Title: The Positron Emission Tomography ligand \([^{11}C]5\)-Hydroxy-Tryptophan can be used as a surrogate marker for the human endocrine pancreas

Short title: A surrogate PET marker for the endocrine pancreas

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**Abstract:** In humans a well-developed serotonin system is localized to the pancreatic islets while being absent in exocrine pancreas. Assessment of pancreatic serotonin biosynthesis could therefore be used to estimate the human endocrine pancreas. Proof of concept was tested in a prospective clinical trial by comparisons of type 1 diabetic (T1D) patients, with extensive reduction of beta cells, with healthy volunteers (HV).

C-peptide negative (i.e. insulin-deficient) T1D subjects (n=10) and HV (n=9) underwent dynamic Positron Emission Tomography with the radiolabeled serotonin precursor $[^{11}\text{C}]5$-Hydroxy-Tryptophan ($[^{11}\text{C}]5$-HTP).

A significant accumulation of $[^{11}\text{C}]5$-HTP was obtained in the pancreas of the HV, with large inter-individual variation. A substantial and highly significant reduction (66%) in the pancreatic uptake of $[^{11}\text{C}]5$-HTP in T1D subjects was observed, and this was most evident in the corpus and caudal regions of the pancreas where beta-cells normally are the major constituent of the islets.

$[^{11}\text{C}]5$-HTP retention in the pancreas was reduced in T1D compared to non-diabetic subjects. Accumulation of $[^{11}\text{C}]5$-HTP in the pancreas of both HV and subjects with T1D were in agreement with previously reported morphological observations on the beta cell volume implying that $[^{11}\text{C}]5$-HTP retention is a useful non-invasive surrogate marker for the human endocrine pancreas.
Introduction

The ability to repeatedly quantify the amount of remaining beta-cells by non-invasive methods would be of significant importance to obtain further insights of the pathophysiology of diabetes, as well as a tool to evaluate new therapies aiming to prevent beta cell loss.

Significant efforts have been made in order to develop such a method (1-5), but so far no clinically validated technique has been described.

Despite originating from different germinal layers, pancreatic islet cells share many common developmental features with neurons, especially serotonin-producing neurons in the hindbrain (6-7). As shown in mice, a common transcriptional cascade drives the differentiation of beta-cells and serotonergic neurons, which imparts the shared ability to produce serotonin (8).

Several studies in mice and guinea pigs have also demonstrated the localization of serotonin to the islets of Langerhans, particularly to the granules or microvesicles of the beta cells (8-11). In humans similar to mice, guinea-pigs, cats and dogs the serotonin system is restricted to islets with no expression in the exocrine pancreas (12). In mice, pancreatic serotonin biosynthesis has recently been implicated in insulin secretion (13) and beta cell proliferation during pregnancy (14).

$[^{11}]$C$[^5]$Hydroxy-tryptophan ($[^{11}]$C$[^5]$HTP) was originally developed as a positron emission tomography (PET) tracer for assessing the rate of serotonin biosynthesis in health and disease (15), and is currently in clinical use for localization of neuroendocrine tumors (NETs) including insulinomas (16-17). $[^{11}]$C$[^5]$HTP is readily taken up by both endocrine and exocrine tissue by the Large Amine Transporters (LATs) present in most cell types. However, $[^{11}]$C$[^5]$HTP rapidly exit the cell by the same mechanism unless it is further metabolized. This is evident in the pancreas of non human primate, where rapid washout ensues within minutes when the enzyme Dopa Decarboxylase (DDC, which converts 5-HTP into serotonin) is inhibited (18) as well as in streptozotocin diabetic rats. Hence, retention of $[^{11}]$C$[^5]$HTP
demands the presence of the entire molecular machinery involved in serotonin synthesis present in the islet cells, but not in the exocrine cells (having only LATs). \[^{11}\text{C}]5\text{-HTP will after decarboxylation share the fate of endogenous serotonin, including uptake into secretory vesicles by the Vesicular Monamine Transporter 2 (VMAT2) or biodegradation by Monoamine Oxidase-A (MAO-A) into 5-Hydroxyindoleacetic acid (HIAA), which is excreted into urine (15; 18-21). Radiolabeled 5-HTP therefore accumulates in the form of serotonin in islets of Langerhans as opposed to the exocrine pancreatic parenchyma (9; 22). In vitro studies of \[^{11}\text{C}]5\text{-HTP show high selective retention (8-55 times) in human islets compared to exocrine cells (18). The selectivity in vivo is likely a magnitude higher, due to a small remaining number of islets in density purified exocrine preparations examined in vitro as well as to a significantly higher blood perfusion in islets (10 times that of acinar pancreas (23)) resulting in proportionally higher tracer delivery. In mice, a similar uptake was found in both alpha- and beta-cells, but the retention in beta-cells was markedly higher resulting in an increased relative accumulation in beta-cells 60 minutes after intravenous administration with 75% of the tracer found within the beta cells (9). These findings in mice have been corroborated by us in non-human primates (18) and humans (24) by the observations that the pancreatic \[^{11}\text{C}]5\text{-HTP uptake is almost exclusively through serotonergic biosynthesis mediated by DDC. Also, in studies of human islets mixed with exocrine tissue in different ratios, a linear correlation between \[^{11}\text{C}]5\text{-HTP uptake and the percentage of islets present was obtained (18). Importantly, the pancreatic uptake of \[^{11}\text{C}]5\text{-HTP in streptozotocin-diabetic rats, with only 10-20% beta cells remaining as assessed by morphometric studies, was reduced by 66% when compared to non-diabetic animals (18). We therefore hypothesized that pancreatic \[^{11}\text{C}]5\text{-HTP uptake could serve as a non-invasive in vivo surrogate marker for the amount of remaining pancreatic endocrine cells in humans.}
Initially, we performed a retrospective study in non-diabetic subjects and subjects with type 2 diabetes (T2D) who were examined by \(^{11}\text{C}\)5-HTP due to NET.

Based on the encouraging results obtained a prospective PET/CT study measuring pancreatic uptake of \(^{11}\text{C}\)5-HTP in healthy volunteers (HV) and C-peptide negative individuals with type 1 diabetes (T1D) was initiated.

**Materials and Methods**

*Retrospective study of subjects with T2D and non-diabetic subjects with NETs*

\(^{11}\text{C}\)5-HTP is regularly used for localization or staging of NETs. The routine procedure is to examine the patient by static whole-body PET/CT (Discovery ST, GE Healthcare, Milwaukee, USA) 25 minutes following an intravenous administration of 2-5 MBq/kg \(^{11}\text{C}\)5-HTP. Patients are given 100-200mg carbidopa orally 1 hour prior to tracer administration to decrease renal and urinary radioactivity concentration of tracer metabolites.

In a retrospective study, we analyzed the pancreatic uptake in ten patients who were examined by \(^{11}\text{C}\)5-HTP-PET/CT because of known or suspected NETs. The inclusion criterion was >2 examination by \(^{11}\text{C}\)5-HTP-PET/CT between January 2010 and July 2011. The pancreatic uptake of \(^{11}\text{C}\)5-HTP was measured by delineating the pancreas on sequential co-registered CT images by using the computer software PMOD (PMOD Technologies Ltd., Zurich, Switzerland). The pancreatic uptake was expressed as the Standardized Uptake Value (SUV), which is the concentration of \(^{11}\text{C}\)5-HTP in tissue, normalized for subject weight and administered radioactivity.

Eight of the subjects were considered as having a normal pancreas, and were diagnosed with carcinoids (n=5), carcinoma (n=1) or NET of unknown origin (n=2) (Table 1). The remaining two subjects (gastrinoma n=1 and carcinoid n=1) had been previously diagnosed by T2D prior to being examined for suspected NET.
Prospective study of T1D and healthy subjects

Research subjects

This study was approved by the regional ethical board in Uppsala (EPN 2011/439). All study participants provided written informed consent before participation in any experiments. The studies were conducted according to the principles expressed in the Declaration of Helsinki.

T1D subjects (n=10) were recruited from the diabetes center at Uppsala University Hospital. Inclusion criteria were >10 years history of T1D, and fasting C-peptide concentration <0.003 nmol/l (lower range for detection with hospital standard assay). Two of the T1D subjects have received a whole-pancreas transplant; both have lost the graft function and require the same amounts of exogenous insulin as prior to transplantation.

HV (n=9) without any medications and first-degree relatives with T1D were recruited by advertising.

Beta-cell function

All HV undertook a mixed meal tolerance test (MMTT) in order to exclude subclinical impairment of glucose metabolism. All examinations took place after overnight fasting and at bed rest. Fasting plasma samples for HbA1C, plasma glucose (P-glucose) and C-peptide concentrations were taken prior to the test. Following oral administration of 360 ml Resource protein Novartis®, P-glucose and c-peptide concentrations were measured after 15, 30, 60, 90 and 120 minutes.

PET examination

Prior to the PET examination all participants were fasting for more than 4 hours, and insulin doses to T1D patients were modified accordingly. During the PET-examination P-glucose levels were measured at three times and targeted between 3 and 12 mmol/l (mean 8.1 ± 0.62
mmol/l). In one subject P-glucose dropped to 3.6 mmol/l during the examination and a slow infusion of glucose was started in order to prevent hypoglycaemia.

The subjects were positioned to include pancreas in the center of the 15cm axial field of view of a Discovery ST PET/CT scanner (GE Healthcare, Milwaukee, MI, USA) by assistance of a low dose CT scout view (140 kV, 10 mAs). Attenuation correction was acquired by a 140 kV, Auto mA 10-80 mA CT examination. Prior to administration of $^{11}$C-5-HTP a 6 minute dynamic PET examination with oxygen15 labeled water ($^{15}$O WAT) was performed in order to assess pancreatic perfusion. Thereafter, 2-5 MBq/kg $^{11}$C-5-HTP was administered intravenously and subjects underwent a dynamic PET protocol for 60 min (33 frames; 12x10s, 6x30s, 5x120s, 5x300s, 5x600s).

Image acquisition was performed in 3D and reconstructed using an iterative OSEM VUEPOINT algorithm (2 iterations/21subsets) in a 128 x 128 matrix, zoom 50 cm diameter). Reconstructed data were analyzed using the VOIager 4.0.7 computer software (GE Healthcare, Uppsala, Sweden). Regions of Interest (ROIs) were delineated on transaxial CT slices. Entire organs were delineated on sequential slices and combined into VOIs. Pancreatic volumes were assessed by applying a (VOI) with VOIager on the CT images.

CT VOIs were transferred to $^{11}$C-5-HTP-PET images and modified (due to breathing motion) to match the pancreatic signal within the existing CT VOI. Perfusion was assessed by a one-tissue compartment model from dynamic pancreatic WAT-PET data, using an aortic VOI as the input function (4 pixels in 10 consecutive slices to avoid partial volume effects) (25). Pancreas was further subdivided into its three main components: caput, corpus and cauda. The border for cauda was applied at the medial border of the left kidney and caput was defined as left lateral of the superior mesenteric artery. Mean VOI values for each timeframe were used to calculate percentage of injected dose (%ID) in each pancreatic region and for the entire pancreas. To assess background uptake of $^{11}$C-5-HTP, VOIs were applied to muscle tissue
(erector spinae). In each individual, the muscle VOI volume was identical to that of the pancreas.

Statistical analysis

All data were expressed as means±SD. Difference between groups were assessed by a two-tailed student’s t-test (GraphPad Prism 5, San Diego, CA, USA) where \( p<0.05 \) was considered significant. Normal distribution was confirmed by the D'Agostino & Pearson omnibus normality test.

Results

Retrospective study of subjects with T2D and non-diabetic subjects with NETs

Ten patients underwent repeated (n=2-8) \([^{11}C]5\)-HTP-PET/CT during the period 1999-2008 for staging or detection of recurrent NET (Table 1). None of the non-diabetic patients had reported hyperglycemia or diabetes. A whole-body (proximal thighs to skull base) PET/CT was performed 25 minutes after tracer administration. Pancreas was readily visible on PET/CT in all patients (Figure 1A-B). The uptake in pancreas was high compared to that of other abdominal tissues, but varied between patients (%ID/g= 0.0048±0.001, Figure 1E). The within subject variation between examinations was low (Coefficient of Variation, CV, in this group was 6.7%, Figure 1F), demonstrating a high test-retest reproducibility.

Importantly, we were also able to identify two subjects (T2D:a and T2D:b) with T2D among the patients with NET. The uptake of \([^{11}C]5\)-HTP in the pancreas of these subjects was markedly lower (%ID/g =0.0016±0.0005) than that observed in non-diabetic subjects (Figure 1C-D and E). No overlapping values between the two groups were found. One of the individuals with diabetes (T2D:a) was followed over time with repeated \([^{11}C]5\)-HTP-PET/CT examinations (Figure 1G). T2D was diagnosed in May 2008 (month 0), and subsequent \([^{11}C]5\)-HTP-PET/CT examinations (month 1, 16, 24 and 30) show a substantial and
progressive decrease of tracer uptake in the pancreas over time. In December 2008 (month 7) patient T2D started medication with glimepiride. However, her diabetes progressed and in September 2009 (month 15) exogenous insulin treatment was initiated.

**Prospective study of T1D and healthy subjects**

**Research subjects**

The mean HbA1C in T1D subjects was 7.2 ± 0.2 % (55 ± 2 mmol/mol) as compared to 5.3 ± 0.1 % (34 ± 0.7 mmol/mol) in HV (p<0.05). There were no significant differences in age (26 ± 2 vs. 22 ± 0.6 years), BMI (23 ± 0.7 vs. 25 ± 1 kg/m²) or gender distribution (5/10 vs. 4/9 female). The HV had a normal fasting- and 2-hour P-glucose (5.5 ± 0.1 resp. 5.4 ± 0.2 mmol/l).

**[^11]C-5-HTP uptake and retention in the pancreas**

During the initial minute(s) after[^11]C-5-HTP administration we measured high uptake in kidney cortex, pancreas and spleen, reflecting the rich perfusion of these tissues, which was paralleled by intense tracer concentration in the aorta (Figure 2, left panels). After 1-10 minutes, we observed retention of the tracer in the pancreas of HV, but not in the non-endocrine tissues kidney cortex and spleen, or in the pancreas of T1D patients (Figure 2, middle panels). Finally, up to 60 minutes after[^11]C-5-HTP administration, we recorded that the uptake had faded in most abdominal tissues besides the pancreas of HV and the kidney medulla (Figure 2, right panels). The latter likely represented the rapid excretion of the metabolite[^11]C-HIAA.

When quantified, the total pancreatic uptake of[^11]C-5-HTP, measured as percentage of injected dose (%ID), was markedly reduced throughout the dynamic scan in T1D subjects (Figure 3A). The reduction was most prominent at the end of the examination, with a mean decrease of 66% (p<0.001) in[^11]C-5-HTP uptake in T1D subjects when compared to HV (Figure 3B). There was no difference in the amount of un-metabolized[^11]C-5-HTP in blood.
plasma between groups, except for a tendency for higher initial plasma concentration in subjects with T1D (Figure 4A).

When subdividing the pancreas into its three anatomical regions, we observed that the reduced $^{11}$C-5-HTP uptake in the pancreas of T1D patients was mainly due to lower uptake in the corpus (Figure 3D, 68% decrease, p<0.001) and cauda (Figure 3E, 70% decrease, p<0.01), and to a lesser extent to decreased uptake in the caput region (Figure 3C, 60% decrease, p<0.01).

When correcting for the pancreatic volume in each individual (%ID/g), i.e. tracer accumulation per g pancreas, we found that the decreased pancreatic tracer accumulation in T1D patients persisted (0.0028±0.001 %ID/g in T1D compared to 0.0046±0.001 %ID/g in HV corresponding to a 39% decrease 60 min after tracer administration, p<0.05). Again, tracer accumulation was reduced in the corpus (41%, p<0.01) and caudal parts of the pancreas (47%, p<0.01), and to a lesser extent in the caput part (34%, p<0.05). The differences between groups in %ID/g were seen only between 40-60 minutes post administration (Figure 4B).

*Size and perfusion of the healthy and diabetic pancreas*

Pancreatic volumes assessed by CT scans were on average 89.4±23.5 cc in HV and 54.9±14.6 in T1D subjects, corresponding to 61% of HV volume (Figure 5A, p<0.01). There was a tendency towards reduced pancreatic blood flow (ml blood/min/ml tissue) (p=0.068) in the T1D group (28% reduction compared to HV, Figure 5B).

**Discussion**

In this clinical study, we present evidence that $^{11}$C-5-HTP can be used as an imaging biomarker for the native endocrine pancreas. In the prospective clinical study accumulation of the tracer was assayed 60 min after injection in order to preferentially target the beta cells
utilizing their prolonged retention-time of the tracer when compared with non-beta cells within the islets and lack of retention in the exocrine pancreas.

Pancreatic $[^{11}\text{C}]$5-HP accumulation in subjects with T1D was substantially reduced (mean reduction of 66%) when compared to that recorded in HV. We stress that all remaining pancreatic neuroendocrine tissue should retain $[^{11}\text{C}]$5-HP, not only beta cells. Thus, complete loss of all beta cells would only partly reduce the endocrine signal. The contribution of the beta cell mass to the mass of individual islets is still a matter of disagreement, due to differences in study design, sample size and whether number or volume contribution was measured. The volumetric contribution is likely the suitable parameter when assessing beta cell mass and can be approximated to 62% (range 46-75%) by averaging previously reported values from nine studies using human pancreatic sections (26). Thus, a complete loss of all beta cells should reduce the $[^{11}\text{C}]$5-HP signal by approximately 62%.

There are about 1.5 million islets within the human endocrine pancreas, of these the absolute majority has a diameter less than 50 µm and only 200 -300 islets have a diameter of 250 µm or more (27). With the current resolution of PET technology individual islets cannot be detected, instead the integrated signal from the pancreas (%ID) represents a composite value from the total endocrine pancreas. However, sub-analysis of the signal from different regions of the pancreas is feasible, which enabled us to compare changes in $[^{11}\text{C}]$5-HP accumulation in regions differing in their fraction of beta-cells within the islets. We recorded a more substantial decrease in $[^{11}\text{C}]$5-HP accumulation in the beta-cell rich corpus and cauda of the pancreas of T1D subjects than in the caput region where Pancreatic Polypeptide (PP) and to a lesser extent alpha cells dominate. Interestingly, in autopsy studies the total endocrine mass in C-peptide negative subjects with long-standing T1D has been reported to be about 30% of that in non-diabetic controls (28). Noteworthy, similar to our observations by $[^{11}\text{C}]$5-HP
PET, no changes in endocrine density were found in the ventral PP cell rich segment of the pancreas (28).

The total alpha and beta cell mass in non-diabetic subjects has been described in carefully conducted morphometric studies and show a surprisingly high inter-individual variation from 0.35 to 2.4 gram, with a strong correlation between the alpha (0.1 – 1 gram) and beta cell (0.25-1.5 gram) masses (29). The pancreatic $[^{11}\text{C}]$5-HTP accumulations of the HV in the present study are in good agreement with previously reported morphological findings, with a large inter-individual variation in total pancreatic uptake of $[^{11}\text{C}]$5-HTP (0.2 to 0.7 %ID). The retrospective study, based on an analysis of static $[^{11}\text{C}]$5-HTP images of NET patients, should be interpreted with caution due to the lack of tracer pharmacokinetics and information on pancreatic perfusion as well as because of the non-standardization of the investigated subjects. The high uptake of $[^{11}\text{C}]$5-HTP in NETs, which is the basis of using serotonergic biosynthesis as a diagnostic biomarker, may also increase the endogenous levels of 5-HTP or serotonin in plasma. This could potentially induce changes in uptake of $[^{11}\text{C}]$5-HTP in pancreatic endocrine tissue due to competition for DDC.

Therefore, a prospective study in subjects with T2D, repeatedly examined over a period of several years, will be required to confirm these initial results. However, NET patients diagnosed with T2D in the herein reported retrospective study had markedly low pancreatic accumulation of $[^{11}\text{C}]$5-HTP. A progressive loss of $[^{11}\text{C}]$5-HTP uptake in the pancreas was observed in one patient (T2D:a), who was repeatedly examined during a 2 year period. In is not known how the chemotherapy, including STZ, in combination with glimepiride and concurrent beta cell exhaustion may affect the beta cells and their uptake and retention of $[^{11}\text{C}]$5-HTP, in subjects with T2D. At the time of the PET examination, none of the other retrospectively investigated subjects were diabetic. These subjects could potentially have been
pre-diabetic and to have later developed T2D. Unfortunately, this information was unavailable.

It would be of interest to compare the pancreatic uptake in T1D and T2D subjects, but several factors make it difficult to draw conclusions from a direct comparison between the retrospective and the prospective study. Firstly, the %ID/g values are reported at different time points (T2D; 25 minutes post tracer admin, and T1D; 60 minutes post admin) and secondly the T2D and non-diabetic subjects in the retrospective study received carbidopa 1h prior to $^{11}$C-5-HTP which also affects the pancreatic uptake. It is therefore possible to compare between groups within each study as they were treated similarly, but not between the studies.

Di Gialleonardo et al. recently showed that absolute uptake of $^{11}$C-5-HTP in non-endocrine PANC1 cells was comparable to that of INS-1 cells, but not mediated by normal serotonergic metabolism (22). Based on these results, they posited that the specific endocrine signal in vivo would not be higher than the non-specific signal from exocrine pancreas. Following this line of reasoning, the pancreatic signal would therefore be non-specific in nature thereby reducing the possibilities of visualizing the specific uptake, mediated by serotonin biosynthesis, in endocrine pancreas.

Thus, the conclusion by Di Gialleonardo et al. should, as pointed out by the authors, be interpreted with caution since it relies on in vitro observations in cell-lines. By selective inhibition of DDC in non-human primate, as well as in STZ diabetic rats, we have shown that the entire $^{11}$C-5-HTP uptake in pancreas is specific for serotonergic biosynthesis and thus consists of $^{11}$C-serotonin or its metabolites (18). Based on these preclinical studies, as well as on the herein presented clinical studies showing a correlation between plasma C-peptide and
pancreatic uptake of $[^{11}\text{C}]$5-HTP, we conclude that in humans the non-specific uptake in vivo is negligible and washed out within minutes after injection of the tracer.

Patients with T1D are known to have a marked reduction in the total exocrine volume, concomitant with a decrease in the exocrine functional capacity (30-31). Also in this study, we observed a mean substantial reduction of the total pancreatic volume in T1D patients. However, this measurement could not discriminate between non-diabetic and diabetic subjects due to a large overlap between the two groups (Figure 5A and 6A). Moreover, the uptake of $[^{11}\text{C}]$5-HTP/gram pancreas was significantly decreased in subjects with T1D when compared with that in HV, which excludes mere non-specific uptake of the tracer in exocrine pancreas to explain the observed differences between HV and T1D patients.

Pancreatic blood flow comprises a sum of the blood perfusion of the exocrine and endocrine parts. Rodent studies show that the islet blood flow normally constitutes about 10 % of the total pancreatic blood flow even though the islet tissue comprises only 1-2% of the total pancreatic volume (23). In humans, no techniques are available for specific studies of islet blood perfusion, but the perfusion of the whole pancreas in HV and T1D subjects as measured by magnetic resonance perfusion imaging did not differ (32). In the present study, a tendency to a decrease in mean pancreatic blood flow by 20% was recorded. There was no clear correlation ($p=0.28$) between the uptake of $[^{11}\text{C}]$5-HTP and the blood perfusion as measured by $[^{15}\text{O}]$WAT (Figure 6B). There was an apparent increase in initial tracer delivery in non-diabetic subjects during the first minutes. This could be considered as a flow-dependent effect and would govern the subsequent difference in the uptake over the course of the examination. However, in this case this was likely largely dependent on pancreatic size rather than perfusion, since no difference in initial delivery remained when correcting for pancreatic volume (Figure 4B).
The lack of a correlation between [\(^{11}\)C]5-HTP accumulation and blood flow does not rule out that endocrine blood flow may be partly or entirely responsible for the differences seen between non-diabetic and diabetic subjects. PET studies in primates show that the pancreatic uptake of [\(^{11}\)C]5-HTP is an active process which can be enhanced or diminished by pharmaceutical intervention, i.e. carbidopa or clorgyline (18), but it is unclear if these results are directly translatable to humans. Full quantification by compartmental models using the metabolite corrected arterial plasma input functions are required to answer this question, which is important for defining the potential and limitation of the use of [\(^{11}\)C]5-HTP in PET studies of the endocrine pancreas.

VMAT2, which mediates serotonin uptake into secretory vesicles, has been identified as a tissue-restricted transcript in human islets and suggested as a suitable target for imaging of beta-cells (33). PET studies using radiolabeled dihydrotetabenazine (DTBZ), a ligand for VMAT2, have been conducted in a range of in vitro and in vivo studies. While DTBZ has many attributes that suggest that it may provide a suitable probe for PET imaging, recent published communications raise severe questions concerning the specificity of this probe in vitro and in vivo (34-37) and conclude that the pancreatic uptake of DTBZ is mainly due to nonspecific binding in the exocrine pancreas. In line with these observations the signal response in subjects with manifest T1D was reduced with only 14% of the pancreatic signal in healthy volunteers (2). Significant overlap between subjects with T1D and HV was also found in a recent study both when measuring radiotracer concentration and total uptake in the pancreas (1). Importantly, these studies did not correct for the impact of reduced blood flow to the diabetic pancreas, which should reduce pancreatic accumulation of a perfusion dependent tracer in subjects with T1D. Our results demonstrate that the reduction of blood perfusion in T1D approaches 30%, which mirror the decrease of DTBZ uptake found in subjects with
manifest T1D in the aforementioned studies (1-2). Notably, VMAT2 may still constitute a suitable target for beta cell imaging but DTBZ in its current form has not been shown to be a suitable PET-tracer to quantify the BCM (38).

The ability to measure the amount of remaining beta cells by non-invasive methods such as $[^{11}C]5$-HTP PET will most likely have no role in the diagnosis of T1D. However, it would be of immense value for our understanding of the pathophysiology of both T1D and T2D and as a tool for evaluation of new therapies aiming to prevent or reverse beta cell loss or to regenerate or replace beta cells. As indicated in the retrospective study, optimal utilization of the herein presented $[^{11}C]5$-HTP PET technology will most likely entail repeated examinations within the same subject, which should enable detection of even small changes in the endocrine pancreas when considering its low CV for within subject variation. The PET technology can be combined with either CT or magnetic resonance imaging (MRI) to provide an anatomical correlate to the PET signal. We have developed a noise-reduction filter based on Principal Component Analysis technology, which allows for reducing the radioactivity dose of the PET tracer by a factor of 4 without compromising spatial resolution or quantification (38). The use of radioisotopes with short half-lives and optimal data acquisition using PET-MRI instead of PET-CT allows further reduction of the total radiation dose to the extent that repeated examinations can be frequently performed (3-4 times annually) in the same subject and also allow for examinations of children. The results from the herein presented prospective and retrospective clinical studies show that $[^{11}C]5$-HTP constitutes a promising PET tracer for quantitative imaging of the human endocrine pancreas that readily can discriminate between non-diabetic and diabetic subjects. The dependency on blood flow on tracer uptake will require further studies to define the potential and limitations of the use of $[^{11}C]5$-HTP in this setting.
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O.E. researched data and wrote the manuscript. D.E. researched data and wrote the manuscript. RK.S. researched data and reviewed/edited the manuscript. E.J. researched data and reviewed/edited the manuscript. G.A. contributed to discussion and reviewed/edited the manuscript. J.S. contributed to discussion and reviewed/edited the manuscript. M.L. researched data and reviewed/edited the manuscript. A.B. researched data and reviewed/edited the manuscript. JW. E. contributed to discussion and reviewed/edited the manuscript. A.S. contributed to discussion and reviewed/edited the manuscript. H.A. researched data, contributed to discussion and reviewed/edited the manuscript. B.E. researched data, contributed to discussion and reviewed/edited the manuscript. L.J. contributed to discussion and reviewed/edited the manuscript. P-O.C. researched data and wrote the manuscript. OK researched data and wrote the manuscript.

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Figure Legends

Figure 1. Retrospective analysis of uptake of $[^{11}C]5$-HTP in non-diabetic subjects and subjects with T2D (A-D) $[^{11}C]5$-HTP-PET maximum intensity projection (MIP) images (frontal views) showing that the pancreas is clearly visualized (encircled) in representative non-diabetic patients (A (C3), B (C8)), whereas the pancreatic uptake is lower in two patients with T2D (C (T2D:a), D (T2D:b)). K and B in images denote kidneys and bladder, respectively. Subjects were examined 25 minutes post tracer administration (E) Semi-quantitative analysis of $[^{11}C]5$-HTP-PET/CT show substantial pancreatic tracer accumulation in non-diabetic subjects when compared with the two subjects with T2D. (F) The test-retest reproducibility within non-diabetic subjects as measured as Coefficient of Variation (CV) was 6.4±1.7% (range 1.5-14.9%). (G) Repeated $[^{11}C]5$-HTP-PET/CT scans in patient T2D:a developing T2D over a period of 2.5 years show a decrease in $[^{11}C]5$-HTP uptake in the pancreas (expressed as SUV) in parallel with progression of the disease. The third examination, 24 months post diagnosis of T2D, is the same one as shown in panels C and E.

Figure 2. Prospective study on $[^{11}C]5$-HTP uptake in the human endocrine pancreas. (A) Transaxial images of $[^{11}C]5$-HTP uptake in pancreas (white arrows) in HV and T1D subjects. Initial high uptake in both HV and T1D pancreas during the perfusion/uptake by LAT phase (0-1min, left panels). The difference in pancreatic retention of $[^{11}C]5$-HTP between the two individuals is seen already between 1-10 minutes (middle panels), but is more pronounced 60 minutes after tracer administration (right panels). A and K denote aorta and kidneys respectively.

Figure 3. Prospective study on $[^{11}C]5$-HTP uptake in the different anatomical regions of human endocrine pancreas (A) The caput, the corpus and the cauda was delineated
separately in CT images to assess regional differences in pancreatic uptake. The red lines
denote the approximate boundary between caput (left arrow), corpus (middle arrow) and
cauda (right arrow) (B) Uptake and retention of $[^{11}C]5$-HTP in pancreas over time as
measured by %ID. There is a marked decrease in retention in subjects with T1D, which
approaches background levels after 60 minutes. The background uptake in muscle is
presented as a dotted line in each panel. (C) Pancreatic uptake at individual level after 60
minutes. %ID is reduced in corpus (68%, E) and cauda (70%, F). The caput is decreased to a
lesser extent (60%, D) and exhibit significant overlap between the T1D and HV groups. The
two subjects with T1D who received a pancreas graft are shown as circles.

**Figure 4. Input functions for the prospective study and pancreatic uptake corrected for
volume** (A) Input functions based on un-metabolized $[^{11}C]5$-HTP in arterial blood plasma
normalized for tracer concentration in blood, subject weight and administered radioactivity.
There are no differences between non-diabetic subjects and subjects with T1D. (B) Pancreatic
uptake and retention of $[^{11}C]5$-HTP corrected for pancreatic volume. There is lower retention
in subject with T1D 35-60 minutes post administration of $[^{11}C]5$-HTP.

**Figure 5. Impact of the diabetic condition on pancreatic size and perfusion** (A) Pancreas
volume is decreased in subjects with T1D (61% of HV, p<0.01). However, pancreatic size
alone cannot discriminate between T1D and HV on an individual level due to significant
overlap between groups. (B) Pancreatic blood flow as assessed by $[^{15}O]WAT$ has a tendency
to decrease in the T1D group (28% reduction compared to HV, p=0.068).

**Figure 6. Uptake of $[^{11}C]5$-HTP in the human pancreas is not dependent on perfusion**
(A) Correlation between pancreatic size and %ID in all individuals. %ID complete separates
between the TID and HV groups, while there is a substantial overlap in pancreatic volume despite the difference at group level. (B) There is no correlation between the perfusion of pancreas and the uptake of $[^{11}\text{C}]$5-HTP as measured by %ID.
Table 1. Data on 10 patients referred for 2 or more HTP-PET/CT examinations.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Primary tumor</th>
<th>Location</th>
<th>Metastasis</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>C1</td>
<td>F</td>
<td>37</td>
<td>64</td>
<td>Carcinoid, atypical</td>
<td>Lung</td>
<td>-</td>
<td>Resection of lower left lobe.</td>
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<td>NET</td>
<td>Neck</td>
<td>Skin</td>
<td>Surgery of lymph nodes.</td>
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<tr>
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<td>F</td>
<td>23</td>
<td>46</td>
<td>Medullary Thyroid Carcinoma</td>
<td>Thyroid</td>
<td>-</td>
<td>Thyroidectomy and bilateral lymph node dissection.</td>
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<td>M</td>
<td>22</td>
<td>93</td>
<td>Carcinoid, atypical</td>
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<td>-</td>
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<tr>
<td>C6</td>
<td>F</td>
<td>17</td>
<td>54</td>
<td>NET</td>
<td>Appendix</td>
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<td>Right hemicolecctomy.</td>
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<td>F</td>
<td>71</td>
<td>57</td>
<td>NET</td>
<td>Rectum</td>
<td>-</td>
<td>Preoperative radiation therapy, then surgery (amputation of rectum).</td>
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<tr>
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<td>64</td>
<td>NET</td>
<td>Unknown</td>
<td>Lymph nodes</td>
<td>Surgery of lymph nodes (neck and abdomen), chemotherapy with temozolomide.</td>
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<tr>
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<td>F</td>
<td>54</td>
<td>102</td>
<td>Gastrinoma</td>
<td>Duodenum</td>
<td>Lymph nodes</td>
<td>Gastrinoma removed by duodenotomy and resection of lymph node conglomerate; chemotherapy with STZ plus 5-FU; Sandostatin-LAR.</td>
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<tr>
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<td>64</td>
<td>96</td>
<td>Carcinoid, atypical</td>
<td>Thymus</td>
<td>Lymph nodes (thorax)</td>
<td>Surgery of thymic tumor plus postop radiation therapy and chemotherapy</td>
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</table>
A (C3)  B (C8)  C (T2D:a)  D (T2D:b)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>T2D</th>
<th>Examination</th>
<th>%ID/g</th>
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Input functions

Pancreas

Diabetes

**A**

- **SUV**
- **Time (min)**

**B**

- **%ID/g**
- **Time (min)**

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<td>60</td>
<td>0.015</td>
<td>0.015</td>
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</tbody>
</table>

* Indicates statistically significant differences.
A  

\[ R^2 = 0.51 \]
\[ p = 0.0014 \]

B  

\[ R^2 = 0.08 \]
\[ p = 0.28 \]