1) [33]. After incubation for 1.5 h at 37°C, bound radioactivity was harvested by vacuum filtration onto Millipore MultiScreenHTS 96-well glass fibre FB filter plates using a Millipore MultiScreenHTS Vacuum Manifold (Millipore, Billerica, MA, USA). Filters containing bound [^{18}F]florbetapir were assayed for radioactivity in a γ counter.

Animals hIAPP transgenic mice on a C57BL/6J background were bred with DBA/2J mice obtained from Jackson Laboratories (Bar Harbor, ME, USA), and hemizygous hIAPP transgenic mice on an F1 C57BL/6J $\times$ DBA/2J background were studied with non-transgenic littermates as a control group [34]. Male mice, 8–10 weeks old, from both genotypes were housed in a specific-pathogen-free vivarium and received a diet containing 60% kJ energy from fat (HFD; Research Diets, New Brunswick, NJ, USA) for 12 months. This study was approved by and conducted in accordance with the Institutional Animal Care and Use Committee at the Veterans Affairs Puget Sound Health Care System.

In vitro binding of florbetapir to pancreas sections and autoradiography Archived paraffin-embedded pancreas tissue sections from hIAPP transgenic and non-transgenic mice were selected for in vitro florbetapir binding assays. Blinded experimenters added [^3H]florbetapir (370 kBq/section) with or without cold florbetapir, to pancreas sections for 1 h at room temperature and sections were then washed. Tissue sections were placed in a phosphor imager cassette in which a phosphor screen was exposed overnight to the tissue. The

phosphor screen was then scanned with a GE Typhoon FLA7000 Bio-Imaging System (GE, Fairfield, CT, USA). Densitometry was performed on autoradiographic images using Image J (NIH, Bethesda, MD, USA) to quantify the degree of florbetapir binding.

In vivo florbetapir administration and PET Non-transgenic and hIAPP transgenic mice were anaesthetised with isoflurane and a carotid catheter was placed. ExiTron Nano 12000 contrast agent (Milenyi, Bergisch Gladbach, Germany) was administered via the carotid catheter. An Inveon MultiModality PET/computed tomography (CT) scanner (Siemens, Cary, NC, USA) conducted dynamic scans every 30 s throughout the experimental procedure. A CT scan was obtained for co-registration and scatter correction purposes before injection of the isotope. Florbetapir dose volume was calculated and 184.0 ± 6.9 kBq/g body weight was injected via the carotid catheter. PET image reconstruction was done by ordered subset expectation maximisation/3D map protocol and the co-registered PET/CT images were evaluated by selecting regions of interest in pancreas, colon (positive control) and seminal vesicle (negative control). Pancreas was identified on CT images, left of midline, above the spleen and even with the stomach. Within this area, a conservative 3 mm³ region of interest was defined for each mouse (by T. Wolden-Hanson, blinded to mouse genotype) to ensure that only pancreas tissue was included. Blinded intra- and inter-observer repeated measurements of the PET pancreas region of interest were highly correlated (Spearman rank correlations: $r = 0.987$, $p < 0.0001$, slope, 0.95 and $r = 0.966$, $p < 0.0001$, slope 0.86, respectively). Given that clearance/metabolism of florbetapir in vivo is rapid by design [32, 35], the primary analysis of standardised uptake values (SUVs) were done over the first 5 and 10 min, and scanning then continued up to 90 min. The PET signal was quantified for each mouse by calculating AUC (SUV $\times$ min) at the indicated time points. Two mice (one hIAPP transgenic and one non-transgenic) died early during the PET scan and did not complete the procedure. Although we collected pancreases from these mice, for consistency, data were reported only from mice that completed all portions of the in vivo analyses (PET scan, ex vivo radioquantification, histological measurement).

Ex vivo florbetapir radioquantification Mice were euthanised immediately following PET scanning. Pancreases were rapidly excised, placed into 10% (vol./vol.) neutral buffered formalin, and weighed. ^{18}F radiolabel uptake was determined for 1 min in a γ counter. Pancreases were then processed for histology as described below.

Histological measurements Whole pancreases were fixed overnight in formalin, paraffin-embedded, then 4 μm sections were cut longitudinally. Sections were stained with thioflavin

S (Sigma-Aldrich, St Louis, MO, USA) to visualise amyloid deposits and then incubated with mouse monoclonal anti-insulin antibody (no. I2018; Sigma-Aldrich, 1:500) followed by goat anti-mouse Cy3 (no. 115-165-146; Jackson ImmunoResearch, West Grove, PA, USA, 1:500) to visualise beta cell area and Hoechst 33258 to visualise nuclei. Morphometric analyses were then performed by individuals blinded to sample genotypes on an average of 118 ± 11 islets per mouse using an E800 Eclipse epifluorescence microscope and NIS Elements Software (Nikon USA, Melville, NY, USA) [36]. For whole pancreas section imaging, sections were stained with thioflavin S to visualise amyloid deposits or labelled with guinea pig polyclonal anti-insulin antibody (no. A0564; Dako, Santa Clara, CA, USA, 1:100) followed by rabbit anti-guinea pig IgG linker and Leica BOND detection reagents (no. DS9800; Leica, Wetzlar, Germany, no dilution) to visualise beta cell area [37]. Large area scans were acquired using DeltaVision Elite Imaging System (GE Life Sciences, Issaquah, WA, USA).

Statistical analysis Data are presented as mean $\pm$ SEM with each data point represented in a dot plot to depict the distribution of data within each group. Non-parametric statistical tests were used throughout to analyse data that were not normally distributed. Statistical differences between groups were determined using unpaired, two-tailed Mann–Whitney *U* tests, and Spearman rank correlation tests were used to determine the correlation between measurements. Values of $p \leq 0.05$ were considered significant.

Results

Florbetapir binds synthetic hIAPP fibrils in vitro To determine whether the radiopharmaceutical florbetapir (Fig. 1a) may be

useful as an islet amyloid imaging probe, we first asked whether florbetapir binds fibrillar hIAPP in vitro. Saturation binding assays determined that florbetapir binds synthetic hIAPP fibrils in vitro with a dissociation constant (K_d) of 7.9 nmol/l (Fig. 1b), similar to the K_d of 7 nmol/l that we observed for florbetapir's interaction with amyloid- β 42 fibrils (data not shown).

Florbetapir binds endogenous islet amyloid deposits in vitro

To examine whether florbetapir binds endogenous islet amyloid deposits in vitro, pancreas sections from hIAPP transgenic and non-transgenic control mice were incubated with [3 H]florbetapir (Fig. 2a). For comparison, serial pancreas sections were labelled histochemically for the presence of amyloid (thioflavin S) and insulin (Fig. 2a; insets show amyloid-free and amyloid-laden islets). Quantification of autoradiograph signal from hIAPP transgenic mouse and control mouse pancreas sections showed that florbetapir bound amyloid-laden pancreatic tissue to a significantly greater extent than amyloid-free control tissue ($16,475 \pm 5581$ vs 5762 ± 575 density/unit area, $p < 0.05$, Fig. 2b). Within these pancreas sections, the florbetapir autoradiograph signal was correlated to the degree of islet amyloid deposition assessed by histology (Fig. 2c). Furthermore, the binding of florbetapir to islet amyloid in hIAPP transgenic mouse pancreas sections was reduced by incubation with excess non-radiolabelled (cold) florbetapir to a level similar to that noted in control tissue (Fig. 2d). Together, these data suggest that florbetapir specifically binds islet amyloid in vitro.

hIAPP transgenic mice form islet amyloid deposits in vivo following HFD feeding We next used our in vivo model of islet amyloid deposition [10, 30, 31, 36] to test whether florbetapir allows in vivo detection of islet amyloid using PET scanning. After 1 year of HFD feeding, body weight

Fig. 1 The amyloid imaging probe [18 F]florbetapir binds synthetic hIAPP fibrils in vitro. **(a)** The chemical structure of [18 F]florbetapir. **(b)** Saturation binding assay of florbetapir and probe-sonicated synthetic hIAPP fibrils was carried out following empirical determination of optimal radiolabel and hIAPP fibril concentrations (described in Methods). [18 F]florbetapir

and hIAPP fibrils were incubated, then bound radioactivity was quantified by γ counting to determine total binding (black circles). [18 F]florbetapir and 2-(4'-methylaminophenyl)benzothiazole were used to quantify non-specific binding (white circles), and specific binding (grey circles) was calculated; $B_{\max}$ = total radioligand binding; $n = 2$ samples

Fig. 2 [^3H]Florbetapir binds endogenous islet amyloid deposits in vitro. **(a)** Serial pancreas sections from non-transgenic control mice and hAPP transgenic mice were subjected to autoradiography with [^3H]florbetapir to assess radioligand binding, histochemical staining with thioflavin S to quantify islet amyloid deposition or immunohistochemistry to visualise insulin. Insets show amyloid-free non-transgenic or amyloid-laden hAPP transgenic islets. No contrast adjustment was made to any of the panels. Scale bars, 2500 μm . **(b)** Densitometry was performed on autoradiographs from non-transgenic and hAPP transgenic pancreas sections to quantify [^3H]florbetapir binding; $n=4$ for non-transgenic mouse islets and $n=5$ for hAPP transgenic mouse islets, $*p<0.05$, Mann–Whitney

U test, values presented as mean $\pm$ SEM. **(c)** Pancreas section [^3H]florbetapir autoradiograph density is correlated with amyloid severity (amyloid area/islet area, %) as assessed by quantitative microscopy in these samples; $n=4$ for non-transgenic mouse pancreas sections and $n=5$ for hAPP transgenic mouse pancreas sections, $p<0.01$, $r=0.88$, Spearman rank correlation test. **(d)** Binding of [^3H]florbetapir in pancreas sections from hAPP transgenic mice was blocked by non-radiolabelled florbetapir to a level similar to that noted in non-transgenic pancreas sections; magnification $\times 10$. No contrast adjustment was made to any of the panels. Thio S, thioflavin S

and pancreas mass were not different between hAPP transgenic and control genotypes (Table 1). In line with previous studies, hAPP transgenic mice developed significant islet amyloid deposition (electronic supplementary material [ESM] Fig. 1a) and displayed decreased islet beta cell area (ESM Fig. 1b) compared with non-transgenic control mice.

Furthermore, islet amyloid area was inversely correlated to beta cell area (ESM Fig. 1c), showing the expected relationship between islet amyloid deposition and beta cell loss, and demonstrating the potential utility of in vivo islet amyloid detection as a means to identify pathological changes in islet protein aggregation.

Table 1 After 1 year of HFD feeding, body weight, pancreas mass and florbetapir dose were equivalent in *hAPP* transgenic and non-transgenic mice

Genotype	Non-transgenic mice	<i>hAPP</i> transgenic mice
<i>n</i>	8	10
Body weight (g)	64.2 ± 2.1	67.6 ± 4.1
Pancreas mass (g)	0.52 ± 0.04	0.51 ± 0.04
Florbetapir dose (kBq/g body weight)	191 ± 9	178 ± 11

Data are means ± SEM

Florbetapir enables PET detection of islet amyloid in vivo in *hAPP* transgenic mice To investigate whether [^{18}F]florbetapir enables PET detection of islet amyloid in vivo, we administered an equivalent dose of this radioligand (target dose 185 kBq/g body weight) to both *hAPP* transgenic and non-transgenic mice (Table 1). Concurrent with florbetapir administration, PET scanning was conducted to determine the presence of florbetapir in the pancreas (Fig. 3a, b), colon (Fig. 3c; positive control) and seminal vesicle (Fig. 3d; negative control). The regions of interest for PET analysis are illustrated in Fig. 3e. The florbetapir signal was greater in *hAPP* transgenic mouse pancreases than in control mouse pancreases during the first 5 min of the PET scan (36.83 ± 2.22 vs 29.34 ± 2.03 standardised uptake value $\times$ min, $p < 0.05$, Fig. 3a, b). After 10 min, the pancreatic PET signal was no longer significantly different between groups ($p = 0.06$) and from 20 min on the signal did not differ between *hAPP* transgenic and non-transgenic mice. Conversely, the florbetapir signal in control tissues, namely colon (Fig. 3c) and seminal vesicle (Fig. 3d), did not differ between *hAPP* transgenic and non-transgenic mice during the first 5 min, nor did they differ over 10 or 20 min intervals.

Florbetapir administered in vivo accumulates preferentially in islet amyloid-laden *hAPP* transgenic mouse pancreases

Finally, we sought to determine whether in vivo-administered florbetapir accumulated preferentially in amyloid-containing pancreases of these same mice by ex vivo γ counting of excised pancreas following the PET scan. The amyloid-containing *hAPP* transgenic mouse pancreases exhibited significantly greater radiolabel uptake compared with amyloid-free non-transgenic control tissue (Fig. 4a). We next quantified the degree of islet amyloid deposition in each pancreas using our established histochemical method. We found that the degree of radioactivity present in the pancreases was strongly correlated to the amount of islet amyloid present for both *hAPP* transgenic and non-transgenic mice ($r = 0.74$, $p < 0.001$; Fig. 4b), and also when data from *hAPP* transgenic mice were analysed alone ($r = 0.81$, $p < 0.005$). In Fig. 4c, three representative pancreas images are

shown to illustrate this relationship between islet amyloid deposition and florbetapir uptake.

Discussion

Type 2 diabetes develops over a period of years, during which time pancreatic islet beta cells undergo morphological and functional changes leading to beta cell loss and inadequate insulin secretion [38, 39]. Islet amyloid deposition is a significant contributor to beta cell loss and alpha cell proliferation in humans [1–3] as well as in non-human primates [40] and has also been linked with delta cell death in the latter [40, 41]. Due to the prolonged time course of changes to islet cell abundance and function, techniques to detect pathological changes in the islet in vivo could aid in the treatment of type 2 diabetes. However, no such methods are currently being employed clinically.

Previous studies have provided histological and autoradiographic evidence for in vitro binding of amyloid- β imaging probes to synthetic hIAPP aggregates [26] and, when administered intravenously, these agents accumulated in implanted hIAPP aggregates in mice [27]. However, these studies did not determine whether amyloid imaging probes bind to endogenous islet amyloid or whether they allow identification of islet amyloid deposits in vivo using PET. Our study is the first to examine whether administration of a radiopharmaceutical agent allows PET detection of endogenous islet amyloid in vivo.

In this study, we took advantage of the available ^{18}F -labelled radiopharmaceutical florbetapir, which was developed for PET imaging of amyloid- β deposits in the brain, and now provide evidence that this compound binds islet amyloid in vitro, in vivo and ex vivo. In vitro, we observed that florbetapir binds synthetic hIAPP fibrils with a K_d of 7.9 nmol/l, similar to that seen for amyloid- β 42 fibrils. When administered to pancreas sections in vitro, florbetapir bound pancreases with islet amyloid deposits to a greater extent than those lacking these deposits and this binding was blocked by unlabelled florbetapir. Intravenous administration of florbetapir in mice resulted in a transient increase in pancreatic PET signal in amyloid-laden pancreases in vivo, and florbetapir retention in excised pancreases from these mice following PET was significantly correlated with the degree of islet amyloid deposition assessed by histology. Although further studies are required to demonstrate that in vivo-administered florbetapir binds specifically to islet amyloid, our in vitro and ex vivo studies suggest that this is the case. Together, these results strongly imply that florbetapir binds specifically and quantitatively to islet amyloid deposits.

The relationship between pancreatic florbetapir binding and islet amyloid deposition both in vitro and ex vivo indicates that we can distinguish pancreatic tissue with an amyloid

Fig. 3 Florbetapir enables PET detection of islet amyloid in vivo in *hAPP* transgenic mice. PET scanning was used to determine the presence of [^{18}F]florbetapir (SUV) in non-transgenic (white circles) and *hAPP* transgenic (black squares) mice from 0 to 85 min after administration of the radio-agent. **(a)** Time course of [^{18}F]florbetapir uptake in the pancreases of non-transgenic (white circles) and *hAPP* transgenic (black squares) mice, values presented as mean $\pm$ SEM. **(b)** Quantification of [^{18}F]florbetapir uptake by the pancreas expressed as AUC (SUV $\times$ min) for the first 5 and 10 min of the scan; $n = 8$ for non-transgenic mouse pancreas and $n = 10$ for *hAPP* transgenic mouse pancreas, $*p < 0.05$, Mann–Whitney U test, values presented as mean $\pm$ SEM. **(c, d)** [^{18}F]Florbetapir uptake was also quantified in the colon (c) and seminal vesicle (d); $n = 8$ for non-transgenic mice and $n = 10$ for *hAPP* transgenic mice, values presented as mean $\pm$ SEM. Vertical lines indicate 5 min and 10 min PET intervals. **(e)** Images illustrating PET/CT regions of interest for pancreas (red), colon (green) and seminal vesicle (blue) as assigned by a blinded observer

burden of $>10\%$ of islet area from amyloid-free tissue. Thus, florbetapir may not be capable of detecting small amounts of islet amyloid but it has potential to identify islet amyloid and concurrent beta cell loss in a considerable proportion of the population. Specifically, the average degree of islet amyloid deposition in individuals with type 2 diabetes is near 10% of islet area and ranges from 0 to $\sim 50\%$ [2]. Analysis of data from Jurgens et al [2] indicates that 45% of individuals with type 2 diabetes possess amyloid deposition $>10\%$ of islet area; 36% of amyloid-prone mice in our in vivo study met this criteria. Therefore, the approach used in this study could allow identification of pathological islet protein aggregation in nearly half of all individuals with type 2 diabetes.

The findings in this study may also have clinical relevance for the treatment of human type 2 diabetes. It is well recognised that the disease is associated with a progressive loss of beta cell function and that this process is more rapid when patients are treated with a sulfonylurea vs either

metformin or a thiazolidinedione [42, 43]. In vivo mouse studies have shown that both metformin and rosiglitazone reduce islet amyloid deposition when administered to amyloid-prone *hAPP* transgenic mice [36, 44] and this decrease in islet amyloid was associated with reduced beta cell loss [36]. Thus, should it be possible to image islet amyloid in humans, this technique could be used to determine the effect of glucose-lowering interventions on amyloid formation over time in people.

Certain aspects of this in vivo islet amyloid imaging approach warrant further consideration. Specifically, the magnitude of increase in florbetapir signal we observed in amyloid-laden pancreases in vivo using PET was smaller than that observed ex vivo by γ counting. This discrepancy likely reflects the modest resolution of mouse islets achievable using PET and may result in part from the significant intra-abdominal and intrapancreatic fat deposits that were evident in all mice in this study. Since florbetapir is lipophilic [32, 45],

Fig. 4 [¹⁸F]Florbetapir administered in vivo accumulates in islet amyloid-laden hAPP transgenic mouse pancreas. (a) [¹⁸F]florbetapir uptake was determined ex vivo by γ counting in non-transgenic control and hAPP transgenic pancreases 90 min after administration of the radioligand for PET scanning; $n = 8$ for non-transgenic and $n = 10$ for hAPP transgenic mouse pancreases, $**p < 0.01$, Mann–Whitney U test, values presented as mean $\pm$ SEM. (b) Islet amyloid severity (amyloid

area/islet area, %) is positively correlated with [¹⁸F]florbetapir uptake in non-transgenic (white circles) and hAPP transgenic (black squares) mouse pancreases; $n = 8$ for non-transgenic mice and $n = 10$ for hAPP transgenic mice, $p < 0.001$, $r = 0.74$, Spearman rank correlation test. (c) Representative images of islet amyloid deposition with [¹⁸F]florbetapir uptake values in three mice that underwent PET scanning. Scale bars, 2500 μ m. Thio S, thioflavin S

non-specific signal was likely emitted from fat-containing organs physically close to the pancreas, such as the liver and intestine [35]. These limitations may have contributed to a high background PET signal and hampered our efforts to quantify islet amyloid deposits in vivo. However, our study represents a realistic assessment of florbetapir's utility for individuals with type 2 diabetes, where high levels of non-specific abdominal signal may present a challenge to capturing PET signal specifically from islet amyloid.

Further, issues with resolving islet amyloid in vivo using PET are likely compounded by the small size and diffuse nature of pancreatic islets, which comprise 1–4% of pancreas mass [46]. This is a challenge that has also been noted in studies attempting to image islet beta cells using PET [17, 20]. Because amyloid accounts for only a fraction of islet volume and can be present in fewer than 50% of islets, reliable in vivo imaging of islet amyloid may be even more difficult to achieve.

Metabolism of florbetapir in the peripheral circulation is rapid, by design, with 95% being cleared from the blood in the first 20 min after injection [32, 35]. This property of

florbetapir may limit its ability to optimally bind islet amyloid and emit a stable radiologic signal from it. In the future, examination of pancreas autoradiography ex vivo following intravenous administration of florbetapir could shed more light on the dynamics of florbetapir and islet amyloid interaction in vivo. New amyloid-binding radiopharmaceuticals designed specifically to image islet amyloid may provide more robust or prolonged PET detection in vivo.

Despite the challenges discussed, we were successful in detecting increased [¹⁸F]florbetapir signal in amyloid-laden vs amyloid-free pancreases for the first 5 min of the PET scan. One benefit of imaging islet amyloid vs beta cells is reduced variability among populations of interest. Previous studies using PET to quantify beta cell mass found highly variable beta cell radioligand uptake in healthy individuals [17, 21]. Some of this variability may stem from technical difficulties or a lack of ligand specificity but it is also partially attributable to the wide variation of and overlap in beta cell mass present in healthy control individuals and those with diabetes [4, 47]. Although islet amyloid is also variable in those with type 2 diabetes [3], we previously found only one out of 39 healthy

individuals to have islet amyloid deposition exceeding 10% [2], a finding in concordance with earlier studies [48, 49]. Therefore, imaging of islet amyloid would allow detection of pathological islet protein aggregates in the setting of type 2 diabetes.

In conclusion, we found that the amyloid imaging probe florbetapir binds islet amyloid in vitro in a specific and quantitative manner and observed increased florbetapir uptake in amyloid-laden pancreases in vivo using PET. Furthermore, the amount of florbetapir uptake in the pancreas was highly correlated to the degree of islet amyloid deposition ex vivo. Although advances in PET scanning methodologies or islet amyloid radioligand properties may be needed to make in vivo imaging of islet amyloid a clinical reality, our studies suggest that this is a possibility. If successful, in vivo detection of islet amyloid would provide a valuable tool for identification of these deposits in individuals with type 2 diabetes and the development and use of therapies aimed at decreasing their impact.

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Data availability Data supporting the conclusions of this article are included within the article and are available from the corresponding author on reasonable request.

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References

1. Westermark P, Grimelius L (1973) The pancreatic islet cells in insular amyloidosis in human diabetic and non-diabetic adults. *Acta Pathol Microbiol Scand* 81:291–300
2. Jurgens CA, Toukatly MN, Fligner CL et al (2011) β -Cell loss and β -cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *Am J Pathol* 178:2632–2640
3. Hull RL, Westermark GT, Westermark P, Kahn SE (2004) Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* 89:3629–3643
4. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β -Cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110
5. Westermark GT, Davalli AM, Secchi A et al (2012) Further evidence for amyloid deposition in clinical pancreatic islet grafts. *Transplantation* 93:219–223
6. Davalli AM, Perego L, Bertuzzi F et al (2008) Disproportionate hyperproinsulinemia, β -cell restricted prohormone convertase 2 deficiency, and cell cycle inhibitors expression by human islets transplanted into athymic nude mice: insights into nonimmune-mediated mechanisms of delayed islet graft failure. *Cell Transplant* 17:1323–1336
7. Westermark P, Wernstedt C, Wilander E, Hayden DW, O'Brien TD, Johnson KH (1987) Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a neuro peptide-like protein also present in normal islet cells. *Proc Natl Acad Sci U S A* 84:3881–3885
8. Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB (1987) Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad Sci U S A* 84:8628–8632
9. Kahn SE, D'Alessio DA, Schwartz MW et al (1990) Evidence of cosecretion of islet amyloid polypeptide and insulin by β -cells. *Diabetes* 39:634–638
10. Verchere CB, D'Alessio DA, Palmer RD et al (1996) Islet amyloid formation associated with hyperglycemia in transgenic mice with pancreatic beta cell expression of human islet amyloid polypeptide. *Proc Natl Acad Sci U S A* 93:3492–3496
11. Masters SL, Dunne A, Subramanian SL et al (2010) Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1 β in type 2 diabetes. *Nat Immunol* 11: 897–904
12. Zraika S, Hull RL, Udayasankar J et al (2009) Oxidative stress is induced by islet amyloid formation and time-dependently mediates amyloid-induced beta cell apoptosis. *Diabetologia* 52:626–635
13. Zraika S, Aston-Mourmey K, Marek P et al (2010) Neprilysin impedes islet amyloid formation by inhibition of fibril formation rather than peptide degradation. *J Biol Chem* 285:18177–18183
14. Aston-Mourmey K, Zraika S, Udayasankar J et al (2013) Matrix metalloproteinase-9 reduces islet amyloid formation by degrading islet amyloid polypeptide. *J Biol Chem* 288:3553–3559
15. Potter KJ, Scrocchi LA, Warnock GL et al (2009) Amyloid inhibitors enhance survival of cultured human islets. *Biochim Biophys Acta* 1790:566–574
16. Hopping G, Kellock J, Barnwal RP et al (2014) Designed α -sheet peptides inhibit amyloid formation by targeting toxic oligomers. *Elife* 3:e01681
17. Eriksson O, Laughlin M, Brom M et al (2016) In vivo imaging of beta cells with radiotracers: state of the art, prospects and recommendations for development and use. *Diabetologia* 59:1340–1349
18. Li J, Karunanathan J, Pelham B, Kandeel F (2016) Imaging pancreatic islet cells by positron emission tomography. *World J Radiol* 8:764–774

19. Frey KA, Koeppe RA (2016) PET amyloid analyses. *J Nucl Med* 57:1168–1169
20. Selvaraju RK, Velikyan I, Johansson L et al (2013) In vivo imaging of the glucagonlike peptide 1 receptor in the pancreas with 68Ga-labeled DO3A-exendin-4. *J Nucl Med* 54:1458–1463
21. Normandin MD, Petersen KF, Ding Y-S et al (2012) In vivo imaging of endogenous pancreatic β -cell mass in healthy and type 1 diabetic subjects using 18F-fluoropropyl-dihydrotetrabenazine and PET. *J Nucl Med* 53:908–916
22. Guo T, Brendel M, Grimmer T, Rominger A, Yakushev I (2016) Predicting regional pattern of longitudinal beta-amyloid accumulation by baseline positron emission tomography. *J Nucl Med* 58:639–645
23. Insel PS, Mattsson N, Mackin RS et al (2016) Accelerating rates of cognitive decline and imaging markers associated with β -amyloid pathology. *Neurology* 86:1887–1896
24. Nakamura T, Iwata A, Ueda K, Namiki C (2016) Clinical implications and appropriate use of amyloid imaging with florbetapir (^{18}F) in diagnosis of patients with Alzheimer disease. *Brain Nerve* 68:1215–1222
25. Kaye R, Head E, Thompson JL et al (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300:486–489
26. Yoshimura M, Ono M, Watanabe H, Kimura H, Saji H (2014) Feasibility of amylin imaging in pancreatic islets with β -amyloid imaging probes. *Sci Rep* 4:6155
27. Yoshimura M, Ono M, Watanabe H, Kimura H, Saji H (2016) Development of $^{99\text{m}}\text{Tc}$ -labeled pyridyl benzofuran derivatives to detect pancreatic amylin in islet amyloid model mice. *Bioconjug Chem* 27:1532–1539
28. Romano M, Buratti E (2013) Florbetapir F 18 for brain imaging of β -amyloid plaques. *Drugs Today (Barc)* 49:181–193
29. Yang L, Rieves D, Ganley C (2012) Brain amyloid imaging—FDA approval of florbetapir F18 injection. *N Engl J Med* 367:885–887
30. Hull RL, Andrikopoulos S, Verchere CB et al (2003) Increased dietary fat promotes islet amyloid formation and beta-cell secretory dysfunction in a transgenic mouse model of islet amyloid. *Diabetes* 52:372–379
31. Meier DT, Morcos M, Samarasekera T, Zraika S, Hull RL, Kahn SE (2014) Islet amyloid formation is an important determinant for inducing islet inflammation in high-fat-fed human IAPP transgenic mice. *Diabetologia* 57:1884–1888
32. Choi SR, Golding G, Zhuang Z et al (2009) Preclinical properties of 18F-AV-45: a PET agent for A β plaques in the brain. *J Nucl Med* 50:1887–1894
33. Klunk WE, Wang Y, Huang G et al (2003) The binding of 2-(4'-methylaminophenyl)benzothiazole to postmortem brain homogenates is dominated by the amyloid component. *J Neurosci* 23:2086–2092
34. D'Alessio DA, Verchere CB, Kahn SE et al (1994) Pancreatic expression and secretion of human islet amyloid polypeptide in a transgenic mouse. *Diabetes* 43:1457–1461
35. Trembath L, Newell M, Devous MD (2015) Technical considerations in brain amyloid PET imaging with 18F-florbetapir. *J Nucl Med Technol* 43:175–184
36. Hull RL, Shen Z-P, Watts MR et al (2005) Long-term treatment with rosiglitazone and metformin reduces the extent of, but does not prevent, islet amyloid deposition in mice expressing the gene for human islet amyloid polypeptide. *Diabetes* 54:2235–2244
37. Hull RL, Willard JR, Struck MD et al (2017) High fat feeding unmasks variable insulin responses in male C57BL/6 mouse substrains. *J Endocrinol* 233:53–64
38. Prentki M, Nolan CJ (2006) Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116:1802–1812
39. Rhodes CJ (2005) Type 2 diabetes—a matter of β -cell life and death? *Science* 307:380–384
40. Guardado-Mendoza R, Davalli AM, Chavez AO et al (2009) Pancreatic islet amyloidosis, β -cell apoptosis, and α -cell proliferation are determinants of islet remodeling in type-2 diabetic baboons. *Proc Natl Acad Sci* 106:13992–13997
41. Guardado-Mendoza R, Perego C, Finzi G et al (2015) Delta cell death in the islet of Langerhans and the progression from normal glucose tolerance to type 2 diabetes in non-human primates (baboon, *Papio hamadryas*). *Diabetologia* 58:1814–1826
42. Kahn SE, Haffner SM, Heise MA et al (2006) Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 355:2427–2443
43. Kahn SE, Lachin JM, Zinman B et al (2011) Effects of rosiglitazone, glyburide, and metformin on beta-cell function and insulin sensitivity in ADOPT. *Diabetes* 60:1552–1560
44. Aston-Mourney K, Subramanian SL, Zraika S et al (2013) One year of sitagliptin treatment protects against islet amyloid-associated β -cell loss and does not induce pancreatitis or pancreatic neoplasia in mice. *Am J Physiol Endocrinol Metab* 305:E475–E484
45. Nemmi F, Saint-Aubert L, Adel D et al (2014) Insight on AV-45 binding in white and grey matter from histogram analysis: a study on early Alzheimer's disease patients and healthy subjects. *Eur J Nucl Med Mol Imaging* 41:1408–1418
46. Maclean N, Ogilvie RF (1955) Quantitative estimation of the pancreatic islet tissue in diabetic subjects. *Diabetes* 4:367–376
47. Hanley SC, Austin E, Assouline-Thomas B et al (2010) β -Cell mass dynamics and islet cell plasticity in human type 2 diabetes. *Endocrinology* 151:1462–1472
48. Westermark P (1972) Quantitative studies on amyloid in the islets of Langerhans. *Ups J Med Sci* 77:91–94
49. Clark A, Wells CA, Buley ID et al (1988) Islet amyloid, increased α -cells, reduced β -cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. *Diabetes Res* 9:151–159